ASSESSING ENTERIC METHANE EMISSIONS IN FEEDLOT BEEF HEIFERS: EFFECTS OF SEAWEED SUPPLEMENTATION AND INDIVIDUAL VARIATION IN METHANE YIELD

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DEDICATION

For my family and friends who supported me throughout this journey.

ABSTRACT

Enteric methane (CH_4) emissions from ruminants is an important greenhouse gas (GHG) and contribute to global warming, as well as representing energy loss for the animal. Consequently, there is growing interest in developing mitigation strategies that reduce CH_4 emissions without compromising cattle health and productivity. This body of work was carried out to investigate whether dietary intervention or selecting low CH_4-producing cattle technologies may potentially reduce enteric CH_4 in feedlot systems across backgrounding (high-forage diet) and finishing (high-grain diets) phases. Initially, this study evaluated the effect of adding 0.60% dry matter (DM) of a multispecies seaweed blend (SWblend) in a total mixed ration (TMR) on growth performance, feeding behavior, ruminal fermentation, protozoa count, ruminal pH, GreenFeed (GF) system variables, and hematological parameters of feedlot heifers fed backgrounding and finishing diets. One hundred and twelve beef heifers (100 intact heifers + 12 cannulated heifers) were enrolled in a completely randomized block design with two treatments (control and SWblend) across two blocks. Supplementation with SWblend did not affect ($P > 0.05$) dry matter intake (DMI; kg/d), average daily gain (ADG; kg), feed efficiency (gain:feeding; G:F), feeding behavior patterns, or hematological indicators in either backgrounding or finishing phases. Although the seaweed blend altered ($P < 0.05$) ruminal fermentation during the finishing phase, including total volatile fatty acids (VFA) and ruminal ammonia (NH_3) concentration, it did not reduce CH_4 production (g/d), CH_4 yield (CH_4y; g CH_4/kg DMI), or CH_4 intensity (Mcal/% GEI). These results suggest that the tested multispecies seaweed blend may be safe for inclusion in feedlot diets but has limited potential to mitigate enteric CH_4 emissions under the conditions of this study. Subsequently, the 100 intact feedlot beef heifers were ranked into low (<0.5 SD from the mean), intermediate (within

±0.5 SD of the mean), or high (>0.5 SD of the mean) CH_4y groups (MYG) in each period by phase, to evaluate how MYG affects growth performance, feeding behavior, GF system visitation patterns, CH_4 emissions, and compare the rumen microbiota of heifers in low and high MYG. As treatment did not affect CH_4 emissions; treatment and pen (within block) were used as random effects. Throughout both backgrounding and finishing phases, considerable variability was observed in the GF system visitation and CH_4 emissions both within groups and by individual heifers. Low MYG had lower ($P < 0.05$) CH_4 production, CH_4y, and CH_4 intensity in both dietary phases. Heifers classified as low MYG demonstrated greater ($P < 0.05$) DMI in contrast to other MYG during both phases. There were no differences ($P \geq 0.12$) between low and high MYG in rumen microflora alpha- diversity estimated by observed ASVs or in β-diversity based on Bray-Curtis dissimilarity. However, a temporal difference ($P \leq 0.04$) between low and high MYG was found in alpha diversity of the last period (period 4) of backgrounding and generalized β-diversity based on UniFrac distance ($\alpha = 0.5$) in the last period of the finishing phase. These findings emphasize the complexity and temporal variability of CH_4 traits in feedlot systems and demonstrate the importance of evaluating CH_4 emissions across and within productive phases to identify consistently low-emitting cattle. Overall findings underscore that sustainably mitigating enteric CH_4 in beef production will likely require a combination of nutritional strategies, genetic selection, and improved measurement technologies, anchored in a deep understanding of rumen microbial ecology and animal behavior.

CONTRIBUTION OF AUTHORS

Tatiane R. Ramos is the primary author of Chapters 1-4. The candidate was responsible for designing and conducting the feedlot experiment, performing laboratorial analysis, and analyzing data for Chapters 2-3. Dr. Stephanie A. Terry contributed to the conceptualization and guidance of experimental design, formal analysis, data curation, and writing review of Chapters 1-4. Dr. Kim Stanford contributed to the guidance formal analysis, data curation, and writing review of Chapters 1-4.

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USE OF GENERATIVE AI

Microsoft Copilot AI was used to search for academic literature and aided with grammar correction and synonym suggestions.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADG	Average daily gain
CHBr3	Bromoform
BW	Body weight
CHO	Carbohydrate
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
GE	Gross energy
GEI	Gross energy intake
G:F	Gain:feeding
GF	GreenFeed
GHG	Greenhouse gas
GRAN	Granulocyte concentration
HCT	Hematocrit
HGT	Horizontal gene transfer
LYMF	Lymphocytes concentration
MCH	Mean cell hemoglobin
MCV	Mean cell volume of rbcs

MONO	Mid-sized cells
MYG	Methane yield group
NDF	Neutral detergent fibre
OM	Organic matter
PCR	Polymerase chain reaction
RBC	Total red blood cell count
RFID	Radio-frequency identification
RNA	Ribonucleic acid
SBW	Shrunk body weight
SD	Standard deviation
SWblend	Multispecies seaweed blend
TMR	Total mixed ration
VFA	Volatile fatty acids
WBC	Total white blood cell count

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Given the significant contribution of greenhouse gases (GHGs), mainly carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), to rising global temperature, numerous studies have focused on strategies to reduce anthropogenic emissions. The world-wide livestock sector accounts for 14.5% of total anthropogenic GHG emissions, with enteric CH_4 from ruminant production contributing 6.0% to this total (Beauchemin et al., 2020). While CH_4 has a shorter half-life (~12 years) in the atmosphere compared to CO_2 (~100-1000 years), its global warming potential is 28 times greater than CO_2 (IPCC, 2022), making its mitigation important to decelerate climate change. In response, an international agreement (Global Methane Pledge; https://www.globalmethanepledge.org/) among 160 countries and organizations was signed in 2021 during COP26, committing to a collective reduction of CH_4 emissions by 30% by 2030.

In line with concerns about CH_4 emission, there has been a growing emphasis on food security and ensuring an adequate supply of high-quality, nutrient-dense food. According to the United Nations (2024), the global population is projected to reach a peak of 10.3 billion in the mid-2080s. Consequently, investments in technologies that enhance productivity while minimizing environmental impacts in the dairy and beef sector are essential, as ruminants play a key role in converting plant biomass into nutrient-rich food for humans (Abbott et al., 2020). Likewise, enteric CH_4 may represent a metabolic energy loss for ruminants, ranging from 2-12% of the gross energy intake (Hoque et al., 2017). Therefore, strategies such as production systems management, genetic selection, and diet modifications can contribute to increasing the efficiency and sustainability of the livestock sector (Beauchemin et al., 2020; Terry et al., 2021).

Canada plays a significant role in global efforts to reduce agricultural GHG emissions, as livestock production is a key part of the national economy. Beef and dairy sectors together generate an estimated 24 billion CAD annually (Government of Canada, 2025a; Government of Canada, 2025b). As of July 2025, Canada had approximately 11.9 million cattle, including 10.0 million beef cattle and 1.9 million dairy cattle (Statistics Canada, 2025). In 2023, total national GHG emissions reached 694 megatons of carbon dioxide equivalents (Mt CO_2-eq), with the agricultural sector accounting for 10% of the total with livestock production being responsible for 23% of anthropogenic CH_4 emissions (ECCC, 2025). In response, the Government of Canada, through initiatives led by Agriculture and Agri-Food Canada (AAFC) and Environment and Climate Change Canada (ECCC), have developed national strategies to reduce agricultural emissions and enhance environmental sustainability. These strategies include the Agricultural Climate Solutions Program and the On-Farm Climate Action Fund, which encourage the adoption of best management practices, innovative feed additives, and carbon-smart technologies to improve feed efficiency and lower CH_4 emissions from cattle production. These efforts support the national commitment to achieving net-zero GHG emissions by 2050 from all economic sectors (ECCC, 2025).

Furthermore, the Canadian livestock industry has also increasingly adopted technological innovations to enhance productivity while substantially reducing GHG emissions, including precision nutrition with CH_4 inhibitors and feed alternatives (e.g., food processing by-products), automated manure and air management systems, precision livestock tools such as GPS and sensor technologies, and genetic selection for more resilient and lower CH_4-producing animals (Neethirajan, 2024; Ominski et al., 2021). Together, these initiatives provide a strong framework that could be expanded internationally, thereby supporting global efforts to mitigate agricultural GHG emissions while promoting sustainable livestock production systems.

A comprehensive understanding of ruminal microbial ecology and the synergistic interactions of the rumen microflora is fundamental to developing effective mechanisms to reduce enteric CH_4 emissions without compromising animal health and productivity. In this context, the objective of this literature review is to examine (i) the rumen system, ruminal microbiota, fermentation, and enteric CH_4 synthesis; (ii) current and emerging strategies to mitigate enteric CH_4 production, with a focus on seaweed supplementation, and (iii) techniques available to measure CH_4 emissions *in vivo*.

1.2 Ruminal system

Through evolution, ruminants have developed a complex digestive system that enables them to utilize nutrients from fibrous plant materials through microbial fermentation (Figure 1.1). Ruminants have a stomach divided into four compartments: rumen, reticulum, omasum, and abomasum, with the rumen functioning as an anaerobic digestion chamber, containing a diverse microbial community (Cholewińska et al., 2021; Hungate, 1975). This microbiota is composed primarily of bacteria, along with protozoa, archaea, fungi, and a smaller proportion of viruses (Huws et al., 2018). It may be influenced by factors such as breed, age, external environment, and nutrition, which are essential for maintaining the animal's homeostasis, health, and productivity (Henderson et al., 2015; Keum et al., 2024; Liu et al., 2021).

In a collaborative and synergistic network, bacteria, protozoa, and fungi break down cellulose, hemicellulose, starch, and other carbohydrates into their basic monomers. These monomers are then fermented, producing volatile fatty acids (VFAs) that are absorbed through the rumen wall and used as energy by the host. As a result of this microbial digestion, CO_2 and H_2 are produced and used by methanogenic archaea to synthesize CH_4 (Bergman, 1990; Cholewińska et al., 2021; Dijkstra et al., 2005). Methanogenesis functions as the primary H_2 sink, preventing increases in H_2 partial pressure that could hinder the normal functioning of microbial enzymes involved in electron transfer reactions. This is particularly important for NADH dehydrogenase, which might lead to an accumulation of NADH and consequently reduce rumen fermentation (Morgavi et al., 2010).

1.2.1. Ruminal microbiome composition

Bacteria

Bacteria are the most abundant microorganisms in the ruminal environment, with a population of 10^{10} to 10^{11} cells/mL of ruminal content (Perez et al., 2024). Firmicutes and Bacteroidetes phyla make up over 90% of the bacterial population, along with smaller amounts of Proteobacteria, Actinobacteria, and Tenericutes phyla (Jami & Mizrahi, 2012). Bacteria are typically classified according to their substrate preference and metabolic function (Table 1.1). Thus, specific bacteria can be classified as fibrolytic (fibre-degrading), amylolytic (starch-degrading), proteolytic (protein-degrading), lactic acid metabolizing, hydrogen-producing or consuming, and those that form syntrophic partnerships (Henderson et al., 2015; Mizrahi et al., 2021). Additionally, some bacteria can display both specific and general activity, breaking down various substrates and carrying out diverse metabolic functions (Mizrahi et al., 2021).

Fibrolytic bacteria are capable of hydrolyzing the structural polysaccharides from plant cell walls (cellulose, hemicellulose, and xylan) to release VFAs and H_2 and are important for the ruminal functional and anatomical development, helping the growth of ruminal papillae and providing energy for host cattle (Morgavi et al., 2013). Among the major fibrolytic bacteria, *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* have a strong cellulolytic capacity. Other genera, such as *Butyrivibrio*, recognized for the xylanolytic activity, and *Prevotella*, associated with starch and protein metabolism, also exhibit cellulolytic and hemicellulolytic abilities (Morgavi et al., 2013). Different than other fibrolytic bacteria, *F. succinogenes* degrades structural carbohydrates to produce succinate and formate rather than H_2 (Henderson et al., 2015). Additionally, research has shown that *F. succinogenes*, *R. flavefaciens*, and *Prevotella ruminicola* can be detected in the gastrointestinal tract of newborn calves minutes after birth, even before solid feed intake. This indicates that these populations utilize alternative substrates, possibly derived from colostrum or microbial interactions, for their early establishment (Guzman et al., 2015; Huws et al., 2015; Weimer, 2022). Fibrolytic function is not exclusive to bacteria; anaerobic fungi and some protozoa can also utilize structural carbohydrates and have an important synergistic relationship with bacteria that is essential for fibre degradation (Huws et al., 2015; Morgavi et al., 2013; Weimer, 2022). In addition, cellulolytic bacteria depend on close associations with hydrogenotrophic archaea, which use the hydrogen released during fiber degradation to produce CH_4. This syntrophic relationship prevents hydrogen accumulation, thus maintaining optimal fermentation efficiency (Morgavi et al., 2013).

Amylolytic bacteria are those specialized in the degradation and utilization of starch and soluble sugars, producing mainly propionate and lactate through the action of α-amylases and other glycoside hydrolases (Cotta, 1988; Hua et al., 2022). Within the representatives of this group, *Streptococcus bovis*, *Ruminobacter amylophilus*, and genera like *Succinivibrio, Succinimonas,* and *Olsenella* primarily utilize starch and soluble sugars to produce propionate, lactate, and succinate as end-products (Cotta, 1988). Additionally, genera such as *Prevotella* and *Butyrivibrio*, known for primarily utilizing protein, amino acids, and lipid as substrates, can also degrade starch (Henderson et al., 2015; Keum et al., 2024). These bacteria are metabolically important for the rumen ecosystem, as they efficiently compete for energy substrates, influence rumen pH, and modulate fermentative dynamics related to feed efficiency (Russell & Rychlik, 2001). As rapidly fermenting microorganisms, amylolytic populations tend to respond quickly to dietary changes, potentially altering the fermentation profile and microbial interactions that influence CH_4 production (Tapio et al., 2017; Ungerfeld, 2020).

Proteolytic bacteria are responsible for hydrolyzing dietary and endogenous proteins into peptides, amino acids, NH_3, and branched-chain VFAs (Cotta & Hespell, 1986). Among the most important proteolytic species, *Prevotella spp.* and *Butyrivibrio fibrisolvens* play central roles in hydrolyzing a wide variety of protein sources. Their metabolic activity contributes to nitrogen turnover in the rumen, influencing microbial protein synthesis and the host's nitrogen use efficiency (Griswold et al., 1999; Wallace & Brammall, 1985). Additionally, proteolytic activity contributes to other functional groups; for example, the end-products of *P. ruminicola* and *B. fibrisolvens* fermentation support the growth of *Selenomonas ruminantium* and *Megasphaera elsdenii*. This indirectly influences cellulolytic populations and hydrogenotrophic archaea through H_2 suppression (Cabral & Weimer, 2024; Wallace & Brammall, 1985).

Succinate- and propionate-producing bacteria, including *S. ruminantium*, *M. elsdenii*, *Prevotella spp.*, and *Succinivibrio spp.*, produce end products that can act as alternative hydrogen sinks, offering potential pathways to mitigate CH_4 emissions by redirecting reducing equivalents away from methanogenesis (Liu et al., 2021; Ungerfeld, 2020).

Overall, rumen bacteria supply approximately 70% of the energy and up to 85% of the amino acids required by ruminants (Henderson et al., 2015; Mizrahi et al., 2021). This microbial community also contributes to B vitamin production, including vitamin B_{12}, crucial for methyl group metabolism in both microbes and the host (Liu et al., 2021). Furthermore, studies have shown that despite ruminal microbial community variation, there is a consistent core bacterial population, including genera like *Prevotella*, *Butyrivibrio*, *Fibrobacter*, *Olsenella*, *Ruminococcus*, and *Selenomas* (Cholewińska et al., 2021; Keum et al., 2024; Liu et al., 2021).

Archaea

Ruminal archaea, which belong to the phylum Euryarchaeota, are primarily methanogenic (Morgavi et al., 2010; Patra et al., 2017). Within the rumen ecosystem, archaea help maintain fermentation balance through utilising H_2 and CO_2 produced by fermentative microorganisms, preventing the accumulation of reducing equivalents that could inhibit other microbial activities (Morgavi et al., 2010). Archaea are present in the rumen at $10^{8–9}$ cells/mL, representing less than 4% of the microbial community. This group is composed mainly of species from the genus *Methanobrevibacter* (the most common group), *Methanobacterium*, *Methanomicrobium*, and *Methanosarcina* (Keum et al., 2024; Patra et al., 2017). These microorganisms may exist as free-floating cells in rumen fluid, as part of biofilms attached to feed particles, or as symbionts associated with ciliated protozoa that produce hydrogen using hydrogenosomes (Ferry, 1992; Hook et al., 2010; Thauer et al., 2008).

Different from bacteria, archaea do not contain peptidoglycan in their cell walls, which are replaced by other compounds depending on the genus. For example, *Methanobrevibacter* and *Methanobacterium* genera have pseudomurein, a peptidoglycan-like polymer that provides structural support and protection, while *Methanosarcina* has heteropolysaccharides, and *Methanomicrobium* contains protein-based cell walls (Hook et al., 2010). Furthermore, cellular and metabolic characteristics also vary among the genera. For instance, *Methanobrevibacter ruminantium* and *Methanobacterium formicicum* are rod-shaped methanogens that utilize H_2, CO_2, and formate as substrates, whereas *Methanomicrobium mobile* uses similar substrates but exhibits motility. In contrast, *Methanosarcina barkeri* and *M. mazeii* have a coccoid morphology and greater metabolic versatility. Members of the order Methanosarcinales, such as *M. barkeri*, are the only methanogens that contain cytochromes. This allows them to oxidize methyl groups and grow on several substrates, including acetate, methylamines, and methanol, making them highly versatile within the ruminal archaeal community (Hook et al., 2010; Thauer et al., 2008). Thus, ruminal archaea exhibit a high metabolic diversity among taxa, emphasizing their role in maintaining redox balance and facilitating efficient fermentation in the rumen.

Protozoa, fungi, and viruses

Protozoa are unicellular eukaryotes with an abundance of 10^{5-6} cells/mL. However, due to their large size (10 – 100 μm), they constitute 40 - 50% of the total microbial biomass in the rumen (Hungate, 1975). The community of ruminant protozoa is predominantly composed of ciliates rather than flagellates, including genera like *Entodinium*, *Epidinium*, *Enoploplastron*, and *Ophryoscolex* (Keum et al., 2024; Newbold et al., 2015). They are widely spread throughout the rumen, influencing the structure and function of the bacterial community by preying, shaping nutrient metabolism, and altering fermentation patterns (Mizrahi et al., 2021; Newbold et al., 2015). These organisms engulf starch, fibre fragments, and bacteria. They can degrade complex carbohydrates, which is in part attributed to horizontal gene transfer (HGT) from their bacterial prey. The HGT provides enzymes that enhance survival in the anaerobic and carbohydrate-rich environment of the rumen (Keum et al., 2024; Newbold et al., 2015). Protozoa are an important source of H_2; species such as *Entodinium*, *Polyplastron*, *Epidinium*, and *Ophryoscolex* may have physical associations with methanogenic archaea, which can colonize protozoa intracellularly and extracellularly (Mizrahi et al., 2021). The association between protozoa and methanogenic archaea enhances CH_4 production; thus, eliminating protozoa (defaunation) leads to a reduction in methanogenesis. As a result of defaunation, there may be a reduction in the degradation rate of organic matter, including neutral and acid detergent fibre, and the abundance of *Ruminococcus spp.*, and free-living methanogenic archaea may increase (Elghandour et al., 2020; Keum et al., 2024).

Rumen fungi are strictly anaerobic microorganisms from the phylum Neocallimastigomycota. They account for between 8-12% of total microbial biomass and are present in concentrations at around 10^{3-6} zoospores/mL, with their abundance greatly influenced by host diet (Elghandour et al., 2020). Currently, six genera have been described, including *Anaeromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Orpinomyces*, and *Piromyces*. These fungi possess fibrolytic enzymes, such as cellulases, hemicellulases, and xylanases, and many of these enzymes result from bacterial HGT, enhancing fungal capacity to degrade plant structural fibre (Elghandour et al., 2020; Gruninger et al., 2014). The invasive fungal hyphae penetrate lignified plant tissues, disrupting cell walls and facilitating bacterial access to cellulose and hemicellulose (Mizrahi et al., 2021). In addition, rumen fungi exhibit amylolytic and proteolytic activities and contain hydrogenosomes capable of producing H_2 during carbohydrate fermentation, providing substrates for methanogenic archaea. Furthermore, the fermentation patterns of the ruminal fungi can shift depending on the presence of methanogens (Gruninger et al., 2014; Mizrahi et al., 2021).

Viruses, specifically bacteriophages, represent a small portion of the ruminal microbiome. Recent metagenomic studies have shown a highly diverse virome, including families of *Siphoviridae*, *Myoviridae*, and *Podoviridae* (Lobo & Faciola, 2021). Their activity contributes to microbial cell lysis and nutrient cycling, releasing cellular components and enzymes that can increase substrate availability and fermentation (Lobo & Faciola, 2021). Rumen viruses can infect bacteria, archaea, fungi, and protozoa, influencing ruminal population dynamics through specific interactions with the microbial host mediated by cell surface receptors (lipopolysaccharides, carbohydrates, proteins, and fimbriae structures) (Gilbert et al., 2020; Lobo & Faciola, 2021). Furthermore, they can modulate microbial diversity by selective predation of dominant populations and facilitate HGT (Gilbert et al., 2020; Lobo & Faciola, 2021). Similar to bacteria, protozoa, and fungi, the ruminant diet has a direct impact on the rumen virus community (Anderson et al., 2017).

1.2.2. Ruminal fermentation

Feed particles consumed by ruminants are directed to the rumen, where they are colonized, degraded, and fermented by the microbial community. Microorganisms associate with feed particles either randomly or through chemoattractants in response to compounds released from damaged plant tissue. Colonization begins with microbial adhesion, in which bacteria, fungi, and protozoa rapidly attach to feed particles through specific mechanisms, involving adhesins and receptors, or non-specific mechanisms based on physicochemical forces (McAllister et al., 1994). Digestive bacteria such as *R. albus*, *R. flavefaciens*, *F. succinogenes*, and *R. amylophilus* firmly attach to the feed surface using glycocalyx and binding proteins (Miron et al., 2001). Once attached, a microcolony starts to form, dividing and secreting enzymes to break down the insoluble substrate, creating digestive crypts and releasing soluble nutrients, which attract secondary colonizers from rumen fluid. As colonization progresses, a diverse and complex multispecies microbial biofilm forms and often completely envelops the feed particles (McAllister et al., 1994; Miron et al., 2001). This microbial consortium comprises physiologically complementary species essential for the degradation of chemically and structurally complex plant tissues. Within the biofilm, nutrients are concentrated, and substrates, such as succinate, isobutyrate, and H_2, are exchanged among microbial members, accelerating digestion (McAllister et al., 1994; Miron et al., 2001; Wang & McAllister, 2002).

The primary end products of ruminal fermentation are VFAs, generated mainly from the breakdown of complex carbohydrates (Bergman, 1990; Morgavi et al., 2010). Structural polysaccharides such as cellulose, hemicellulose, and pectin serve as substrates for microbial fermentation (Morgavi et al., 2010). The degradation of these polysaccharides relies on a variety of microbial enzymes (cellulases, xylanases, and pectinases), produced by specialized bacteria in the rumen (Dehority, 1993; Weimer, 2022). Released fermentable sugars are quickly processed through glycolysis to form pyruvate, which is then rapidly converted into acetate, propionate, and butyrate. Acetate and butyrate share metabolic pathways that involve acetyl-CoA, and their interconversion is energetically favorable for microorganisms. Propionate, which is produced via the succinate or acrylate pathways, is the only VFA that significantly contributes to gluconeogenesis in the host. Branched-chain VFAs (isobutyrate, isovalerate, and 2-methylbutyrate) occur from the fermentation of amino acids, while valerate is formed through the condensation of acetate and propionate (Bergman, 1990; Miron et al., 2001; Morgavi et al., 2010).

In addition to VFAs, ruminal fermentation also generates CH_4 through the utilization of CO_2, H_2, formate, methanol, and methylamines, a process performed exclusively by methanogenic archaea (Hook et al., 2010; Keum et al., 2024; Morgavi et al., 2010). Functionally, methanogens are essential for removing H_2 produced during bacterial fermentation, preventing feedback inhibition of other fermentative pathways (Beauchemin et al., 2020; Morgavi et al., 2010). Three pathways support CH_4 production: hydrogenotrophic, methylotrophic, and acetoclastic methanogenesis (Figure 1.2). Hydrogenotrophic methanogenesis is the main metabolic pathway in the rumen, where CO_2 is progressively reduced to CH_4 using H_2 as an electron donor ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$). This process involves a series of redox reactions catalyzed by specialized enzymes and cofactors, including coenzyme F_{420}, coenzyme M (CoM - 2-

mercaptoethanesulfonate), coenzyme B (CoB - 7-mercaptoheptanoylthreonine phosphate), and coenzyme F_{430} (Ferry, 1992; Thauer et al., 2008).

Methylotrophic and acetoclastic methanogenesis play a minor role in CH_4 production in the rumen. In the methylotrophic pathway, methylated compounds (methanol and methylamines) are reduced to CH_4, with CO_2 as a byproduct ($3CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$) (Ferry, 1992; Króliczewska et al., 2023). During this process, the methyl group of methanol or methylamines is transferred to coenzyme M (HS-CoM), resulting in Methyl-S-CoM, an intermediate common to all methanogenesis pathways. This intermediate is subsequently reduced to CH_4 by the methyl-coenzyme M reductase, using HS-CoB as an electron donor (Ferry, 1992; Króliczewska et al., 2023). Acetoclastic methanogenesis involves the utilization of acetate as the substrate to generate CH_4 and CO_2 ($CH_3COO^+ + H^+ \rightarrow CH_4 + CO_2$) (Ferry, 1992; Króliczewska et al., 2023). In this pathway, acetate is activated to acetyl-CoA, followed by decarbonylation and methyl transfer to HS-CoM, resulting in Methyl-S-CoM, which is then reduced to CH_4. Importantly, acetoclastic methanogenesis is insignificant in the rumen environment (Hook et al., 2010).

1.3 Enteric CH_4 mitigation strategies

Several approaches have been explored to reduce enteric CH_4 production from ruminants, including the selection of lower CH_4-producing cattle by animal breeding, and dietary manipulation, which is one of the most effective approaches (Beauchemin et al., 2022). Studies have demonstrated that adjusting the concentrate to forage ratio, as well as supplementing plant bioactive compounds, 3-nitrooxypropanol, seaweed, and other feed additives into the diet, can decrease enteric CH_4 production (Abbott et al., 2020; Beauchemin et al., 2022; Terry et al., 2023).

1.3.1. Concentrate *vs.* forage diets

The composition of a diet has a significant impact on the structure of the rumen bacterial community. Diets rich in forage tend to promote the growth of fibrolytic bacteria, especially those with cellulolytic and hemicellulolytic activity. Alternatively, diets rich in concentrates tend to encourage the proliferation of amylolytic and lactic acid-producing species (Henderson et al., 2015). Starch fermentation results in a greater concentration of propionate and butyrate, different than fibrolytic fermentation, which results in higher production of acetate and H_2 available for methanogenesis. Likewise, high starch intake can decrease ruminal pH, inhibiting the growth of protozoa and methanogenic archaea (Beauchemin et al., 2022; Beauchemin et al., 2020). Together, reduced H_2 availability and a ruminal environment less favorable for protozoa and methanogenic archaea contribute to the lower enteric CH_4 production (Beauchemin et al., 2022; Beauchemin et al., 2020; Henderson et al., 2015).

It is important to note that the rapid changes in diet composition, such as an increase in rapidly fermentable carbohydrates, can disrupt the microbial balance and fermentation pathways, resulting in acidosis and decreased fibre digestibility (Nagaraja & Titgemeyer, 2007). Furthermore, although increasing the utilization of concentrate-based diets may reduce enteric CH_4 production, this strategy has significant limitations. Its large-scale adoption must consider the world's capacity for expansion of grain production for ruminant nutrition, which could intensify competition with the human food system. This approach also compromises one of the main advantages of ruminant production sector, the ability of these animals to convert fibrous plant material (indigestible for humans) into high-quality nutrients, such as milk and meat. Additionally, the environmental implications of this strategy must also be considered, as it may elevate land-use pressure and GHG emissions associated with crop cultivation, harvesting, processing, and transportation.

1.3.2. Plant bioactive compounds

Plant bioactive compounds, known as secondary metabolites, act as natural defense mechanisms against pathogenic microorganisms, herbivores, pests, and environmental stressors affecting the plant (Wallace, 2004). These metabolites occur in multiple plant structures, such as leaves, roots, rhizomes, seeds, bark, and fruits, and can be obtained in plant extracts, oil, or essential oils. Their chemical composition is highly variable and influenced by plant species, plant structure, growth stage, environmental factors, and extraction methods (Lambo et al., 2024; Wallace, 2004). These compounds are classified into three chemical groups: terpenoids, phenolics, and alkaloids (nitrogen-containing), which can exhibit antimicrobial, antioxidant, anti-inflammatory, and immunomodulator activity (Benchaar et al., 2008; Lambo et al., 2024; Wallace, 2004).

