THESIS

GENETIC PARAMETERS FOR BLOOD UREA NITROGEN, METHANE EMISSIONS, AND FEED INTAKE IN HEREFORD BEEF CATTLE

Submitted by

MacKenzie Griffin

Department of Animal Science

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Master's Committee:

Advisor: Scott E. Speidel
Co-Advisor: R. Mark Enns

Nathan DeLay

ABSTRACT

GENETIC PARAMETERS FOR BLOOD UREA NITROGEN, METHANE EMISSIONS, AND FEED INTAKE IN HEREFORD BEEF CATTLE

The objective of this project was to estimate heritabilities and evaluate genetic and phenotypic correlations among three key environmental impact traits: dry matter intake (DMI), methane (CH_4) emissions, and blood urea nitrogen (BUN)—a proxy for nitrogen metabolism—in a population of Hereford cattle. Enteric methane (CH_4) emissions and nitrogen excretion from beef cattle are major contributors to agriculture's environmental footprint. DMI is a critical trait because it influences the amount of nutrients consumed and metabolized by the animal, thereby affecting both CH_4 production and nitrogen excretion. Data were collected from 766 animals across six trials from 2021 to 2024, with phenotypes obtained using the Vytelle intake system (Vytelle, Greeley, CO), GreenFeed head-chamber system (C-Lock Inc., Rapid City, SD), and colorimetric BUN assays (Thermo Fisher Scientific, Waltham, MA). A three-trait animal model was fitted using ASReml 3.0 to estimate genetic parameters. Fixed effects in the model included trait-specific contemporary groups (CG; DMI, CH_4, BUN) and animal age at the time of measurement to account for environmental and developmental variation. Heritability estimates were moderate for BUN (0.17 ± 0.17) and CH_4 (0.14 ± 0.16), and high for DMI (0.50 ± 0.14), indicating meaningful potential for genetic improvement.

A strong genetic correlation was observed between BUN and DMI (0.71 ± 0.45), suggesting overlapping genetic control of nitrogen metabolism and feed intake, while genetic

correlations between CH_4 and the other traits were weak or negative. Residual correlations revealed environmental drivers strongly influencing CH_4 and DMI (0.71 ± 0.19) and a potential trade-off between BUN and DMI (-0.13 ± 0.12). These results support the feasibility of genetic selection for reduced environmental impact through reduced CH_4 emissions and improved nitrogen efficiency. Further validation in larger, multi-breed populations is warranted. Incorporating traits like BUN and CH_4 into breeding programs may enable long-term reductions in livestock-related environmental impacts without compromising productivity.

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CHAPTER 1: INTRODUCTION

Since the 1800s, human activities have been the primary driver of climate change (IPCC, 2021). The Earth's climate is a complex, interconnected system, and changes in one area can have widespread effects on ecosystems, economies, and societies. This highlights the urgent need for immediate and comprehensive action.

There are six major greenhouse gases (GHGs), each with different atmospheric lifespans and heat-trapping abilities (Mohican, 2011). In livestock production, the most significant GHGs are methane (CH_4), nitrous oxide, and carbon dioxide (CO_2), which are produced through enteric fermentation, respiration, and manure decomposition (Stackhouse et al., 2011). Among them, CH_4 has more than doubled in atmospheric concentration over the past two centuries, primarily due to agriculture, waste management, and fossil fuel production (Mohican, 2011; NASA, 2024). While livestock systems have historically played a role in converting plant-based nitrogen (N) into valuable animal protein, their low efficiency in nutrient utilization can strain ecosystems (Steinfeld & Wassenaar, 2007). This makes it critical to address emissions from both CH_4 and N to create a more sustainable beef industry with a lower environmental impact.

Recognizing the urgency of CH_4 reduction, the EPA introduced a final rule in November 2024 to curb CH_4 emissions from the oil and gas sector (EPA, 2024). This raises an important question—when will similar policies or incentives be applied to the beef cattle industry? Reducing CH_4 emissions is one of the most cost-effective ways the U.S. can slow global warming in the short term (EPA, 2024). At the same time, N losses from livestock production pose another environmental challenge. Excess N, whether retained in the soil or released into the

air and water, contributes to pollution and ecosystem degradation (Kanter et al., 2019). Agriculture remains the primary driver of N pollution, primarily due to rising global food demand and inefficiencies throughout the supply chain, from fertilizer production to waste management (Kanter et al., 2019). Given these challenges, CH_4 and N emissions from beef cattle must be a research priority to develop strategies that improve sustainability while maintaining productivity in the livestock sector.

Enteric methanogenesis is a natural process in which CH_4 is produced in the rumen of cattle (Morgavi et al., 2010). This process contributes to digestive efficiency by utilizing hydrogen and CO_2 produced during feed fermentation by bacteria (Baker, 1999; Morgavi et al., 2010). Various methods have been developed to measure CH_4 emissions, each differing in accuracy, cost, behavioral impact, and repeatability. These methods include respiration chambers, GreenFeed systems (C-Lock Inc., Rapid City, SD, USA), sulfur hexafluoride tracer techniques, breath sampling during milking or feeding, and laser CH_4 detectors (Garnsworthy et al., 2019). While all measurement techniques contain some degree of error, respiration chambers are considered the gold standard due to their precision and repeatability (Garnsworthy et al., 2019). However, no method can capture the exact CH_4 output of an animal with complete accuracy (Garnsworthy et al., 2019).

Although genetic selection has not been widely explored as a strategy for reducing CH_4 emissions, it holds significant potential. Heritability estimates for CH_4 production (MP) range from 0.29 to 0.40 under general conditions and from 0.13 to 0.19 when dry matter intake (DMI) is included in the model (Pickering et al., 2015). However, selecting for lower CH_4 production presents challenges due to unfavorable genetic correlations with key production traits, such as milk yield (Beauchemin et al., 2020). Genetic selection for CH_4 reduction must be carefully

considered, as livestock traits are interconnected, meaning that altering one trait can unintentionally affect others, potentially impacting overall performance and producer profitability. In beef cattle, CH_4-related traits with genetic influence generally fall into four categories: MP, CH_4 yield, CH_4 intensity (MI), and residual CH_4 production (RMP). Methane yield represents CH_4 output per unit of feed intake (DMI), while MI accounts for CH_4 output relative to the animal's productivity. Residual CH_4 production reflects CH_4 emissions after adjusting for other production traits, whereas MP represents total CH_4 emitted without an inputoutput relationship. Accurate heritability estimates are essential for improving the prediction of breeding values for CH_4 emissions (Brito et al., 2018).

Nitrogen excretion can be estimated using milk urea N (MUN), blood urea N (BUN), or urine N (UN). Milk urea N is proportional to BUN, which, in turn, correlates with UN levels (Eggleston et al., 2006; Broderick, 2003). While the existing literature does not necessarily have a research gap, the limited number of studies makes it difficult to draw concrete conclusions or compare methods of measurement. Unlike BUN, MUN can be collected non-invasively, making it a convenient tool for monitoring N metabolism. However, this method is impractical for beef cattle since they are not routinely milked, limiting its usefulness in this sector.

While N excretion is undoubtedly important for environmental sustainability, direct measurement remains challenging, and its genetic correlations with economically relevant traits are not well understood. There is a clear gap in research regarding the relationship between N excretion and economically significant traits in beef cattle. To date, the only relevant publication is by Waldrip et al. (2013), with most research focusing on dairy cattle and monogastric livestock.

Heritability estimates for BUN and MUN range from low to moderate, between 0.07 and 0.32, though Wood et al. (2003) reported higher estimates between 0.44 and 0.59 for MUN. Notably, all heritability estimates found in the literature have been conducted in dairy cattle, further emphasizing the research gap in beef cattle. By integrating N excretion into genetic selection strategies, the beef industry could take a proactive approach to improving sustainability without compromising production efficiency.

Addressing GHG emissions and N excretion in beef cattle is essential for improving the sustainability of livestock production. Methane emissions from enteric fermentation and N losses from manure contribute significantly to environmental challenges, yet both present opportunities for genetic selection as a long-term mitigation strategy. While heritability estimates suggest that CH_4 production and N excretion have a genetic basis, the complexity of these traits and their correlations with economically important production traits must be carefully considered. Genetic selection for reduced CH_4 emissions must balance environmental benefits with potential tradeoffs in productivity, while N excretion remains an understudied area in beef cattle genetics. Existing research has primarily focused on dairy cattle, underscoring the need for further studies specific to beef cattle systems. By integrating genetic selection into sustainability efforts, the beef industry has the potential to reduce its environmental impact without compromising efficiency. However, closing research gaps, refining measurement techniques, and evaluating the economic feasibility of breeding strategies will be critical for successful implementation.

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CHAPTER 2: METHANOGENISIS AND NITROGEN EXCRETION IN CATTLE REVIEW OF LITERATURE

SECTION 1: INTRODUCTION TO METHANOGENESIS IN CATTLE

Enteric methanogenesis is a normal process of methane (CH_4) being produced in the gastrointestinal tract in the ruminant (Morgavi et al., 2010) (Figure 2.1). Eight methanogenic archaea have been identified as specialized groups of microbes. These microbes search for and

utilize hydrogen (H_2) and carbon dioxide (CO_2) produced by other microbes in the rumen (Janssen & Kirs, 2008). They produce CH_4 out of the H_2 and CO_2 for electron transportation, prohibiting the buildup of these chemical compounds (Morgavi et al., 2010). The eight methanogenic archaea are *Methanoculleus olentangyi*, *Methanobacterium formicicum*, *Methanosarcina barkeri Methanobacterium bryantii*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile*, and *Methanobrevibacter ruminantium* (Janssen & Kirs, 2008). Without them, there is the possibility of lost efficiency and a rise in the partial pressure of H_2 (Morgavi et al., 2010).

Methanogens carry out metabolic functions crucial for the animal's growth, health, and nutrition (Morgavi et al., 2010). They are found in association with the liquid and solid phases of the rumen, as well as the rumen epithelium (Pei et al., 2009). As shown in Figure 2.1, these CH_4producing microbes utilize H_2 and CO_2 that are produced from feed fermentation by bacteria (Baker, 1999). Carbon dioxide is the primary electron receptor, while H_2 is the main donor for methanogenesis (Morgavi et al., 2010). Compounds like formate and acetate serve as additional H_2 sources in the process (Baker, 1999). Since the rumen is most active soon after feeding (Figure 2.1, step 1), naturally, that is when methanogens are the most abundant in the rumen (Baker, 1999; Pei et al., 2009). Overall, methanogenesis enhances the system's efficiency (Morgavi et al., 2010). As depicted in Figure 2.1, it achieves this by preventing an increase in the partial pressure of H_2, which could inhibit the normal function of microbial enzymes involved in the electron transport chain, particularly NADH dehydrogenase, ultimately reducing rumen fermentation (Morgavi et al., 2010).