The utilization of plant bioactive compounds as ruminant dietary supplements has been extensively studied due to their potential to modulate ruminal fermentation and mitigate enteric CH_4 emission (Benchaar et al., 2008). These compounds can influence a wide variety of microorganisms, including both Gram-positive and Gram-negative bacteria, by targeting diverse components within bacterial cells (Benchaar et al., 2008; Rochfort et al., 2008; Wallace, 2004). Among them, saponins and tannins have demonstrated potential effects in reducing enteric CH_4 when used as dietary supplements.

Saponins are naturally terpenoid compounds with detergent properties, whereas tannins are polyphenolic compounds that can be classified as hydrolysable and condensed (Goel & Makkar, 2012). Both compounds can suppress fibrolytic bacteria that reduce H_2 available for methanogenesis and further promote the growth of propionate producers. Hence, there are fewer substrates for archaea, resulting in a decrease in enteric CH_4 production (Lambo et al., 2024;

Lileikis et al., 2023). In addition, direct inhibition of methanogenic archaea can occur by interfering with essential enzymes involved in methanogenesis (Sari et al., 2022). Tannins may also bind to the cell membranes of archaea, impairing their growth and disrupting their association with protozoa. As well, both saponins and tannins disrupt protozoan membranes, leading to a decrease in protozoal population and their associated methanogenic archaea (Lileikis et al., 2023).

Essential oils (EOs) may also exhibit potential CH_4 mitigation activity. They are a complex and variable mixture composed primarily of terpenoids, although alcohols, acyclic esters, and lactones may also be present (Dorman & Deans, 2000). Their chemical composition can vary substantially among plant species and cultivars, as well as according to plant maturity and environmental growing conditions (Benchaar et al., 2008). Research has demonstrated that the antimicrobial effects of EOs are associated with the disruption of bacterial cell membranes, including electron transport, maintenance of ionic gradients, protein translocation, phosphorylation, and other enzyme-dependent reactions (Ambrosio et al., 2020; Benchaar et al., 2008; Hook et al., 2010). As a result, they may inhibit Gram-positive bacteria, leading to reduced hydrogen availability for methanogenesis (Hook et al., 2010). Nevertheless, due to their chemical complexity and compositional variability, the mechanisms of action of EOs are likely multifaceted, involving multiple cellular targets rather than a single specific pathway (Beauchemin & McGinn, 2006; Benchaar et al., 2008).

Despite the potential of plant bioactive compounds as dietary additives for ruminants to reduce enteric CH_4 production, their efficacy remains highly variable (Lambo et al., 2024). Compounds like tannins and saponins may reduce feed intake by ruminants due to their lack of palatability and alteration in ruminal fermentation patterns, resulting in a negative impact on animal performance (Beauchemin et al., 2022; Rochfort et al., 2008). Furthermore, long-term *in vitro* and *in vivo* studies indicate that the initial benefits of those compounds often diminish over time, likely as a result of shifts in microbial populations or the adaptation of individual microbes (Benchaar et al., 2008). Also, the large quantities required and the high costs associated with extraction and purification represent additional barriers to their practical adoption by the livestock industry (Lileikis et al., 2023). Collectively, these challenges highlight the necessity for further research to develop consistent, cost-effective, and animal-friendly approaches to implementing plant bioactive compounds in CH_4 mitigation strategies.

1.3.3. 3-Nitrooxypropanol

3-Nitrooxypropanol, also known as 3-nitrooxypropan-1-ol or 1,3-propanediol mononitrate (3-NOP), is a synthetic organic compound used as a feed supplement for ruminants to reduce enteric CH_4 production (Romero-Perez et al., 2014). This molecule is a structural analog of methyl-coenzyme M; thus, 3-NOP acts as a highly specific inhibitor by oxidizing the nickel ion from the enzyme methyl-coenzyme M reductase (MCR). As a result, it inhibits the final step of CH_4 synthesis in methanogenic archaea, where methyl-S-CoM is reduced by MCR to generate CH_4 (Alemu et al., 2021; Duin et al., 2016; Romero-Perez et al., 2014).

Studies in dairy and beef cattle demonstrated a consistent reduction in CH_4 production, with results varying between 7% to 60% depending upon the type of animal, diet composition, dose, and method of supplementation (Haisan et al., 2014; Reynolds et al., 2014). In several studies, 3-NOP is typically supplemented at a dose of 1-2 g/animal/day, equivalent to 60-280 mg/kg dry matter (DM) in the diet, with a consistent linear reduction in CH_4 production (g/d) of approximately 2.56% for every 10 mg/kg DM supplemented (Dijkstra et al., 2018). Nevertheless, the effectiveness of 3-NOP is dependent on the neutral detergent fibre (NDF) content of the diet. As demonstrated by Dijkstra et al. (2018) in a meta-analysis using data from 11 experiments across dairy and beef cattle, every 10 g/kg of DM increase in dietary NDF content compromised the 3-NOP effect on CH_4 production by 1.64 ± 0.33% and on CH_4 yield (g/kg DM) by 1.52 ± 0.41%.

In a meta-analysis, Kebreab et al. (2023) related that including an average dose of 70.5 mg/kg (DM basis) of 3-NOP in a dairy cattle diet, resulted in a significant reduction of 32.7%, 30.9%, and 32.6% for CH_4 production, yield, and intensity (g/kg energy-corrected milk), respectively. The authors also related a reduction in the efficiency of mitigating CH_4 emission by the compound when NDF content in the diet increased (Kebreab et al., 2023). In addition, Orzuna-Orzuna et al. (2024) also used a meta-analysis approach to assess the effect of 15 h 3-NOP studies on growth performance, ruminal fermentation, and enteric CH_4 emissions in beef cattle. The authors demonstrated a reduction in DMI in response to 3-NOP supplementation, but average daily gain (ADG) was not affected, and feed efficiency (FE) was increased (Orzuna-Orzuna et al., 2024). In addition, 3-NOP supplementation altered ruminal fermentation patterns and led to dose-dependent reductions in both daily CH_4 emissions and CH_4 yield (Orzuna-Orzuna et al., 2024).

It is important to note that utilizing this supplement in pasture systems may be a limiting factor. Furthermore, the effect of 3-NOP on DMI is controversial, while some studies revealed no impact (Alemu et al., 2023; Kim et al., 2019), others demonstrated a trend or significant DMI reductions (Alemu et al., 2021; Jayanegara et al., 2018; Orzuna-Orzuna et al., 2024; Vyas et al., 2016).

1.3.4. Seaweeds

Interest in using seaweed as a feed additive for ruminants has increased in recent years, driven by positive results from both controlled laboratory studies (*in vitro*) and experiments with live animals (*in vivo*; Table 1.2). There are several seaweed species divided into three main groups: red (*Rhodophyceae*), brown (*Phaeophyceae*), and green (*Chlorophyceae)* (Makkar et al., 2016). Seaweeds grow in saltwater or freshwater environments and contain diverse compounds, including amino acids, minerals, sulfur-based polysaccharides, proteins, peptides, polyunsaturated fat, phlorotannins, saponins, alkaloids, and bioactive compounds, which may reduce enteric CH_4 production through the suppression of archaea and protozoa (Króliczewska et al., 2023).

Red seaweeds constitute the largest and most diverse group of seaweeds, including families such as Gigartinales, Ceramiales, Bangiales, and Palmariales. Most red species live in coastal environments from intertidal zones to depths of approximately 100 m. They are characterized by their pink-to-red color due to high pigment levels, and are often rich in proteins, carbohydrates, and minerals (Makkar et al., 2016). Brown seaweeds, in contrast, are distinguished by their high content of complex polysaccharides like fucoidan, laminaran, and alginate, involving families such as Laminariales, Fucales, Dictyotales, and Ectocarpales. Green seaweeds are characterized by chlorophyll and cellulose-rich cell walls, but they also contain a variety of bioactive compounds with potential antimethanogenic activity. For example, families such as Ulvales, Cladophorales, Bryopsidales, and Trentepohliales are commonly used as dietary supplements, due to their frequent abundance in flavonoids, phenols, saponins, and sulfated polysaccharides (Abbott et al., 2020; Makkar et al., 2016; Wanapat et al., 2024).

The greatest effect on CH_4 mitigation has been found in studies examining the red seaweed *Asparagopsis spp.* (Machado et al., 2016; Roque et al., 2019a; Roque et al., 2019b). Both *in vitro* and *in vivo* studies have demonstrated that supplementation with *A. taxiformis* and *A. armata* may reduce enteric CH_4 production by up to 90% and 50%, respectively. These effects are attributed to the high content of bromoform, a halogenated secondary compound (Machado et al., 2016; Roque et al., 2019a; Roque et al., 2019b). Bromoform reduces CH_4 by interfering with vitamin B_{12}-dependent enzymes, inhibiting the cobalamide-dependent methyltransferase responsible for the final step of methanogenesis (Beauchemin et al., 2022; Machado et al., 2016). However, despite its effectiveness, concerns have emerged regarding the safety of bromoform. This compound exhibits carcinogenic properties; thus, prolonged exposure to high concentrations may induce hepatic and intestinal tumors in animals (Beauchemin et al., 2022).

A study of Muizelaar et al. (2021) investigated the effects of bromoform in *A. taxiformis* on the ruminal epithelium of non-pregnant Holstein-Friesian dairy cows supplemented with three different doses (67 g, 133 g, and 333 g DM basis). The authors noted abnormalities in the rumen wall and histological signs of inflammation in two cows euthanized from the lowest treatment (Muizelaar et al., 2021). However, it remains unclear whether the abnormalities in the ruminal epithelium can be directly attributed to bromoform, underscoring the necessity for further targeted investigations. Additionally, bromoform also has a detrimental impact on the atmosphere; it may destroy the ozone layer, primarily in the troposphere and stratosphere (Beauchemin et al., 2022; Glasson et al., 2022; Jia et al., 2022). Consequently, current research is increasingly focused on evaluating non-halogenated seaweeds as a safer alternative to reduce enteric CH_4 emissions.

In a long-term *in vitro* study using the Rumen Simulation Technique (RUSITEC), Künzel et al. (2022) evaluated the inclusion of 2.5% and 5% (DM basis) of two brown seaweeds (*Ascophyllum nodosum* and *Fucus vesiculosus*) into a high-forage total mixed ration. The supplementation led to a reduction in total gas production (mL/g) for both seaweeds and inclusion doses. Likewise, inclusion at 5% of *A. nodosum* and *F. vesiculosus* led to a decrease in CH_4/degraded organic matter (mL/g) by 16.9% and 11.2%, respectively (Künzel et al., 2022). This effect was attributed to changes in the ruminal microbial community, including a decrease in the growth of *F. succinogenes*. However, there was also a decrease in nutrient degradation, emphasizing the relationship between CH_4 production and digestive efficiency (Künzel et al., 2022).

Further illustrating the chemical and biological diversity of seaweed, Ramos et al. (2025) evaluated the effect of 5% inclusion (DM basis) of 30 individual species of seaweeds (including red, brown, and green species), on *in vitro* degradability, gas production (GP), CH_4, and VFA production using *in vitro* batch cultures. This study demonstrated a high variation in the composition of seaweeds. Notably, Shoreblend (a blend with *Chondrus crispus*, *F. vesiculosus*, *Furcellaria lumbricalis*, and *Laminaria longicruris*) decreased CH_4 yield (mL/g DMI, mL/g DMD) by 12.2% without modifying VFA production (Ramos et al., 2025). In addition, the authors reported that there was no effect on CH_4 yield by the red seaweeds (*Calliarthron tuberculosum*, *Gracilaria sp.*, *Odonthalia floccose*, and *Prionitis lanceolata* - juvenile and mature), brown seaweeds (*Macrocystis pyrifera*, *Nereocystis luetkeana* - blades, *Postelsia palmaeformis*, *Saccharina groenlandica* - wild, and *F. vesiculosus*), or green seaweed (*Phyllospadix scouleri*) (Ramos et al., 2025).

The chemical composition of seaweeds can change depending on the species, harvest, and environmental conditions (Makkar et al., 2016). Molina-Alcaide et al. (2017) studied the effect of seven different seaweeds: three red species (*Mastocarpus stellatus*, *Palmaria palmata,* and *Porphyra sp.*), three brown species (*Alaria esculenta*, *Laminaria digitata*, and *Pelvetia canaliculata*), and one green species (*Acrosiphonia sp.*) harvested in autumn and spring seasons, using 24-h *in vitro* batch cultures. This study found a substantial variation in nutritional composition among species and seasons. For example, the effective DM degradability was greatest for *M. stellatus* and *Porphyra sp.*, and least for *P. canaliculate* and *Acrosiphonia sp. P. palmata* resulted in the greatest production of VFA and CH_4 production. Overall, seaweeds harvested in autumn showed lower degradability, CH_4 and VFA production, compared with those harvested in spring (Molina-Alcaide et al., 2017). Differences in nutrient degradability across seasons can be linked to variations in seaweed morphology and maturity, including changes in size, structural complexity, and functional form (Tayyab et al., 2016). These characteristics influence surface area:volume ratio and, consequently, mineral storage capacity (Tayyab et al., 2016). Additionally, environmental factors such as desiccation stress during low tide, fluctuations in salinity, and sunlight exposure may affect photosynthesis and nutrient assimilation by seaweed (Tayyab et al., 2016).

It is important to note that the high variability in the chemical composition of different seaweed species, harvest times, and storage conditions can limit their applicability to the livestock sector. Additionally, concerns about the safety of bioactive compounds, such as toxicity, environmental implications, and the accumulation of heavy metals in animal products, should also be taken into account, as they can affect animal, human, and environmental health. Therefore, future research is necessary to identify the potential bioactive compounds in several seaweed species and their diverse effects on animal performance and health, ruminal fermentation and microbial communities, CH_4 mitigation, and environmental impact.

1.4 Enteric methane measurement techniques

Quantification of enteric CH_4 emission from ruminants *in vivo* requires reliable measurement techniques. Currently, diverse tools are available for research, each with different levels of precision, cost, applicability, and feasibility. These methods range from high-precision systems, such as respiration chambers (RC), to more flexible techniques, like the sulfur hexafluoride (SF_6) tracer technique, which can measure methane emissions from individual animals on pasture. In addition, there are some spot sampling methods, including GreenFeed system (GF), gas detection devices, laser detectors, and facemasks, which offer practical and high-throughput alternatives for estimating CH_4 emissions under commercial or field conditions (Hammond et al., 2016a; Storm et al., 2012; Zhao et al., 2020). The choice of method ultimately depends on the research objective, the required precision, the animal management system, and the available resources. The RC and GF systems are the two most commonly used methods in current research studies to estimate enteric CH_4 production from ruminants (Alemu et al., 2017; Hammond et al., 2016a).

1.4.1. Respiration chamber

Respiration chambers have been used extensively for energy metabolism and gas emissions studies. They are recognized for accuracy, low coefficient of variation (CV, %), and reliability in measuring enteric CH_4 production (Bekele et al., 2022; Storm et al., 2012). Respiration chambers are based on the complete collection of air exhaled by the animal, allowing for precise measurement of CH_4, CO_2, and O_2 concentration and total airflow. Typically, the RCs are used for 24-h periods over 1 to 7 consecutive days, with the animal housed individually in a sealed room, large enough to ensure its comfort, with a feeder and automatic waterer (Hammond et al., 2016a).

The system is usually maintained under slightly negative pressure to ensure that any small leaks draw air in rather than allowing gases to escape (Hammond et al., 2016a). Fresh air enters through an inlet, and the outgoing air is continuously pumped through a flow meter and gas analyzers for ongoing monitoring of gas concentration (Storm et al., 2012). Therefore, enteric CH_4 emissions are calculated by multiplying the airflow rate by the difference in gas concentrations between the inlet and outlet air, with corrections for temperature, pressure, and humidity (Bekele et al., 2022; Zhao et al., 2020).

The utilization of RC systems allows continuous, high-resolution measurements and is frequently used to characterize daily emission patterns and to validate alternative CH_4 measurement techniques. However, their application is limited due to costs for implementation and maintenance, limited capacity in the number of animals, and artificial measurement conditions.

Additionally, animals must be adapted to confinement, which may affect their feeding behavior and intake. As a result, RC systems are more suitable for controlled research trials rather than large-scale studies or pasture-based applications.

1.4.2. GreenFeed system (GF)

The GF is an automated head chamber technology that is integrated into a portable feeding station, allowing estimation of enteric CH_4 and CO_2 emission from individual ruminants in controlled or commercial environments (French et al., 2025; Hammond et al., 2015; Hammond et al., 2016b). The system captures multiple short and repeated gas emission events during the day and aggregates them to estimate the individual daily CH_4 emission average over a determined period (ranging from days to months). The main principle is that accurate CH_4 estimates require sufficiently numerous and well-distributed measurements that capture the animal's natural daytime feeding, rumination and emission patterns (Bekele et al., 2022; French et al., 2025).

When an animal approaches the GF, it is identified by a radio frequency identification ear tag (RFID). Then, a small amount of palatable pellets are released to encourage consistent and repeated visits, which generally last 3 to 7 minutes, depending on system configuration (Zhao et al., 2020). During each visit, a high-capacity fan draws air from around the muzzle into an airflow duct, where the total volumetric airflow is continuously measured. A proportional subsample of this air is then directed to gas analyzers, which quantify CH_4, O_2, H_2, and CO_2 concentrations using non-dispersive infrared spectroscopy (NDIR) (Bekele et al., 2022; French et al., 2025; Zhao et al., 2020). Furthermore, the GF system includes a fractional capture rate, which is the portion of exhaled air that is effectively collected, ensuring precise measurement. This rate is evaluated using automated injections of tracer gases, such as SF_6 or CO_2. The system can perform recovery tests to verify the efficiency of exhaled gas collection periodically by injecting tracer gas into the upper chamber, as incomplete recovery directly impacts the calculation of emission rates. Therefore, by using the measured gas concentrations, airflow, and fractional capture rate, the system can calculate CH_4 g/min flow (Hammond et al., 2016b). All raw data is uploaded to a cloud-based platform managed by the manufacturer, where algorithms integrate gas flow data with visit duration, bait patterns, animal identification, and instrument diagnostics to generate estimates of emissions over 24-h (French et al., 2025; Hammond et al., 2015; Hammond et al., 2016b).

GreenFeed is an important tool for CH_4 measurement due to its portability, automation, and applicability. However, it is important to note that GF only captures emissions during brief visits; it does not directly quantify continuous respiration over 24-h. Therefore, its accuracy largely depends on collecting a sufficient number of visits that are evenly spread throughout the day. Irregular visit patterns, especially in grazing conditions, can cause significant variability. Additionally, the systems can also be influenced by environmental factors like wind direction, turbulence, and temperature changes, affecting airflow stability and gas collection efficiency.

1.5 Conclusions

The worldwide effort to reduce GHG emissions is a major concern across various economic sectors, especially regarding enteric CH_4 production by ruminant industries. This concern stems not only from its role in global warming but also from the energy losses associated with enteric CH_4, which can impact productivity. As this review emphasized, CH_4 formation is biologically driven by complex interactions among ruminal microorganisms, fermentation pathways, and host physiology. Therefore, effective mitigation strategies must consider the balance within the ruminal ecosystem, ensuring that mitigation measures reduce CH_4 emissions without harming animal health, nutrient absorption, or overall productivity. Nutritional interventions, such as seaweed and 3-NOP, have shown promising results. Seaweeds, particularly species rich in halogenated compounds like *Asparagopsis spp.*, have become one of the most effective natural inhibitors of methanogenesis identified. However, several key limitations remain, including variability in bioactive compound concentrations, cultivation challenges, concerns about long-term safety, and the need for scalable, cost-effective production systems. Moving forward, addressing several research gaps is essential to support the global goal of decreasing CH_4 emissions and improving the sustainability of livestock farming. Future research should focus on long-term evaluations of the most promising mitigation strategies to determine their sustained effectiveness, impact on rumen microbial communities, animal performance, product quality, and potential environmental benefits.

1.6 Objectives

The present study was conducted to improve the understanding of CH_4 emissions in feedlot systems and to evaluate potential mitigation strategies across two productive phases: backgrounding (high-forage diet) and finishing (high-grain diet). This research aimed to investigate nutritional interventions and the selection of low CH_4-producing cattle to identify mechanisms that contribute to sustainable mitigation without compromising animal productivity.

1.6.1. Specific objectives

Nutritional strategy – Seaweed supplementation: to evaluate the effectiveness of supplementing 0.60% (on dry matter [DM] basis) of a commercial multispecies seaweed blend (SWblend) in a total mixed ration (TMR) on:

- Growth performance: evaluating initial and final shrunk body weight (SBW; kg), DMI (kg/d), weight gain (kg), average daily gain (ADG; kg/d), and gain:feeding (G:F).

- Feeding behavior by the GrowSafe system: measuring DMI (kg/d), day-to-day variation of DMI (SD of DMI), meal duration (min/meal), inter-meal interval (min), meal size (kg/meal), and eating rate (g/min).
- Ruminal fermentation: assessing total VFA and individual VFA concentrations, ammonia (NH_3), ruminal pH, and protozoa counts.
- GF system patterns: evaluating total DMI, gross energy intake (GEI, Mcal/d), CH_4 production (g/d), CH_4 yield (CH_4y; g/kg DMI), and CH_4 intensity (Mcal/%GEI).
- Hematological parameters: measuring total red blood cell count (RBC), mean cell volume of RBCs (MCV), hematocrit (HCT), mean cell hemoglobin (MCH), total white blood cell count (WBC), lymphocyte concentration in absolute number and percentage (LYMF), mid-sized cells in absolute number and percentage (MONO), and granulocyte concentration in absolute number and percentage (GRAN).

Selecting low CH_4-producing cattle – CH_4 yield rank: to rank feedlot beef heifers into low, intermediate, and high CH_4 yield groups (MYG) and evaluate their differences on:

- Growth performance: evaluating initial and final SBW, DMI, weight gain, ADG, and G:F.
- Feeding behavior by the GrowSafe system: measuring DMI, SD of DMI, meal duration, intermeal interval, meal size, and eating rate.
- GF system patterns: evaluating total DMI, GEI, CH_4 production (g/d), CH_4y, and CH_4 intensity (Mcal/%GEI).
- Coefficient of variation of GF system visitation.
- Ruminal microbial diversity: analyzing alpha and beta diversity.

1.6.2. Hypotheses

The present study is based on two specific hypotheses: (1) supplementing feedlot beef heifers with SWblend will reduce enteric CH_4 production during both the backgrounding and finishing phases, without compromising animal health and productivity; and (2) classifying feedlot beef heifers according to their MYG could help to explain individual variations in enteric CH_4 emissions. These approaches aim to determine whether CH_4 mitigation in feedlot systems can be achieved through the use of seaweed supplementation or by selecting cattle that emit less CH_4.

1.7 References

Abbott, D. W., Aasen, I. M., Beauchemin, K. A., Grondahl, F., Gruninger, R., Hayes, M., Huws, S., Kenny, D. A., Krizsan, S. J., Kirwan, S. F., Lind, V., Meyer, U., Ramin, M., Theodoridou, K., Von Soosten, D., Walsh, P. J., Waters, S., & Xing, X. (2020). Seaweed and Seaweed Bioactives for Mitigation of Enteric Methane: Challenges and Opportunities. *Animals*, *10*(12), 2432. https://www.mdpi.com/2076-2615/10/12/2432

Alemu, A. W., Gruninger, R. J., Zhang, X. M., O'Hara, E., Kindermann, M., & Beauchemin, K. A. (2023). 3-Nitrooxypropanol supplementation of a forage diet decreased enteric methane emissions from beef cattle without affecting feed intake and apparent total-tract digestibility. *Journal of Animal Science*, *101*. https://doi.org/10.1093/jas/skad001

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*, *Volume 2 - 2021*. https://doi.org/10.3389/fanim.2021.641590

Alemu, A. W., Vyas, D., Manafiazar, G., Basarab, J. A., & Beauchemin, K. A. (2017). Enteric methane emissions from low– and high–residual feed intake beef heifers measured using GreenFeed and respiration chamber techniques1,2. *Journal of Animal Science*, *95*(8), 3727-3737. https://doi.org/10.2527/jas.2017.1501

Ambrosio, C. M. S., Contreras-Castillo, C. J., & Da Gloria, E. M. (2020). In vitro mechanism of antibacterial action of a citrus essential oil on an enterotoxigenic Escherichia coli and Lactobacillus rhamnosus. *Journal of Applied Microbiology*, *129*(3), 541-553. https://doi.org/10.1111/jam.14660

Anderson, C. L., Sullivan, M. B., & Fernando, S. C. (2017). Dietary energy drives the dynamic response of bovine rumen viral communities. *Microbiome*, *5*(1), 155. https://doi.org/10.1186/s40168-017-0374-3

Beauchemin, K. A., & McGinn, S. M. (2006). Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. *Journal of Animal Science*, *84*(6), 1489-1496. https://doi.org/10.2527/2006.8461489x

Beauchemin, K. A., Ungerfeld, E. M., Abdalla, A. L., Alvarez, C., Arndt, C., Becquet, P., Benchaar, C., Berndt, A., Mauricio, R. M., McAllister, T. A., Oyhantçabal, W., Salami, S. A., Shalloo, L., Sun, Y., Tricarico, J., Uwizeye, A., De Camillis, C., Bernoux, M., Robinson, T., & Kebreab, E. (2022). Invited review: Current enteric methane mitigation options. *Journal of Dairy Science*, *105*(12), 9297-9326. https://doi.org/https://doi.org/10.3168/jds.2022-22091

Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J., & Wang, M. (2020). Review: Fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation. *Animal*, *14*(S1), s2-s16. https://doi.org/10.1017/S1751731119003100

Bekele, W., Guinguina, A., Zegeye, A., Simachew, A., & Ramin, M. (2022). Contemporary methods of measuring and estimating methane emission from ruminants. *Methane*, *1*(2), 82-95. https://www.mdpi.com/2674-0389/1/2/8

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G. R., Colombatto, D., McAllister, T. A., & Beauchemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology*, *145*(1), 209-228. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2007.04.014

Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews*, *70*(2), 567-590. https://doi.org/10.1152/physrev.1990.70.2.567

Cabral, L. S., & Weimer, P. J. (2024). Megasphaera elsdenii: Its Role in Ruminant Nutrition and Its Potential Industrial Application for Organic Acid Biosynthesis. *Microorganisms*, *12*(1), 219. https://www.mdpi.com/2076-2607/12/1/219

Cholewińska, P., Górniak, W., & Wojnarowski, K. (2021). Impact of selected environmental factors on microbiome of the digestive tract of ruminants. *BMC Veterinary Research*, *17*(1), 25. https://doi.org/10.1186/s12917-021-02742-y

Cotta, M. A. (1988). Amylolytic activity of selected species of ruminal bacteria. *Applied and Environmental Microbiology*, *54*(3), 772-776. https://doi.org/doi:10.1128/aem.54.3.772-776.1988

Cotta, M. A., & Hespell, R. B. (1986). Proteolytic activity of the ruminal bacterium Butyrivibrio fibrisolvens. *Applied and Environmental Microbiology*, *52*(1), 51-58. https://doi.org/doi:10.1128/aem.52.1.51-58.1986

Dehority, B. A. (1993). *Laboratory manual for classification and morphology of rumen ciliate protozoa*. CRC Press. https://doi.org/https://doi.org/10.1201/9781351073912

Dijkstra, J., Bannink, A., France, J., Kebreab, E., & van Gastelen, S. (2018). Short communication: Antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type. *Journal of Dairy Science*, *101*(10), 9041-9047. https://doi.org/https://doi.org/10.3168/jds.2018-14456

Dijkstra, J., Forbes, J. M., & France, J. (2005). *Quantitative aspects of ruminant digestion and metabolism* (pp. xi+-734).

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, *88*(2), 308-316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

Duin, E. C., Wagner, T., Shima, S., Prakash, D., Cronin, B., Yáñez-Ruiz, D. R., Duval, S., Rümbeli, R., Stemmler, R. T., Thauer, R. K., & Kindermann, M. (2016). Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. *Proceedings of the National Academy of Sciences*, *113*(22), 6172-6177. https://doi.org/doi:10.1073/pnas.1600298113

ECCC. (2025). *Canadian Environmental Sustainability Indicators: Greenhouse gas emissions*. Accessed date: 2025-11-6. www.canada.ca/en/environment-climate-change/services/environmental-indicators/greenhouse-gas-emissions.html

Elghandour, M. M. Y., Khusro, A., Adegbeye, M. J., Tan, Z., Abu Hafsa, S. H., Greiner, R., Ugbogu, E. A., Anele, U. Y., & Salem, A. Z. M. (2020). Dynamic role of single-celled fungi in ruminal microbial ecology and activities. *Journal of Applied Microbiology*, *128*(4), 950-965. https://doi.org/10.1111/jam.14427

Ferry, J. G. (1992). Biochemistry of Methanogenesis. *Critical Reviews in Biochemistry and Molecular Biology*, *27*(6), 473-503. https://doi.org/10.3109/10409239209082570

French, E. A., Beck, M. R., Kalscheur, K. F., Jaramillo, D. M., Derner, J. D., Moffet, C. A., Neville, B. W., Soder, K. J., O'Connor, R. C., Koziel, J. A., Vadas, P., Moeller, S., & Gunter, S. A. (2025). Experimental and experiential recommendations for using the GreenFeed systems to measure gas flux in grazing and confined cattle. *MethodsX*, *15*, 103667. https://doi.org/https://doi.org/10.1016/j.mex.2025.103667

Gilbert, R. A., Townsend, E. M., Crew, K. S., Hitch, T. C. A., Friedersdorff, J. C. A., Creevey, C. J., Pope, P. B., Ouwerkerk, D., & Jameson, E. (2020). Rumen virus populations: technological advances enhancing current understanding. *Frontiers in Microbiology*, *Volume 11 - 2020*. https://doi.org/10.3389/fmicb.2020.00450

Glasson, C. R. K., Kinley, R. D., de Nys, R., King, N., Adams, S. L., Packer, M. A., Svenson, J., Eason, C. T., & Magnusson, M. (2022). Benefits and risks of including the bromoform containing seaweed Asparagopsis in feed for the reduction of methane production from ruminants. *Algal Research*, *64*, 102673. https://doi.org/https://doi.org/10.1016/j.algal.2022.102673

Goel, G., & Makkar, H. P. S. (2012). Methane mitigation from ruminants using tannins and saponins. *Tropical Animal Health and Production*, *44*(4), 729-739. https://doi.org/10.1007/s11250-011-9966-2

Government of Canada (2025a). *Canada's dairy industry at a glance*. Government of Canada. Accessed date: 12/05/2025. https://agriculture.canada.ca/en/sector/animal-industry/canadian-dairy-information-centre/dairy-industry

Government of Canada (2025b). *Canada's red meat and livestock industry at a glance*. Government of Canada. Accessed date: 12/05/2025. https://agriculture.canada.ca/en/sector/animal-industry/red-meat-livestock-market-information/industry-profile

Griswold, K. E., White, B. A., & Mackie, R. I. (1999). Diversity of extracellular proteolytic activities among Prevotella species from the rumen. *Current Microbiology*, *39*(4), 187-194. https://doi.org/10.1007/s002849900443

Gruninger, R. J., Puniya, A. K., Callaghan, T. M., Edwards, J. E., Youssef, N., Dagar, S. S., Fliegerova, K., Griffith, G. W., Forster, R., Tsang, A., McAllister, T., & Elshahed, M. S. (2014). Anaerobic fungi (phylum Neocallimastigomycota): advances in understanding their taxonomy, life cycle, ecology, role and biotechnological potential. *FEMS Microbiology Ecology*, *90*(1), 1-17. https://doi.org/10.1111/1574-6941.12383

Guzman, C. E., Bereza-Malcolm, L. T., De Groef, B., & Franks, A. E. (2015). Presence of selected methanogens, fibrolytic bacteria, and proteobacteria in the gastrointestinal tract of neonatal dairy calves from birth to 72 hours. *PLoS One*, *10*(7), e0133048. https://doi.org/10.1371/journal.pone.0133048

Haisan, J., Sun, Y., Guan, L. L., Beauchemin, K. A., Iwaasa, A., Duval, S., Barreda, D. R., & Oba, M. (2014). The effects of feeding 3-nitrooxypropanol on methane emissions and productivity of Holstein cows in mid lactation. *Journal of Dairy Science*, *97*(5), 3110-3119. https://doi.org/https://doi.org/10.3168/jds.2013-7834

Hammond, K. J., Crompton, L. A., Bannink, A., Dijkstra, J., Yáñez-Ruiz, D. R., O'Kiely, P., Kebreab, E., Eugène, M. A., Yu, Z., Shingfield, K. J., Schwarm, A., Hristov, A. N., & Reynolds, C. K. (2016a). Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. *Animal Feed Science and Technology*, *219*, 13-30. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2016.05.018

Hammond, K. J., Humphries, D. J., Crompton, L. A., Green, C., & Reynolds, C. K. (2015). Methane emissions from cattle: Estimates from short-term measurements using a GreenFeed system compared with measurements obtained using respiration chambers or sulphur hexafluoride tracer. *Animal Feed Science and Technology*, *203*, 41-52. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2015.02.008

Hammond, K. J., Waghorn, G. C., & Hegarty, R. S. (2016b). The GreenFeed system for measurement of enteric methane emission from cattle. *Animal Production Science*, *56*(3), 181-189. https://doi.org/10.1071/an15631

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., Abecia, L., Angarita, E., Aravena, P., Nora Arenas, G., Ariza, C., Attwood, G. T., Mauricio Avila, J., Avila-Stagno, J., Bannink, A., Barahona, R., Batistotti, M., Bertelsen, M. F., Brown-Kav, A., Carvajal, A. M., … & Global Rumen Census Collaborators (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*, *5*(1), 14567. https://doi.org/10.1038/srep14567

Hook, S. E., Wright, A.-D. G., & McBride, B. W. (2010). Methanogens: methane producers of the rumen and mitigation strategies. *Archaea*, *2010*(1), 945785. https://doi.org/https://doi.org/10.1155/2010/945785

Hoque, S. A. M., Islam, M. M., Md Selim, A. S., Ahmed, S., & Rahman, M. M. (2017). Estimation of total methane emission from enteric fermentation of ruminant livestock in Bangladesh. *Asian Journal of Medical and Biological Research*, *3*(2), 245-253. https://doi.org/10.3329/ajmbr.v3i2.33577

Hua, D., Hendriks, W. H., Xiong, B., & Pellikaan, W. F. (2022). Starch and cellulose degradation in the rumen and applications of metagenomics on ruminal microorganisms. *Animals*, *12*(21), 3020. https://www.mdpi.com/2076-2615/12/21/3020

Hungate, R. E. (1975). The Rumen Microbial Ecosystem. *Annual Review of Ecology, Evolution, and Systematics*, *6*(Volume 6, 1975), 39-66. https://doi.org/https://doi.org/10.1146/annurev.es.06.110175.000351

Huws, S. A., Creevey, C. J., Oyama, L. B., Mizrahi, I., Denman, S. E., Popova, M., Munoz-Tamayo, R., Forano, E., Waters, S. M., Hess, M., Tapio, I., Smidt, H., Krizsan, S. J., Yanez-Ruiz, D. R., Belanche, A., Guan, L., Gruninger, R. J., McAllister, T. A., Newbold, C. J., … & Morgavi, D. P. (2018). Addressing global ruminant agricultural challenges through understanding the rumen microbiome: past, present, and future. *Frontiers in Microbiology*, *9*, 2161. https://doi.org/10.3389/fmicb.2018.02161

Huws, S. A., Edwards, J. E., Creevey, C. J., Rees Stevens, P., Lin, W., Girdwood, S. E., Pachebat, J. A., & Kingston-Smith, A. H. (2015). Temporal dynamics of the metabolically active rumen bacteria colonizing fresh perennial ryegrass. *FEMS Microbiology Ecology*, *92*(1). https://doi.org/10.1093/femsec/fiv137

IPCC. (2022). *Climate Change 2022 – Impacts, Adaptation and Vulnerability: Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press. https://doi.org/10.1017/9781009325844

Jami, E., & Mizrahi, I. (2012). Composition and similarity of bovine rumen microbiota across individual animals. *PLoS One*, *7*(3), e33306. https://doi.org/10.1371/journal.pone.0033306

Jayanegara, A., Sarwono, K. A., Kondo, M., Matsui, H., Ridla, M., Laconi, E. B., & Nahrowi. (2018). Use of 3-nitrooxypropanol as feed additive for mitigating enteric methane emissions from ruminants: a meta-analysis. *Italian Journal of Animal Science*, *17*(3), 650-656. https://doi.org/10.1080/1828051X.2017.1404945

Jia, Y., Quack, B., Kinley, R. D., Pisso, I., & Tegtmeier, S. (2022). Potential environmental impact of bromoform from Asparagopsis farming in Australia. *Atmospheric Chemistry and Physics*, *22*(11), 7631-7646. https://doi.org/10.5194/acp-22-7631-2022

Kebreab, E., Bannink, A., Pressman, E. M., Walker, N., Karagiannis, A., van Gastelen, S., & Dijkstra, J. (2023). A meta-analysis of effects of 3-nitrooxypropanol on methane production, yield, and intensity in dairy cattle. *Journal of Dairy Science*, *106*(2), 927-936. https://doi.org/https://doi.org/10.3168/jds.2022-22211

Keum, G. B., Pandey, S., Kim, E. S., Doo, H., Kwak, J., Ryu, S., Choi, Y., Kang, J., Kim, S., & Kim, H. B. (2024). Understanding the Diversity and Roles of the Ruminal Microbiome. *Journal of Microbiology*, *62*(3), 217-230. https://doi.org/10.1007/s12275-024-00121-4

Kim, S.-H., Lee, C., Pechtl, H. A., Hettick, J. M., Campler, M. R., Pairis-Garcia, M. D., Beauchemin, K. A., Celi, P., & Duval, S. M. (2019). Effects of 3-nitrooxypropanol on enteric methane production, rumen fermentation, and feeding behavior in beef cattle fed a high-forage or high-grain diet1. *Journal of Animal Science*, *97*(7), 2687-2699. https://doi.org/10.1093/jas/skz140

Krizsan, S. J., Ramin, M., Chagas, J. C., Halmemies-Beauchet-Filleau, A., Singh, A., Schnürer, A., & Danielsson, R. (2023). Effects on rumen microbiome and milk quality of dairy cows fed a grass silage-based diet supplemented with the macroalga Asparagopsis taxiformis. *Frontiers in Animal Science*, *4*, 1112969. https://doi.org/10.3389/fanim.2023.1112969

Królíczewska, B., Pecka-Kiełb, E., & Bujok, J. (2023). Strategies used to reduce methane emissions from ruminants: controversies and issues. *Agriculture*, *13*(3), 602. https://www.mdpi.com/2077-0472/13/3/602

Künzel, S., Yergaliyev, T., Wild, K. J., Philippi, H., Petursdottir, A. H., Gunnlaugsdottir, H., Reynolds, C. K., Humphries, D. J., Camarinha-Silva, A., & Rodehutscord, M. (2022). Methane Reduction Potential of Brown Seaweeds and Their Influence on Nutrient Degradation and Microbiota Composition in a Rumen Simulation Technique. *Frontiers in Microbiology*, *Volume 13 - 2022*. https://doi.org/10.3389/fmicb.2022.889618

Lambo, M. T., Ma, H., Liu, R., Dai, B., Zhang, Y., & Li, Y. (2024). Review: Mechanism, effectiveness, and the prospects of medicinal plants and their bioactive compounds in lowering ruminants' enteric methane emission. *animal*, *18*(4), 101134. https://doi.org/https://doi.org/10.1016/j.animal.2024.101134

Lileikis, T., Nainienė, R., Bliznikas, S., & Uchockis, V. (2023). Dietary ruminant enteric methane mitigation strategies: current findings, potential risks and applicability. *Animals*, *13*(16), 2586. https://www.mdpi.com/2076-2615/13/16/2586

Liu, K., Zhang, Y., Yu, Z., Xu, Q., Zheng, N., Zhao, S., Huang, G., & Wang, J. (2021). Ruminal microbiota–host interaction and its effect on nutrient metabolism. *Animal Nutrition*, *7*(1), 49-55. https://doi.org/https://doi.org/10.1016/j.aninu.2020.12.001

Lobo, R. R., & Faciola, A. P. (2021). Ruminal Phages – A Review. *Frontiers in Microbiology*, *Volume 12 - 2021*. https://doi.org/10.3389/fmicb.2021.763416

Machado, L., Magnusson, M., Paul, N. A., Kinley, R., de Nys, R., & Tomkins, N. (2016). Dose-response effects of Asparagopsis taxiformis and Oedogonium sp. on in vitro fermentation and methane production. *Journal of Applied Phycology*, *28*(2), 1443-1452. https://doi.org/10.1007/s10811-015-0639-9

Maia, M. R. G., Fonseca, A. J. M., Oliveira, H. M., Mendonça, C., & Cabrita, A. R. J. (2016). The potential role of seaweeds in the natural manipulation of rumen fermentation and methane production. *Scientific Reports*, *6*(1), 32321. https://doi.org/10.1038/srep32321

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, *212*, 1-17. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2015.09.018

McAllister, T. A., Bae, H. D., Jones, G. A., & Cheng, K.-J. (1994). Microbial attachment and feed digestion in the rumen. *Journal of Animal Science*, *72*(11), 3004-3018. https://doi.org/10.2527/1994.72113004x

Miron, J., Ben-Ghedalia, D., & Morrison, M. (2001). Invited Review: Adhesion Mechanisms of Rumen Cellulolytic Bacteria. *Journal of Dairy Science*, *84*(6), 1294-1309. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(01)70159-2

Mizrahi, I., Wallace, R. J., & Moraïs, S. (2021). The rumen microbiome: balancing food security and environmental impacts. *Nature Reviews Microbiology*, *19*(9), 553-566. https://doi.org/10.1038/s41579-021-00543-6

Molina-Alcaide, E., Carro, M. D., Roleda, M. Y., Weisbjerg, M. R., Lind, V., & Novoa-Garrido, M. (2017). In vitro ruminal fermentation and methane production of different seaweed species. *Animal Feed Science and Technology*, *228*, 1-12. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2017.03.012

Morgavi, D. P., Forano, E., Martin, C., & Newbold, C. J. (2010). Microbial ecosystem and methanogenesis in ruminants. *animal*, *4*(7), 1024-1036. https://doi.org/https://doi.org/10.1017/S1751731110000546

Morgavi, D. P., Kelly, W. J., Janssen, P. H., & Attwood, G. T. (2013). Rumen microbial (meta)genomics and its application to ruminant production. *animal*, *7*(s1), 184-201. https://doi.org/10.1017/S1751731112000419

Muizelaar, W., Groot, M., van Duinkerken, G., Peters, R., & Dijkstra, J. (2021). Safety and transfer study: transfer of bromoform present in Asparagopsis taxiformis to milk and urine of lactating dairy cows. *Foods*, *10*(3), 584. https://www.mdpi.com/2304-8158/10/3/584

Muizelaar, W., Van Duinkerken, G., Khan, Z., & Dijkstra, J. (2023). Evaluation of 3 northwest European seaweed species on enteric methane production and lactational performance of Holstein-Friesian dairy cows. *Journal of Dairy Science*, *106*(7), 4622-4633. https://doi.org/10.3168/jds.2022-22749

Nagaraja, T. G., & Titgemeyer, E. C. (2007). Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *Journal of Dairy Science*, *90*, E17-E38. https://doi.org/https://doi.org/10.3168/jds.2006-478

Neethirajan, S. (2024). Innovative strategies for sustainable dairy farming in canada amidst climate change. *Sustainability*, *16*(1), 265. https://www.mdpi.com/2071-1050/16/1/265

Newbold, C. J., de la Fuente, G., Belanche, A., Ramos-Morales, E., & McEwan, N. R. (2015). The role of ciliate protozoa in the rumen. *Frontiers in Microbiology*, *Volume 6 - 2015*. https://doi.org/10.3389/fmicb.2015.01313

Ominski, K., Gunte, K., Wittenberg, K., Legesse, G., Mengistu, G., & McAllister, T. (2021). The role of livestock in sustainable food production systems in Canada. *Canadian Journal of Animal Science*, *101*(4), 591-601. https://doi.org/10.1139/cjas-2021-0005

Orzuna-Orzuna, J. F., Godina-Rodríguez, J. E., Garay-Martínez, J. R., Granados-Rivera, L. D., Maldonado-Jáquez, J. A., & Lara-Bueno, A. (2024). A meta-analysis of 3-nitrooxypropanol dietary supplementation on growth performance, ruminal fermentation, and enteric methane emissions of beef cattle. *Fermentation*, *10*(6), 273. https://www.mdpi.com/2311-5637/10/6/273

Patra, A., Park, T., Kim, M., & Yu, Z. (2017). Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. *Journal of Animal Science and Biotechnology*, *8*(1), 13. https://doi.org/10.1186/s40104-017-0145-9

Perez, H. G., Stevenson, C. K., Lourenco, J. M., & Callaway, T. R. (2024). understanding rumen microbiology: an overview. *Encyclopedia*, *4*(1), 148-157. https://www.mdpi.com/2673-8392/4/1/13

Ramos, T. R., Dash, S. S., Prado, I. N. d., McAllister, T. A., Stanford, K., & Terry, S. A. (2025). Evaluating the efficacy of red, brown, and green seaweeds in reducing in vitro methane production from cattle. Canadian *Journal of Animal Science*, *105*, 1-12. https://doi.org/10.1139/cjas-2025-0009

Reyes, D. C., Meredith, J., Puro, L., Berry, K., Kersbergen, R., Soder, K. J., Quigley, C., Donihue, M., Cox, D., & Price, N. N. (2023). Maine organic dairy producers' receptiveness to seaweed supplementation and effect of Chondrus crispus on enteric methane emissions in lactating cows. *Frontiers in Veterinary Science*, *10*, 1153097. https://doi.org/10.3389/fvets.2023.1153097

Reynolds, C., Humphries, D., Künzel, S., Rodehutscord, M., Lignou, S., Fagan, C., Methven, L., Norton, G., Alzahrani, A., & Feldmann, J. (2025). Effects of feeding dairy cows seaweed mixtures on feed intake, methane emission, milk production, and milk mineral and fatty acid composition. *Journal of Dairy Science*, *108*(9), 9487-9500. https://doi.org/10.3168/jds.2025-26577

Reynolds, C. K., Humphries, D. J., Kirton, P., Kindermann, M., Duval, S., & Steinberg, W. (2014). Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. *Journal of Dairy Science*, *97*(6), 3777-3789. https://doi.org/https://doi.org/10.3168/jds.2013-7397

Rochfort, S., Parker, A. J., & Dunshea, F. R. (2008). Plant bioactives for ruminant health and productivity. *Phytochemistry*, *69*(2), 299-322. https://doi.org/https://doi.org/10.1016/j.phytochem.2007.08.017

Romero-Perez, A., Okine, E. K., McGinn, S. M., Guan, L. L., Oba, M., Duval, S. M., Kindermann, M., & Beauchemin, K. A. (2014). The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle1. *Journal of Animal Science*, *92*(10), 4682-4693. https://doi.org/10.2527/jas.2014-7573

Roque, B. M., Brooke, C. G., Ladau, J., Polley, T., Marsh, L. J., Najafi, N., Pandey, P., Singh, L., Kinley, R., Salwen, J. K., Eloe-Fadrosh, E., Kebreab, E., & Hess, M. (2019a). Effect of the macroalgae Asparagopsis taxiformis on methane production and rumen microbiome assemblage. *Animal Microbiome*, *1*(1), 3. https://doi.org/10.1186/s42523-019-0004-4

Roque, B. M., Salwen, J. K., Kinley, R., & Kebreab, E. (2019b). Inclusion of Asparagopsis armata in lactating dairy cows' diet reduces enteric methane emission by over 50 percent. *Journal of Cleaner Production*, *234*, 132-138. https://doi.org/https://doi.org/10.1016/j.jclepro.2019.06.193

Roque, B. M., Venegas, M., Kinley, R. D., de Nys, R., Duarte, T. L., Yang, X., & Kebreab, E. (2021). Red seaweed (Asparagopsis taxiformis) supplementation reduces enteric methane by over 80 percent in beef steers. *PLoS One*, *16*(3), e0247820. https://doi.org/10.1371/journal.pone.0247820

Russell, J. B., & Rychlik, J. L. (2001). Factors That Alter Rumen Microbial Ecology. *Science*, *292*(5519), 1119-1122. https://doi.org/doi:10.1126/science.1058830

Sari, N. F., Ray, P., Rymer, C., Kliem, K. E., & Stergiadis, S. (2022). Garlic and Its Bioactive Compounds: Implications for Methane Emissions and Ruminant Nutrition. *Animals*, *12*(21), 2998. https://www.mdpi.com/2076-2615/12/21/2998

Statistics Canada (2025). Number of cattle, by class and farm type Table 32-10-0130-01 Number of cattle, by class and farm type (x 1,000). https://doi.org/doi.org/10.25318/3210013001-eng

Storm, I. M. L. D., Hellwing, A. L. F., Nielsen, N. I., & Madsen, J. (2012). Methods for measuring and estimating methane emission from ruminants. *Animals*, *2*(2), 160-183. https://www.mdpi.com/2076-2615/2/2/160

Tapio, I., Fischer, D., Blasco, L., Tapio, M., Wallace, R. J., Bayat, A. R., Ventto, L., Kahala, M., Negussie, E., Shingfield, K. J., & Vilkki, J. (2017). Taxon abundance, diversity, co-occurrence and network analysis of the ruminal microbiota in response to dietary changes in dairy cows. *PLoS One*, *12*(7), e0180260. https://doi.org/10.1371/journal.pone.0180260

Tayyab, U., Novoa-Garrido, M., Roleda, M. Y., Lind, V., & Weisbjerg, M. R. (2016). Ruminal and intestinal protein degradability of various seaweed species measured in situ in dairy cows. *Animal Feed Science and Technology*, *213*, 44-54. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2016.01.003

Terry, S. A., Basarab, J. A., Guan, L. L., & McAllister, T. A. (2021). Strategies to improve the efficiency of beef cattle production. *Canadian Journal of Animal Science*, *101*(1), 1-19. https://doi.org/10.1139/cjas-2020-0022

Terry, S. A., Coates, T., Gruninger, R., Abbott, D. W., & Beauchemin, K. A. (2023). Evaluation of the red seaweed Mazzaella japonica as a feed additive for beef cattle. *Frontiers in Animal Science*, *Volume 4 - 2023*. https://doi.org/10.3389/fanim.2023.1181768

Thauer, R. K., Kaster, A. K., Seedorf, H., Buckel, W., & Hedderich, R. (2008). Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Reviews Microbiology*, *6*(8), 579-591. https://doi.org/10.1038/nrmicro1931

United Nations Organization (2024). *World Population Prospects 2024: Summary of Results*. United Nations Organization. https://desapublications.un.org/publications/world-population-prospects-2024-summary-results?_gl=1*1jh80et*_ga*NDY5NTg2MzI4LjE3NTgwNTA5MTU.*_ga_TK9BQL5X7Z*czE3NTgwNTIwNzIkbzEkZzAkdDE3NTgwNTIwNzIkajYwJGwwJGgw

Ungerfeld, E. M. (2020). Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. *Frontiers in Microbiology*, *11*, 589. https://doi.org/10.3389/fmicb.2020.00589

Vyas, D., McGinn, S. M., Duval, S. M., Kindermann, M., & Beauchemin, K. A. (2016). Effects of sustained reduction of enteric methane emissions with dietary supplementation of 3-nitrooxypropanol on growth performance of growing and finishing beef cattle1. *Journal of Animal Science*, *94*(5), 2024-2034. https://doi.org/10.2527/jas.2015-0268

Wallace, R. J. (2004). Antimicrobial properties of plant secondary metabolites. *Proceedings of the Nutrition Society*, *63*(4), 621-629. https://doi.org/10.1079/PNS2004393

Wallace, R. J., & Brammall, M. L. (1985). The role of different species of bacteria in the hydrolysis of protein in the rumen. *Microbiology*, *131*(4), 821-832. https://doi.org/https://doi.org/10.1099/00221287-131-4-821

Wanapat, M., Prachumchai, R., Dagaew, G., Matra, M., Phupaboon, S., Sommai, S., & Suriyapha, C. (2024). Potential use of seaweed as a dietary supplement to mitigate enteric methane emission in ruminants. *Science of The Total Environment*, *931*, 173015. https://doi.org/https://doi.org/10.1016/j.scitotenv.2024.173015

Wang, Y., & McAllister, T. (2002). Rumen microbes, enzymes and feed digestion-a review. *Asian-Australasian Journal of Animal Sciences*, *15*(11), 1659-1676.

Weimer, P. J. (2022). Degradation of cellulose and hemicellulose by ruminal microorganisms. *Microorganisms*, *10*(12), 2345. https://www.mdpi.com/2076-2607/10/12/2345

Zhao, Y., Nan, X., Yang, L., Zheng, S., Jiang, L., & Xiong, B. (2020). A review of enteric methane emission measurement techniques in ruminants. *Animals*, *10*(6), 1004. https://www.mdpi.com/2076-2615/10/6/1004

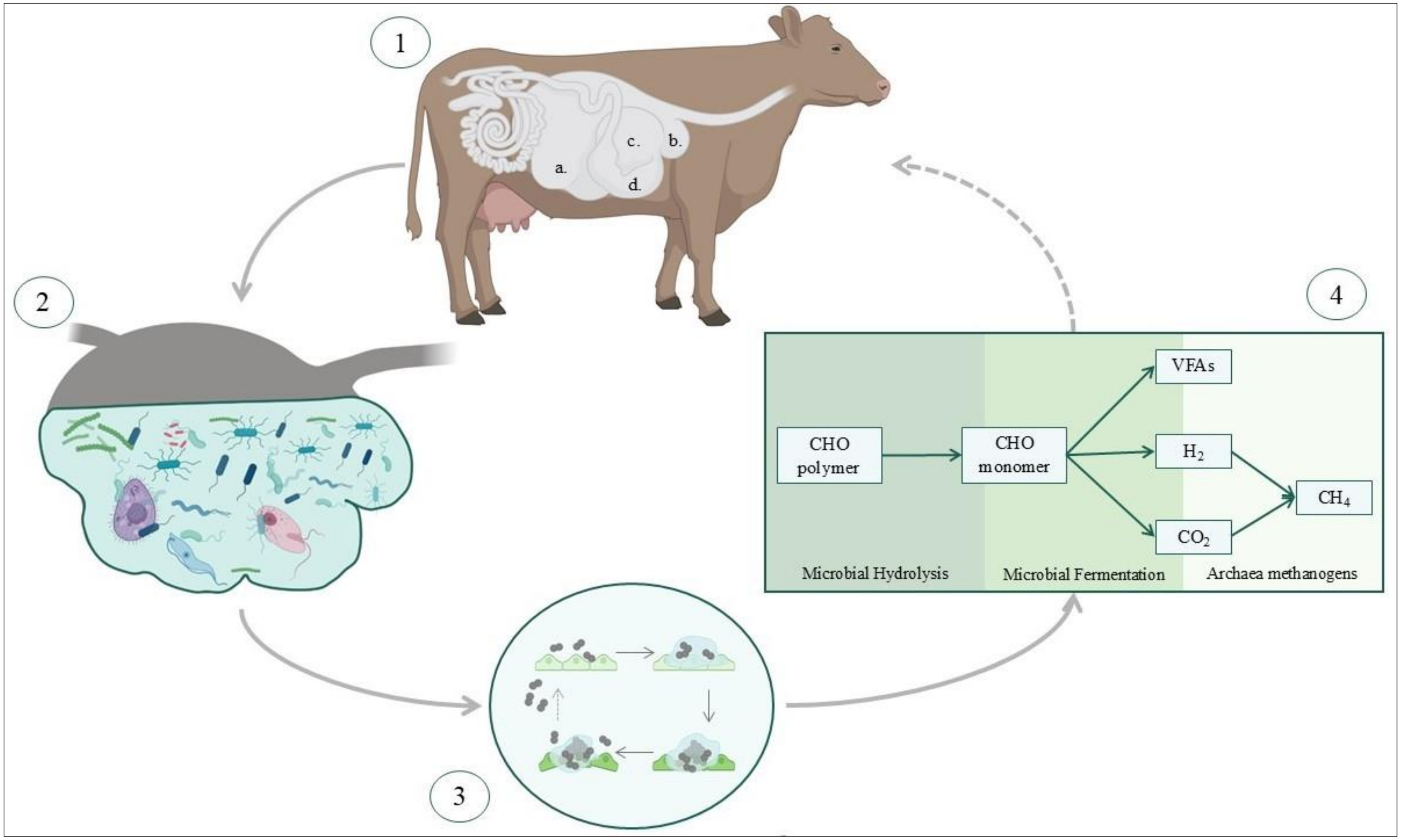

Figure 1.1. Diagram of the ruminant digestive tract and feed degradation cycle
1. Ruminant stomach compartments: **a.** rumen, **b.** reticulum, **c.** omasum, and **d.** abomasum; **2.** Representation of the ruminal anaerobic compartment containing bacteria, archaea, protozoa, and fungi; **3.** microbial attachment to the feed particle, followed by colonization and biofilm formation; **4.** Ruminal microorganisms hydrolyze carbohydrate polymers (CHO polymer) into monomers (CHO monomer), which are subsequently fermented into volatile fatty acids (VFAs), CO_2, and H_2. Methanogenic archaea use CO_2 and H_2 to produce CH_4. VFAs are absorbed through the rumen wall, and CH_4 is released by eructation, normal respiration, and small amounts as flatus into the environment. Source: Figure created by the author with BioRender.com and Microsoft PowerPoint.