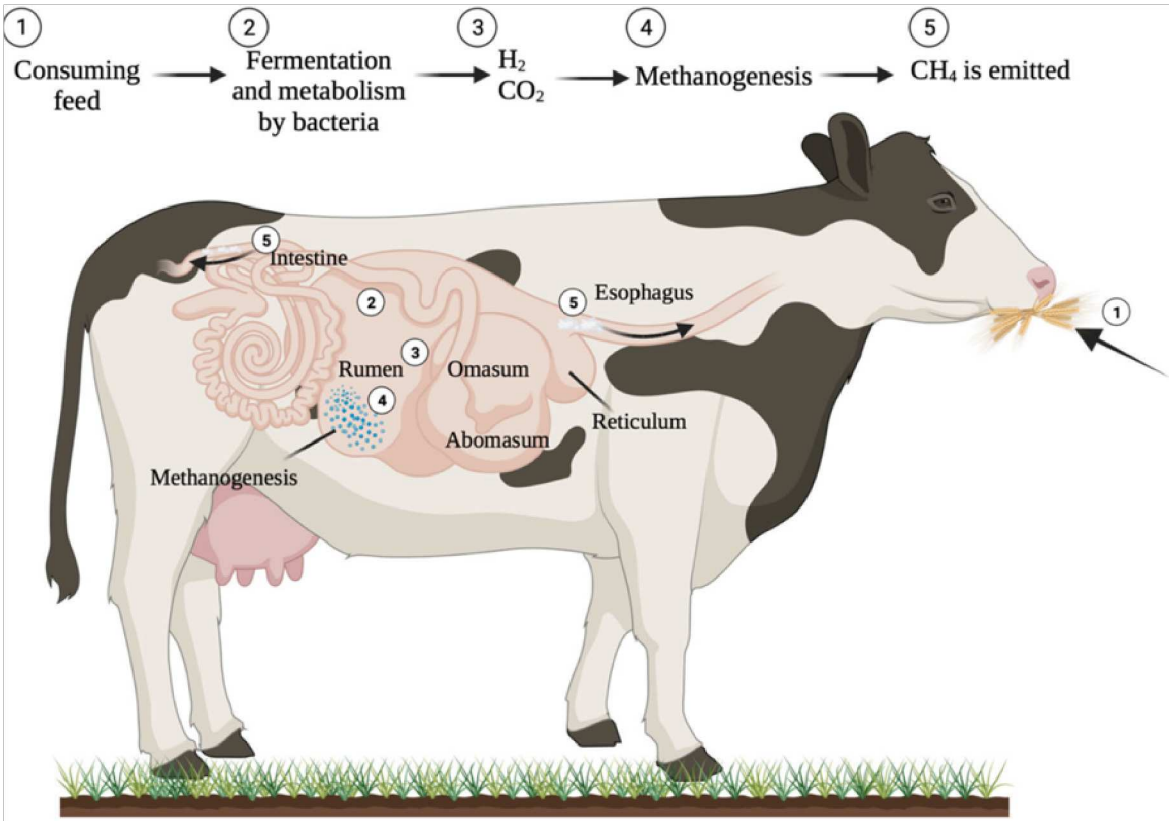

Figure 2.1 Mechanisms involved in the generation of methane in ruminants. Adapted from Džermeikaitė et al., 2024.

Methane is produced by hydrogenotrophic methanogenesis pathways, which convert CO_2 to CH_4, and studies have provided proof that the animal has an additive genetic influence on the microbiome (Roehe et al., 2016). Metagenomics has allowed the identification of the microbial community, thus making it possible to identify genes linked to higher or lower CH_4 emissions (Auffret et al., 2018; Roehe et al., 2016). A greater abundance of bacteria involved in propionate metabolism is linked to lower CH_4 emissions. As propionate metabolism consumes more H_2, it leaves less available to be converted into CH_4 (Auffret et al., 2018). Acetate metabolism has the opposite effect (Auffret et al., 2018). With insight into the rumen microbiome, Wallace et al. (2015), Roehe et al. (2016), and Auffret et al. (2018) used a combination of metagenomics and partial least squares analysis to identify 51 genes serving as biomarkers for CH_4 emissions.

SECTION 2: INTRODUCTION TO NITROGEN PROCESSES IN CATTLE

Nitrogen metabolism in cattle is a complex biological process that plays a crucial role in animal growth, protein turnover, waste management, and microbial function. Figure 2.2 illustrates the primary pathways of nitrogen (N) intake, transformation, and excretion; thus highlighting the connections between dietary protein, microbial digestion, ammonia production, and N metabolic efficiency (Owens & Zinn, 1988).

Briefly, cattle acquire N through dietary protein, which enters the rumen and is fractionated into degradable and undegradable components (Owens & Zinn, 1988). The N group on amino acids (AA) associated with degradable protein, along with non-protein N, is rapidly hydrolyzed by rumen microbes into free ammonia (NH_3) and carbon skeletons from AA (Owens & Zinn, 1988). Ammonia in the rumen exists within a dynamic pool with multiple inputs, including microbial breakdown of dietary N (Owens & Zinn, 1988). As stated earlier, N exits through microbial uptake, absorption across the rumen wall, or passage to the omasum (Owens & Zinn, 1988; Huntington & Archibeque, 2000). Ammonia serves as a critical N source for microbial protein synthesis, supporting microbial populations that contribute to fiber digestion (Owens & Zinn, 1988). However, excess ammonia not incorporated into microbial protein is absorbed through the rumen wall and enters the bloodstream (Owens & Zinn, 1988).

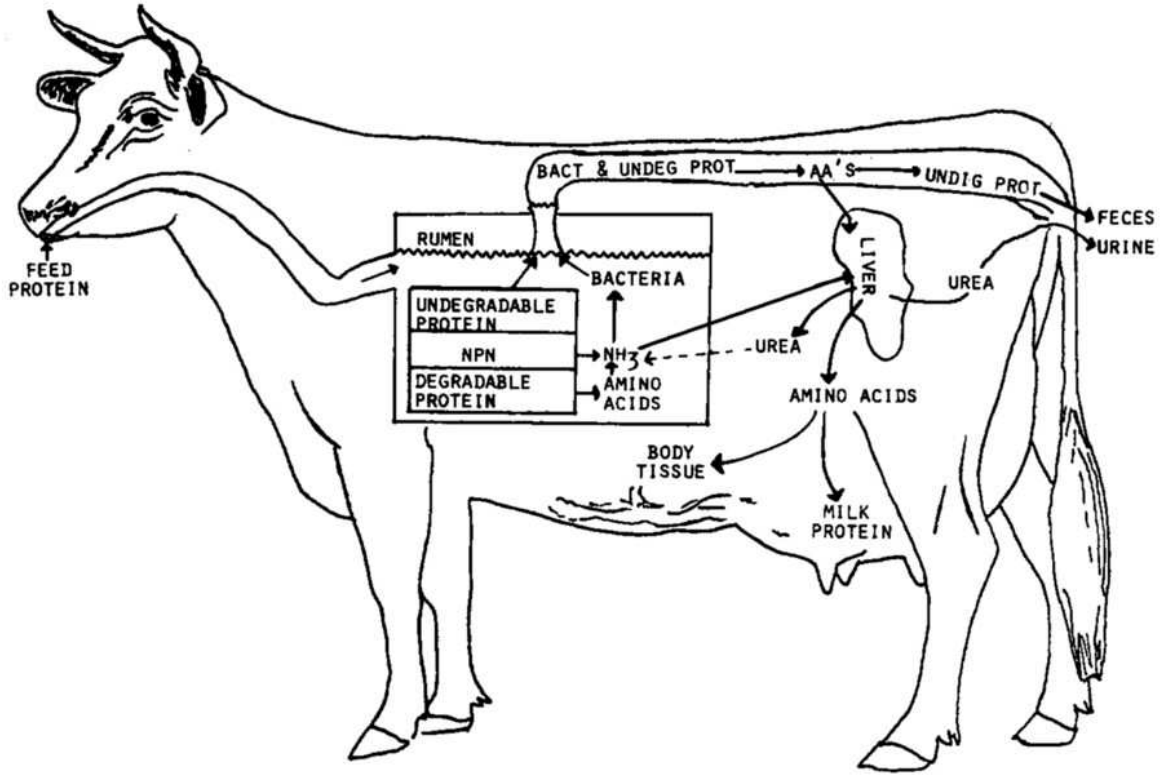

Figure 2.2 Protein metabolism in a lactating cow. Adapted from Owens & Zinn, 1988.

To prevent excess NH_3 in the bloodstream from becoming toxic, ruminants synthesize urea, primarily in the liver, though other tissues have enzymatic activity for urea production (Emmanuel, 1980). Once in the bloodstream, urea is either excreted in urine or recycled back to the digestive tract via diffusion into saliva or directly across the rumen wall (Huntington & Archibeque, 2000). This N recycling provides a continuous supply of ammonia to support microbial growth and fermentation (Huntington & Archibeque, 2000). Plasma urea enters the rumen by both saliva and passive diffusion, contributing to the N available for microbial metabolism (Huntington & Archibeque, 2000). As mentioned earlier, the breakdown of both energy and protein in the rumen incurs metabolic costs, with energy losses occurring as heat or

CH_4 (Owens & Zinn, 1988). Efficient N recycling and utilization are essential to maintaining microbial function and minimizing N waste (Owens & Zinn, 1988).

Once synthesized, urea circulates in the bloodstream as blood urea N (BUN), with portions either excreted or recycled to the rumen (Owens & Zinn, 1988; Figure 2.2). Blood urea N concentration provides insight into the efficiency of N metabolism and serves as a valuable indicator of genetic variation in N utilization (Owens & Zinn, 1988). Elevated BUN concentrations may suggest an imbalance between N intake and microbial assimilation, often reflecting inefficient N use or increased protein catabolism (Owens & Zinn, 1988).

SECTION 3: OVERVIEW OF CATTLE AND THE ENVIRONMENT

Deep cuts in global GHG emissions are essential to addressing climate change and supporting global environmental efforts (Mohican, 2011). Climate change, driven primarily by human activity since the 1800s, represents a long-term shift in temperature and weather patterns (IPCC, 2021). The Earth is an interconnected system, and the effects of climate change ripple across ecosystems, economies, and societies, underlining the urgency of taking comprehensive and immediate action.