Table 1.1. Main ruminal bacterial phyla, families, and genera associated with different metabolic functions, substrate utilization, and end-product synthesis

Phylum	Family	Genus	Function	Substrate	End-product
Firmicutes	Lachnospiraceae	*Butyrivibrio*	Fibrolytic Proteolytic Biohydrogenation	Cl, Hc, xylan, Fa, protein	C1, C2, C4, H_2, Br, NH_3
		Pseudobutyrivibrio	Fibrolytic	Hc, Ss	C4; H_2
	Ruminococcaceae	*Ruminococcus*	Fibrolytic	Cl, Hc, Ss	C2, C3, H_2
	Selenomonadaceae	*Selenomonas*	Fibrolytic Lactate user	Cl	C2, C3, C4, H_2
	Streptococcaceae	*Streptococcus*	Amylolytic	Starch, Ss	Lac, C1, C2
	Veillonellaceae	*Megasphaera*	Lactate user	Lac	C2, C3, C4, Br, H_2
Bacteroidetes	Prevotellaceae	*Prevotella*	Proteolytic Fibrolytic Amylolytic	Protein, Hc, Pp, Ss, xylans, starch	Suc, C1, C2, C3, Br, NH_3
Proteobacteria	Succinivibrionaceae	*Ruminobacter*	Fibrolytic Amylolytic	Starch, Ss, Hc	C3, Lac, Suc, H_2
		Succinimonas	Amylolytic	Starch	Suc, C2, C3
		Succinivibrio	Amylolytic	Starch, Ss	Suc, C1, C2, Lac
Actinobacteria	Atopobiaceae	*Olsenella*	Amylolytic	Ss	Lac
	Bifidobacteriaceae	*Bifidobacterium*	Fibrolytic	Ss, Ol	C2, Lac
Fibrobacteres	Fibrobacteraceae	*Fibrobacter*	Fibrolytic	Cl, Hc, xylan	C1, C2, Suc
Spirochaetes	Spirochaetaceae	*Treponema*	Fibrolytic Amylolytic	Hc, Ss	C2, C3, H_2

Cl: Cellulose; Hc: Hemicellulose; Ss: Soluble sugar; Fa: Fatty acid; Lac: Lactate; Pp: peptides; Suc: Succinate; Ol: Oligosaccharides; C1: Formate; C2: Acetate; C3: Propionate; C4: Butyrate; C5: Valerate; C6: Caproate; Br: branched-chain volatile fatty acids. Source: Huws et al. (2015); Mizrahi et al. (2021); Russell & Rychlik (2001).

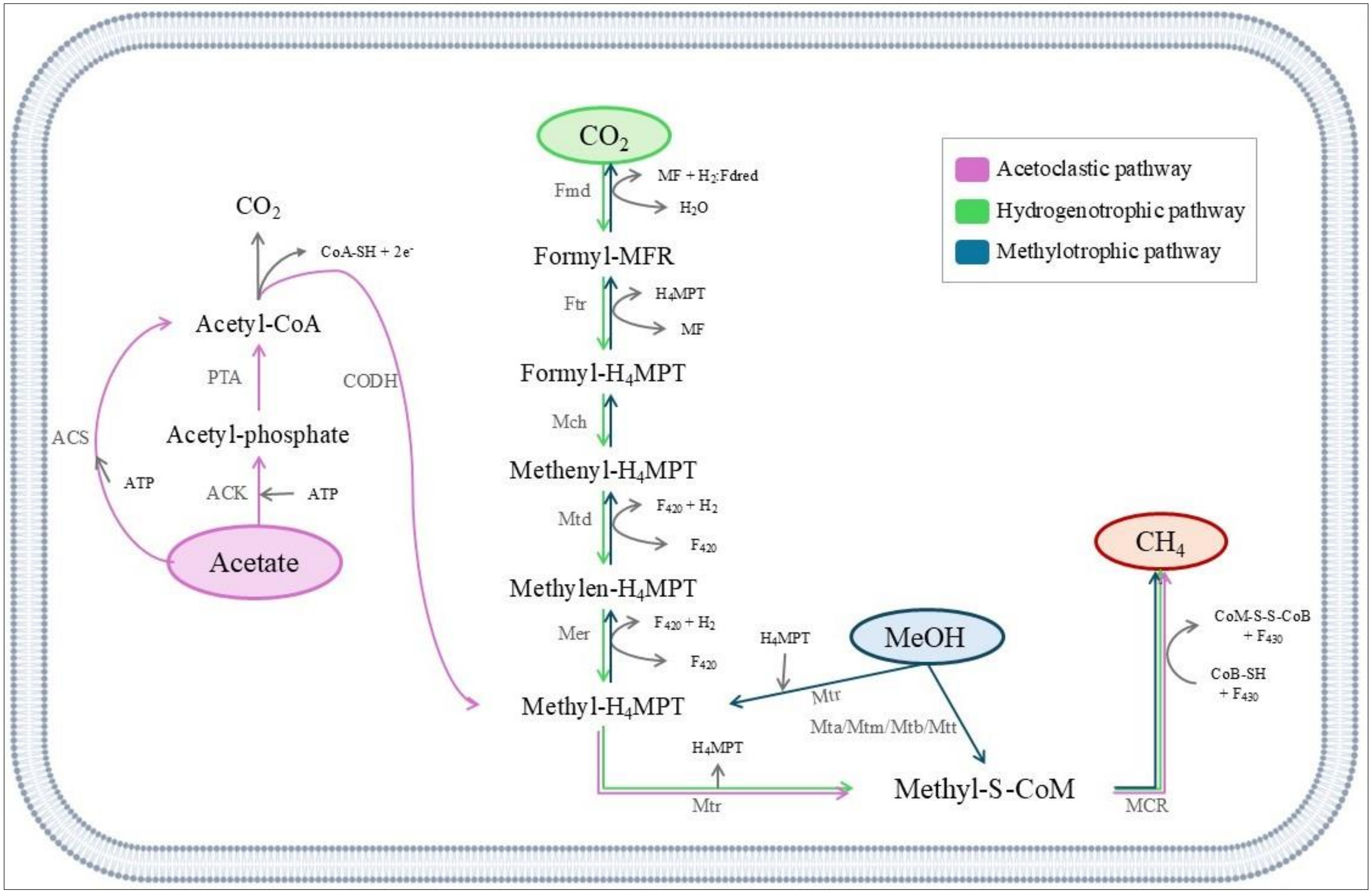

Figure 1.2. Overview of the three methanogenic pathways: acetoclastic (purple), hydrogenotrophic (green), and methylotrophic (blue). The figure illustrates how different substrates are converted into CH_4, with enzymes and cofactors involved in these processes
ACS: acetyl-CoA synthase; ACK: acetate kinase; PTA: phosphotransacetylase; CODH: CO dehydrogenase; Fmd: formylmethanofuran dehydrogenase; Ftr: formylmethanofuran:H_4MPT formyltransferase; Mch: Methenyl-H4MPT cyclohydrolase; Mtd: Methylen-H_4MPT dehydrogenase (F_{420}-dependent); Mer: methylen-H_4MPT reductase; Mtr: Methyl-H_4MPT (Coenzyme M methyltransferase and Na^+ translocating); MCR: Methyl-coenzyme M reductase; Coenzyme M Methyltransferase: Mta system (methanol utilization), Mtm system

(monomethylamine utilization), Mtb system (dimethylamine utilization), and Mtt system (trimethylamine utilization); Fdred: ferredoxin (reduced); H_4MPT: tetrahydromethanopterin; MFR: methanofuran; CoM: coenzyme M (2-mercaptoethanesulfonate); CoB: coenzyme B (7-mercaptoheptanoylthreonine phosphate); CoM-S-S-CoB: CoM-CoB heterodisulfide; HS-CoM: reduced coenzyme M; HS-CoB: Reduced coenzyme B; F_{420}: Coenzyme F_{420} (a deazaflavin electron carrier; reduced form is $F_{420}H_2$); F_{430}: Coenzyme F_{430} (nickel-containing tetrapyrrole in MCR). Source: Figure adapted from Ferry (1992) by the author with BioRender.com and Microsoft PowerPoint.

Table 1.2. Summary of the effect of seaweed supplementation in ruminant diets on methane and volatile fatty acids

Seaweed species	Group	Study	Animal	Diet	Doses level	CH_4 effect[1]	VFA effect*	Citation
Asparagopsis taxiformis	Red	*In vitro*	Brahman beef steers	Rhodes grass hay	Dose-response from 0 to 16.7% OM basis	↓ 84.7% CH_4 mL/g OM at 1% of inclusion ↓ >99% CH_4 mL/g OM at ≥ 2% of inclusion	↓ Total VFA	Machado et al. (2016)
Oedogonium sp.	Green	*In vitro*	Brahman beef steers	Rhodes grass hay	Dose-response from 0 to 100% OM basis	↓ ~ 50% CH_4 mL/g OM at ≥ 75% of inclusion ↓ 61.6% CH_4 mL/g OM at 100% of inclusion	↓ Total VFA	Machado et al. (2016)
Gigartina sp.	Red	*In vitro*	Holstein cows	Two substrates: 1. Meadow hay 2. Corn silage	25% DM basis	1. ↓ ~ 44% CH_4 mL/g DM 2. N/E	1. ↑ C2:C3 2. ↓ Total VFA, ↑ Iso-C5	Maia et al. (2016)
Gracilaria vermiculophylla	Red					1. ↓ ~ 59% CH_4 mL/g DM 2. ↓ ~ 63% CH_4 mL/g DM	1. ↑ C2:C3 2. ↓ Total VFA	
Laminaria ochroleuca	Brown					1. N/E 2. ↑ 148% CH_4 mL/g DM	1. ↑ C2:C3, ↓ Iso-C5 2. ↓ Total VFA	

Table 1.2. Continued

Seaweed species	Group	Study	Animal	Diet	Doses level	CH_4 effect[1]	VFA effect*	Citation
Saccharina latissimi	Brown	*In vitro*	Holstein cows	Two substrates: 1. Meadow hay 2. Corn silage	25% DM basis	1. N/E 2. N/E	1. ↑ C2:C3 2. N/E	Maia et al. (2016)
Ulva sp.	Green					1. ↓ ~ 55% CH_4 mL/g DM 2. N/E	1. ↑ C2:C3 2. N/E	
Mastocarpus stellatus	Red	*In vitro*	Segureña ewes	Seaweed as substrate	100% (500g of lyophilized seaweed)	*P. palmata*: ↑ 49.8% CH_4, mmol ≠ other seaweeds	*P. palmata*: ↓Total VFA & C3; ↑ C2:C3 ≠ other seaweeds	Molina-Alcaide et al. (2017)
Palmaria palmata	Red							
Porphyra sp.	Red							
Alaria esculenta	Brown							
Laminaria digitata	Brown							
Pelvetia canaliculata	Brown							
Asparagopsis taxiformis	Red	*In vitro*	Jersey & Holstein cows	SBR forage-based	5.0% OM basis	↓ 95.1% CH_4 mL/g OM	↑ C2:C3	Roque et al. (2019a)
Asparagopsis armata	Red	*In vivo*	Holstein cow	TMR	0.5% & 1.0% OM basis	↓ 20.3% & 42.7% CH_4 g/kg DMI	N/A	Roque et al. (2019b)
Asparagopsis taxiformis	Red	*In vivo*	Angus-Hereford cross beef steers	1. TMR high forage 2. TMR medium forage 3. TMR low forage	0.25% & 0.5% OM basis	1. ↓ 32.6% & 52.0% 2. ↓ 44.8% & 79.6% 3. ↓ 69.8% & 79.8% CH_4 g/kg DMI	N/A	Roque et al. (2021)

Table 1.2. Continued

Seaweed species	Group	Study	Animal	Diet	Doses level	CH_4 effect[1]	VFA effect*	Citation
Ascophyllum nodosum	Brown	*In vitro*	Jersey cow	TMR forage-based	2.5% & 5.0% DM basis	↓ 16.9% CH_4 mL/g OMD at 5% of inclusion	↓ Total VFA, C2, C3, C4, C5, Iso-C4, Iso-C5, & C2:C3	Künzel et al. (2022)
Fucus vesiculosus	Brown					↓ 11.2% CH_4 mL/g OMD at 5% of inclusion	↓ Total VFA, C2, C3, C4, C5, Iso-C4, Iso-C5, & C2:C3	
Asparagopsis taxiformis	Red	*In vivo*	Nordic Red dairy cows	TMR forage-based	0.5% OM basis	↓ 51.6% CH_4 g/kg DMI	↓ C2 & C2/C3; ↑ C3, C4 & C5	Krizsan et al. (2023)
Mazzaella japonica	Red	*In vivo*	Angus cross beef heifers	TMR forage-based	1.0% & 2.0% %, DM basis	↓ 9.2% CH_4 g/d at 2.0% of inclusion N/E on CH_4 g/kg DMI	N/E	Terry et al. (2023)
Chondrus crispus	Red	*In vivo*	Jersey & Holstein cows	Forage diet-based	6.0% DM basis	↓ 13.9% CH_4 g/d	N/A	Reyes et al. (2023)

Table 1.2. Continued

Seaweed species	Group	Study	Animal	Diet	Doses level	CH_4 effect[1]	VFA effect*	Citation
Chondrus crispus	Red	*In vivo*	Holstein-Friesian dairy cows	PMR forage-based	0.6% DM basis	N/E	N/A	Muizelaar et al. (2023)
Saccharina latissima	Brown					N/E	N/A	
Blend: *Fucus serratus* & *Saccharina latissima*	Brown					N/E	N/A	
Blend: *Ascophyllum nodosum* (5%), *Fucus vesiculosus* (45%), & *Asparagopsis taxiformis* (50%)	Brown Brown Red	*In vivo*	Holstein dairy cows	TMR - 56:44 forage:concentrate	1.5% DM basis	↓ 11.9% CH_4 g/kg DMI	N/A	Reynolds et al. (2025)
Blend: *Ascophyllum nodosum* (90%) & *Fucus vesiculosus* (10%)	Brown Brown				6.5% DM basis	N/E	N/A	
Blend: *Ascophyllum nodosum* (10%) & *Fucus vesiculosus* (90%)	Brown Brown				17.5% DM basis	↓ 11.2% CH_4 g/kg DMI	N/A	

Table 1.2. Continued

Seaweed species	Group	Study	Animal	Diet	Doses level	CH_4 effect[1]	VFA effect*	Citation
Asparagopsis taxiformis	Red	*In vitro*	Angus cross beef heifers	Alfalfa hay	5.0% DM basis	↓ 100% CH_4 mL/g DMD	↓ Total VFA, C2 & C2:C3; ↑ C3, C4 & C5	Ramos et al. (2025)
Calliarthron tuberculosum	Red					N/E	↓ Total VFA	
Gracilaria sp.	Red					N/E	↓ Iso-acids &C5	
Odonthalia floccosa (juvenile)	Red					N/E	↑ C2:C3	
Prionitis lanceolata (juvenile)	Red					N/E	↑ C2 & C2:C3	
Prionitis lanceolata (mature)	Red					N/E	↑ C2 & C2:C3; ↓ Iso-acids, C5 & C6	
Prionitis sp.	Red					N/E	↓ Total VFA, Iso-acids, C5 & C6	
Macrocystis pyrifera	Brown					N/E	↑ C6	
Nereocystis luetkeana (blades)	Brown					N/E	↓ C5	
Postelsia palmaeformis	Brown					N/E	↓ C5	

Table 1.2. Continued

Seaweed species	Group	Study	Animal	Diet	Doses level	CH_4 effect[1]	VFA effect*	Citation
Saccharina groenlandica (wild)	Brown	*In vitro*	Angus cross beef heifers	Alfalfa hay	5.0% DM basis	N/E	↓ Total VFA & Iso-acids	Ramos et al. (2025)
Phyllospadix scouleri	Green					N/E	↑ C2	
Chondracanthus corymbiferus	Red				0.5%, 1.0%, 2.0% & 5% DM basis	↓ 12.4% CH_4 mL at 5% of inclusion N/E on CH_4 mL/g DMD	↑ C3 at 2% of inclusion	
Halosaccion glandiforme	Red					N/E	↑ C3 at 2% & 5% of inclusion	
Fucus vesiculosus	Brown					N/E	N/E	
Blend: *Laminaria longicruris*, *Chondrus crispus*, *Fucus vesiculosus*, & *Furcellaria lumbricalis*	Red Red Brown Brown					↓ 9.7% CH_4 mL/g DMD	N/E	

*Reduction (↓) or increase (↑), values were contrasted against control treatment (without seaweed addition); SBR: super basic ration; TMR: total mixed ration; PMR: partial mixed ration; C2: Acetate; C3: Propionate; C4: Butyrate; C5: Valerate; C6: Caproate; N/A: not analyzed; N/E: No effect; OM: organic matter; DM: dry matter; DMI: dry matter intake; DMD: dry matter degraded; OMD: organic matter degraded.

CHAPTER 2: EVALUATING THE EFFECT OF A SEAWEED BLEND ON GROWTH PERFORMANCE, FEEDING BEHAVIOR, METHANE EMISSIONS, AND HEMATOLOGICAL PROFILE OF BACKGROUNDING AND FINISHING FEEDLOT BEEF HEIFERS

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Abstract: The growing interest in reducing greenhouse gas emissions from ruminants has highlighted the potential of seaweed to mitigate enteric methane (CH_4) production. This study evaluated the effect of adding 0.60% dry matter (DM) of a multispecies seaweed blend (SWblend) in a total mixed ration (TMR) on growth performance, feeding behavior, ruminal fermentation, protozoa count, ruminal pH, CH_4 production, and hematological parameters of feedlot heifers fed backgrounding and finishing diets. One hundred and twelve beef heifers were enrolled in a completely randomized block design with two treatments (control and SWblend) across two blocks. Supplementation of SWblend did not affect ($P \geq 0.23$) dry matter intake (DMI), weight gain, average daily gain (ADG), gain to feed ratio (G:F), or initial and final shrunk body weight (SBW). There was a tendency ($P = 0.06$) for SWblend fed heifers to have a greater eating rate during the backgrounding phase. There was no effect ($P \geq 0.25$) of treatment on ruminal NH_3, total volatile fatty acids (VFA), individual VFA proportions, protozoa counts and mean and maximum ruminal pH during backgrounding. However, heifers fed the SWblend tended (*P* < 0.10) to have a greater minimum ruminal pH, and the standard deviation (SD) of mean pH was reduced (*P* = 0.03) by 12.8% in SWblend fed heifers. During the finishing phase, SWblend heifers had decreased (*P* < 0.05) total VFA and propionate production, and increased (*P* < 0.05) ruminal NH_3, butyrate, isobutyrate, and isovalerate levels. There was no effect (*P* ≥ 0.23) of SWblend on GreenFeed system (GF) variables, such as number of visits (total and daily), total DMI, CH_4 production (g/d), CH_4 yield (g/kg DMI), gross energy intake (GEI; Mcal/d), or CH_4 (Mcal/% GEI) during the backgrounding phase. Alternatively, SWblend heifers had shorter (*P* = 0.02) mean duration and lower (*P* = 0.01) pellet DMI in the GF system. There was no effect (*P* ≥ 0.17) of treatment on GF variables during the finishing phase. Hematological parameters were not affected (*P* ≥ 0.10) by treatment in either the backgrounding or finishing phase. In conclusion, supplementing feedlot beef heifers with 0.60% of SWblend during backgrounding and finishing phases did not negatively impact animal performance, feeding behavior, or hematological parameters but did not decrease enteric CH_4 emissions. While the seaweed blend altered the ruminal fermentation profile in the finishing phase, no adverse effects were observed on ADG or feed efficiency. Overall, SWblend appears to be a safe feed additive for ruminants and may contribute to enhanced ruminal stability, although it has limited potential for reducing enteric CH_4 emissions in feedlot heifers.

2.1. Introduction

Concerns about climate change have intensified in recent years, prompting the development of strategies to reduce greenhouse gas (GHG) emissions (Beauchemin et al., 2022). In response, more than 110 countries and organizations have signed the Global Methane Pledge (created during COP26 in 2021; https://www.globalmethanepledge.org/), committing to a collective reduction of methane (CH_4) emissions by 30% from 2020 levels by 2030. Currently, enteric CH_4 emissions from ruminant livestock contribute to 30% of global anthropogenic CH_4 emissions (Beauchemin et al., 2022; Shindell et al., 2021). To address this, several studies have focused on dietary strategies and feed additives to reduce enteric CH_4 production without compromising ruminant health and productivity (Almeida et al., 2021; Beauchemin et al., 2022; Beauchemin et al., 2020; Hristov et al., 2022).

Enteric CH_4 is produced as a by-product of carbohydrate fermentation by ruminal microorganisms. The fermentation process plays a crucial role in enabling ruminants to digest fibrous plant material and convert it into energy for maintenance, growth, and reproduction (Abbott et al., 2020; Chen et al., 2022). Overall, complex carbohydrates are converted into volatile fatty acids (VFAs), carbon dioxide (CO_2), and hydrogen (H_2) by ruminal microbial activity. Simultaneously, methanogenic archaea produce CH_4 using CO_2 and H_2 mainly via the hydrogenotrophic pathway, with minor contributions from methylotrophic routes. Furthermore, methanogenesis is the primary mechanism for H_2 removal, helping to prevent the accumulation of NADH, which would otherwise inhibit carbohydrate fermentation and fibre degradation (Morgavi et al., 2010).

Given the critical role of methanogenesis in rumen fermentation and its environmental impact, there is increasing interest in identifying natural compounds capable of modulating this process, especially those that can reduce CH_4 production without disrupting rumen function. In this context, seaweeds have emerged as promising feed additives due to their potential anti-methanogenic properties (Kinley et al., 2020). This interest has been linked to their unique chemical composition, including carbohydrates, proteins, amino acids (AA), lipids, minerals, and vitamins, as well as secondary metabolites, such as phlorotannins, saponins, and halogenated compounds (Abbott et al., 2020; Fouts et al., 2022). Seaweeds are generally classified into three main groups: green

(Chlorophyta), red (Rhodophyta), and brown (Phaeophyta), and their composition and effectiveness of bioactive compounds can vary depending on the seaweed species, harvest methods, season, and growth conditions (Makkar et al., 2016).

Red seaweeds, such as *Asparagopsis spp.*, have gained significant attention as a potent feed additive for mitigating enteric CH_4 due to the presence of bromoform (CHBr3), a halogenated CH_4 analogue (Kinley et al., 2020; Machado et al., 2016; Roque et al., 2021). The feeding of halogenated compounds to ruminants raises concerns about toxicity, carcinogenicity, and environmental risks, which may include potential effects on the stratospheric ozone layer (Glasson et al., 2022). As a result, there is a need to investigate the anti-methanogenic properties of non-halogenated seaweeds and multispecies blends as safer and more sustainable alternatives for CH_4 mitigation, without posing adverse effects on the environment and human health. In parallel, it is essential to understand their effect on feed intake, performance, and health indicators to ensure that mitigation strategies do not compromise animal productivity or welfare.

Therefore, the objective of this study was to evaluate the effect of supplementing 0.60% (dry matter (DM) basis) of a commercial multispecies seaweed blend (SWblend) in a total mixed ration (TMR) on growth performance, feeding behavior, ruminal fermentation, protozoa counts, ruminal pH, CH_4 production, and hematological parameters of feedlot heifers fed backgrounding and finishing diets.

2.2. Materials and methods

This study was conducted at Agriculture and Agri-Food Canada’s Lethbridge Research and Development Centre in Lethbridge, Alberta. Heifers were cared for following the guidelines of the Canadian Council on Animal Care (CCAC, 2009). The study was reviewed and approved by the Institutional Animal Care Committee at the centre (#2309).

2.2.1. Animals, experimental design, and diets

This study was conducted as a completely randomized block design with two treatments (control and SWblend) replicated across two blocks. A total of 112 Angus× beef heifers with an initial body weight of 324.5 ± 32.0 kg were blocked by BW and randomly allocated to four pens, each of which was equipped with the GrowSafe system. Each pen housed 25 intact and 3 ruminally cannulated heifers. The experiment was conducted over both backgrounding and finishing phases, with each phase divided into four periods (4 weeks per period). Prior to data collection, heifers underwent a 4-week adaptation to acclimate to pens, diet, and use of Growsafe (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) and Greenfeed (GF; C-Lock Inc., Rapid City, SD) systems.

Upon arrival, each heifer was equipped with an RFID ear tag and treated with Bovimectin Pour-on (5 mg of ivermectin/mL; 500 µg of ivermectin per kg of body weight (BW); Vetoquinol N.-A. Inc., Lavaltrie, QC, Canada) and vaccinated with Ultrabac 7/Somubac (Clostridium vaccine; Zoetis Canada Inc., Kirkland, QC, Canada) and Pyramid FP5 (infectious bovine rhinotracheitis and bovine viral diarrhea vaccine; Boehringer Ingelheim Ltd., Burlington, ON, Canada).

Heifers were fed a typical Canadian feedlot diets throughout backgrounding and finishing phases (Table 2.1 and Table 2.2). During the backgrounding, the control group received a total mixed ration (TMR) consisting of 65.0% corn silage, 20.0% barley grain, 10.0% canola meal, and 5.0% mineral supplement on a dry matter (DM) basis. The SWblend pens received that same TMR as control pens, except 0.60% of corn silage was replaced by the commercial seaweed blend (OceanFeed™ Bovine) on a DM basis. This blend comprises red, brown, and green species from Alariaceae, Bangiaceae, Fucaceae, Gelidiaceae, Gigartinaceae, Gracilariaceae, Laminariaceae, Lessoniaceae, Monostromataceae, Palmariaceae, Sargassaceae, Solieriaceae, and Ulvaceae, as well as calcified red seaweed, harvested in different coastal regions of Europe, North America, and Southeast Asia. The 0.60% inclusion of the SW blend was formulated to supply 50 g/heifer/day of the additive as recommended by the manufacturer.

A 4-week transition period was used to gradually adapt the cattle to the finishing diet, with increasing increments of concentrates replacing the forage in the TMR. The control finishing diet consisted of 9.0% corn silage, 86.0% barley grain, and 5.0% mineral supplement (on DM basis) with SWblend replacing 0.60% of the corn silage. All diets were formulated to meet or exceed the nutrient requirements for feedlot cattle during backgrounding and finishing (NASEM, 2016). Feed was delivered once daily at 0900 h using a slick bunk management approach, targeting less than 5% of feed refusal, and the heifers had *ad libitum* access to water.

2.2.2. Measurements and sampling

Live body weight and feeding behavior

All heifers were weighed (non-fasted) before feeding at the initiation and completion of each phase on 2 consecutive days. Throughout the study, cattle were also weighed every 4 weeks prior to feeding. Average daily gain (ADG) was calculated for each month and over the duration of the experiment. Gain:feed (G:F) was calculated as ADG divided by dry matter intake (DMI). To provide a more accurate estimate of BW, shrunk initial and final BWs (SBW) were calculated by dividing the recorded BW by 0.96, correcting for gastrointestinal fill (Ribeiro et al., 2016).

Individual heifer intake and feeding behavior was measured using the GrowSafe system, which recorded individual heifer feed consumption. Distinct feeding events were grouped into meals using a 300-sec meal criterion (Schwartzkopf-Genswein et al., 2002). The amount of feed consumed during a visit in the GrowSafe bunks was used to calculate meal size (kgof DM per meal), individual DMI, day-to-day variation of DMI (SD of DMI), meal frequency (meals per day), duration of each meal (min per meal), and the interval between meals (min per day). Feeding time was calculated as the sum of the length of all meals within a day (min per day), and the feeding rate was determined as the sum of the mass of all meals within a day divided by daily feeding time (g of DM per min). The DMI of each animal was calculated as the sum of dietary DM consumed from the basal diets offered in the GrowSafe system and the DMI from the GF system.

Rumen samples and pH measurements

Samples from the reticulum and rumen (ventral, caudal, and dorsal-ventral sacs) were collected from cannulated heifers, and the pooled samples were used to measure volatile fatty acids (VFA) and ammonia (NH_3) concentrations, protozoa counts, and microbial profiles. Pooled rumen content was squeezed through two layers of PECAP nylon. Two 1-mL subsamples were placed in microtubes prefilled with 0.2 mL of 25% (wt/vol) metaphosphoric acid for VFA analysis, and 0.2 mL of 1% (wt/vol) sulfuric acid for NH_3 analysis and stored at −20 °C until analyses. Furthermore, 5 mL of rumen fluid was stored in a scintillation vial containing 5 mL of methyl green-formalin-saline solution for protozoa enumeration and subsequently stored at room temperature in the dark (Ogimoto & Imai, 1981).

During each period, indwelling pH loggers were inserted into the 12 cannulated heifers for continuous rumen pH monitoring over five consecutive days. Rumen pH was recorded using the LRCpH data logger system (Dascor, Escondido, CA), with sensors standardized in pH 4 and 7 solutions before and after use. The pH data were summarized for daily minimum, maximum, and mean pH values, as well as the standard deviation for each animal.

Greenhouse gas emissions

Two of the four pens were equipped with GF to measure individual CH_4 emissions. The heifers accessed the GF system voluntarily, which dispensed a pelleted supplement to encourage visits. To allow for CH_4 measurements from both treatment groups, heifers were rotated every two wk between two pens within each block, with each block having one GF. This rotation ensured that each treatment was exposed to the GF monthly and eliminated potential pen effects by allowing all heifers to spend an equal amount of time in each pen within a block (Alemu et al., 2021). The GF pellet supplied to the heifers during the backgrounding phase was composed of 43.2% of ground barley, 11.5% canola meal, 1.0% limestone ($CaCO_3$), 13.0% dried molasses, 0.15% salt, 30.4% beet pulp, and 0.75% canola oil (DM basis). The GF pellet in the finishing phase was comprised of 45.1% ground barley, 7.5% canola meal, 1.0% limestone ($CaCO_3$), 13.0% dried molasses, 0.15% salt, 32.5% beet pulp, and 0.75% canola oil (DM basis). The GF pellet was made to target the chemical composition of the TMR, and be highly palatable to ensure visitation (Table 2.1).

The calculation of gaseous emissions from the GF system was based on data from non-cannulated heifers to maintain accuracy and avoid potential gas loss through the cannula. Only visits in which the animal's head was close to the sensor for at least 3 min were used in the analysis (described as useful/good visits), ensuring capture of a minimum of three eructation events per sampling. To calculate the average daily CH_4 emission rate (production, g/day) for each heifer during a 14-d measurement period, the flux data from useful visits were compiled into six 4-h blocks corresponding to time of day (i.e., time bins). Only heifers with ≥ 10 useful/good visits spread in at least five of the six 4-h time blocks within a measurement period were used in the final analysis to ensure that the full circadian cycle of emissions was represented. Pellet DMI (kg/d) was calculated by dividing the mean visitation duration by 30 sec interval per pellet drop and multiplying by the pellet drop mass (45 g), adjusted to DM basis. Total DMI (kg/d) was determined as the sum of DMI from GrowSafe bunks and daily pellet DMI. Gross energy intake (GEI, Mcal/d) was obtained by multiplying total DMI by the gross energy content of the diet. Methane yield (g/kg DMI) was calculated as daily CH_4 production (g/d) divided by total DMI (kg/d). Methane intensity (Mcal/%GEI) was estimated as a proportion of GEI, by converting CH_4 mass to energy equivalents [mean CH_4 production (kg/d) multiplied by energy equivalency (13.3 Mcal/kg CH_4); (Blaxter & Clapperton, 1965)], and divided by GEI (Alemu et al., 2021; Terry & Beauchemin, 2025). All CH_4 variables were averaged per heifer over the 14-d measurement period and then analyzed as repeated measures within each experimental phase.

Hematological Parameters

At the beginning of the study and subsequently in four-wk intervals, blood samples were taken from the jugular vein of each heifer to assess immune status. Samples were taken before feed delivery and collected into a 10 mL EDTA tube. Complete blood counts were performed on a HemaTrue blood analyzer (Heska, Des Moines, IA) within 45 min of collection, measuring total red blood cell count (RBC), mean cell volume of RBCs (MCV), hematocrit (HCT), mean cell hemoglobin (MCH), total white blood cell count (WBC), lymphocyte concentration in absolute number and percentage (LYMF), mid-sized cells in absolute number and percentage (MONO), and granulocyte concentration in absolute number and percentage (GRAN).

2.2.3. Laboratory analysis

Chemical composition

Dried ingredients, feed, and refusals were ground in a Wiley mill through a 1-mm screen (Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for analytical DM (AOAC (2005); method 930.15), organic matter (OM) (method 942.05), and ash (method 942.05). Neutral detergent fibre (NDF) was determined using an ANKOM200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA), and heat-stable α-amylase (Termamyl® 120; Sigma-Aldrich, St. Louis, MO, USA). Sodium sulfite was used for NDF analysis (Mertens et al., 2002). Subsequently, acid detergent fibre (ADF) was analyzed (AOAC (2005); method 973.18). Subsamples (5 g) were further ground with a ball grinder (Retsch MM 400; Retsch Inc., Newtown, PA) and analyzed for N using flash combustion (method 990.03; Carlo Erba Instruments, Milan, Italy). Crude protein was calculated as N × 6.25. Starch concentration was determined as described by Herrera-Saldana et al. (1990), and absorbance was read on a Thermo Scientific Appliskan 1.437 (SkanIt Software 2.3 RE) microplate reader at a wavelength of 490 nm. Gross energy content of samples was determined using a bomb calorimeter (model 6200 Isoperibol Calorimeter, Parr Instrument Company, Illinois, USA).

Volatile fatty acids, ammonia concentrations, and protozoa counts

Samples were analyzed for VFA concentrations using a gas-liquid chromatograph (model 6890; Agilent, Wilmington, DE, USA) equipped with a 30-m Zebron free fatty acid phase fused silica capillary column (0.32-mm i.d., and 1.0-µm film thickness; Phenomenex, Torrance, CA, USA). Individual VFA concentrations were expressed as proportions (mmol/mmol total VFA concentration), and the acetate to propionate ratio (C2/C3) was calculated. The concentration of

NH_3 was determined by the salicylate–nitroprusside–hypochlorite method using a flow injection analyser (Sims et al. 1995). Protozoa were enumerated under a light microscope using a Levy-Hausser counting chamber (Hausser Scientific, Horsham, PA) with a 1-mm depth, as described by Dehority (1993).

2.2.4. Statistical analysis

Data was analyzed as a completely randomized block design with the fixed effect of treatment (control or SWblend), random effect of pen within block, and block. The distribution and homogeneity of variance were determined using the UNIVARIATE procedure of SAS (SAS Inst., Inc., Cary, NC). Data for DMI, BW, ADG, feeding behavior, rumen fermentation, CH_4 production (g/d), and blood parameters were then analyzed using the MIXED procedure of SAS. Data from each phase were analyzed separately, except for overall DMI, weight gain, ADG, and G:F, which were also evaluated across the entire experiment, excluding the adaptation and transition period. Individual heifer was considered as the experimental unit for all variables. False discovery rate corrected *P* values were calculated using Tukey's test, with differences between means declared significant at $P < 0.05$, and a trend at $P < 0.10$.

2.3. Results

Overall, backgrounding and finishing DMI, weight gain, ADG, and G:F were not affected by addition of SWblend to the diet of feedlot heifers ($P \geq 0.23$; Table 2.3). Similarly, initial and final SBW did not differ ($P \geq 0.26$) between treatments across phases. There were no effects of SWblend s on DMI or feeding behavior during either phase (Table 2.4). Heifers fed SWblend tended ($P = 0.06$) to have a greater eating rate during the backgrounding phase.

Ruminal NH_3, total VFA, individual VFA proportions, protozoa counts and mean and maximum ruminal pH values were not affected ($P \geq 0.25$) by the inclusion of the SWblend during the backgrounding phase (Table 2.5). Minimum ruminal pH tended ($P = 0.07$) to be greater in heifers fed SWblend compared to control. The standard deviation (SD) of mean pH was 12.8% lower ($P = 0.03$) in the SWblend group than in the control group. Conversely, greater ($P = 0.008$) ruminal NH_3 concentrations were observed in the SWblend group during the finishing phase compared to control. Heifers fed the SWblend had decreased ($P < 0.05$) total VFA and propionate concentration, and greater ($P < 0.05$) concentration of butyrate, isobutyrate, and isovalerate compared to control, in the finishing phase. There was no effect ($P \geq 0.19$) of SWblend on the proportions of acetate, valerate, or caproate, the C2/C3 ratio, protozoa counts, or ruminal pH in the finishing phase.

During the backgrounding phase, there were no effect ($P \geq 0.23$) of SWblend on total or average visits to GrowSafe, total DMI, GEI, or CH_4 production (g/d), yield (g/kg DMI), or intensity (% GEI; Table 2.6). Heifers fed SWblend had shorter ($P < 0.05$) mean visit duration to the GF and lower pellet DMI compared to control. There was no effect ($P \geq 0.17$) of treatment on any GF variables during the finishing phase. Supplementation of the SWblend did not affect ($P \geq 0.10$) any hematological parameters in either the backgrounding or finishing phase (Table 2.7).

2.4. Discussion

There is increasing interest in using seaweeds as a feed additive for ruminants due to their rich and diverse composition, which includes iodine, vitamins, minerals, PUFA, and secondary metabolites like phlorotannin and halogenated compounds (Makkar et al., 2016; Min et al., 2021; Wanapat et al., 2024). These bioactive components have been associated with modulation of ruminal fermentation, potential reductions in enteric CH_4 emissions, and effects on animal performance (Wanapat et al., 2024). Despite this potential, few studies have specifically evaluated the effects of seaweed blends, which contain multiple species and variable chemical profiles, on ruminant growth parameters, feeding behavior, ruminal fermentation, and blood variables. To contribute to this body of knowledge, the present study evaluated the effect of a commercial seaweed blend (OceanFeed™ Bovine) in the diets of feedlot heifers.

Supplementing SWblend into the diet of beef heifers fed either high forage backgrounding or high grain finishing feedlot diets did not influence animal performance parameters, including DMI or growth performance. These results contrast with findings from previous *in vivo* studies using *Asparagopsis taxiformis*, a red seaweed from the Bonnemaisoniaceae family known for its strong antimethanogenic properties. Kinley et al. (2020) reported that including *A. taxiformis* at 0.05% OM in the diets of beef steers led to a trend toward a 10.8% decrease in DMI. Similar findings were reported by Roque et al. (2021), who found a 14.5% decrease in DMI when 0.5% OM of *A. taxiformis* was included in low, medium, and high forage diets fed to beef steers. Interestingly, ADG remained unaffected, resulting in a 13.8% improvement in G:F, suggesting that *A. taxiformis* enhanced nutrient utilization, likely due to reduced energy losses through CH_4 production (Roque et al., 2021). Furthermore, the linear reduction in DMI observed with increasing doses of *A. taxiformis* may be explained through changes in ruminal fermentation, H_2 accumulation, and alterations in feed palatability associated with the presence of bromoform (Beauchemin et al., 2022; Min et al., 2021).

In contrast to the consistent DMI reduction reported for *A. taxiformis*, other non-halogenated seaweeds have shown more variable effects. Terry et al. (2023) demonstrated a quadratic effect on DMI with the addition of 0%, 1%, and 2% (on DM basis) of *Mazzaella japonica* from the Gigartinaceae family in a TMR, further suggesting that seaweed species and inclusion levels can differentially influence intake, but with no effect on body weight. Similarly, Oretomiloye et al. (2025) observed no effect of *Ascophyllum nodosum* and *Himanthalia elongata* included at 2% DM on DMI or the body weight of beef heifers fed a high-forage diet. Collectively, the results of the present study are consistent with previous findings on non-bromoform containing seaweeds, indicating that SWblend supplementation did not impair nutrient intake or utilization.

Feeding behavior variables were also unaffected by SWblend supplementation, which may be attributed to the absence of changes in the diet's sensory qualities. Although seaweed inclusion may influence palatability or feed texture, resulting in smaller or shorter meals, and a reduction in feed intake (Nyløy et al., 2023), this response were not evidenced in my study. Additionally, the lower SD of mean ruminal pH observed in the SWblend fed heifers during the backgrounding phase suggests a more stable ruminal environment, which could improve digestive function and promote a more consistent feeding pattern. Similar effects have been reported by Rafferty et al. (2019), who demonstrated that supplementation with a marine-based rumen buffer (including calcified seaweed) reduced the amount of time that ruminal pH remained below critical thresholds (pH < 5.8 and 6.0) in dairy cows grazing highly digestible perennial ryegrass-based pastures.

Calcified seaweed, a natural source of calcium and magnesium, has been studied as a dietary supplement for ruminants, especially for dairy cattle, due to its buffering capacity and potential to improve ruminal fermentation (Abd-El Razek et al., 2024; Cruywagen et al., 2015; Kumar et al., 2024). Furthermore, including calcified seaweed to replace sodium bicarbonate in the diet of steers consuming a high-energy TMR improved ruminal pH regulation, ADG, and feed efficiency (Rossi et al., 2019). This effect is likely related to the buffering action of calcium and magnesium carbonates naturally present in calcified seaweed, which can act similarly to sodium bicarbonate in stabilizing ruminal pH. Thus, the finding of the present study suggests that the inclusion of

SWblend may have contributed to reducing the SD of mean ruminal pH as observed during the backgrounding phase. However, this effect was not observed in the finishing phase, which may be attributed to the high fermentability and low fibre content in the diet, reducing chewing activity, saliva flow, and rumen retention time, limiting the buffering capacity of the diet (Allen, 1997; Plaizier et al., 2008). Furthermore, SWblend supplementation promoted changes in ruminal fermentation during the finishing phase, including changes in NH_3 and VFA profile, which together contribute to the absence of treatment effect on SD of mean ruminal pH.

In ruminants, ruminal NH_3 is mainly produced through the breakdown of crude proteinaceous substrates and removed from the rumen via passage of digesta, absorption through the ruminal wall, or use by microbes as a nitrogen source for microbial protein synthesis (Parker et al., 1995; Russell et al., 1992). The elevated ruminal NH_3 noted in the SWblend treatment may suggest either enhanced protein degradation or a reduction in microbial uptake of NH_3 for microbial protein synthesis. Similar findings were reported by Terry et al. (2023), who observed an increase in ruminal NH_3 concentration in beef heifers supplemented with *M. japonica*, which was attributed to the degradation of protein fractions in this seaweed. Furthermore, research using *in vitro* batch cultures showed that 25% (DM basis) inclusion of *Ulva sp.*, *Gigartina sp.*, and *Gracilaria vermiculophylla* with two different substrates (meadow hay or corn silage) increased NH_3-N production by 30.3%, 77.0%, and 21.9%, respectively (Maia et al., 2016). These responses were attributed to the protein and solubility properties of the seaweed (Maia et al., 2016). In the present study, the CP content was similar between treatments (13.9 ± 1.0% for the control and 13.5 ± 0.55% for the SWblend), suggesting that the difference in NH_3 concentration may be attributed to an increase in soluble protein from the SWblend and reduced microbial capture efficiency of available NH_3-N.

Total VFA production was reduced by the inclusion of SWblend during the finishing phase, supported by a corresponding decrease in propionate and an increase in butyrate, isobutyrate, and isovalerate concentration, supporting a shift in ruminal fermentation. These alterations indicate a deviation from glucogenic to more ketogenic and proteolytic fermentation pathways, which can reflect a less energy-efficient fermentation pattern and greater H_2 release (Abbott et al., 2020; Morgavi et al., 2010). Butyrate synthesis is typically linked to the activity of fibrolytic and lactate-fermenting bacteria such as *Butyrivibrio fibriosolvens*, which utilize structural carbohydrates and lactate as substrates (Huws et al., 2018; Mizrahi et al., 2021). Conversely, isobutyrate and isovalerate, branched-chain volatile fatty acids, are produced through deamination of valine and leucine, respectively, and are commonly associated with the activity of proteolytic bacteria such as *Clostridium spp.* and *Peptostreptococcus spp.* (Bergman, 1990). These microbial shifts are consistent with the higher NH_3 concentrationss observed in the SWblend group in this study, further supporting the hypothesis of an imbalance between amino acid deamination and microbial protein turnover. Although the SWblend altered the ruminal fermentation profile in the finishing phase, no adverse effects were observed on animal performance.

The lack of effect on CH_4 emissions by the SWblend may be due to the blending of seaweed families that are not known to produce bromoform (Al-Adilah et al., 2022; Keng et al., 2020). Species like *Asparagopsis spp.* produce bromoform, a compound that inhibits CH_4 production by targeting methyl-coenzyme M reductase (Wood et al., 1968). This compound has shown a strong anti-methanogenic effect both *in vivo* and *in vitro*, reducing CH_4 production up to 100% (Ramos et al., 2025; Roque et al., 2019; Roque et al., 2021). Nevertheless, the seaweeds used in SWblend may influence enteric CH_4 production due to the presence of phlorotannin, polyphenols, polysaccharides, and minerals, which can alter ruminal microbial community or directly affect methanogenic archaea (Beauchemin et al., 2020; Makkar et al., 2016). Collectively, although the SWblend influenced fermentation dynamics, CH_4 emissions remained unchanged, suggesting that the concurrent reduction in total VFA production and propionate, along with the increase in butyrate during the finishing phase, did not change H_2 availability for methanogenesis.

A reduction in average visit duration and pellet DMI at the GF system was observed in heifers receiving SWblend supplementation in the backgrounding phase. Research using GF system demonstrated that reliable CH_4 emission estimates require a minimum visit duration of 2-3 mins and consistent visitation across at least five of six 4-h time blocks (Alemu et al., 2017; Hammond et al., 2015; Hammond et al., 2016). Thus, numbers and distribution of visits between treatments met the GF system data criteria, ensuring adequate representation of each group. Terry and Beauchemin (2025) reported variation in GF visitation patterns across treatment and feeding phases, with cattle in the backgrounding phase exhibiting a coefficient of variation of 34.7% in visit frequency, highlighting the natural behavioral variability among animals. Therefore, the results found in the present study reflect normal behavioral variation in visitation of heifers to the GF system.

Red and white blood cell counts, hematocrit, hemoglobin levels, and leukocytes (lymphocytes, granulocytes, and mid-size cells) remained within normal physiological ranges for beef cattle in this study (Roland et al., 2014). Analyzing the hematological profile is important to assess the overall health and immune status of cattle, ensuring that dietary treatments do not cause physiological stress and compromise health (Alfaro et al., 2021). In addition, the results found in the present study align with previous research evaluating a variety of seaweed species as feed additives for growing crossbred calves (Anderson et al., 2023), and lactating Holstein cows

(Bošnjaković et al., 2024; Newton et al., 2023), with no demonstrated alteration in the hematological profile.

2.5. Conclusions

Supplementing feedlot beef heifers with 0.60% of SWblend during backgrounding and finishing phases did not negatively impact animal performance, feeding behavior, or hematological parameters, but was ineffective at decreasing enteric CH_4 emissions. While the seaweed blend altered the ruminal fermentation profile in the finishing phase, no adverse effects were observed on ADG or feed efficiency. Additionally, a reduction in variability of ruminal pH during the backgrounding phase suggests a potential improvement in fermentation stability. Therefore, SWblend appears to be a safe feed additive for ruminants and may contribute to enhanced ruminal stability, although it has limited potential for reducing CH_4 enteric emissions or improving growth efficiency.

2.6. References

Abbott, D. W., Aasen, I. M., Beauchemin, K. A., Grondahl, F., Gruninger, R., Hayes, M., Huws, S., Kenny, D. A., Krizsan, S. J., Kirwan, S. F., Lind, V., Meyer, U., Ramin, M., Theodoridou, K., von Soosten, D., Walsh, P. J., Waters, S., & Xing, X. (2020). Seaweed and Seaweed Bioactives for Mitigation of Enteric Methane: Challenges and Opportunities. *Animals, 10*(12), 2432. https://doi.org/10.3390/ani10122432

Abd-El Razek, A. A., Abdo, S. G., Abd-Allah, M., & Abouamra, W. K. (2024). Effect of supplement some feed additives to mitigate the risk of subacute ruminal acidosis in fatting calves on performance, rumen fermentation activity, and some blood parameters. *Archives of Agriculture Sciences Journal, 7*(2), 80-89. https://doi.org/10.21608/aasj.2024.287109.1165

Al-Adilah, H., Feiters, M. C., Carpenter, L. J., Kumari, P., Carrano, C. J., Al-Bader, D., & Küpper, F. C. (2022). Halogens in Seaweeds: Biological and Environmental Significance. *Phycology,* 2(1), 132-171. https://doi.org/10.3390/phycology2010009

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*, Volume 2 - 2021. https://doi.org/10.3389/fanim.2021.641590

Alemu, A. W., Vyas, D., Manafiazar, G., Basarab, J. A., & Beauchemin, K. A. (2017). Enteric methane emissions from low– and high–residual feed intake beef heifers measured using GreenFeed and respiration chamber techniques1,2. *Journal of Animal Science, 95*(8), 3727-3737. https://doi.org/10.2527/jas.2017.1501

Alfaro, G. F., Rodriguez-Zas, S. L., Southey, B. R., Muntifering, R. B., Rodning, S. P., Pacheco, W. J., & Moisá, S. J. (2021). Complete Blood Count Analysis on Beef Cattle Exposed to Fescue Toxicity and Rumen-Protected Niacin Supplementation. *Animals, 11*(4), 988. https://doi.org/10.3390/ani11040988

Allen, M. S. (1997). Relationship Between Fermentation Acid Production in the Rumen and the Requirement for Physically Effective Fiber. *Journal of Dairy Science, 80*(7), 1447-1462. https://doi.org/10.3168/jds.S0022-0302(97)76074-0

Almeida, A. K., Hegarty, R. S., & Cowie, A. (2021). Meta-analysis quantifying the potential of dietary additives and rumen modifiers for methane mitigation in ruminant production systems. *Animal Nutrition, 7*(4), 1219-1230. https://doi.org/10.1016/j.aninu.2021.09.005

Anderson, P., Malik, R., Ojha, L., Adjei-Mensah, B., & Naliyapara, H. B. (2023). Investigations on modulating effect of three tropical red seaweed by-products on growth performance, immune response, antioxidant status and endocrine variables in crossbred calves. *Journal of Applied Phycology, 35*(1), 445-457. https://doi.org/10.1007/s10811-022-02871-3

AOAC. (2005). *Official methods of analysis* (Vol. 18th ed).

Beauchemin, K. A., Ungerfeld, E. M., Abdalla, A. L., Alvarez, C., Arndt, C., Becquet, P., Benchaar, C., Berndt, A., Mauricio, R. M., McAllister, T. A., Oyhantçabal, W., Salami, S. A., Shalloo, L., Sun, Y., Tricarico, J., Uwizeye, A., De Camillis, C., Bernoux, M., Robinson, T., & Kebreab, E. (2022). Invited review: Current enteric methane mitigation options. *Journal of Dairy Science, 105*(12), 9297-9326. https://doi.org/https://doi.org/10.3168/jds.2022-22091

Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J., & Wang, M. (2020). Review: Fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation. *animal, 14*(S1), s2-s16. https://doi.org/10.1017/S1751731119003100

Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological reviews, 70*(2), 567-590. https://doi.org/10.1152/physrev.1990.70.2.567

Blaxter, K. L., & Clapperton, J. L. (1965). Prediction of the amount of methane produced by ruminants. British *Journal of Nutrition, 19*(1), 511-522. https://doi.org/10.1079/BJN19650046

Bošnjaković, D., Nedić, S., Arsić, S., Prodanović, R., Vujanac, I., Jovanović, L., Stojković, M., Jovanović, I. B., Djuricic, I., & Kirovski, D. (2024). Effects of Brown Seaweed (Ascophyllum nodosum) Supplementation on Enteric Methane Emissions, Metabolic

Status and Milk Composition in Peak-Lactating Holstein Cows. *Animals, 14*(11), 1520. https://www.mdpi.com/2076-2615/14/11/1520

CCAC. (2009). *The care and use of farm animals in research, teaching and testing*. Canadian Council on Animal Care.

Chen, X., Yan, F., Liu, T., Zhang, Y., Li, X., Wang, M., Zhang, C., Xu, X., Deng, L., Yao, J., & Wu, S. (2022). Ruminal Microbiota Determines the High-Fiber Utilization of Ruminants: Evidence from the Ruminal Microbiota Transplant. *Microbiology Spectrum, 10*(4), e00446-00422. https://doi.org/doi:10.1128/spectrum.00446-22

Cruywagen, C. W., Taylor, S., Beya, M. M., & Calitz, T. (2015). The effect of buffering dairy cow diets with limestone, calcareous marine algae, or sodium bicarbonate on ruminal pH profiles, production responses, and rumen fermentation. *Journal of Dairy Science, 98*(8), 5506-5514. https://doi.org/10.3168/jds.2014-8875

Dehority, B. A. (1993). *Laboratory manual for classification and morphology of rumen ciliate protozoa*. CRC Press. https://doi.org/https://doi.org/10.1201/9781351073912

Fouts, J. Q., Honan, M. C., Roque, B. M., Tricarico, J. M., & Kebreab, E. (2022). Enteric methane mitigation interventions. *Translational Animal Science, 6*(2). https://doi.org/10.1093/tas/txac041

Glasson, C. R. K., Kinley, R. D., de Nys, R., King, N., Adams, S. L., Packer, M. A., Svenson, J., Eason, C. T., & Magnusson, M. (2022). Benefits and risks of including the bromoform containing seaweed Asparagopsis in feed for the reduction of methane production from ruminants. *Algal Research, 64*, 102673. https://doi.org/https://doi.org/10.1016/j.algal.2022.102673

Hammond, K. J., Humphries, D. J., Crompton, L. A., Green, C., & Reynolds, C. K. (2015). Methane emissions from cattle: Estimates from short-term measurements using a GreenFeed system compared with measurements obtained using respiration chambers or sulphur hexafluoride tracer. *Animal Feed Science and Technology, 203*, 41-52. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2015.02.008

Hammond, K. J., Waghorn, G. C., & Hegarty, R. S. (2016). The GreenFeed system for measurement of enteric methane emission from cattle. *Animal Production Science, 56*(3), 181-189. https://doi.org/10.1071/an15631

Herrera-Saldana, R. E., Huber, J. T., & Poore, M. H. (1990). Dry Matter, Crude Protein, and Starch Degradability of Five Cereal Grains1. *Journal of Dairy Science, 73*(9), 2386-2393. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(90)78922-9

Hristov, A. N., Melgar, A., Wasson, D., & Arndt, C. (2022). Symposium review: Effective nutritional strategies to mitigate enteric methane in dairy cattle. *Journal of Dairy Science, 105*(10), 8543-8557. https://doi.org/10.3168/jds.2021-21398

Huws, S. A., Creevey, C. J., Oyama, L. B., Mizrahi, I., Denman, S. E., Popova, M., Munoz-Tamayo, R., Forano, E., Waters, S. M., Hess, M., Tapio, I., Smidt, H., Krizsan, S. J., Yanez-Ruiz, D. R., Belanche, A., Guan, L., Gruninger, R. J., McAllister, T. A., Newbold, C. J., . . . Morgavi, D. P. (2018). Addressing Global Ruminant Agricultural Challenges Through

Understanding the Rumen Microbiome: Past, Present, and Future. *Frontiers in Microbiology, 9*, 2161. https://doi.org/10.3389/fmicb.2018.02161

Keng, F. S.-L., Phang, S.-M., Abd Rahman, N., Leedham Elvidge, E. C., Malin, G., & Sturges, W. T. (2020). The emission of volatile halocarbons by seaweeds and their response towards environmental changes. *Journal of Applied Phycology, 32*(2), 1377-1394. https://doi.org/10.1007/s10811-019-02026-x

Kinley, R. D., Martinez-Fernandez, G., Matthews, M. K., de Nys, R., Magnusson, M., & Tomkins, N. W. (2020). Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. *Journal of Cleaner Production, 259*, 120836. https://doi.org/https://doi.org/10.1016/j.jclepro.2020.120836

Kumar, B. S. B., Tariq, H., Mohanta, R. K., Yaqoob, M. U., Nampoothiri, V. M., Mahesh, M. S., Kumar, D., Kumar, B., & Datt, C. (2024). Rumen Buffers to Harness Nutrition, Health and Productivity of Ruminants. In M. S. Mahesh & V. K. Yata (Eds.), Feed Additives and Supplements for Ruminants (pp. 495-518). *Springer Nature Singapore*. https://doi.org/10.1007/978-981-97-0794-2_23

Machado, L., Magnusson, M., Paul, N. A., Kinley, R., de Nys, R., & Tomkins, N. (2016). Dose-response effects of Asparagopsis taxiformis and Oedogonium sp. on in vitro fermentation and methane production. *Journal of Applied Phycology, 28*(2), 1443-1452. https://doi.org/10.1007/s10811-015-0639-9

Maia, M. R. G., Fonseca, A. J. M., Oliveira, H. M., Mendonça, C., & Cabrita, A. R. J. (2016). The Potential Role of Seaweeds in the Natural Manipulation of Rumen Fermentation and Methane Production. *Scientific reports, 6*(1), 32321. https://doi.org/10.1038/srep32321

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology, 212*, 1-17. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2015.09.018

Mertens, D. R., & Collaborators. (2002). Gravimetric Determination of Amylase-Treated Neutral Detergent Fiber in Feeds with Refluxing in Beakers or Crucibles: Collaborative Study. *Journal of AOAC International, 85*(6), 23. https://doi.org/10.1093/jaoac/85.6.1217Journal

Min, B. R., Parker, D., Brauer, D., Waldrip, H., Lockard, C., Hales, K., Akbay, A., & Augyte, S. (2021). The role of seaweed as a potential dietary supplementation for enteric methane mitigation in ruminants: Challenges and opportunities. *Animal Nutrition, 7*(4), 1371-1387. https://doi.org/10.1016/j.aninu.2021.10.003

Mizrahi, I., Wallace, R. J., & Moraïs, S. (2021). The rumen microbiome: balancing food security and environmental impacts. Nature Reviews Microbiology, 19(9), 553-566. https://doi.org/10.1038/s41579-021-00543-6

Morgavi, D. P., Forano, E., Martin, C., & Newbold, C. J. (2010). Microbial ecosystem and methanogenesis in ruminants. *animal, 4*(7), 1024-1036. https://doi.org/https://doi.org/10.1017/S1751731110000546

NASEM. (2016). *Nutrient requirements of beef cattle*. National Academies of Sciences Medicine Division on Earth Life Studies Committee on Nutrient Requirements of Beef Cattle.