Since 1992, the world has taken both significant and incremental steps to mitigate environmental impact, including reducing greenhouse gas (GHG) emissions (Mohican, 2011). There are six key GHGs, each with varying atmospheric lifespans and heat-trapping capabilities (Mohican, 2011). Among them, CO_2 is the most abundant and long-lasting. At the same time, CH_4, though less prevalent, is 28 times more potent over a 100-year period because of its heat trapping ability, making it a critical target for reduction due to its relatively short atmospheric lifespan and high potency (EPA, 2024). Over the past two centuries, CH_4's atmospheric

concentration has more than doubled, primarily driven by human activities such as agriculture, waste management, and fossil fuel production (Mohican, 2011; NASA, 2024).

Over the past decade, GHG emissions from cattle have gained significant political and public attention. While manure, when properly managed, serves as a valuable resource for improving soil quality through crop fertilization, its mismanagement contributes to environmental concerns. The primary GHGs associated with livestock production include CH_4, nitrous oxide (N_2O), and carbon dioxide, which originate from enteric fermentation, animal respiration, and microbial decomposition of manure (Stackhouse et al., 2011). Among these, N_2O is particularly potent, with approximately 5% of its agricultural emissions stemming from manure management in livestock facilities (AGLED, 2023). This gas is produced through incomplete microbial processes of nitrification and denitrification (Stackhouse et al., 2011), although ruminants contribute only minor amounts via enteric fermentation (Kaspar & Tiedje, 1981).

While extensive livestock production has historically played a role in converting N fixed in grass and forage into animal products for human consumption, its low efficiency in utilizing these nutrients can strain ecosystems (Steinfeld & Wassenaar, 2007). Thus, addressing emissions from both CH_4 and N is essential in creating a sustainable livestock sector that minimizes environmental impact.

Human actions have consequences that extend far beyond the emissions they directly produce. Rising atmospheric temperatures have led to an increase in water temperatures, causing thermal expansion and contributing to sea-level rise (Mohican, 2011). Additionally, permafrost in regions like Alaska and Siberia is melting for the first time in over 11,000 years since it began forming (Mohican, 2011). The thawing Siberian bog alone has the potential to release up to 70 billion tons of CH_4, a staggering amount equivalent to nearly a quarter of the CH_4 currently

present in the atmosphere (Mohican, 2011). Methane and N also contribute to ozone production, further heightening its environmental impact (EPA, 2024; EPA, 2024).

Researchers have discovered that livestock and agricultural practices are responsible for 37% of CH_4 emissions resulting from human activities worldwide (EPA, 2020). Globally, however, cattle's enteric fermentation was estimated to account for 27% of anthropogenic CH_4 emissions in 2020, surpassing the 24% due to the oil and gas sector (Bruns, 2024). Enteric fermentation occurs during digestion when microbial fermentation in the rumen generates excess H_2, which methanogenic archaea utilize to produce CH_4 (Roehe et al., 2016). This CH_4 is then expelled through eructation, resulting in a loss of 2-12% of the energy cattle consume (Johnson & Johnson, 1995; Roehe et al., 2016). While CH_4 is considered a pollutant, it is also a natural byproduct of anaerobic microbial activity (Wallace et al., 2015).

Recognizing the importance of reducing CH_4 emissions, the EPA announced a final rule in November 2024 to cut CH_4 emissions from the oil and gas sector (EPA, 2024). This initiative includes providing financial and technical assistance to support CH_4 reduction efforts (EPA, 2024). However, it raises the question of when similar incentives or regulations might be applied to the beef cattle industry. Methane reduction is one of the most cost-effective and impactful actions the U.S. can take to mitigate global warming in the short term (EPA, 2024). While CH_4 reduction remains a priority, addressing N pollution is equally critical, as its widespread environmental impact continues to grow alongside global food production, as depicted in Figure 2.3.

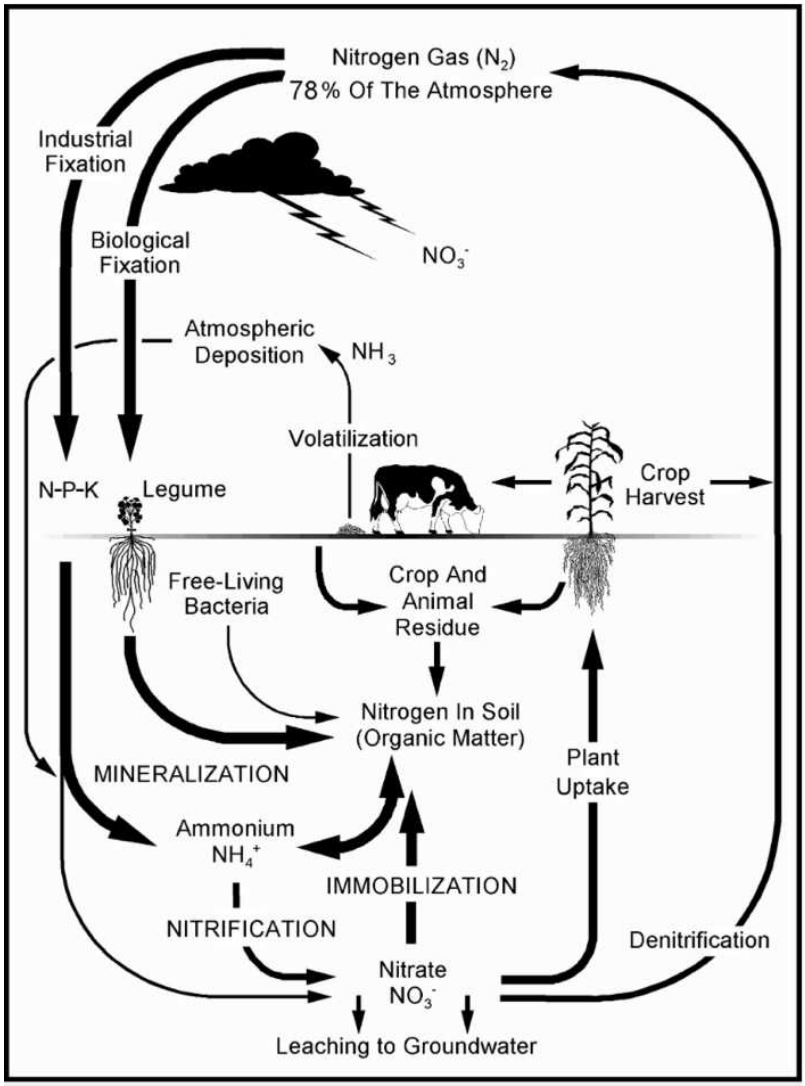

Figure 2.3 Nitrogen forms and pathways within an agriculture production system. Adapted from Environmental impacts of nitrogen use in agriculture., 2022.

Nitrogen is a fundamental element for life and food production, yet its use in agriculture is highly inefficient (Zhang et al., 2015). On average, crops absorb only about half of the N applied, and once consumed, both humans and animals utilize only a tiny portion, excreting the remainder (Zhang et al., 2015). This excess N either remains in the soil or is released back into the environment, contributing to N loss and subsequent pollution (Kanter et al., 2019).

Agriculture is the primary driver of N pollution, fueled by increasing global food demand and inefficiencies across the food supply chain, from synthetic fertilizer production to waste management (Kanter et al., 2019). Consequently, policy discussions often center on improving N management at the farm level, as it remains the dominant source of N-related environmental impacts (Bouwman, 2013). Effectively addressing N pollution will require comprehensive solutions that integrate improved agricultural management with broader regulatory policies.

While efforts to curb CH_4 emissions have been relatively more developed, strategies to reduce N_2O emissions are still critical due to their significant environmental consequences. The EPA has taken an active role in addressing N pollution by collaborating with organizations such as the Water Research Foundation, the USDA, Clean Water Act programs, and the Natural Resources Conservation Service (EPA, 2025). These partnerships enable the agency to provide technical and programmatic support while overseeing regulatory programs related to water quality, drinking water standards, wastewater management, and the reduction of N_2O air emissions (EPA, 2025). Managing N pollution is not only a national concern but also a global imperative, highlighting the need for coordinated international efforts like the Paris Agreement.

Addressing climate change requires coordinated international efforts, as seen in the Paris Agreement. On December 12, 2015, the Paris Agreement was adopted as a legally binding international treaty addressing climate change (UNFCCC, 2016). Its primary goal is to limit the global average temperature increase to well below 2 degrees Celsius above pre-industrial levels, with efforts to restrict it further to 1.5 degrees Celsius (UNFCCC, 2016). The United Nations Intergovernmental Panel on Climate Change warns that exceeding the 1.5 degrees Celsius threshold could trigger significantly more severe climate impacts, including extreme droughts, heatwaves, and intensified rainfall (UNFCCC, 2016). To prevent this, GHG emissions needed to peak before 2025 and decline by 43% by 2030 (UNFCCC, 2016).

The Paris Agreement operates on a five-year cycle (UNFCCC, 2016). Starting in 2020, countries submitted Nationally Determined Contributions (NDC), which outline their plans to reduce GHG emissions and actions to build resilience and adapt to climate change (UNFCCC, 2016). Each NDC is expected to reflect increased ambition over time (UNFCCC, 2016). In addition to NDCs, countries are encouraged but not required to submit Long-Term LowEmission Development Strategies, which provide a longer-term perspective on climate goals (UNFCCC, 2016).

To ensure accountability, the Enhanced Transparency Framework (ETF) was established to track countries' progress (UNFCCC, 2016). Beginning in 2024, countries are required to report on their actions and progress in mitigating climate change, adapting to its effects, and providing or receiving support (UNFCCC, 2016). The data collected through the ETF feeds into the Global Stocktake, a comprehensive assessment of collective progress toward long-term climate goals (UNFCCC, 2016).

SECTION 4: METHODS TO MEASURE METHANE EMISSIONS IN CATTLE

Livestock CH_4 emissions are a significant contributor to global CH_4 levels, accounting for approximately 17% of total emissions (Knapp et al., 2014). The amount of CH_4 cattle produce is directly related to the organic matter digested in the rumen, and this CH_4 production (MP) varies considerably between individual animals (Garnsworthy et al., 2019). Evidence shows inherent differences in CH_4 emissions among animals, which presents unique challenges and opportunities for research.

The primary goal of CH_4-related research is to minimize variability among animals when comparing treatment effects while having genetic variation to explore heritable traits

(Garnsworthy et al., 2019). Respiration chambers are the gold standard for CH_4 measurement, offering unmatched accuracy and repeatability (Garnsworthy et al., 2019). These chambers provide the benchmark against which all alternative methods should be assessed (Barnhart et al., 2007). However, all measurement methods contain some level of error, meaning the true value of CH_4 emissions is never fully known (Garnsworthy et al., 2019). As measurement error increases, so does the likelihood of bias (Garnsworthy et al., 2019).

When comparing two methods with significant error, a phenomenon known as "attenuation of errors" can occur (Spearman, 1904). This reduces the correlation between the methods and diminishes the ability to detect significant differences in accuracy (Adolph & Hardin, 2006). In linear regression terms, higher observed coefficients of variation in an alternative method compared to the gold standard result in a decreased slope and an upward bias in the intercept (Garnsworthy et al., 2012; Huhtanen et al., 2015).

Various methods are available to measure CH_4 emissions from cattle, including respiration chambers, GreenFeed systems (C-Lock Inc., Rapid City, SD, USA), sulfur hexafluoride (SF_6) tracer techniques, breath sampling during milking or feeding, and laser CH_4 detectors (Garnsworthy et al., 2019). Each method captures a specific aspect of CH_4 emissions and varies in cost, behavioral impact, repeatability, and other factors, as shown in Table 2.1. Notably, only respiration chambers measure total CH_4 emissions, while all other methods focus exclusively on oral emissions (Garnsworthy et al., 2019).

Table 2.1Summary of the main features of methods for measuring methane output by individual animals.

Method	Purchase Cost[2]	Running cost[2]	Labor[2]	Repeatability	Behavior Alteration[3]	Throughput

Respiration Chamber	High	High	High	High	High	Low	SF_6	Medium	High	High	Medium	Medium	Medium
Breath sampling	Low [4]	Low	Low	Medium	None	High						
GreenFeed	Medium	Medium	Low	Medium	Low	Medium	Laser methane detector	Low	Low	High	Low	Low-Medium	Medium

[1]Consensus views based on experiences of methanagene WG2 members. [2]Per measuring unit group of animals. [3] Compared to no methane recording: low = measuring in situ; medium = some handling, training or change in routine; high = confinement. [4] Medium if using FTIR analyzer. Adapted from Garnsworthy et al., 2019.

In respiration chambers, cattle are confined for 2 to 7 days while CH_4 concentrations are measured at the air inlet and outlet vents (Garnsworthy et al., 2019). One of the primary challenges with this method is ensuring stable airflow, which represents the most significant source of measurement uncertainty (Garnsworthy et al., 2019). Additionally, the cost of operating respiration chambers is significant, and only one animal can be measured at a time (Garnsworthy et al., 2019). This method is not representative of grazing conditions, and the practical maximum number of animals that can be measured per year is limited to 30–50 (Garnsworthy et al., 2019).

The SF_6 tracer technique was developed to measure CH_4 emissions without the need to confine animals (Johnson et al., 1994). In this method, air samples are collected near the animal's nostrils using a tube attached to a halter, which is connected to an evacuated canister worn by the animal on its neck or back (Garnsworthy et al., 2019). The airflow into the canister is restricted so that it fills to 50–70% capacity over a 24-hour period (Garnsworthy et al., 2019). A permeation tube containing SF_6 is placed in the rumen of each animal, releasing the gas at a predetermined rate, and CH_4 emissions are calculated by multiplying this release rate by the ratio of CH_4 to SF_6 concentrations in the canister (Garnsworthy et al., 2019). The most significant source of variation in this method is the accuracy of determining the SF_6 release rate from the permeation tube (Garnsworthy et al., 2019). Another potential source of error, which has yet to

be thoroughly evaluated, is the impact of animal interactions and shared CH_4 emissions (Garnsworthy et al., 2019). While the SF_6 tracer technique produces results that generally align with respiration chamber measurements, it has greater variability. The cost of canisters and labor is high, and the method requires 5–7 days of data collection (Garnsworthy et al., 2019). Measuring fewer than 15 animals per pen is recommended, with a maximum capacity of approximately 750 animals per year (Garnsworthy et al., 2019).

Breath sampling, often referred to as "sniffer methods," uses devices initially designed to detect gas leaks (Garnsworthy et al., 2019). In this approach, air is collected near the nostrils of the animal through a feed bin connected to a gas analyzer, with sampling sessions typically lasting 3–10 minutes (Garnsworthy et al., 2019). Some systems also measure CO_2 levels, which are then used as a tracer to calculate CH_4 emissions (Garnsworthy et al., 2019). These methods have shown good repeatability and offer several advantages, such as being non-invasive and allowing animals to remain unaware of the equipment and continue their normal activities (Garnsworthy et al., 2019). Additionally, no changes to diet, handling, or training are required, and the method is relatively inexpensive, with minimal operating costs (Garnsworthy et al., 2019). However, there are challenges and compromises associated with breath sampling. The concentration of measured gases can be influenced by the animal's head position relative to the sampling tube, though this issue can be mitigated using head position sensors and data-filtering algorithms (Garnsworthy et al., 2019; Huhtanen et al., 2015). Variability in measurements can also arise from factors such as wind, barn airflow, head position, and between-animal differences, all increasing error rates (Garnsworthy et al., 2019). While using CO_2 as a tracer has addressed some of these issues, it does not eliminate them entirely (Garnsworthy et al., 2019). Spot sampling introduces additional variability due to differences in the time of day each animal is measured. However, this can be resolved by fitting a model that accounts for all animals and their

sampling times (Lassen et al., 2012). To obtain reliable data, trials are typically conducted over 7–10 days, with an annual capacity predicted to measure 2,000–3,000 animals (Garnsworthy et al., 2019; Pszczola et al., 2017). Despite its limitations, breath sampling remains a practical and cost-effective method for measuring CH_4 emissions.

GreenFeeds, produced by C-Lock Inc. in Rapid City, SD, USA, are advanced sniffer systems designed to collect animal breath samples during brief visits to a bait station (Huhtanen et al., 2015). These systems allow multiple daily measurements, with each visit lasting approximately 5–7 minutes (Garnsworthy et al., 2019). GreenFeed is a portable, stand-alone unit that can operate in both barn and pasture environments. It features an extractor fan to maintain active airflow and utilizes head position sensors to ensure accurate sampling (Hammond et al., 2016). Data is preprocessed by C-Lock and made available in real-time through their online platform (Hammond et al., 2015). The system captures exhaled air, measures airflow, and can be calibrated using CO_2 (Garnsworthy et al., 2019). Methane emissions are measured as a flux during each visit, and continuous monitoring enables CH_4 output to be estimated directly in grams per day (Garnsworthy et al., 2012; Huhtanen et al., 2015). However, some limitations exist. Animals may require training to use the system effectively; while some animals visit frequently, others may visit only once (Garnsworthy et al., 2019). Dietary changes can also influence visit frequency (Hammond et al., 2016). According to the manufacturer, each GreenFeed unit is recommended for use with 15–25 animals, and it is possible to measure up to 750 animals per year per unit, depending on trial duration (Garnsworthy et al., 2019).

The Laser Methane Detector (LMD) is a highly responsive, handheld device designed to measure CH_4 column density along the length of a laser beam directed at an animal's nostrils (Garnsworthy et al., 2019). It detects CH_4 emissions with each exhalation (Chagunda et al., 2009). For optimal accuracy, animals are typically restrained during measurements (Garnsworthy

et al., 2019). The operator must stand 1–3 meters away and maintain continuous alignment of the laser with the nostrils (Garnsworthy et al., 2019). While the LMD can be used in an animal's typical environment, restraining the animal ensures greater consistency in measurements (Garnsworthy et al., 2019). Several factors influence the accuracy of LMD readings, including the distance from the animal, laser pointing angle, head orientation and movement, air temperature, and flow, the presence of other animals, and operator variability—the latter being the most significant source of variation (Garnsworthy et al., 2019; Sorg et al., 2017). If the operator maintains consistency and avoids fatigue, up to 10 animals can be measured per hour, three times a day, over three consecutive days, allowing for the measurement of approximately 1,000 animals annually (Garnsworthy et al., 2019; Sorg et al., 2018). To minimize variation, it is recommended that the same operator conduct all measurements (Garnsworthy et al., 2019).

Further research is needed to compare methods and assess systematic differences in CH_4 emission measurements. Methane emissions in cattle fluctuate throughout the day and over time, with respiration chambers recognized as the gold standard for accuracy (Garnsworthy et al., 2019). While concentration-based methods are less precise and accurate than flux-based methods, they offer a practical solution for large-scale measurements, a crucial requirement for genetic evaluations (Garnsworthy et al., 2019). Over the past two decades, CH_4 measurement technologies have advanced significantly, enabling routine measurements of individual animals (Arthur et al., 2018). However, sniffer methods, which measure CH_4 and CO_2 in exhaled air, may not reliably rank animals due to factors like head position, contributing to higher variability among cows (Huhtanen et al., 2015).

SECTION 5: METHODS TO ESTIMATE THE EFFICIENCY OF NITROGEN UTILIZATION IN CATTLE

In research, as in any field, cost-effective technologies and mitigation strategies are crucial, particularly for reducing GHG emissions. Nitrogen utilization in cattle can be assessed through various methods, with milk urea N (MUN) serving as a reliable indicator. Milk urea N is directly proportional to BUN, which, in turn, correlates with urine N (UN) levels (Eggleston et al., 2006; Broderick, 2003). These N levels are typically measured by analyzing milk, blood, or urine samples collected from cattle. However, no universally accepted gold standard exists for measuring N concentration, as few studies have compared different methods. Unlike CH_4 measurement tools, N measurement is less influenced by environmental factors such as wind or weather. Instead, its accuracy primarily depends on the lab technician's consistency and the equipment used. While the literature does not necessarily have a research gap, the limited number of studies makes it difficult to draw concrete conclusions or compare methods. Additionally, since cattle obtain N primarily from dietary protein, diet plays a key role in the accuracy and relevance of N measurement techniques. As shown in Figure 2.4, N intake is directly related to N excretion in feces, urine, and milk.

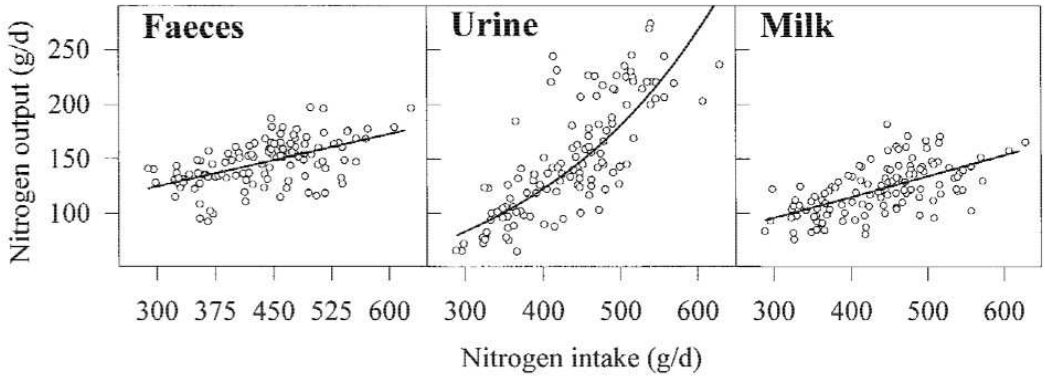

Figure 2.4 Relationships between N intake and the proportion of subsequent output in faeces, urine, or milk. Adapted from Kebreab et al., 2001.