Newton, E. E., Theodoridou, K., Terré, M., Huws, S., Ray, P., Reynolds, C. K., Prat, N., Sabrià, D., & Stergiadis, S. (2023). Effect of dietary seaweed (Ascophyllum nodosum) supplementation on milk mineral concentrations, transfer efficiency, and hematological parameters in lactating Holstein cows. *Journal of Dairy Science, 106*(10), 6880-6893. https://doi.org/https://doi.org/10.3168/jds.2022-23074

Nyløy, E., Prestløkken, E., Eknæs, M., Eikanger, K. S., Heldal Hagen, L., & Kidane, A. (2023). Inclusion of red macroalgae (Asparagopsis taxiformis) in dairy cow diets modulates feed intake, chewing activity and estimated saliva secretion. *Animals, 13*(3), 489. https://doi.org/10.3390/ani13030489

Ogimoto, K., & Imai, S. (1981). Atlas of rumen microbiology.

Oretomiloye, F., Coates, T., Hayes, M., & Terry, S. A. (2025). Effect of whole seaweed and seaweed extract on rumen fermentation, nutrient digestibility, and methane emissions of beef heifers fed a high-forage diet. *Canadian Journal of Animal Science, 105*, 1-9. https://doi.org/https://doi.org/10.1139/cjas-2024-0129

Parker, D. S., Lomax, M. A., Seal, C. J., & Wilton, J. C. (1995). Metabolic implications of ammonia production in the ruminant. *Proceedings of the Nutrition Society, 54*(2), 549-563. https://doi.org/10.1079/PNS19950023

Plaizier, J. C., Krause, D. O., Gozho, G. N., & McBride, B. W. (2008). Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *The Veterinary Journal, 176*(1), 21-31. https://doi.org/https://doi.org/10.1016/j.tvjl.2007.12.016

Rafferty, D. M., Fahey, A. G., Grace, C., Donaldson, G., Whelan, S. J., Lynch, M. B., Pierce, K. M., & Mulligan, F. J. (2019). Feeding a marine-based rumen buffer increases milk production and decreases time of low reticulo-rumen pH in grazing dairy cows offered perennial ryegrass-based pasture. *Animal Feed Science and Technology, 256*, 114255. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2019.114255

Ramos, T. R., Dash, S. S., Prado, I. N. d., McAllister, T. A., Stanford, K., & Terry, S. A. (2025). Evaluating the efficacy of red, brown, and green seaweeds in reducing in vitro methane production from cattle. *Canadian Journal of Animal Science, 105*, 1-12. https://doi.org/10.1139/cjas-2025-0009

Ribeiro, G. O., Jr., Swift, M. L., & McAllister, T. A. (2016). Effect of diastatic power and processing index on the feed value of barley grain for finishing feedlot cattle. *Journal of Animal Science, 94*(8), 3370-3381. https://doi.org/10.2527/jas.2015-0068

Roland, L., Drillich, M., & Iwersen, M. (2014). Hematology as a diagnostic tool in bovine medicine. *Journal of Veterinary Diagnostic Investigation, 26*(5), 592-598. https://doi.org/10.1177/1040638714546490

Roque, B. M., Brooke, C. G., Ladau, J., Polley, T., Marsh, L. J., Najafi, N., Pandey, P., Singh, L., Kinley, R., Salwen, J. K., Eloe-Fadrosh, E., Kebreab, E., & Hess, M. (2019). Effect of the macroalgae Asparagopsis taxiformis on methane production and rumen microbiome assemblage. *Animal Microbiome, 1*(1), 3. https://doi.org/10.1186/s42523-019-0004-4

Roque, B. M., Venegas, M., Kinley, R. D., de Nys, R., Duarte, T. L., Yang, X., & Kebreab, E. (2021). Red seaweed (Asparagopsis taxiformis) supplementation reduces enteric methane by over 80 percent in beef steers. *PLoS One, 16*(3), e0247820. https://doi.org/10.1371/journal.pone.0247820

Rossi, C. A. S., Compiani, R., Baldi, G., Taylor, S. J., Righi, F., Simoni, M., & Quarantelli, A. (2019). Replacing sodium bicarbonate with half amount of calcareous marine algae in the diet of beef cattle. *Revista Brasileira de Zootecnia, 48*, e20180129. https://doi.org/10.1590/rbz4820180129

Russell, J. B., O'Connor, J. D., Fox, D. G., Van Soest, P. J., & Sniffen, C. J. (1992). A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *Journal of Animal Science, 70*(11), 3551-3561. https://doi.org/10.2527/1992.70113551x

Schwartzkopf-Genswein, K. S., Atwood, S., & McAllister, T. A. (2002). Relationships between bunk attendance, intake and performance of steers and heifers on varying feeding regimes. Applied *Animal Behaviour Science, 76*(3), 179-188. https://doi.org/https://doi.org/10.1016/S0168-1591(02)00009-6

Sims, G. K., Ellsworth, T. R., & Mulvaney, R. L. (1995). Microscale determination of inorganic nitrogen in water and soil extracts. *Communications in Soil Science and Plant Analysis, 26*(1-2), 303-316. https://doi.org/10.1080/00103629509369298

Shindell, D., Ravishankara, A., Kuylenstierna, J. C., Michalopoulou, E., Höglund-Isaksson, L., Zhang, Y., Seltzer, K., Ru, M., Castelino, R., & Faluvegi, G. (2021). *Global methane assessment: Benefits and costs of mitigating methane emissions* (9280738542). https://www.ccacoalition.org/resources/global-methane-assessment-full-repor

Terry, S. A., & Beauchemin, K. A. (2025). Essential oil blend and monensin for feedlot steers consuming backgrounding and finishing diets: growth performance, methane emissions using the GreenFeed system, and feeding behavior. *Translational Animal Science, 9*. https://doi.org/10.1093/tas/txae182

Terry, S. A., Coates, T., Gruninger, R., Abbott, D. W., & Beauchemin, K. A. (2023). Evaluation of the red seaweed Mazzaella japonica as a feed additive for beef cattle. Frontiers in Animal Science, Volume 4 - 2023. https://doi.org/10.3389/fanim.2023.1181768

Wanapat, M., Prachumchai, R., Dagaew, G., Matra, M., Phupaboon, S., Sommai, S., & Suriyapha, C. (2024). Potential use of seaweed as a dietary supplement to mitigate enteric methane emission in ruminants. *Science of The Total Environment, 931*, 173015. https://doi.org/https://doi.org/10.1016/j.scitotenv.2024.173015

Wood, J., Kennedy, F. S., & Wolfe, R. (1968). Reaction of multihalogenated hydrocarbons with free and bound reduced vitamin B12. *Biochemistry, 7*(5), 1707-1713.

Table 2.1. Ingredients and chemical composition (% of dry matter) of experimental TMR with or without a seaweed blend used in backgrounding and finishing feedlot trials

Ingredient	Control, DM % of TMR	SWblend, DM % of TMR	DM, %	OM, %	CP, %	Starch, %	NDF, %	ADF, %	GE, Mcal/kg DM
Seaweed blend	-	0.60	91.7 ± 0.84	51.4 ± 3.69	9.63 ± 0.95	< 1.0	30.6 ± 3.23	33.2 ± 2.02	2.29 ± 0.14
Backgrounding									
Corn Silage	65.0	64.4	92.9 ± 0.54	94.6 ± 0.35	8.82 ± 0.24	25.0 ± 2.65	44.4 ± 3.34	23.4 ± 1.82	5.38 ± 0.12
Barley Grain	20.0	20.0	94.9 ± 0.62	97.6 ± 0.54	13.2 ± 0.80	54.8 ± 1.71	20.8 ± 2.18	5.08 ± 0.78	4.79 ± 0.05
Canola Meal	10.0	10.0	94.6 ± 0.63	92.7 ± 0.30	46.2 ± 1.29	4.58 ± 0.37	34.3 ± 7.05	21.5 ± 4.70	5.29 ± 0.10
Supplement	5.00	5.00	95.8 ± 0.45	61.1 ± 1.47	17.6 ± 0.93	28.6 ± 1.57	12.5 ± 0.75	4.11 ± 0.27	3.05 ± 0.12
GF pellet[1]			95.0 ± 0.57	92.8 ± 0.33	17.0 ± 0.60	21.8 ± 2.39	26.9 ± 0.91	14.3 ± 0.83	4.67 ± 0.05
Finishing									
Corn Silage	9.00	8.40	93.4 ± 0.22	94.8 ± 0.18	7.86 ± 0.24	26.4 ± 2.79	49.3 ± 4.46	26.5 ± 1.55	5.75 ± 0.97
Barley Grain	86.0	86.0	93.7 ± 1.74	97.6 ± 0.23	13.1 ± 0.25	53.6 ± 4.38	22.2 ± 2.31	5.35 ± 1.14	5.19 ± 0.73
Supplement	5.00	5.00	95.2 ± 0.79	65.2 ± 1.48	17.6 ± 0.77	31.0 ± 3.09	18.0 ± 6.32	5.36 ± 0.41	3.60 ± 0.78
GF pellet[1]			94.6 ± 0.80	92.7 ± 0.27	13.9 ± 0.45	27.7 ± 3.96	29.2 ± 2.40	15.1 ± 0.71	5.19 ± 1.17

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; GE: gross energy; [1]GF pellet in backgrounding phase include 43.2% ground barley, 11.5% canola meal, 1.0% limestone ($CaCO_3$), 13.0% dried molasses, 0.15% salt, 30.4% beet pulp, and 0.75% canola oil (DM basis); GF pellet in finishing phase include 45.1% ground barley, 7.50% canola meal, 1.0% limestone ($CaCO_3$), 13.0% dried molasses, 0.15% salt, 32.5% beet pulp, and 0.75% canola oil (DM basis).

Table 2.2. Chemical composition (% of dry matter) of experimental TMR with or without a seaweed blend used in a backgrounding and finishing feedlot trials.

	Backgrounding		Finishing	
	Control	SWblend	Control	SWblend
DM, %	94.0 ± 0.40	93.9 ± 0.39	93.7 ± 1.05	93.6 ± 0.88
OM, %	92.4 ± 0.84	92.4 ± 0.89	96.0 ± 0.59	95.8 ± 0.68
CP, %	16.4 ± 0.73	16.6 ± 0.62	13.9 ± 1.01	13.5 ± 0.55
Starch, %	29.1 ± 1.62	27.5 ± 1.56	55.1 ± 4.34	54.4 ± 3.40
NDF, %	34.9 ± 1.89	36.1 ± 2.33	21.0 ± 4.11	21.8 ± 3.80
ADF, %	18.0 ± 0.98	18.5 ± 1.30	5.14 ± 0.70	5.64 ± 0.61
GE, Mcal/kg DM	5.01 ± 0.08	5.08 ± 0.08	4.88 ± 0.37	4.98 ± 0.48

Control: TMR with no additives; SWblend: supplemented at 50 g/heifer/day of a seaweed blend; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; GE: gross energy.

Table 2.3. Dry matter intake and growth performance of heifers fed a TMR with or without a seaweed blend in a backgrounding and finishing feedlot trials

Variables	Treatment[1]		SEM	*P* value
	Control	SWblend		
Overall				
DMI, kg/d	10.2	10.2	0.13	0.94
Weight gain, kg	349.8	344.2	5.56	0.55
ADG, kg/d	1.45	1.41	0.024	0.23
G:F	0.14	0.14	0.003	0.37
Backgrounding				
Shrunk initial body weight, kg	307.1	306.6	2.45	0.87
Shrunk final body weight, kg	446.1	448.4	3.25	0.63
DMI kg/d	9.66	9.79	0.138	0.59
Weight gain, kg	139.0	141.8	2.36	0.40
ADG	1.09	1.11	0.018	0.40
G:F	0.11	0.11	0.002	0.76
Finishing				
Shrunk initial body weight, kg	482.7	481.2	4.03	0.80
Shrunk final body weight, kg	656.6	646.4	6.39	0.26
DMI, kg/d	10.7	10.6	0.17	0.59
Weight gain, kg	173.9	165.2	3.73	0.10
ADG	1.53	1.45	0.033	0.10
G:F	0.14	0.14	0.003	0.25

[1] Treatments include Control (no additives) and SWblend (supplemented at 0.60% on DM); DMI: dry matter intake; ADG: average daily gain; G:F: gain:feed.

Table 2.4. Feed intake behavior of heifers fed a TMR with or without a seaweed blend in backgrounding and finishing feedlot trials

Variables	Treatment[1]		SEM	*P* value
	Control	SWblend		
Backgrounding				
DMI, kg/d	10.1	10.2	0.12	0.57
SD of DMI, kg/d	1.67	1.69	0.035	0.49
Duration, min/meal	23.0	22.8	0.35	0.73
Intermeal interval, min	152.6	156.1	2.20	0.25
Meal size, kg/meal	1.22	1.25	0.025	0.31
Eating rate, g/min	53.7	55.4	0.63	0.06
Finishing				
DMI, kg/d	10.8	10.7	0.09	0.64
SD of DMI, kg/d	1.85	1.87	0.040	0.83
Duration, min/meal	16.5	16.9	0.23	0.51
Intermeal interval, min	266.4	256.2	7.67	0.44
Meal size, kg/meal	2.13	2.00	0.118	0.48
Eating rate, g/min	143.3	140.9	3.54	0.16

[1] Treatments include Control (no additives) and SWblend (supplemented at 0.60% on DM of a seaweed blend); DMI: dry matter intake.

Table 2.5. Ruminal ammonia, volatile fatty acids and proportions, protozoa count, and rumen pH of heifers fed a TMR with or without a seaweed blend in backgrounding and finishing feedlot trials

Variables	Treatment[1]		SEM	*P* value
	Control	SWblend		
Backgrounding				
NH_3, mM	7.02	6.76	0.564	0.75
Total VFA, mM	138.3	133.6	6.89	0.34
Acetate, %	66.2	67.0	2.29	0.44
Propionate, %	19.3	18.0	0.79	0.25
Butyrate, %	10.4	10.9	0.81	0.85
Isobutyrate, %	1.03	1.03	0.089	0.66
Valerate, %	1.28	1.17	0.314	0.44
Isovalerate, %	1.56	1.61	0.257	0.62
Caproate, %	0.268	0.298	0.110	0.72
C2/C3	3.44	3.73	0.161	0.29
Protozoa count	5.55	5.58	0.046	0.80
Ruminal pH				
Mean	6.17	6.20	0.033	0.59
Minimum	5.42	5.52	0.048	0.07
Maximum	6.90	6.89	0.035	0.84
SD of mean pH	0.39^a	0.34^b	0.022	0.03
Finishing				
NH_3, mM	11.6^b	15.8^a	2.28	0.008
Total VFA, mM	171.8^a	127.4^b	7.66	<0.001
Acetate, %	48.3	50.1	1.00	0.20
Propionate, %	36.0^a	31.6^b	4.02	0.03
Butyrate, %	9.73^b	11.2^a	1.92	0.02
Isobutyrate, %	0.86^b	1.25^a	0.065	<0.001
Valerate, %	3.38	3.58	0.352	0.69
Isovalerate, %	1.42^b	1.85^a	0.137	0.03
Caproate, %	0.400	0.433	0.123	0.40
C2/C3	1.34	1.58	0.502	0.19
Protozoa count	2.21	2.26	0.462	0.94
Ruminal pH				
Mean	5.76	5.75	0.030	0.94
Minimum	5.15	5.13	0.022	0.65
Maximum	6.58	6.62	0.051	0.68
SD of mean pH	0.37	0.39	0.014	0.45

[1] Treatments include Control (no additives) and SWblend (supplemented at 0.60% on DM); $^{a,\ b}$: means within the same row with different letters differ significantly ($P < 0.05$).

Table 2.6. Visitation to the GreenFeed Monitoring System, dry matter intake, and CH_4 production, yield, and energetic intensity of heifers fed a TMR with or without a seaweed blend in a backgrounding and finishing feedlot trials

Variables	Treatment[1]		SEM	*P* value
	Control	SWblend		
Backgrounding				
No. visit total	32.1	31.9	1.32	0.88
No. visit day	2.30	2.28	0.095	0.89
Mean duration, min	297.3[a]	284.3[b]	10.43	0.02
Pellet DMI, kg	0.41[a]	0.39[b]	0.014	0.01
DMI total, kg	9.98	10.2	0.108	0.23
GEI, Mcal/d	50.1	50.9	0.62	0.36
CH_4, g/d	194.6	194.7	1.98	0.98
CH_4 yield, g/kg DMI	19.7	19.4	0.37	0.39
CH_4, Mcal/%GEI	5.23	5.17	0.112	0.54
Finishing				
No. visit total	20.0	20.3	0.72	0.81
No. visit day	1.43	1.45	0.052	0.80
Mean duration, min	228.9	224.3	6.17	0.25
Pellet DMI, kg	0.32	0.32	0.009	0.28
DMI total, kg	10.8	10.9	0.20	0.69
GEI, Mcal/d	55.4	56.6	4.63	0.34
CH_4, g/d	144.6	148.7	10.79	0.31
CH_4 yield, g/kg DMI	13.4	14.0	0.83	0.17
CH_4, Mcal/%GEI	3.48	3.56	0.072	0.46

[1] Treatments include Control (no additives) and SWblend (supplemented at 0.60% of DM); DMI: dry matter intake; GEI: gross energy intake; [a, b]: means within the same row with different letters differ significantly ($P < 0.05$).

Table 2.7. Hematological parameters of heifers fed a TMR with or without a seaweed blend in backgrounding and finishing feedlot trials

Variables	Treatment[1]		SEM	*P* value
	Control	SWblend		
Backgrounding				
Total red blood cell, 10^{12}/L	8.52	8.63	0.067	0.26
Mean cell volume of RBCs, fl	42.9	42.4	0.26	0.11
Hematocrit, L/L	0.36	0.36	0.003	0.91
Hemoglobin, g/L	122.8	123.5	0.97	0.55
Total white blood cell, 10^{9}/L	9.93	10.2	0.13	0.10
Lymphocyte, 10^{9}/L	6.13	6.27	0.087	0.26
Mid-sized cell, 10^{9}/L	0.81	0.82	0.019	0.46
Granulocyte, 10^{9}/L	2.99	3.09	0.083	0.35
Lymphocyte, %	62.6	62.0	0.58	0.44
Mid-sized cell, %	7.63	7.64	0.150	0.81
Granulocyte, %	29.80	30.3	0.60	0.63
Finishing				
Total red blood cell, 10^{12}/L	7.85	7.96	0.049	0.14
Mean cell volume of RBCs, fl	49.1	48.6	0.43	0.50
Hematocrit, L/L	0.38	0.39	0.004	0.91
Hemoglobin, g/L	125.5	126.2	1.24	0.75
Total white blood cell, 10^{9}/L	7.85	7.74	0.139	0.61
Lymphocyte, 10^{9}/L	4.49	4.46	0.075	0.58
Mid-sized cell, 10^{9}/L	0.67	0.65	0.016	0.59
Granulocyte, 10^{9}/L	2.69	2.63	0.086	0.78
Lymphocyte, %	58.0	57.8	0.77	0.87
Mid-sized cell, %	7.89	7.98	0.088	0.50
Granulocyte, %	34.1	34.2	0.71	0.92

[1] Treatments include Control (no additives) and SWblend (supplemented at 0.60% of DM).

CHAPTER 3: UNDERSTANDING METHANE EMISSION VARIABILITY OF FEEDLOT BEEF HEIFERS RANKED INTO LOW, INTERMEDIATE, AND HIGH METHANE YIELD GROUPS DURING BACKGROUNDING AND FINISHING USING THE GREENFEED SYSTEM

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Abstract: This study aimed to (1) categorize feedlot beef heifers fed backgrounding and finishing diets into low, intermediate, and high CH_4 yield groups (g CH_4/ kg DMI; MYG), (2) evaluate how MYG effects growth performance, feeding behavior, GF system visitation patterns, CH_4 emissions, and (3) compare the rumen microbiota of heifers differing between low and high MYG. One hundred Angus-cross beef heifers were randomly assigned to four pens and fed a total mixed ration (TMR) with a control (no feed additive) or a multispecies seaweed supplemented at 0.60% dry matter (DM) basis. Treatment was assessed as a covariate and did not affect any measured parameter; therefore, treatments and pen (within block) were used as random effects. Heifers were classified into low (<0.5 SD from the mean), intermediate (within ±0.5 SD of the mean), or high (>0.5 SD of the mean) MYG in each period by phase. Heifers classified as low and intermediate MYG had greater DMI ($P < 0.0001$) in both phases. During the finishing phase, low MYG heifers had greater ($P < 0.01$) initial and final shrunk body weight (SBW) than the intermediate MYG heifers. During the backgrounding phase, feeding behavior variables, including DMI, meal duration, and meal size, were greater ($P < 0.01$) for heifers ranked as low and intermediate compared to the high MYG. In the finishing phase, the low MYG heifers had greater DMI ($P < 0.001$). Low MYG had lower ($P < 0.0001$) CH_4 production, CH_4y, and CH_4 intensity in both dietary phases. Mean CV for the number of visits to the GF system ranged from 44.6% to 55.6% among MYG. Mean CV of CH_4 production (g/d) ranged from 21.3% to 25.3% in both dietary phases, and the mean CV of CH_4y ranged from 6.55% to 19.6%. There were no differences ($P \geq 0.12$) between low and high MYG in rumen microflora alpha- diversity estimated by observed ASVs or in β-diversity based on Bray-Curtis dissimilarity. However, a temporal difference ($P \leq 0.04$) between low and high MYG was found in alpha diversity determined by Shannon index of the last period (period 4) of backgrounding and generalized β-diversity based on UniFrac distance ($\alpha = 0.5$) in the last period of the finishing phase. In conclusion, the findings emphasize the complexity and temporal variability of CH_4 traits in feedlot systems and demonstrate the importance of evaluating the CH_4 emission across and within productive phases to identify consistently low-emitting cattle.

3.1. Introduction

Methane (CH_4) is one of the three most significant greenhouse gases (GHGs), along with carbon dioxide (CO_2) and nitrous oxide (N_2O). The livestock sector accounts for 14.5% of total anthropogenic GHG emissions, with enteric CH_4 from ruminant production contributing 6.0% of that total (Beauchemin et al., 2020). Enteric CH_4 is produced by methanogenic archaea through the utilization of CO_2 and hydrogen (H_2) derived from carbohydrate fermentation in the gastrointestinal tract of ruminants (Morgavi et al., 2010). Methanogenesis is essential for removing H_2 produced during bacterial fermentation, preventing feedback inhibition of other fermentative pathways (Beauchemin et al., 2020; Morgavi et al., 2010). However, it also represents from 2% to 12% of the ingested feed energy loss (Johnson & Johnson, 1995).

Several methods have been explored to reduce enteric CH_4 production from ruminants, including the selection of low CH_4-producing cattle, animal breeding, and dietary manipulation (Beauchemin et al., 2022a). There is a variation in enteric CH_4 production among animals within the same herd and under the same feeding management, suggesting a meaningful host genetic component (de Haas et al., 2017). Methane yield (CH_4y; g/kg dry matter intake [DMI]) is a heritable trait, with heritability for absolute CH_4 production (g/d) estimated at 0.40, and CH_4y ranging from 0.13 to 0.19 (Króliczewska et al., 2023). Notably, genetic gains through selection of low CH_4-producing ruminants are permanent and cumulative (de Haas et al., 2017; Króliczewska et al., 2023), making this an attractive approach for long-term mitigation. Additionally, recent evidence suggests that CH_4y rank in beef cattle shows substantial variability over time (Hammond et al., 2016a; Vargas et al., 2025; Williams et al., 2025). Cattle categorized by CH_4 production, yield, intensity, or residual CH_4 emissions may shift categories within or across diets (Vargas et al., 2025; Williams et al., 2025). Factors such as physiological characteristics, individual feeding behavior, and diet composition can also affect this variability (Hammond et al., 2016a; Vargas et al., 2025; Williams et al., 2025). Nevertheless, the selection of low CH_4-producing cattle depends on the availability of methods capable of accurately quantifying individual animal emissions under experimental and commercial conditions.

Currently, diverse tools are available for research, each with different levels of precision, cost, applicability, and feasibility. The use of the respiration chamber and GreenFeed (GF) systems are the two most commonly used research methods to estimate enteric CH_4 production from ruminants (Alemu et al., 2017; Hammond et al., 2016a). The GF is an automated head chamber technology that is integrated into a portable feeding station, allowing estimation of enteric CH_4, oxygen (O_2), and CO_2 emission from individual ruminants in controlled or commercial environments (French et al., 2025; Hammond et al., 2015; Hammond et al., 2016a). This system captures multiple short and repeated gas emission events during the day and aggregates them to estimate the individual daily CH_4 emission average over a determined period, ranging from days to months (French et al., 2025). However, its accuracy largely depends on collecting a sufficient number of visits that are evenly spread throughout the day.

The objectives of this study were to (1) categorize feedlot beef heifers fed backgrounding and finishing diets into low, intermediate, and high CH_4y groups (MYG), (2) evaluate how MYG affects GF system visitation patterns, CH_4 emissions, growth performance, feeding behavior, and (3) compare the rumen microflora of heifers in low and high MYG across and within backgrounding and finishing diets.

3.2. Materials and Methods

This study was conducted at Agriculture and Agri-Food Canada's Research and Development Centre in Lethbridge, Alberta. Heifers were cared in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009). The study protocol (#ACC2309) was reviewed and approved by the Institutional Animal Care Committee at Lethbridge Research and Development Centre.

3.2.1. Animals, experimental design, and diets

The present study employed a completely randomized block design with two treatments (control and SWblend) replicated across two blocks. A total of 100 Angus× beef heifers with uniform body weight (320.2 ± 17.8 kg) were randomly assigned to four pens (25 heifers/pen). The experiment spanned backgrounding and finishing phases, with each phase divided into four periods (28-d per period). Before data collection, heifers underwent a 4-week adaptation period to acclimate to the pens, diet, and use of Growsafe (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) and Greenfeed (GF; C-Lock Inc., Rapid City, SD) systems.

Upon arrival, each heifer was equipped with an RFID ear tag that allowed for continuous recording of individual animal feed intake and feeding behavior throughout the experiment. At arrival heifers were also treated with Bovimectin Pour-on (5 mg of ivermectin/mL; 500 µg of ivermectin per kg of body weight (BW); Vetoquinol N.-A. Inc., Lavaltrie, QC, Canada) and vaccinated with Ultrabac 7/Somubac (Clostridium vaccine; Zoetis Canada Inc., Kirkland, QC, Canada) and Pyramid FP5 (infectious bovine rhinotracheitis and bovine viral diarrhea vaccine; Boehringer Ingelheim Ltd., Burlington, ON, Canada)

Heifers were fed a typical Canadian feedlot diet throughout the backgrounding and finishing phases (Table 1). During the backgrounding, the control group received a total mixed ration (TMR) consisting of 65.0% corn silage, 20.0% barley grain, 10.0% canola meal, and 5.0% mineral supplement on dry matter (DM) basis. The SWblend pens received that same TMR as control pens, except 0.60% of corn silage was replaced by the commercial seaweed blend (OceanFeed™ Bovine) on a DM basis. The 0.60% inclusion of the SWblend was formulated to supply 50 g/heifer/day of the additive as recommended by the manufacturer. The SWblend is a blend of red, brown, and green seaweeds harvested from Europe, North America, and Southeast Asia supplied by Ocean Harvest Technologies® (Castle Court, Reigate, London, UK).

A 4-week transition period was used to gradually adapt the cattle to the finishing diet, with increasing concentrate amount replacing forage in the TMR. The control finishing diet consisted of 9.0% corn silage, 86.0% barley grain, and 5.0% mineral supplement (on DM basis), with

SWblend replacing 0.60% of the corn silage. All diets were formulated to meet or exceed the nutrient requirements for feedlot cattle during backgrounding and finishing (NASEM, 2016). Feed was delivered once daily at 9:00 AM using a slick bunk management approach, targeting less than 5% of feed refusal, and the heifers had *ad libitum* access to water.

3.2.2. Measurements and laboratory analysis

Methane emissions

Two of the four pens were equipped with GF system to measure individual CH_4 emissions. The heifers accessed the GF system voluntarily, which dispensed a pelleted supplement to encourage visits. To allow for CH_4 measurements from all pens, heifers were rotated every two weeks between two pens within each block, with each block equipped with one GF. This rotation ensured that each pen was exposed to the GF system monthly and eliminated potential pen effects by allowing all heifers to spend an equal amount of time in each pen within a block (Alemu et al., 2021). The GF pellet supplied to the heifers during the backgrounding phase was composed of 43.2% ground barley, 11.5% canola meal, 1.0% limestone ($CaCO_3$), 13.0% dried molasses, 0.15% salt, 30.4% beet pulp, and 0.75% canola oil (DM basis). The GF pellet in the finishing phase was comprised of 45.1% ground barley, 7.5% canola meal, 1.0% limestone ($CaCO_3$), 13.0% dried molasses, 0.15% salt, 32.5% beet pulp, and 0.75% canola oil (DM basis). The GF pellet was made to target the chemical composition of the TMR and be highly palatable to ensure visitation (Table 3.1).