Ammonia not utilized by rumen bacteria is absorbed into the bloodstream and detoxified in the liver into urea, which is then primarily excreted through the kidneys in urine (Broderick & Clayton, 1997). Because urea diffuses quickly in and out of blood cells, the term "plasma urea N" is often used interchangeably with BUN, and both are considered equivalent in the scientific literature (Broderick & Clayton, 1997). While most blood urea is excreted in urine, some is recycled into the rumen or gastrointestinal tract. Blood urea N levels fluctuate throughout the day, typically peaking 4 to 6 hours after feeding (Lavery & Ferris, 2021). Sampling time can affect BUN readings, and this pattern is further influenced by dietary crude protein (CP) content (Lavery & Ferris, 2021). Higher CP levels may increase urea clearance from the blood (Lavery & Ferris, 2021). Additionally, BUN is sensitive to water intake (Burgos et al., 2001), as lower water consumption reduces urine production. Seasonal changes can also influence BUN levels (Meyer et al., 2004). This may be caused by temperature stress, whether from heat or cold.

Milk urea N is widely used as an indirect measure of N utilization in dairy cows (Hof et al., 1997). Unlike BUN, MUN can be collected non-invasively, making it a convenient tool for monitoring N metabolism. Numerous studies (Spek et al., 2013; Gulinski et al., 2016) have extensively reviewed the factors influencing MUN concentrations and their relationship with urinary urea excretion.

Several other methods have been explored to predict N use efficiency (NUE) in cattle, each offering unique advantages. For instance, mid-infrared spectroscopy (MIRS) of milk has been used to predict NUE in early lactation cows (Grelet et al., 2020). Near-infrared spectroscopy (NIRS) has been employed to analyze N fractions in dairy cow feces (Althaus et al., 2013). However, accurately predicting microbial and endogenous N remains challenging due to their significant contribution to fecal N (Mason, 1969). Despite this limitation, fecal analysis through NIRS could still be valuable when dietary CP is lower, as fecal N represents a larger

portion of total N excretion (Dijkstra et al., 2013). Nuclear magnetic resonance spectroscopy of urine is another promising tool, capable of detecting various metabolites and distinguishing between beef production systems based on urine metabolite profiles (Lavery & Ferris, 2021). Bertram et al. (2011) found a strong correlation between urinary metabolite profiles, CP intake, and NUE in dairy cows. Additionally, while research on breath ammonia (BA) in ruminants remains limited, studies in humans show a strong correlation between BA and BUN levels, suggesting BA could serve as a non-invasive measure of N metabolism in ruminants (Lavery & Ferris, 2021). Although still in its early stages, future advancements in precision livestock farming may enable BA sensors to be integrated into automated systems, such as milk or concentrate feeders (Neri et al., 2012).

While BUN remains a strong predictor of NUE, its invasive nature makes it inefficient for widespread on-farm use. Milk urea N provides a non-invasive alternative and is widely used to measure NUE in dairy cows. However, MUN has limitations when applied to individual cows. Direct NUE prediction using MIRS holds the potential for more precise individual assessments, but further research is needed. Currently, MUN remains the most practical and widely used measure of NUE in dairy cows (Lavery & Ferris, 2021). However, it is less useful for beef cattle, as they are not routinely milked, making this technique impractical.

SECTION 6: METHODS TO MITIGATE METHANE EMISSIONS IN CATTLE

Methane mitigation strategies in livestock can be grouped into three key areas: animal breeding (i.e. genetic improvement), nutrition, and microbiome manipulation. For widespread adoption by farmers, these strategies must be cost-effective. Enteric MP represents an environmental challenge and a loss of valuable feed energy (Guan et al., 2006). As a result, significant efforts are focused on developing methods to reduce CH_4 emissions by targeting ruminal microorganisms (Guan et al., 2006).

Although animal breeding is not a widely researched approach to inhibiting methanogenesis, it is a potential strategy. Heritability estimates for CH_4 emissions are moderate, ranging from 0.29 to 0.40 and 0.13 to 0.19 when is included in the model (Pickering et al., 2015). However, selecting for lower MP quickly becomes challenging, because of its observed correlations between CH_4 emissions and production traits. Another limitation is incorporating CH_4 emissions into selection indexes, which are typically designed for sire selection and prioritize traits based on their economic value (Beauchemin et al., 2020). Due to the low economic value of CH_4 mitigation, this trait would carry minimal weight in a multi-trait index (Beauchemin et al., 2020) at least in the current economic situation. Additionally, selecting animals with low CH_4 emissions could inadvertently reduce feed efficiency (Løvendahl et al., 2018). A study on sheep showed that low-emitting sheep have lower feed digestibility compared to high-emitting sheep (Pinares-Patino et al., 2011), which could be indicative of the relationship in cattle.

An alternative approach involves selecting for lower emissions by identifying animals with improved feed conversion efficiency (Kenny et al., 2018). This could be achieved using residual feed intake (RFI), which is moderately heritable (0.26 to 0.43) and moderately repeatable across diets (0.33 to 0.67) (Basarab et al., 2013). However, challenges include animal re-ranking due to diet changes and the need for accurate dry matter intake (DMI) measurements, which is particularly difficult for grazing animals (Kenny et al., 2018). Including RFI in a selection index has the potential to reduce feed consumption for meat and milk production, thereby lowering absolute CH_4 emissions (Beauchemin et al., 2020). Beauchemin et al. (2020) emphasized the need for additional research on range diets to confirm these findings and ensure that no genotype-by-environment interactions occur.

Unlike animal breeding, nutrition is a strategy for inhibiting methanogenesis that has been deeply studied and reviewed. The different aspects range from feed manipulation to feed additives, which have been utilized since ancient times for various purposes, and starting in the 1950s, were applied to mitigate enteric CH_4 emissions (Tseten et al., 2022). Dietary changes are said to be capable of reducing ruminant CH_4 emissions by up to 70%, depending on the method and environmental conditions (Benchaar et al., 2001; Mosier et al., 1998). The most straightforward approach is to modify the concentration, quality, or ratio of forage (Tseten et al., 2022). This could involve feeding younger plants with lower neutral detergent fiber content, resulting in higher digestibility and faster passage rates, which can shift rumen fermentation towards propionate production (Hills et al., 2015). Propionate acts as an alternative H_2 sink, reducing the availability of H_2 for methanogens (Beauchemin et al., 2009). The thought process holds up in theory, but its application becomes challenging, as most animals do not exclusively consume forage unless they are on pasture. Even then, they are often supplemented in some way.

Concentrate-based diets generally result in lower CH_4 yield (g/kg DMI) compared to forage-based diets because of the fermentation of starch (Johnson & Johnson, 1995). This leads to the production of more propionate and butyrate, thereby competing with methanogenesis for H_2 (Beauchemin et al., 2020). Starch is fermented and digested more quickly than cellulose, leading to higher H_2 production; however, high starch intake also lowers ruminal pH, inhibiting methanogen growth (Beauchemin et al., 2020). Conversely, this could reduce fiber digestibility and increase the risk of acidosis (Beauchemin et al., 2020). Additionally, grain-based diets overlook the vital role ruminants play in converting fibrous feeds, which humans cannot usually consume, into high-quality protein sources (Beauchemin et al., 2020).

Lipid supplementation is another approach to CH_4 reduction through low-level additives, which has been shown to decrease CH_4 production while increasing the energy density of diets

(Beauchemin et al., 2020). Lipids reduce methanogenesis by replacing rumen-fermentable organic matter in the diet, lowering the populations of ruminal methanogens and protozoa, and through the biohydrogenation of unsaturated fatty acids (Patra, 2013). Biohydrogenation can act as an alternative H_2 sink in the rumen, competing with methanogenesis, though its contribution is relatively small, accounting for only 1% to 2% of H_2 used in this process (Nagaraja et al., 1997). This effect may become more significant when methanogenesis is suppressed; however, lipid supplementation tends to be expensive and can have several drawbacks, such as reducing fiber digestibility and DMI, inhibiting rumen fermentation, lowering milk fat synthesis, and altering the fatty acid profile of products (Grainger & Beauchemin, 2011; Patra, 2013).

There is a diverse range of feed additives with varying mechanisms for reducing CH_4 emissions. Tannins, for instance, have demonstrated the ability to lower MP; however, they may also reduce fiber digestion, an undesirable outcome for producers (Angelova et al., 2023). Virgin coconut oil, used as a dietary lipid, has shown a potential to decrease CH_4 emissions by inhibiting the growth of methanogenic bacteria (Angelova et al., 2023). Ionophores, approved by the FDA in the 1970s (Russell & Strobel, 1989), are feed additives that enhance animal metabolism by improving efficiency and ruminal N utilization while also reducing the risk of bloat and acidosis (Richardson et al., 1976; Bergen & Bates, 1984). Commercially available ionophores are already widely used throughout the industry (Tseten et al., 2022). Continued research and innovation are essential to optimize these additives for CH_4 mitigation while minimizing negative trade-offs, ensuring they remain practical and beneficial for producers.

Modifying the microbiome early in an animal's life is a promising strategy to support rumen development and boost metabolic efficiency in livestock. This concept, known as *early life programming*, involves influencing an animal’s growth and physiology before it reaches full

maturity (Beauchemin et al., 2020). The goal is to create lasting changes that carry into adulthood, setting the animal apart from those not exposed to such interventions. One potential method involves altering H_2 sinks to support better rumen development (Beauchemin et al., 2020). Research on small ruminants has shown encouraging results. For instance, treating newborn goats and their mothers with the methanogenesis inhibitor bromochloromethane led to lower CH_4 emissions and higher levels of rumen propionate in the young goats, even three months after the treatment had ended (Abecia et al., 2013). These findings suggest that treating the mother can help shape the development of the offspring's rumen microbiota (Abecia et al., 2018). However, not all interventions show lasting effects. For example, giving newborn lambs anti-methanogenic substances like garlic, essential oil, and linseed oil did not result in long-term reductions in CH_4 production (Króliczewska et al., 2023). These mixed results highlight that while early-life treatments have potential, their effectiveness can vary depending on the approach used (Beauchemin et al., 2020).

Chemical inhibitors for methanogenesis have long been shown to be effective (Liu et al., 2010). Coenzyme M, a cofactor found exclusively in methanogens and absent in other bacteria or archaea (Liu & Whitman, 2008), is a commonly used methanogenic inhibitor due to its efficiency at relatively low concentrations (Liu et al., 2010). Hydroxymethylglutaryl-CoA reductase, on the other hand, inhibits the synthesis of mevalonate, a critical compound required by methanogens for isoprenoid synthesis, thereby preventing their growth (Liu et al., 2010). It also does not affect other ruminal bacteria since they are eubacteria (Liu et al., 2010). Mediumchain fatty acids demonstrate strong potential for suppressing methanogenesis (Liu et al., 2010). Long-chain fatty acids are believed to inhibit the growth of gram-positive and methanogenic bacteria by penetrating and disrupting their cell membranes (Galbraith & Miller, 1973; Soliva et al., 2003).

Nitro compounds could also serve as alternative electron acceptors by redirecting electron flow from the reduction of CO_2 (Božic et al., 2009; Conrad et al., 2000).

Efforts to reduce CH_4 emissions span across animal breeding, nutritional strategies, and microbiome manipulations, each offering unique advantages and challenges. While breeding for low CH_4 emissions is promising, it faces trade-offs with production efficiency and economic feasibility. Nutritional strategies, including dietary changes, lipid supplementation, and feed additives, offer immediate and cost-effective solutions but may have unintended consequences like reduced fiber digestibility or increased costs. Meanwhile, microbiome manipulation and chemical inhibitors provide innovative approaches, though further research is needed to ensure their practicality in diverse production systems.

SECTION 7: METHODS TO MITIGATE NITROGEN IN CATTLE

Livestock production is a significant source of N-based emissions, particularly N_2O and ammonia. The release of N_2O within livestock systems is primarily influenced by N transfer efficiency among animals, manure, soil, and crops (Singh et al., 2010). In ruminant production, approximately one-quarter of manure N is transformed into NH_3 and released into the atmosphere (Pinder et al., 2004). The efficiency of N utilization in ruminants is closely tied to the composition of the diet and the structure of microbial populations in the rumen, making it a key area for intervention to reduce N losses (Tan et al., 2021).

Rumen microorganisms play a fundamental role in N metabolism by regulating nutrient utilization and emissions (Tan et al., 2021). Dietary protein is degraded into peptides and AA, which are either incorporated into microbial protein or deaminated into NH_3 (Firkins et al.,

2007). When dietary protein intake surpasses microbial requirements, excess N is converted into NH_3 and ultimately excreted (Parker et al., 1995). This microbial process directly impacts N efficiency, influencing both productivity and environmental N loss (Tan et al., 2021).

Unlike monogastric animals, ruminants do not regulate nutrient intake based on real-time physiological needs but instead rely on hunger and appetite cues (Tan et al., 2021). Therefore, diet formulation and feed management play a critical role in optimizing N utilization. Adjusting feed composition, incorporating active dietary substances, and employing processing treatments can enhance N efficiency while minimizing environmental N losses (Tan et al., 2021). However, rapid improvements in N metabolism at the feed level can sometimes lead to increased N_2O and NH_3 emissions, contributing to environmental pollution (Tan et al., 2021). Inefficient N utilization, particularly of NH_3 or urea, remains a primary concern in ruminant production systems.

Several nutritional strategies have been explored to mitigate N emissions while enhancing N retention (Tan et al., 2021). Plant-based compounds and extracts have shown promising effects in improving N metabolism by reducing emissions, increasing retention, or both (Tan et al., 2021). Additionally, essential oils, such as clove oil and origanum oil, have been found to reduce the abundance of ammoniagenic and proteolytic bacteria, thereby lowering NH_3 production (Patra and Yu, 2014). Tannins, which form complexes with proteins, can increase ruminal pH, promote the growth of N metabolism-related microorganisms, and enhance N retention, making them another valuable tool for N management (Tan et al., 2021). In addition to direct dietary interventions, manure management practices also play a significant role in reducing N emissions. Adjusting and optimizing CP levels in feed is a widely recognized method for lowering NH_3 emissions from ruminants (Tan et al., 2021). By tailoring N intake to meet but not exceed animal

requirements, excess N excretion can be minimized, leading to improved efficiency and reduced environmental impact (Tan et al., 2021).

Early life programming poses another strategy. The rumen microbial community undergoes significant shifts during the pre-ruminant period, characterized by rapid changes in microbial species, unstable community structure, and the gradual establishment of N metabolism (Tan et al., 2021). Understanding these biological dynamics presents an opportunity to manipulate microbial populations in early life stages to enhance N retention and reduce N emissions (Tan et al., 2021). Maintaining protein-producing bacteria and hydrolytic ammoniaproducing bacteria at controlled levels while efficiently utilizing unabsorbed N can potentially improve N metabolism (Tan et al., 2021).

Furthermore, ecological strategies, such as alternative forage planting, soil management, and strategic seasonal grazing, have demonstrated positive effects in mitigating N losses and alleviating environmental pollution (Tan et al., 2021). As ruminant production systems expand, balancing livestock productivity with environmental sustainability is essential. The development of resource-efficient, environmentally friendly production methods has become a key objective for the long-term sustainability of ruminant production.

Before a trait can be incorporated into a breeding program, it should meet three key criteria: (1) it must have economic, social, or environmental importance; (2) it must be under genetic control, meaning it has sufficient heritability; and (3) it must be easily measurable at a reasonable cost or have strong genetic correlations with other measurable traits (Berry, 2013). While N excretion is undoubtedly environmentally important, its direct measurement remains challenging, and our understanding of its genetic correlations with economically relevant traits is still limited, as discussed below. However, with further research into its heritability and genetic relationships, N excretion could become a viable selection criterion in breeding programs.

Identifying genetic markers or correlated traits that serve as reliable indicators of N efficiency would enable producers to select cattle that minimize environmental impact while maintaining productivity. By integrating N excretion into genetic selection strategies, the beef industry could take a proactive approach to improving sustainability without compromising production efficiency.

Reducing N excretion in ruminant production systems is essential for improving environmental sustainability while maintaining economic viability. Current strategies to mitigate N emissions primarily focus on nutritional interventions, manure management, and ecological practices. While these approaches have shown promise in improving N efficiency, they often require continuous management adjustments. Ultimately, a combination of improved management practices and genetic advancements will be necessary to develop a more resourceefficient and environmentally sustainable livestock industry. Producers can optimize N utilization by prioritizing short-term mitigation strategies and long-term genetic improvements while ensuring the continued success of cattle production systems.

SECTION 8: RELATIONSHIPS BETWEEN METHANE PRODUCTION AND ECONOMICALLY IMPORTANT TRAITS IN BEEF CATTLE

Genetic manipulation offers a promising strategy for mitigating MP in cattle. By implementing carefully designed breeding plans, it may be possible to produce animals that are naturally low CH_4 emitters. However, traits in livestock are interconnected, meaning that altering one trait can influence others, potentially impacting producer profitability. In beef cattle production, traits economically relevant to MP include growth, feed efficiency, and carcass characteristics (Lakamp et al., 2022).

Dry matter intake and CH_4 production share a strong positive relationship (See Table 2.2 and 2.3 for correlations). As an animal consumes more feed, its gut microbes have more substrate

to break down, resulting in increased H_2 production. This H_2 is reduced by methanogens, leading to higher CH_4 emissions. Diet composition also plays a significant role; diets high in roughage have been shown to increase MP compared to diets primarily consisting of concentrates (Johnson & Johnson, 1995). The first study examining the relationship between MP and DMI, conducted in 1930 by Kriss, reported extremely high correlations of 0.963 (±0.006) and 0.942 (±0.01). However, these results may have been overestimated due to the lack of mixed-model analysis, which was not yet available, and the combination of data from steers of varying weights and dry dairy cows. While phenotypic correlations provide valuable insights into observable traits, they do not reveal the genetic relationships between traits. Methane production and DMI are correlated both phenotypically (0.48) (Herd et al., 2016) and genetically (0.83) (Donoghue et al., 2016), although the strength of these correlations varies depending on breed, diet, and measurement methods. Despite the fairly robust relationship between these traits, there is a notable gap in the literature regarding CH_4 measurements in pasture-based systems (Lakamp et al., 2022). To date, only two studies have measured CH_4 production on pasture, largely due to the challenges associated with accurately assessing DMI in such environments.
Given that most cow-calf operations globally rely on rangelands, further research in this area is urgently needed (Lakamp et al., 2022).

Methane production and DMI exhibit a moderate to strong phenotypic and genotypic correlation, as depicted in Table 2.2 and 2.3. Additionally, DMI is closely linked to body weight (BW), a key factor in beef cattle production. Body weights are typically recorded at birth, weaning, yearling, and as final live weight, and these measurements have been incorporated into multi-trait analyses with MP to assess potential genetic antagonisms as shown in table 2.3 (Lakamp et al., 2022). Live weight is strongly correlated phenotypically with MP, which is understandable from a logistical perspective—larger animals consume more feed, resulting in

increased CH_4 emissions. To address this, selecting feed-efficient animals offers a pathway to maintaining desired weight gains while reducing CH_4 production.

Average daily gain (ADG) measures the daily increase in an animal's BW over a specific period of time (Lakamp et al., 2022). In beef production, animals are typically fed to achieve a target weight, and those that reach this goal more quickly can enhance profitability. Average daily gain and feed efficiency are key factors influencing the cost of gain and time on feed making their measurement crucial for the cattle industry. As seen in Table 2.2, research indicates that MP is positively correlated with growth and gain at the phenotypic level. Interestingly, MP also appears to be associated with feed efficiency, suggesting that more efficient animals may produce higher levels of CH_4 (Lakamp et al., 2022). This complex relationship highlights the need for strategies that balance growth performance and environmental sustainability.

Residual feed intake is another commonly used measure of feed efficiency, frequently studied in relation to MP. Residual feed intake represents the difference between an animal's observed feed intake and its expected feed requirements based on weight and body composition (Lakamp et al., 2022). Research to date has found significant correlations between RFI and MP primarily in animals fed forage-based diets. However, the literature remains inconclusive on the nature of the relationship between RFI and MP, with studies reporting positive, negative, or no phenotypic correlations. This variability underscores the need for further investigation to clarify the connection and its implications for sustainable cattle production.