The calculation of gaseous emissions from the GF system was based on data from non-cannulated heifers to maintain accuracy and avoid potential gas loss through the cannula. Only visits in which the animal's head was close to the sensor for at least 3 min were used in the analysis (described as useful/good visits), ensuring capture of a minimum of three eructation events per sampling. To calculate the average daily CH_4 emission rate (production, g/day) for each heifer during a 14-day period of measurement/treatment, the flux data from useful visits were compiled into six 4-h blocks corresponding to time of day (i.e., time bins). Only heifers with ≥10 useful/good visits spread in at least five of the six 4-h time blocks within a period of measurement were used in the final analysis to ensure that the full diurnal cycle of emission was represented. Pellet dry matter intake (DMI; kg/d) was calculated as the daily amount of pellet feed offered minus refusals. Total DMI (kg/d) was determined as the sum of DMI from GrowSafe bunks and pellet DMI daily. Gross energy intake (GEI, Mcal/d) was obtained by multiplying total DMI by the gross energy content of the diet. Methane yield (CH_4y; g/kg DMI) was calculated as the ratio of daily CH_4 production (g/d) to total DMI (kg/d). Methane intensity (Mcal/% GEI) was estimated as a proportion of GEI, by converting CH_4 mass to energy equivalents [mean CH_4 production (kg/d) multiplied by energy equivalency (13.3 Mcal/kg CH_4); Blaxter and Clapperton (1965)], and divided by GEI (Alemu et al., 2021; Terry & Beauchemin, 2025). All CH_4 variables were averaged per animal over the 14-d measurement period and then analyzed as repeated measures within each experimental phase.

Live body weight and feeding behavior

Heifers were weighed (non-fasted) before feeding at the initiation and completion of each phase, and every 4 weeks throughout the study, on 2 consecutive days. Average daily gain (ADG) was calculated for each month and over the duration of the experiment. Feed efficiency (G:F) was calculated based on ADG divided by DMI. To provide a more accurate estimate of BW, shrunk initial and final BWs (SBW) were calculated by dividing the recorded BW by 0.96, correcting for the gastrointestinal fill (Ribeiro et al., 2016).

Individual feed intake and feeding behavior were measured using GrowSafe feed bunks, which recorded individual heifer feed consumption, frequency of daily visits, total daily bunk attendance duration, eating rate (g/min), and average duration of each visit. Distinct feeding events were grouped into meals using a 300-second meal criterion (Schwartzkopf-Genswein et al., 2002). The amount of feed consumed during a visit was used to calculate meal size (kg DM/meal), day-to-day variation of DMI (SD of DMI), meal frequency (meals/day), the duration of each meal (min/meal), and the interval between meals (min/day). Feeding time was calculated as the sum of the length of all meals within a day (min/day), and the feeding rate was determined as the sum of the mass of all meals within a day divided by daily feeding time (g DM/min).

Chemical composition

Dried ingredients, feed, and refusals were ground in a Wiley mill through a 1-mm screen (Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for analytical DM (AOAC (2005); method 930.15), organic matter (OM) (method 942.05), and ash (method 942.05). Neutral detergent fibre (NDF) was determined using an ANKOM200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA), and heat-stable α-amylase (Termamyl® 120; Sigma-Aldrich, St. Louis, MO, USA). Sodium sulfite was used for NDF analysis (Mertens & Collaborators, 2002). Subsequently, acid detergent fibre (ADF) was analyzed (AOAC (2005); method 973.18). Subsamples (5 g) were further ground with a ball grinder (Retsch MM 400; Retsch Inc., Newtown, PA) and analyzed for N using flash combustion (method 990.03; Carlo Erba Instruments, Milan, Italy). Crude protein was calculated as N × 6.25. Starch concentration was determined as described by Herrera-Saldana et al. (1990), and absorbance was read on a Thermo Scientific Appliskan 1.437 (SkanIt Software 2.3 RE) microplate reader at a wavelength of 490 nm. Gross energy content of samples was determined using a bomb calorimeter (model 6200 Isoperibol Calorimeter, Parr Instrument Company, Illinois, USA).

Ruminal sampling and microbial profiling

Ruminal fluid was collected through an esophageal tube inserted orally into the rumen. Samples were collected once at the beginning and once at the end of each phase, before feeding. Approximately 50 mL of ruminal fluid was obtained per animal, immediately filtered through a double layer of PICAP (mesh size 355 µm; Sefar Canada Inc., Ville St. Laurent, Canada), transferred to a Falcon tube (15 mL), and frozen in liquid nitrogen for subsequent analysis. The samples were freeze-dried, ground to a fine powder, and DNA was extracted using the QIAamp PowerFecal Pro DNA kit (QIAGEN GmbH, Germany). The concentration and purity of the extracted metagenomic DNA were determined by measuring the ratios of absorbance at wavelengths of 260/280 and 260/230 using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Mississauga, ON).

Sequencing was performed at McGill University and Genome Quebec Innovation Center in Montreal, Canada. The Illumina NextSeq 2000 XLEAP-SBS P2 Reagent Kit (600-cycle) was used following the manufacturer's instructions. The primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) targeting the V4 region of the 16S rRNA gene were used to examine both bacterial and archaeal diversity (Caporaso et al., 2012). A 33 cycle PCR using 1 µL of a 1 in 10 dilution of genomic DNA and the Fast Start High Fidelity PCR System (Roche, Montreal, PQ) was conducted with the following conditions: 94 °C for 2 min, followed by 33 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, with a final elongation step at 72 °C for 7 min. Fluidigm Corporation (San Francisco, CA) barcodes were incorporated in a second PCR reaction using the following conditions: 95 °C for 10 min, followed by 15 cycles of

95 °C for 15 s, 60 °C for 30 s, and 72 °C for 1 min, followed by a final elongation step at 72 °C for 3 min. After amplification, PCR products were assessed in a 2% agarose gel to confirm adequate amplification. All samples were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies, Carlsbad, CA) and were pooled in equal proportions. Pooled samples were then purified using calibrated Ampure XP beads (Beckman Coulter, Mississauga, ON). The resulting sequencing library was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies, Carlsbad, CA) and the Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems, Wilmington, MA). Data were processed using the QIIME2 software package (Bolyen et al., 2019). Reads were de-noised into amplicon sequencing variants (ASVs) using the DADA2 (Callahan et al., 2016) plugin for QIIME2. Amplicon sequencing variants (ASVs) were taxonomically classified using a Native Bayes classifier trained on the V4 region of the 16S rRNA gene. The SILVA SSU (v.138.1) (Quast et al., 2012) database was used to classify bacterial and archaeal sequences. QIIME2 objects (ASV frequency table, taxonomy table, and phylogenetic tree) were imported into R as a Phyloseq (McMurdie & Holmes, 2013) object using the qiime2R package. ASVs that were not assigned to at least a microbial phylum were discarded. Rarefaction curves were generated to identify suitable subsampling thresholds for diversity analyses. Rarified data were used for Alpha- (observed ASVs and Shannon) and β-diversity (Generalized Unifrac and Bray-Curtis dissimilarity) calculations. Permutational Analysis of Variance (PERMANOVA) tests were performed using the vegan R package (Oksanen et al., 2020). Homogeneity of Dispersion (β-dispersion) tests were performed using Vegan.

3.2.3. Statistical analysis

Firstly, variables from GF system, body weight, and feeding behavior were analyzed as a completely randomized block design with the fixed effect of treatment (control or SWblend), random effect of pen within block, and block, using the MIXED procedure. Data distribution and homogeneity of variance were determined using the UNIVARIATE procedure of SAS (SAS Inst., Inc., Cary, NC). As dietary treatments did not affect GF parameters, heifers were ranked into low (<0.5 SD from the mean), intermediate (within ±0.5 SD of the mean), or high (>0.5 SD of the mean) CH_4y group (MYG) within each phase. Then, GF system parameters (mean duration, pellet DMI, total DMI, GEI, CH_4 production, CH_4y, and CH_4 intensity), growth performance (initial and final SBW, weight gain, ADG, and G:F), and feeding behavior (SD of DMI, duration, intermeal interval, meal size, and eating rate) were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Thus, MYG and period (within each phase) were considered fixed effects, and treatments (control or SWblend) and pen (within block) were used as random effects. Individual heifer was considered as an experimental unit, and false discovery rate corrected *P* values were calculated using Tukey's test. Differences between means were declared significant at $P < 0.05$, and a trend was discussed at $P < 0.10$.

3.3. Results

Of the 100 beef heifers enrolled in the study, 94 met visitation criteria in the GF system in at least one period of both phases. Of the 48 heifers that consistently met the GF system visitation criteria across all four periods during the backgrounding phase, only 9 did not fluctuate between groups throughout the phase (Figure 3.1). During the finishing phase, 18 heifers consistently met the GF system visitation criteria across all four periods, with 3 maintaining the same MYG throughout the phase.

In the backgrounding phase, the total number of visits per period to the GF system varied from 29.2 ± 13.3, 33.9 ± 18.2, and 32.5 ± 16.9 for low, intermediate, and high MYG, respectively (Table 3.2). The number of visit day per period averaged 2.09 ± 0.95, 2.42 ± 1.30, and 2.32 ± 1.19 for low, intermediate, and high MYG, respectively. Low and intermediate MYG showed greater (*P* < 0.0001) total DMI and GEI compared with high MYG during the backgrounding phase. As expected, CH_4 production, CH_4y, and CH_4 intensity were lower (*P* < 0.0001) for low MYG. Furthermore, interactions (*P* ≤ 0.04) between MYG and period were observed for CH_4y and CH_4 intensity during the backgrounding phase (Figure 3.2a).

During the finishing phase, the total number of visits to the GF system per period averaged 19.5 ± 7.47, 20.4 ± 7.12, and 21.0 ± 7.60 for low, intermediate, and high MYG, respectively (Table 3.2). The number of visits per day was 1.39 ± 0.53, 1.46 ± 0.51, and 1.50 ± 0.54 for low, intermediate, and high MYG, respectively. Low MYG had greater (*P* < 0.001) total DMI and GEI than intermediate and high MYG. Conversely, CH_4 production, CH_4y, and CH_4 intensity were lower (*P* < 0.0001) for the low MYG. Additionally, there were interactions (*P* ≤ 0.001) between MYG and period for CH_4 production, CH_4y, and CH_4 intensity (Figure 3.2b).

No effects of MYG (*P* ≥ 0.14) were observed on initial or final SBW, and G:F during the backgrounding phase (Table 3.3). However, heifers classified as low and intermediate MYG had greater DMI (*P* < 0.0001) compared with high MYG heifers in the backgrounding phase. Additionally, low and intermediate MYG tended to have greater weight gain (*P* = 0.09) and ADG (*P* = 0.06) than the high MYG. During the finishing phase, low MYG heifers had greater (*P* < 0.01) initial and final SBW than the intermediate MYG heifers, and DMI was greatest for the heifers classified as low MYG. No effects (*P* ≥ 0.16) were detected among MYG on weight gain, ADG, and G:F during the finishing phase. All growth performance parameters were affected by periods in both the backgrounding and finishing phases.

Feeding behavior variables (Table 3.4), including meal duration and meal size, were greater (*P* < 0.01) for heifers ranked as low and intermediate MYG compared to the high MYG during the backgrounding phase. The SD of DMI, intermeal interval, and eating rate were not affected by the MYG ($P \geq 0.12$). Period effects were observed ($P \leq 0.03$) for feeding behavior variables in both phases, except the intermeal interval ($P = 0.15$) during the finishing phase.

The mean CV of the GF system per heifer in each time-of-day block was 49.7% for the low, 44.7% for the intermediate, and 44.6% for the high MYG during the backgrounding phase; and 55.6% for the low, 55.3% for the intermediate, and 55.6% for the high MYG during the finishing phase (Table 3.5). For CH_4 production within heifer across time-of-day blocks (excluding period), the mean CV was 24.6% for the low, 23.1% for the intermediate, and 22.3% for the high MYG during the backgrounding phase and during the finishing phase, it was 28.0% for the low, 26.2% for the intermediate, and 25.1% for the high MYG. In the backgrounding phase, the mean CH_4 production CV within heifer accounting for period (no time-of-day block) was 29.2%, 26.8%, and 25.9% for low, intermediate, and high MYG, respectively. In the finishing phase, the mean CV were 32.4%, 29.4%, and 29.5% for low, intermediate, and high MYG, respectively. The mean CH_4 production CV within heifer, within time-of-day block accounting for period, were of 23.6%, 22.0%, and 21.3% for low, intermediate, and high MYG, respectively, during the backgrounding phase. During the finishing phase, it was 25.3%, 24.6%, and 24.0% for low, intermediate, and high MYG, respectively. The mean CH_4y CV within heifer accounting for period was of 6.37%, 6.69%, and 7.05% for low, intermediate, and high MYG, respectively, during the backgrounding phase. In the finishing phase, it was 16.9%, 7.88%, and 10.5% for low, intermediate, and high MYG, respectively. The mean CH_4y CV between heifers was of 7.77% for the low, 6.55% for the intermediate, and 11.5% for the high MYG during the backgrounding phase; and 19.6% for the low, 10.7% for the intermediate, and 16.7% for the high MYG during the finishing phase.

There were no differences between low and high MYG for Alpha diversity estimated by observed ASVs ($P \geq 0.12$) within both periods sampled of the backgrounding and finishing phases (Figure 3.3a). Similarly, no differences were observed in the Shannon diversity index ($P \geq 0.45$) within periods 1 of the backgrounding and finishing phases, and period 4 in the finishing phase (Figure 3.3b). However, during period 4 of backgrounding, high MYG heifers exhibited greater Shannon diversity ($P = 0.01$) compared with low MYG heifers. The β-diversity based on Bray-Curtis dissimilarity (Figure 3.3c) did not differ between groups within both period sampled in backgrounding and finishing phases. However, generalized β-diversity based on UniFrac distance ($\alpha = 0.5$) (Figure 3.3d) demonstrated differences ($P = 0.04$) between low and high MYG in period 4 of the finishing phase.

3.4. Discussion

Selecting low CH_4-producing cattle to reduce enteric CH_4 emissions from the beef cattle sector is a promising tool for reducing global GHG emissions (Beauchemin et al., 2022a; de Haas et al., 2017). Understanding how to apply this selection technique effectively without compromising herd productivity requires further investigation and a consistent method which is capable of reliably capturing CH_4 measurements from all cattle instead of just a few animals is required. Within this context, the present study ranked feedlot beef heifers as low, intermediate, or high MYG and evaluated group effects on growth performance, feeding behavior, GF system visitation patterns, and rumen microbial population composition across and within backgrounding and finishing diets.

Estimating enteric CH_4 emission using the GF system is one of the two most used techniques in current livestock CH_4 research (Alemu et al., 2017; Hammond et al., 2016b). However, multiple studies conducted in feedlots or grazing have shown that not all animals consistently visit the GF system, particularly when cattle are fed *ad libitum* diets (Alemu et al., 2021; Hammond et al., 2016a). In the present study, 6% of beef heifers did not meet the visitation criteria for the GF system throughout the entire trial, a lower percentage compared with other studies using the GF system (Beauchemin et al., 2022b; Hammond et al., 2016a). This observation reiterates the importance of adapting the cattle to the system and applying "good visitation" criteria on data analysis to obtain reliable observations.

Categorizing beef heifers by MYG demonstrated that it is a dynamic trait, varying across periods and phases, rather than a fixed individual characteristic. Within the number of animals that met the visitation criteria in the GF system during all four periods in the backgrounding (n = 48) and finishing (n = 18), a small proportion maintained a consistent MYG rank (18.8% and 16.7%, respectively), while most heifers transitioned across groups over time. Similar instability in CH_4 emissions rankings has been reported in previous studies. Vargas et al. (2025) reported that from 35% to 83% of beef steers were re-ranked into low, medium, and high CH_4 production, yield, intensity, or residual emissions classifications across the backgrounding to finishing phases. Likewise, Williams et al. (2025) demonstrated that cattle categorized as low CH_4y emitters during the backgrounding phase may shift to intermediate or high rankings in later periods or in the finishing phase. Additionally, these authors related that diet composition can also modulate this variability, as differences in silage sources affected both the magnitude of CH_4 emissions and the consistency of animal rankings (Williams et al., 2025). Thus, classifying beef heifers by MYG emissions should be conducted across different productive phases and diets to identify consistent low CH_4-producing individuals.

Although low MYG heifers had higher DMI and lower CH_4y in both phases, they also had lower absolute CH_4 production and intensity. Higher DMI likely increased rumen fill and stimulated a higher passage rate, reducing the average retention time of feed particles and limiting the extent of ruminal fermentation, consequently reducing hydrogen available for methanogenesis (Buddle et al., 2011; Pinares-Patiño et al., 2003; Ungerfeld, 2020). Pinares-Patiño et al. (2003) evaluated the influence of ruminal retention time and rumen volume on CH_4 emission in sheep and reported that sheep with longer rumen retention time had higher CH_4y. While the passage rate and average retention time of feed particles were not analyzed in the present study, the results found for the low MYG heifers may be due to modifications in ruminal kinetics; however, further investigation is necessary.

The lack of differences in weight gain, ADG, and G:F among MYG, despite the higher DMI in low MYG heifers in the backgrounding and finishing phases, suggests a possible change in rumen kinetics, as the increased DMI did not lead a reduction in feed efficiency. Unfortunately, ruminal fermentation, such as volatile fatty acids and ammonia were not analyzed in the present study. However, a previous study evaluating the relationship between CH_4 emissions, growth, and feed efficiency in beef heifers found a positive correlation among body weight, DMI, ADG, and CH_4 production, indicating that animals with higher DMI and weight gain tend to produce more CH_4 (g/d), but not necessarily more CH_4y (Renand et al., 2019). In addition, prior studies of beef heifers found a negative correlation between CH_4y and feeding behavior parameters, including the number of visits to the feed bin, average meal size, and average daily eating rate (Biswas et al., 2022; Llonch et al., 2018). In the present study, ranking heifers by CH_4y did not consistently affect feeding behavior in the finishing phase and did not affect heifers' feeding efficiency.

The number of visits per heifer to the GF system within each time-of-day block reflects the voluntary, natural behavior of feedlot cattle (Alemu et al., 2017; Hammond et al., 2016a). In the present study, the high CVs of GF system visitation by MYG in both phases are consistent with the natural behavioral variability of cattle. Voluntary visitation to the GF system is unevenly distributed throughout the day, as a result of natural diurnal activity patterns and feeding behavior (Alemu et al., 2017; Hammond et al., 2016a; Manafiazar et al., 2017). Furthermore, the observed CV for CH_4 production within heifers in both backgrounding and finishing phases by MYG also suggests that the dynamics of voluntary visitation to the GF may affect the variability of CH_4 emissions within the same diet and period. Enteric CH_4 emission rates fluctuate significantly throughout the day and are closely associated with the time since the last meal (Hammond et al., 2016a). In general, CH_4 production tends to increase during and shortly after eating (Hammond et al., 2016a). In this study, when emissions were analyzed within heifers, within time-of-day block accounting for period, the mean CVs decreased, indicating that a significant portion of the variability is explained by differences between periods and hours of the day. Thus, combining the data into period and time-of-day block averages reduced CH_4 variability within animal.

Regarding CH_4y, the CVs within heifers among MYG were substantially lower during the backgrounding phase and moderate during finishing. Similar results were reported by Terry & Beauchemin (2025), who evaluated the CVs of GF system patterns of 100 feedlot steers during backgrounding and finishing trials. The authors demonstrated a mean CV of 34.7% and 42.7%, respectively, for the number of visits per steer in each time-of-day block (Terry & Beauchemin, 2025). Furthermore, the authors found a reduction in the CVs of CH_4 production (within steer, within time-of-day block accounting for period), demonstrating that accounting for time-of-day block reduces variability in feedlot studies (Terry & Beauchemin, 2025).

Additionally, the MYG rank was not consistently associated with differences in ruminal microbial richness or community structure in this study. The lack of differences in ruminal microbial population from heifers ranked as low and high MYG to alpha-diversity estimated by observed ASVs and β-diversity by Bray-Curtis dissimilarity are in accordance with prior findings (Fregulia et al., 2024; Smith et al., 2022). However, there were changes in alpha-diversity by Shannon index in period 4 of the backgrounding phase, and generalized β-diversity using UniFrac distance also occurred in period 4 in the present study. A previous study evaluating dairy heifers classified as high and low CH_4y, revealed that differences in CH_4y classification were not associated with changes in overall microbial diversity but rather with shifts in specific microbial taxa and predicted functional pathways (Fregulia et al., 2024). In addition, Smith et al. (2022) also did not report differences in ruminal alpha- and β-diversity from beef cattle ranked as low and high residual methane emissions (RME). The authors also reported compositional shifts in specific bacterial and archaeal taxa, suggesting that CH_4 divergence was associated with functional shifts in hydrogen metabolism rather than broad changes in microbial diversity (Smith et al., 2022). Overall, the findings in the present study indicate that the low MYG phenotype in feedlot beef heifers is not associated with alterations in microbial diversity or community structure but is instead more related to DMI.

3.5. Conclusions

Classifying feedlot beef heifers according to CH_4y demonstrated that this characteristic is dynamic throughout production phases and influenced by feeding behavior. As found in the present study, low MYG heifers consistently showed higher DMI, maintaining similar ADG and feed efficiency, suggesting that the reduction in CH_4 production did not benefit animal productivity. Importantly, those differences between CH_4y groups were not associated with consistent changes in microbial diversity. Collectively, the findings emphasize the complexity and temporal variability of CH_4 traits in feedlot systems and demonstrate the importance of evaluating the CH_4 emission across and within productive phases to identify consistently low-emitting cattle, which based on this study are relatively rare individuals.

3.6. 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, Volume 2* - 2021. https://doi.org/10.3389/fanim.2021.641590

Alemu, A. W., Vyas, D., Manafiazar, G., Basarab, J. A., & Beauchemin, K. A. (2017). Enteric methane emissions from low– and high–residual feed intake beef heifers measured using GreenFeed and respiration chamber techniques1,2. *Journal of Animal Science, 95*(8), 3727-3737. https://doi.org/10.2527/jas.2017.1501

AOAC. (2005). *Official methods of analysis* (Vol. 18th ed).

Beauchemin, K. A., Ungerfeld, E. M., Abdalla, A. L., Alvarez, C., Arndt, C., Becquet, P., Benchaar, C., Berndt, A., Mauricio, R. M., McAllister, T. A., Oyhantçabal, W., Salami, S. A., Shalloo, L., Sun, Y., Tricarico, J., Uwizeye, A., De Camillis, C., Bernoux, M., Robinson, T., & Kebreab, E. (2022a). Invited review: Current enteric methane mitigation options. *Journal of Dairy Science, 105*(12), 9297-9326. https://doi.org/https://doi.org/10.3168/jds.2022-22091

Beauchemin, K. A., Tamayao, P., Rosser, C., Terry, S. A., & Gruninger, R. (2022b). Understanding variability and repeatability of enteric methane production in feedlot cattle. *Frontiers in Animal Science, Volume 3* - 2022. https://doi.org/10.3389/fanim.2022.1029094

Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J., & Wang, M. (2020). Review: Fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation. *animal, 14*(S1), s2-s16. https://doi.org/10.1017/S1751731119003100

Biswas, A., Khan, A., Luo, D., & Jonker, A. (2022). Methane emissions in growing heifers while eating from a feed bin compared with 24-hour emissions and relationship with feeding behavior. *JDS Communications, 3*(4), 255-259. https://doi.org/10.3168/jdsc.2021-0184

Blaxter, K. L., & Clapperton, J. L. (1965). Prediction of the amount of methane produced by ruminants. *British Journal of Nutrition, 19*(1), 511-522. https://doi.org/10.1079/BJN19650046

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37*(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9

Buddle, B. M., Denis, M., Attwood, G. T., Altermann, E., Janssen, P. H., Ronimus, R. S., Pinares-Patiño, C. S., Muetzel, S., & Neil Wedlock, D. (2011). Strategies to reduce methane emissions from farmed ruminants grazing on pasture. *The Veterinary Journal, 188*(1), 11-17. https://doi.org/https://doi.org/10.1016/j.tvjl.2010.02.019

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*(7), 581-583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal, 6*(8), 1621-1624. https://doi.org/10.1038/ismej.2012.8

CCAC. (2009). *The care and use of farm animals in research, teaching and testing*. Canadian Council on Animal Care.

de Haas, Y., Pszczola, M., Soyeurt, H., Wall, E., & Lassen, J. (2017). Invited review: Phenotypes to genetically reduce greenhouse gas emissions in dairying. *Journal of Dairy Science, 100*(2), 855-870. https://doi.org/https://doi.org/10.3168/jds.2016-11246

Fregulia, P., Dias, R. J. P., Campos, M. M., Tomich, T. R., Pereira, L. G. R., & Neves, A. L. A. (2024). Composition of the rumen microbiome and its association with methane yield in dairy cattle raised in tropical conditions. *Molecular Biology Reports, 51*(1), 447. https://doi.org/10.1007/s11033-024-09381-0

French, E. A., Beck, M. R., Kalscheur, K. F., Jaramillo, D. M., Derner, J. D., Moffet, C. A., Neville, B. W., Soder, K. J., O'Connor, R. C., Koziel, J. A., Vadas, P., Moeller, S., & Gunter, S. A. (2025). Experimental and experiential recommendations for using the GreenFeed systems to measure gas flux in grazing and confined cattle. *MethodsX, 15,* 103667. https://doi.org/https://doi.org/10.1016/j.mex.2025.103667

Hammond, K. J., Waghorn, G. C., & Hegarty, R. S. (2016a). The GreenFeed system for measurement of enteric methane emission from cattle. *Animal Production Science, 56*(3), 181-189. https://doi.org/10.1071/an15631

Hammond, K. J., Crompton, L. A., Bannink, A., Dijkstra, J., Yáñez-Ruiz, D. R., O'Kiely, P., Kebreab, E., Eugène, M. A., Yu, Z., Shingfield, K. J., Schwarm, A., Hristov, A. N., & Reynolds, C. K. (2016b). Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. *Animal Feed Science and Technology, 219,* 13-30. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2016.05.018

Hammond, K. J., Humphries, D. J., Crompton, L. A., Green, C., & Reynolds, C. K. (2015). Methane emissions from cattle: Estimates from short-term measurements using a GreenFeed system compared with measurements obtained using respiration chambers or sulphur hexafluoride tracer. *Animal Feed Science and Technology, 203*, 41-52. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2015.02.008

Herrera-Saldana, R. E., Huber, J. T., & Poore, M. H. (1990). Dry Matter, Crude Protein, and Starch Degradability of Five Cereal Grains1. *Journal of Dairy Science, 73*(9), 2386-2393. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(90)78922-9

Johnson, K. A., & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of Animal Science, 73*(8), 2483-2492. https://doi.org/10.2527/1995.7382483x

Króliczewska, B., Pecka-Kiełb, E., & Bujok, J. (2023). Strategies Used to Reduce Methane Emissions from Ruminants: Controversies and Issues. *Agriculture, 13*(3), 602. https://www.mdpi.com/2077-0472/13/3/602

Llonch, P., Somarriba, M., Duthie, C. A., Troy, S., Roehe, R., Rooke, J., Haskell, M. J., & Turner, S. P. (2018). Temperament and dominance relate to feeding behaviour and activity in beef cattle: implications for performance and methane emissions. *Animal, 12*(12), 2639-2648. https://doi.org/10.1017/S1751731118000617

Manafiazar, G., Zimmerman, S., & Basarab, J. A. (2017). Repeatability and variability of short-term spot measurement of methane and carbon dioxide emissions from beef cattle using GreenFeed emissions monitoring system. *Canadian Journal of Animal Science, 97*(1), 118-126. https://doi.org/10.1139/cjas-2015-0190

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One, 8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Mertens, D. R., & Collaborators. (2002). Gravimetric Determination of Amylase-Treated Neutral Detergent Fiber in Feeds with Refluxing in Beakers or Crucibles: Collaborative Study. *Journal of AOAC INTERNATIONAL, 85*(6), 23. https://doi.org/10.1093/jaoac/85.6.1217Journal

Morgavi, D. P., Forano, E., Martin, C., & Newbold, C. J. (2010). Microbial ecosystem and methanogenesis in ruminants. *animal, 4*(7), 1024-1036. https://doi.org/https://doi.org/10.1017/S1751731110000546

NASEM. (2016). *Nutrient requirements of beef cattle*. National Academies of Sciences Medicine Division on Earth Life Studies Committee on Nutrient Requirements of Beef Cattle.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2020). *Community Ecology Package: Ordination methods, diversity analysis and other functions for community and vegetation ecologists*. In vegan. https://rdrr.io/cran/vegan/man/vegan-package.html

Pinares-Patiño, C. S., Ulyatt, M. J., Lassey, K. R., Barry, T. N., & Holmes, C. W. (2003). Rumen function and digestion parameters associated with differences between sheep in methane emissions when fed chaffed lucerne hay. *The Journal of Agricultural Science, 140*(2), 205-214. https://doi.org/10.1017/S0021859603003046

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research, 41*(D1), D590-D596. https://doi.org/10.1093/nar/gks1219

Renand, G., Vinet, A., Decruyenaere, V., Maupetit, D., & Dozias, D. (2019). Methane and Carbon Dioxide Emission of Beef Heifers in Relation with Growth and Feed Efficiency. *Animals (Basel), 9*(12). https://doi.org/10.3390/ani9121136

Ribeiro, G. O., Jr., Swift, M. L., & McAllister, T. A. (2016). Effect of diastatic power and processing index on the feed value of barley grain for finishing feedlot cattle. *Journal of Animal Science, 94*(8), 3370-3381. https://doi.org/10.2527/jas.2015-0068

Schwartzkopf-Genswein, K. S., Atwood, S., & McAllister, T. A. (2002). Relationships between bunk attendance, intake and performance of steers and heifers on varying feeding regimes. *Applied Animal Behaviour Science, 76*(3), 179-188. https://doi.org/https://doi.org/10.1016/S0168-1591(02)00009-6

Smith, P. E., Kelly, A. K., Kenny, D. A., & Waters, S. M. (2022). Differences in the Composition of the Rumen Microbiota of Finishing Beef Cattle Divergently Ranked for Residual Methane Emissions. *Frontiers in Microbiology, Volume 13* - 2022. https://doi.org/10.3389/fmicb.2022.855565

Terry, S. A., & Beauchemin, K. A. (2025). Essential oil blend and monensin for feedlot steers consuming backgrounding and finishing diets: growth performance, methane emissions using the GreenFeed system, and feeding behavior. *Translational Animal Science, 9*. https://doi.org/10.1093/tas/txae182

Ungerfeld, E. M. (2020). Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. *Frontiers in Microbiology, 11*, 589. https://doi.org/10.3389/fmicb.2020.00589

Vargas, J. d. J., Manriquez, D., Swenson, M., Carvalho, P. H. V., & Place, S. E. (2025). Enteric methane emission classification and ranking of growing steers during the backgrounding and finishing phases. *Journal of Animal Science, 103*. https://doi.org/10.1093/jas/skaf046

Williams, M. S., Terry, S. A., & Beauchemin, K. A. (2025). Low methane phenotype of beef feedlot cattle changes throughout production and depends on diet. *9th International Greenhouse Gas and Animal Agriculture (GGAA2025) Conference*. Oral Presentation., Nairobi, Kenya.