Body composition traits, including body fat and ribeye area, are critical factors in determining yield and quality grades. These traits are key to meeting consumer expectations and are especially relevant in grid-based marketing systems (Lakamp et al., 2022). Research suggests that body composition traits have a low to moderate phenotypic correlation with MP (Table 2.2).

However, studies on this relationship remain scarce, with only two published works, both largely based on the same animal population (Herd et al., 2014; Donoghue et al., 2016).

Some genetic antagonisms between MP and economically important traits suggest that directly selecting for reduced MP could negatively affect traits like live weight and marbling. The majority of correlations reported in the literature are phenotypic, which, while informative, have limitations from the perspective of genetic improvements. Expanding phenotypic data collection and conducting large-scale genetic analyses are essential to generate more robust genetic correlations (Lakamp et al., 2022). These genetic correlations are crucial for understanding the broader impacts of selection on novel traits.

Several approaches to genetically reducing MP are possible. While a selection index focused on a purely terminal system could theoretically include MP, greater progress in mitigating enteric CH_4 could be achieved by designing a CH_4 production index tailored for maternal or general-purpose systems (Lakamp et al., 2022). This approach would be especially impactful if phenotypes were collected in pasture-based systems. Targeting CH_4 mitigation efforts in the cow-calf sector, which houses the majority of cattle and accounts for the largest share of GHG emissions, would yield the most significant environmental benefits (Lakamp et al., 2022).

Table 2.2 Phenotypic correlations of economically important traits with methane production (MP), methane yield (MY), and methane intensity (MI) in beef cattle.

Trait	Type	Average Correlation	Minimum	Maximum

Dry Matter Intake (DMI)	MI [1,5,6]	-0.01	-0.21	0.26
	MP [1,2,3,4,5,6,7,8,9]	0.54	0.38	0.71
	MY [1,2,4,5,6]	-0.23	-0.78	-0.01
Birth Weight	MP [2,4]	0.22	0.19	0.26
	MY [2,4]	-0.02	-0.03	-0.01
Weaning Weight	MP [2,4]	0.51	0.50	0.53
	MY [2,4]	0.04	0.03	0.06
Yearling Weight	MP [2,4]	0.59	0.57	0.61
	MY [2,4]	0.1	0.09	0.11
Finished Weight	MP [2,4]	0.52	0.49	0.56
	MY [2,4]	0.08	0.07	0.10
Live Weight	MI [6]	-0.33	0.67	0.69
	MP [6,9]	0.68	0.04	0.04
	MY [6]	0.04	-0.33	-0.33
Average Daily Gain	MI [5]	-0.69	-0.69	-0.69
	MP [5,9,10]	0.29	0.19	0.36
	MY [5]	0.24	0.24	0.24
Rib Fat	MP [2,5]	0.11	0.10	0.13
	MY [2,5]	-0.01	-0.04	0.02
Rump Fat	MP [2,5]	0.15	0.13	0.17
	MY [2,5]	0.03	0.01	0.06
Ribeye Area	MP [2]	0.28	0.28	0.28
	MY [2]	-0.01	-0.01	-0.01
Intramuscular Fat	MP [2,5]	0.22	0.15	0.29
	MY [2,5]	0.01	0.01	0.01

[1]Bird-Gardiner et al., 2017, [2]Donoghue et al., 2016, [3]Fitzsimons et al., 2013, [4]Herd et al., 2014, [5]Herd et al., 2016, [6]Manzanilla-Pech et al., 2016, [7]McDonnel et al., 2016, [8]Nkrumah et al., 2006, [9]Renand et al., 2019, [10]Velazco et al., 2016

Table 2.3 Genetic correlations of economically important traits with methane production (MP), methane yield (MY), and methane intensity (MI) in beef cattle.

Trait	Type	Correlation
Dry Matter Intake (DMI)	MI [2]	-0.34
	MP [1,2]	0.83
	MY [1,2]	0.02
Birth Weight	MP [1]	0.36
	MY [1]	-0.01
Weaning Weight	MP [1]	0.84
	MY [1]	0.27
Yearling Weight	MP [1]	0.32
	MY [1]	-0.21
Finished Weight	MP [1]	0.86
	MY [1]	0.21
Live Weight	MP [2]	0.8
	MY [2]	0.05
	MI [2]	-0.44
Rib Fat	MP [1]	0.11
	MY [1]	-0.14
Rump Fat	MP [1]	0.1
	MY [1]	-0.14
Ribeye Area	MP [1]	0.4
	MY [1]	-0.09
Intramuscular Fat	MP [1]	0.36
	MY [1]	0.1

[1]Donoghue et al., 2016, [2] Manzanilla-Pech et al., 2016

SECTION 9: RELATIONSHIPS BETWEEN NITROGEN PRODUCTION AND ECONOMICALLY IMPORTANT TRAITS

In livestock production, N utilization plays a crucial role in both environmental sustainability and economic efficiency. While extensive research has examined N metabolism, little to no studies have explored how N excretion and utilization correlate with economically important traits in beef cattle. Understanding these relationships could help refine management

strategies, improve predictive models for N emissions, and enhance efficiency in beef production systems (Waldrip et al., 2013). However, due to the limited availability of literature on beef cattle, this review also includes data from dairy cattle and pigs as reference points. While dairy cattle share similar physiological mechanisms, pig data provide additional insight into how N efficiency may relate to performance traits. Pigs are not the primary focus, but they serve as a comparative resource where relevant cattle data is unavailable. A summary of all phenotypic correlations discussed is presented in Table 2.5 below.

Table 2.4 Several urea phenotypes with their definitions.

Trait	Definition	Source
Milk Urea (MU)	Total urea concentration in milk (e.g., mg/dL)	Ishler, 2023
Milk Urea Nitrogen (MUN)	Nitrogen content of MU (e.g., mg/dL)	Ishler, 2023
Milk Urea Yield (MUY)	MU concentration multiplied by average daily milk yield (e.g., g/day)	Villalobos et al., 2018
Blood Urea Nitrogen (BUN)	Urea Nitrogen concentration in blood (e.g., mmol/L)	Lavery & Ferris, 2021

Research suggests that N excretion in beef cattle is more strongly related to N intake than to live weight or DMI (Waldrip et al., 2013). Weighted correlation analysis indicates that urinary and fecal N excretion and the partitioning of N between these two routes are highly dependent on N intake (Waldrip et al., 2013) (Table 2.5). While BW and DMI are also correlated with N excretion, fecal N excretion appears to be slightly more influenced by BW and DMI than UN excretion (Waldrip et al., 2013). Since BW and DMI are key factors in beef production efficiency, understanding their genetic correlations with N excretion could aid in the selection of more efficient cattle, potentially improving feed efficiency and reducing environmental impact through genetic selection (Table 2.5).

In dairy cattle, correlations between N-related traits and performance indicators vary.

Studies have shown a low correlation between milk urea (MU) and milk urea yield (MUY) with BW (Villalobos et al., 2018), suggesting that BW has little influence on N excretion in lactating cows (Table 2.5). Similarly, MU and DMI also show a low correlation, whereas MUY and DMI exhibit a stronger relationship (Villalobos et al., 2018). These findings indicate that N efficiency in dairy cattle is more closely tied to feed intake than to BW (Table 2.5).

Although pigs differ physiologically from ruminants, their N utilization data can provide additional context for interpreting trends in cattle. A negative phenotypic correlation between N utilization (NU) per unit of ADG and NU per unit of DMI suggests that pigs with lower N efficiency also exhibit reduced growth performance (Schmid et al., 2024). Additionally, BUN and DMI show a low correlation, similar to the weak relationship observed between MU and DMI in dairy cattle (Schmid et al., 2024). Blood urea N and ADG also exhibit a low correlation, reinforcing the idea that N excretion alone is not a strong predictor of growth efficiency in monogastric animals (Schmid et al., 2024) (Table 2.5).

Given the lack of published research on correlations between N excretion and economically important traits in beef cattle, pig data were included to provide a broader perspective. Like pigs, cattle that are inefficient in utilizing N—whether due to reduced N recycling to the rumen, increased UN losses, or other metabolic inefficiencies—may also exhibit lower overall production efficiency. While these studies were not conducted in beef cattle, they highlight the need for further research to better understand how N excretion relates to economically relevant traits in cattle production systems.

Table 2.5 Phenotypic correlations of economically important traits with nitrogen excretion in livestock.

Trait	Animal	Measurement	Correlation (SE)	Citation

Dry Matter Intake	Dairy Cattle	MU[1]	0.16 (0.03)	Villalobos et al., 2018
	Dairy Cattle	MUY[1]	0.55 (0.02)	Villalobos et al., 2018
	Pigs	NUE[1]	-0.17 (0.078)	Schmid et al., 2024
	Pigs	BUN[1]	0.24 (0.079)	Schmid et al., 2024
	Beef Cattle	UNE[1]	0.595	Waldrip et al., 2013
	Beef Cattle	FNE[1]	0.681	Waldrip et al., 2013
Live Weight	Dairy Cattle	MU[1]	0.02 (0.04)	Villalobos et al., 2018
	Dairy Cattle	MUY[1]	0.12 (0.04)	Villalobos et al., 2018
	Beef Cattle	UNE[1]	0.479	Waldrip et al., 2013
	Beef Cattle	FNE[1]	0.645	Waldrip et al., 2013
Average Daily Gain	Pigs	NUE[1]	-0.14 (0.078)	Schmid et al., 2024
	Pigs	BUN[1]	0.27 (0.03)	Schmid et al., 2024

[1]MU= Milk urea concentration, MUY= Milk urea yield, NUE= Nitrogen utilization efficiency, BUN= Blood urea nitrogen, UNE= Urine nitrogen excretion, FNE= Feces nitrogen excretion.

SECTION 10: HERITABILITY ESTIMATES OF METHANE IN CATTLE

Heritability estimates for CH_4-related traits generally fall into four categories: MP, CH_4 intensity (MI), CH_4 yield (MY), and residual CH_4 production. Table 2.6, sourced from de Haas, provides detailed definitions of these categories, while Table 2.7 compiles all heritability estimates from 2012 onward. Accurate heritability estimates are essential for improving the prediction of breeding values for CH_4 emissions (Brito et al., 2018). However, CH_4 emissions are challenging and costly to measure accurately, often leading to a limited number of records per population. Over time, various methods have been developed to assess CH_4 production, contributing to significant variation in heritability estimates (Brito et al., 2018).

Table 2.6 Several methane (CH_4) phenotypes with their definitions, strengths, and weaknesses Adapted from Y de Haas et al., 2016.