Table 3.1. Ingredients and chemical composition of experimental TMR with or without seaweed blend (SWblend) supplementation used in backgrounding and finishing feedlot trial

Ingredient, DM % of TMR	Backgrounding			Finishing		
	Control TMR	SWblend TMR	GF pellet[1]	Control TMR	SWblend TMR	GF pellet[2]
Corn Silage	65.0	64.4		9.00	8.40	
Barley Grain	20.0	20.0		86.0	86.0	
Canola Meal	10.0	10.0		-	-	
Supplement	5.00	5.00		5.00	5.00	
Seaweed blend		0.60			0.60	
Chemical composition, % DM						
DM, %	94.0 ± 0.40	93.9 ± 0.39	95.0 ± 0.57	93.7 ± 1.05	93.6 ± 0.88	94.6 ± 0.80
OM, %	92.4 ± 0.84	92.4 ± 0.89	92.8 ± 0.33	96.0 ± 0.59	95.8 ± 0.68	92.7 ± 0.27
CP, %	16.4 ± 0.73	16.6 ± 0.62	17.0 ± 0.60	13.9 ± 1.01	13.5 ± 0.55	13.9 ± 0.45
Starch, %	29.1 ± 1.62	27.5 ± 1.56	21.8 ± 2.39	55.1 ± 4.34	54.4 ± 3.40	27.7 ± 3.96
NDF, %	34.9 ± 1.89	36.1 ± 2.33	26.9 ± 0.91	21.0 ± 4.11	21.8 ± 3.80	29.2 ± 2.40
ADF, %	18.0 ± 0.98	18.5 ± 1.30	14.3 ± 0.83	5.14 ± 0.70	5.64 ± 0.61	15.1 ± 0.71
GE, Mcal/kg DM	5.01 ± 0.08	5.08 ± 0.08	4.67 ± 0.05	4.88 ± 0.37	4.98 ± 0.48	5.19 ± 1.17

Control TMR: with no additives; SWblend TMR: supplemented at 0.60% on DM; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; GE: gross energy; [1]GF pellet in backgrounding phase include 43.2% ground barley, 11.5% canola meal, 1.0% limestone ($CaCO_3$), 13.0% dried molasses, 0.15% salt, 30.4% beet pulp, and 0.75% canola oil (DM basis); [2]GF pellet in finishing phase include 45.1% ground barley, 7.50% canola meal, 1.0% limestone ($CaCO_3$), 13.0% dried molasses, 0.15% salt, 32.5% beet pulp, and 0.75% canola oil (DM basis).

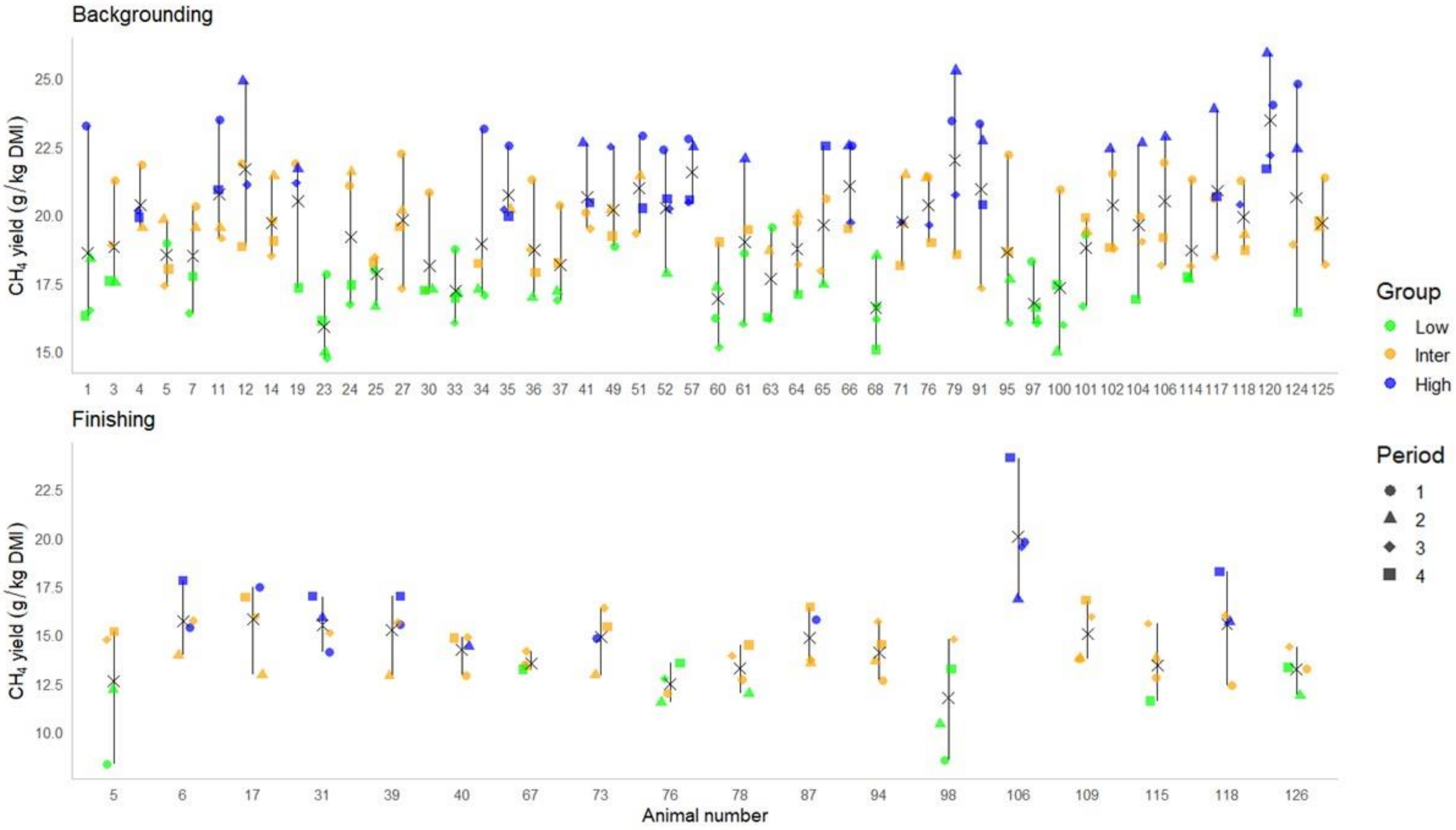


Figure 3.1. Individual variation of beef heifers ranked into low, intermediate, and high MYG[1] during backgrounding (n = 48) and finishing (n = 18) feedlot trials.

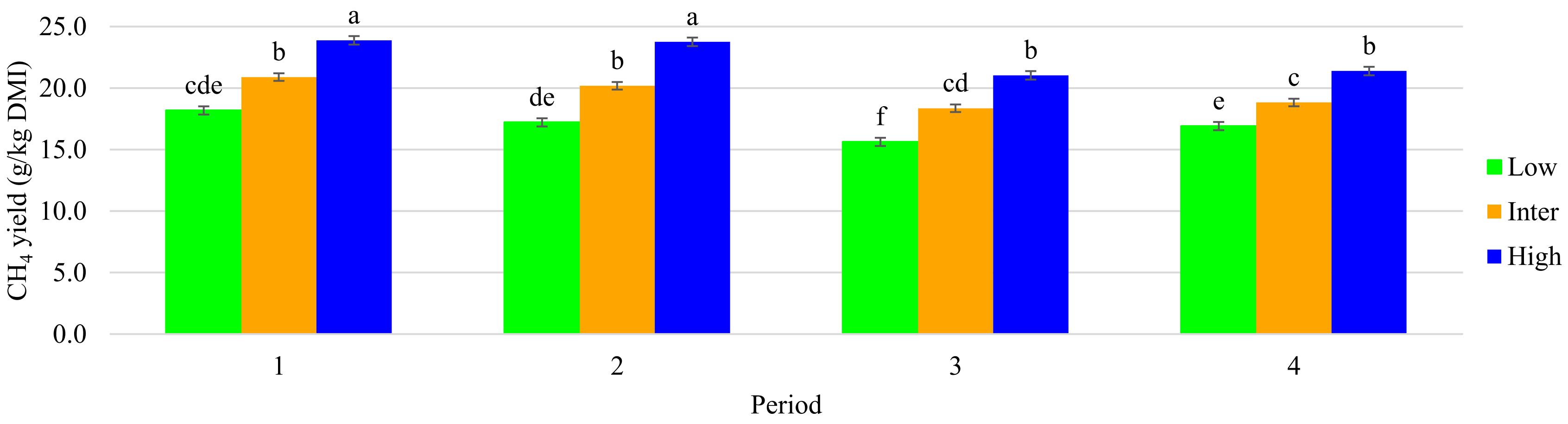
[1]MYG: Methane yield group.

Table 3.2. Visitation to the GreenFeed System, dry matter intake, and CH_4 production, yield, and energetic intensity of heifers divided into low, intermediate, and high MYG[1] during a backgrounding and finishing feedlot trial

Variables	MYG			SEM	*P* value		
	Low	Inter	High		MYG	Period	MYG x Period
Backgrounding							
No. visit total	29.2 ± 13.3	33.9 ± 18.2	32.5 ± 16.7				
No. visit day	2.09 ± 0.95	2.42 ± 1.30	2.32 ± 1.19				
Mean duration, min	294.5	294.1	281.8	12.73	0.12	<.0001	0.81
Pellet DMI, kg	0.40	0.40	0.39	0.018	0.13	<.0001	0.70
DMI total, kg	10.4^{a}	10.2^{a}	9.47^{b}	0.104	<.0001	<.0001	0.91
GEI, Mcal/d	52.3^{a}	51.1^{a}	47.4^{b}	0.49	<.0001	<.0001	0.90
CH_4, g/d	176.2^{c}	198.3^{b}	211.1^{a}	2.39	<.0001	<.0001	0.78
CH_4 yield, g/kg DMI	17.0^{c}	19.6^{b}	22.5^{a}	0.23	<.0001	<.0001	0.04
CH_4, Mcal/% GEI	4.52^{c}	5.21^{b}	5.99^{a}	0.076	<.0001	<.0001	0.01
Finishing							
No. visit total	19.5 ± 7.47	20.4 ± 7.12	21.0 ± 7.60				
No. visit day	1.39 ± 0.53	1.46 ± 0.51	1.50 ± 0.54				
Mean duration, min	221.8	223.4	226.4	11.43	0.73	<.0001	0.26
Pellet DMI, kg	0.31	0.32	0.32	0.016	0.77	<.0001	0.32
DMI total, kg	11.4^{a}	10.8^{b}	10.6^{b}	0.38	<.001	<.001	0.07
GEI, Mcal/d	59.0^{a}	56.0^{b}	54.7^{b}	5.46	0.001	<.0001	0.31
CH_4, g/d	128.7^{c}	152.3^{b}	183.0^{a}	6.74	<.0001	<.0001	<.0001
CH_4 yield, g/kg DMI	11.2^{c}	14.2^{b}	17.3^{a}	0.22	<.0001	<.0001	<.001
CH_4, Mcal/% GEI	2.89^{c}	3.63^{b}	4.47^{a}	0.187	<.0001	<.0001	<.0001

[1] MYG: Methane yield group; DMI: dry matter intake; GEI: gross energy intake; $^{a, b, c}$: means within the same row with different letters differ significantly ($P < 0.05$).

a. Backgrounding



b. Finishing

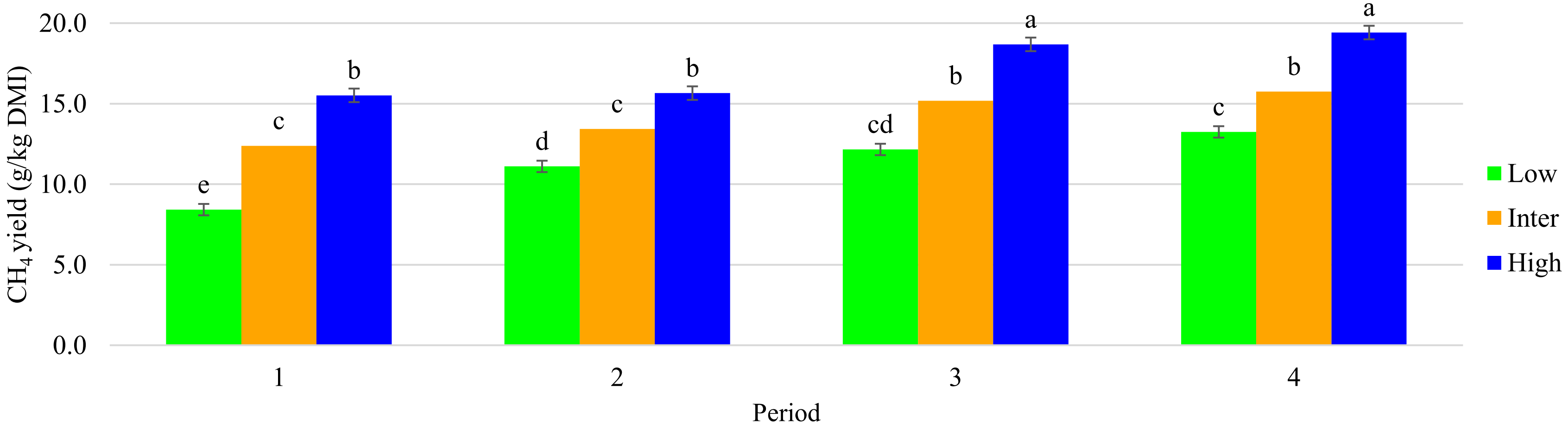


Figure 3.2. Methane yield interaction between MYG and Period of heifers ranked into low, intermediate, and high MYG[1] during backgrounding and finishing feedlot trials

[1]MYG: Methane yield group; $^{a, b, c, d, e, f}$: means within the same row with different letters differ significantly ($P < 0.05$).

Table 3.3. Growth performance and dry matter intake of heifers ranked into low, intermediate, and high MYG[1] during backgrounding and finishing feedlot trials

Parameters	MYG			SEM	*P* value		
	Low	Inter	High		MYG	Period	MYG × Period
Backgrounding							
Shrunk initial body weight, kg	364.6	364.3	363.4	1.92	0.90	<.0001	0.97
Shrunk final body weight, kg	400.4	401.1	398.1	2.12	0.59	<.0001	0.82
DMI, kg/d	10.0^a	9.80^a	9.09^b	0.108	<.0001	<.0001	0.91
Weight gain, kg	35.9	36.9	34.7	1.07	0.09	<.0001	0.41
ADG	1.03	1.05	0.98	0.028	0.06	<.0001	0.33
G:F	0.10	0.11	0.11	0.003	0.14	<.0001	0.64
Finishing							
Shrunk initial body weight, kg	559.4^a	542.9^b	553.8^{ab}	6.04	0.01	<.0001	0.12
Shrunk final body weight, kg	605.4^a	586.0^b	596.2^{ab}	7.36	0.01	<.0001	0.12
DMI, kg/d	11.1^a	10.5^b	10.3^b	0.37	<.001	<.001	0.08
Weight gain, kg	45.8	43.2	42.3	1.72	0.16	<.0001	0.83
ADG	1.62	1.53	1.49	0.06	0.16	<.0001	0.78
G:F	0.15	0.14	0.15	0.004	0.91	<.0001	0.99

[1] MYG: Methane yield group; DMI: dry matter intake; ADG: average daily gain; G:F: gain:feed; $^{a, b}$: means within the same row with different letters differ significantly ($P < 0.05$).

Table 3.4. Feeding behavior of heifers ranked into low, intermediate, and high MYG[1] during backgrounding and finishing feedlot trials.

Parameters	MYG			SEM	*P* value		
	Low	Inter	High		MYG	Period	MYG × Period
Backgrounding							
SD of DMI, kg/d	1.65	1.62	1.59	0.030	0.46	<.0001	0.93
Duration, min/meal	22.5^a	22.7^a	21.0^b	0.65	0.01	<.0001	0.98
Intermeal interval, min	151.1	151.6	154.7	6.55	0.65	<.0001	0.99
Meal size, kg/meal	1.19^a	1.19^a	1.10^b	0.056	0.03	<.0001	0.93
Eating rate, g/min	53.9	53.4	53.0	1.28	0.70	<.0001	0.53
Finishing							
SD of DMI, kg/d	1.75	1.80	1.80	0.050	0.64	0.03	0.43
Duration, min/meal	16.4	16.4	16.2	0.56	0.96	0.01	0.86
Intermeal interval, min	243.0	244.5	238.7	13.76	0.90	0.15	0.69
Meal size, kg/meal	1.93	1.95	2.06	0.106	0.74	<.0001	0.89
Eating rate, g/min	123.9	117.9	113.9	3.41	0.12	0.01	0.82

[1] MYG: Methane yield group; DMI: dry matter intake; $^{a,\,b}$: means within the same row with different letters differ significantly ($P < 0.05$).

Table 3.5. Coefficient of variation of GF system visitation, CH_4 production (g/d), and CH_4y(g/kg DMI) of heifers ranked into low, intermediate, and high MYG[1] during backgrounding and finishing feedlot trials

Variable[2]		Backgrounding		Finishing	
		Mean CV, %	CV range, %	Mean CV, %	CV range, %
GF visit variation					
No. visits per heifer in each time of day block					
	Low	49.7	7.03 to 90.8	55.6	15.5 to 105.1
	Inter	44.7	14.7 to 98.6	55.3	19.6 to 96.7
	High	44.6	11.0 to 84.7	55.6	15.5 to 105.07
CH_4 production variation					
Within heifer across time-of-day blocks (no period)					
	Low	24.6	0.02 to 69.4	28.0	4.05 to 91.2
	Inter	23.1	2.41 to 57.6	26.2	1.83 to 92.1
	High	22.3	0.76 to 51.4	25.1	1.41 to 66.3
Within heifer accounting for period (no time-of-day block)					
	Low	29.2	17.7 to 48.7	32.4	19.0 to 62.6
	Inter	26.8	15.7 to 50.4	29.4	14.9 to 49.8
	High	25.9	10.1 to 38.0	29.5	18.7 to 49.5
Within heifer, within time-of-day block accounting for period					
	Low	23.6	0.02 to 75.5	25.3	0.78 to 91.2
	Inter	22.0	0.25 to 94.7	24.6	0.01 to 92.1
	High	21.3	0.20 to 63.8	24.0	0.04 to 107.4
CH_4 yield variation					
Within heifer accounting for period					
	Low	6.37	0.13 to 18.1	16.9	1.68 to 56.4
	Inter	6.69	0.08 to 13.3	7.88	0.82 to 19.3
	High	7.05	0.90 to 20.4	10.5	0.46 to 28.8
Between heifer					

Low	7.77	-	19.6	-
Inter	6.55	-	10.7	-
High	11.5	-	16.7	-

[1]MYG: Methane yield group; [2]Time of day block consists of six 4-h periods over 24 h; GF: GreenFeed system

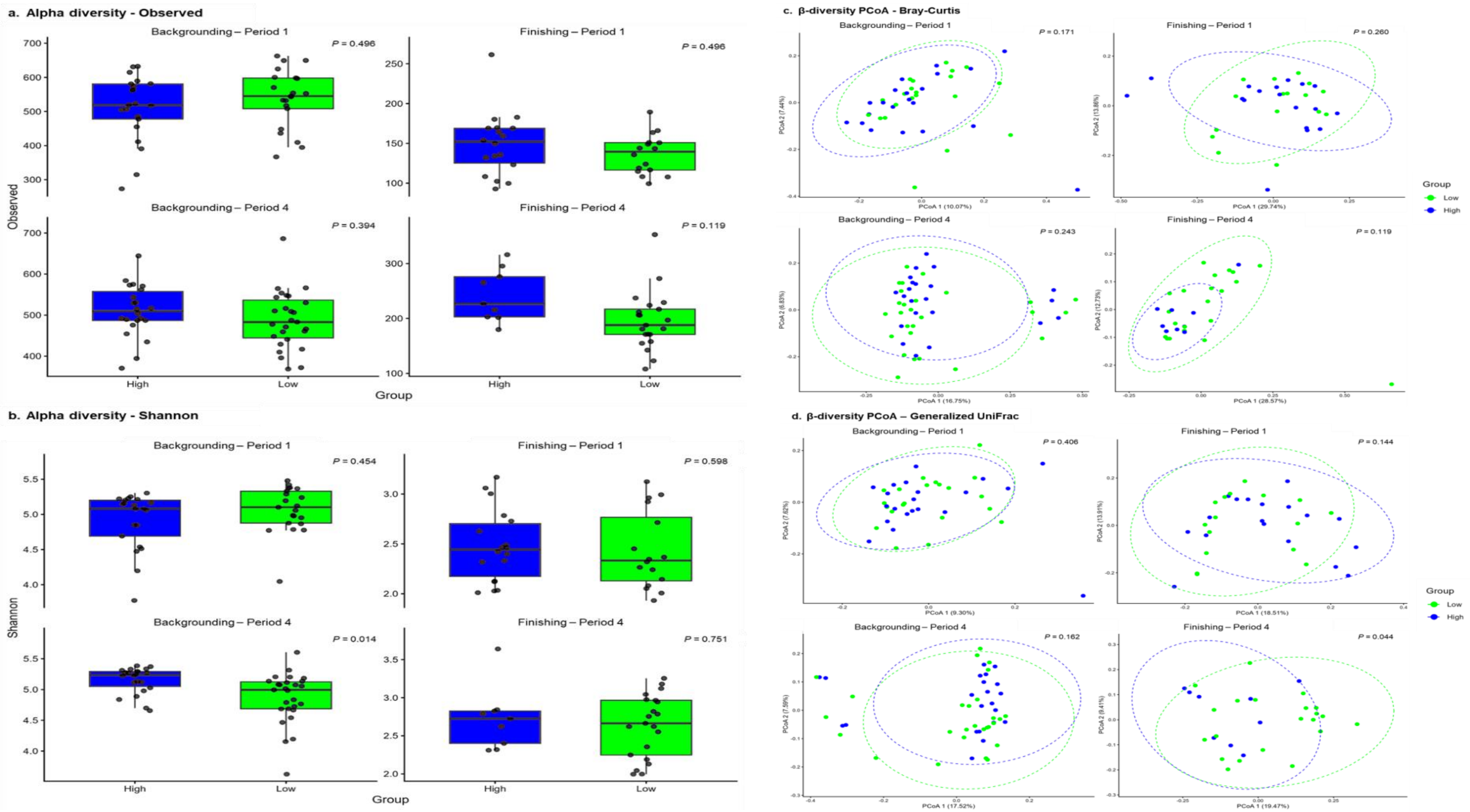


Figure 3.3. Rumen microbial populations from beef heifers in low and high MYG[1] in the first and last periods of the backgrounding and finishing feedlot trial

[1] MYG: Methane yield group; Alpha diversity estimated by (a) Observed ASVs and (b) Shannon diversity index. Boxes represent interquartile range, horizontal lines indicate medians, and points are individual animals. Principal coordinates analysis (PCoA) based on (c) Bray-Curtis dissimilarity and (d) generalized UniFrac distance ($\alpha = 0.5$). Points represent one animal, dashed ellipses indicate 95% confidence intervals for each group, and the percentage of variation explained by each principal coordinate is on the axes; *P* value for the significance of low and high MYG on microbiome composition was calculated using a PERMANOVA test.

CHAPTER 4: GENERAL DISCUSSION

4.1. Reducing methane emissions from the ruminant sector

Concerns about mitigating the effects of global warming are relevant to all production segments worldwide, particularly in the ruminant production sector. As discussed throughout this thesis, methane (CH_4) is one of the three main greenhouse gases (GHGs), and enteric CH_4 emissions from ruminants contributes 6.0% of total anthropogenic GHG emissions. At the same time, with the continued population growth, there is an increasing demand for high-quality nutrient foods, which has reinforced the demand to expand the production of animal-derived products, such as meat and milk. These challenges underscore the importance of developing strategies that improve ruminant productivity while minimizing environmental impacts, especially CH_4 emissions. In this context, the present study was conducted to improve the understanding of CH_4 production in feedlot systems and to evaluate potential mitigation strategies across backgrounding and finishing phases. This research aimed to investigate the supplementation of a multispecies seaweed blend (SWblend) and the selection of low CH_4-producing cattle to identify mechanisms that contribute to sustainable mitigation without compromising animal productivity.

4.2. Utilization of seaweed multispecies blend as a feed additive

Although multiple seaweed species have been widely studied as a dietary supplement to reduce CH_4 emissions from ruminant production, not all species demonstrating positive results. In the present study, supplementing 0.60% (DM basis) of SWblend did not reduce enteric CH_4 production, yield, or intensity during either two dietary phases. Additionally, growth performance, feeding behavior, hematological parameters, and protozoa counts did not change with SWblend.

However, ruminal fermentation was altered during the finishing phase, showing changes in NH_3 and VFA profile.

The absence of CH_4 reduction by the utilization of SWblend reinforces the high variation in seaweed species in terms of bioactive compounds. Importantly, those seaweeds present in the blend are originally from non-halogenated families, necessitating more investigation to determine their main bioactive compounds, doses to include in the diet, and their effect on cattle production. Furthermore, the tendency to improve ruminal pH stability during backgrounding suggests that certain components present in the blend may contribute to the ruminal stability rather than direct CH_4 inhibition.

4.3. Ranking beef heifers by methane yield

Selecting low CH_4-producing cattle is a promising tool to mitigate CH_4 emissions from cattle. However, the application and viability of these methods in the long term demand a deep understanding. Therefore, feedlot beef heifers classified into low, intermediate, and high CH_4 yield groups (MYG) demonstrated a considerable individual variability throughout the study, with heifers shifting groups within phases and from backgrounding to finishing. No heifers were consistently part of the low CH_4 yield group for both phases. Findings in the present study revealed that CH_4 production may be influenced by multiple factors, including individual circadian patterns, feeding behavior, or ruminal kinetics, without necessarily influencing ruminal microbial diversity.

4.4. Future directions

The application of nutritional intervention through supplementing feedlot beef heifers with SWblend and selecting low CH_4-producing cattle techniques in this study, required the use of procedures that allow for highly accurate assessment, such as the use of the GrowSafe system for analyzing feeding behavior; the use of the Greenfeed system to measure individual CH_4 production on a large scale; ruminal fermentation analyses such as VFA and NH_3; measurement of ruminal pH over 5 consecutive days per period; and 16s rRNA sequencing aimed to determine alpha and beta diversity. Thus, the results revealed future research opportunities to improve the applicability of those techniques. For instance, test different doses of the SWblend to determine its effectiveness on CH_4 mitigation or ruminal stability; the utilization of SWblend with other dietary additives (3-NOP, ionophores, or *Asparagopses sp.*), combining multiple mechanisms of action in CH_4 reduction; test the SWblend supplementation in different production systems, for example, grass systems.

In terms of selecting low CH_4-producing cattle, it is important to consider that this method still needs more comprehensive research and an improved approach to classify animals by CH_4 production. Additional GF systems per pen or changes to the GF pellet to make it more palatable especially in the finishing phase may be required. Furthermore, there are a some different approaches to classify cattle that are worthy of investigation, including residual feed intake, residual CH_4 production, or CH_4 yield rank. In addition, as the present study demonstrated, there is also a necessity to include analyses to measure the ruminal kinetics, including ruminal passage rate or feed retention time.

The need for more effective methods to reduce CH_4 production in beef cattle is crucial both in the short and long term, as it can help mitigate global warming. This study explores the importance of understanding rumen dynamics and examines methods to reduce CH_4 emissions, particularly through the application of two promising techniques in feedlot systems. The overall findings underscore that sustainably mitigating enteric CH_4 in beef production will likely require a combination of nutritional strategies, genetic selection, and improved measurement technologies, anchored in a deep understanding of rumen microbial ecology and animal behavior.