Trait	Definition	Strength	Weakness

Methane Production (MP)	Methane production per day (e.g., L/d or g/d).	The clean trait we want to improve.	Highly correlated with feed intake and production level.
Methane Intensity (MI)	Methane production related to output (e.g., per kg milk,	for the user. be difficult to incorporate	The phenotype of interest Ratio trait, so selection can live weight, meat). properly.
Methane Yield (MY)	Methane production related to input (e.g., kg of DMI).	for the user. be difficult to incorporate	The phenotype of interest Ratio trait, so selection can properly.
Residual Methane Production (RMP)	Observed CH_4 production minus predicted CH_4 Production.	Good statistical properties; corrected for traits that influence CH_4 production.	Can be difficult to explain to users.

Heritability estimates for CH_4 traits can be categorized into predicted, direct, and residual measurements. Table 2.7 presents estimates for two of these categories—predicted and direct measurements—including MP, MY, and MI, as defined in Table 2.6. Methane yield and MI are both expressed as ratios of component traits, with MY representing CH_4 output per unit of feed intake or DMI and MI representing CH_4 output per unit of animal product. One advantage of MI and MY is their straightforward calculation and interpretation. However, their strong correlations with the reference traits (as specified in Table 2.6) can be a drawback when selecting for reduced CH_4 emissions (Manzanilla-Pech et al., 2016). Methane intensity and MY have been predicted using various input (e.g.,) or output (e.g., milk fatty acid profile) traits.

In contrast, MP cannot be predicted because it is not dependent on input or output variables; rather, it is a direct phenotypic measurement. Pech et al. (2016) highlighted that selecting for MI in Angus cattle is particularly challenging due to its negative correlation with DMI and BW. As measurement techniques continue to improve, researchers will gain a clearer understanding of CH_4 heritability, enhancing the accuracy of selection for reduced emissions in livestock.

Residual traits have been widely utilized since Koch et al. (1963) introduced RFI and residual daily gain. Residual values represents the difference between actual and predicted values. A key advantage of residual CH_4 (RM), according to Pech et al. (2016), is its zero genetic and phenotypic correlation with its regressors, which in their study were DMI and BW. However, Berry and Crowley (2016) caution that variance in residual traits does not necessarily represent the true trait. This discrepancy can arise due to random noise, measurement, prediction errors, feed losses, or biases in regression coefficients, highlighting the complexity of using RM in selection programs.

Table 2.7 Publication, number of records (N), trait measurement unit (Unit), type of measurement (direct or prediction, and method of measurement), livestock group (Dairy cattle or Beef cattle), trait type (methane production (MP), methane yield (MY), and methane intensity (MI)), heritability estimate with standard error if available.

Citation	N	Unit	Measure	Dairy	Beef	Type	h^2 (SE)
Bittante et al. (2020)	1091	g/kg	Prediction- FAP	✓		MY	0.25

Bittante et al. (2020)	1091	g/d/cow	Prediction- FAP	✓		MI	0.12-0.24
Bittante et al. (2020)	1091	kg/cm	Prediction- FAP	✓		MI	0.13-0.20
Vanrobays et al. (2016)	33555	g/d	Prediction- Milk	✓		MI	0.25 (0.005)
Kandel et al. (2017)	142	g/d	Prediction- Milk	✓		ME	0.12-0.27
Kandel et al. (2017)	142	g/kg	Prediction- Milk	✓		MI	0.17-0.18
Yin et al. (2015)	961	g/d	Prediction- Milk yield	✓		ME	0.15-0.37
Sobrinho et al. (2015)	955	g/d	Prediction- DMI		✓	ME	0.32- 0.47
Van Engelen et al. (2015)	1905	g/kg DMI	Prediction- FAP	✓		MI	0.12-0.44
Visker et al. (2014)	1838	g/kg DMI	Prediction- FAP	✓		MI	0.12 (0.03)
Visker et al. (2014)	1898	g/kg DMI	Prediction- FAP	✓		MI	0.22-0.44
Hayes et al. (2016)	1020	g/d	Direct- RC		✓	MY	0.22 (0.06)
Pech et al. (2016)	1020	g/d	Direct- RC		✓	MP	0.30 (0.6)
Pech et al. (2016)	1020	g/kg DMI	Direct- RC		✓	MY	0.20 (0.05)
Pech et al. (2016)	1020	g/kg weight	Direct- RC		✓	MI	0.25 (0.06)
Pech et al. (2016)	205	g/d	Direct- SF_6	✓		MP	0.23 (0.23)
Pech et al. (2016)	205	g/kg DMI	Direct- SF_6	✓		MY	0.30 (0.23)
Pech et al. (2016)	205	g/kg milk	Direct- SF_6	✓		MI	0.42 (0.23)
Arthur et al. (2016)	1043	g/d	Direct- RC		✓	MP	0.24 (0.06)
Arthur et al. (2016)	1043	g/kg DMI	Direct- RC		✓	MY	0.22 (0.06)
Lassen et al. (2016)	339	g/d	Direct- TIS	✓		MP	0.25 (0.16)
Lassen et al. (2016)	339	g/L milk	Direct- TIS	✓		MI	0.20 (0.16)
Lassen et al. (2016)	339	ppm	Direct- TIS	✓		MY	0.16 (0.15)
Pickering et al. (2015)	274211	g/d	Predicted- DMI	✓		ME	0.13 (0.04)
Pickering et al. (2015)	1308	mg/kg	Direct- LMD	✓		ME	0.05 (0.07)
Lassen et al. (2016)	3121	ppm	Direct- FTI	✓		MY	0.16 (0.04)
Lassen et al. (2016)	1745	g/d	Direct- FTI	✓		MP	0.21 (0.06)
Lassen et al. (2016)	1745	g/L milk	Direct- FTI	✓		MI	0.21 (0.06)
Kandel et al. (2012)	11999	g/d	Prediction- FAP	✓	✓	MI	0.26- 0.46
Kandel et al. (2012)	11999	g/d	Prediction- FAP	✓	✓	MI	0.28-0.39
Kandel et al. (2012)	11999	g/d	Prediction- FAP	✓	✓	MI	0.37-0.66
Kandel et al. (2012)	11999	g/d	Prediction- FAP	✓	✓	MI	0.44-0.63
Kandel et al. (2012)	11999	g/d	Prediction- FAP	✓	✓	MI	0.27-0.46
Kamalanathan et al. (2023)	330	g/d	Direct- Green Feed	✓		MP	0.16 (0.10)
Kamalanathan et al. (2023)	330	g/kg DMI	Direct- Green Feed	✓		MY	0.27 (0.12)

Kamalanathan et al. (2023)	330	g/kg milk	Direct- Green Feed	✓			MI	0.21 (0.14)
Souza et al. (2024)	743	g/d	Direct- SF_6			✓	MP	0.42 (0.09)
Pszczola et al. (2017)	485	g/d	Direct- FTI		✓		MP	0.23-0.30
Sypniewski et al. (2021)	483	g/d	Direct- FTI		✓		MP	0.22
Sypniewski et al. (2021)	483	ppm/d	Direct- FTI		✓		MY	0.085
Pech et al. (2022)	504	g/d	Direct- Infrared CH4 sensor	✓			MP	0.11-0.49
Van Breukelen et al. (2023)	822	g/d	Direct- Green Feed	✓			ME	0.19 (0.02)
Van Breukelen et al. (2023)	822	g/week	Direct- Green Feed	✓			ME	0.33 90.04)
Van Breukelen et al. (2023)	1800	ppm/d	Direct- Sniffer		✓		MY	0.18 (0.01)
Van Breukelen et al. (2023) 1800 ppm/week Direct- Sniffer ✓ MY 0.32 (0.02) Breider et al. (2019) 184 g/d Direct- Infrared CH4 analyzer ✓ MP 0.12-0.45								

FAP= fatty acid profiles, RC = respiration chambers, SF_6 = Sulfur hexafluoride, TIS = Transformed infrared spectra, LMD= laser methane detector, FTI= Fourier transformed infrared,

SECTION 11: HERITABILITY ESTIMATES OF NITROGEN IN CATTLE

Excessive UN in cattle contributes to groundwater contamination, leading to environmental pollution (O'Callaghan et al., 2019). One potential strategy for reducing the environmental impact of cattle is selecting for animals with lower UN excretion. However, because direct measurement of UN is challenging and impractical on a large scale, it is unlikely to be widely available for genomic selection (van den Berg et al., 2021). Instead, BUN and ,

Research has demonstrated a linear phenotypic correlation between BUN, MUN, and UN excretion (Kauffman & St-Pierre, 2001; Kohn et al., 2002, 2005). Additionally, studies indicate that higher feed intake is associated with increased urea levels, whether measured as BUN, MUN, or UN excretion (van den Berg et al., 2021). The heritability of BUN and MUN is estimated to be low to moderate, ranging from 0.07 to 0.32 (Table 2.8), although Wood et al.

(2003) reported higher estimates between 0.44 and 0.59. These heritability estimates suggest that BUN and MUN could potentially be incorporated into genetic selection programs to improve N efficiency in cattle.

Nitrogen excretion has received less attention than CH_4 emissions despite its significant environmental impact. Excess N from livestock production can leach into groundwater, contributing to pollution, and N-based compounds also play a role in GHG emissions. The limited focus on N excretion is evident when comparing the availability of heritability estimates for N-related traits to those for CH_4 emissions. This gap in research highlights the need for further studies to understand better the genetic components of N excretion and its potential for selection in breeding programs. Expanding this knowledge could provide new opportunities to improve N efficiency in cattle while reducing their environmental footprint.

Table 2.8 Publication, number of records (N), unit of measurement, methane trait being measured, heritability estimate with standard error if available.

Citation	N	Unit	Type	h^2 (SE)
Villalobos et al., 2018	468	mg/dL	MU[1]	0.24
Villalobos et al., 2018	468	g/d	MUY[1]	0.19
Buitenhuis & Poulsen, 2023	323,800	mmol/L	MU[1]	0.18-0.24
Peterson et al., 1982	535	mg/dL	BUN[1]	0.17 (0.11)
Mitchell et al., 2005	46,951	mg/dL	MUN[1]	0.09-0.15
Mitchell et al., 2005	26,540	mg/dL	MUN[1]	0.22-0.23
Van Den Berg et al., 2021	18,120	mmol/L	BUN[1]	0.07-0.12
Van Den Berg et al., 2021	18,120	mg/dL	MUN[1]	0.08-0.12
Van Den Berg et al., 2021	18,120	mg/dL	MUN[1]	0.28-0.32
Beatson et al., 2019	133,624	mg/dL	MUN[1]	0.22 (0.01)
Jahnel et al., 2023	261,866	mg/kg	MU[1]	0.24
Jahnel et al., 2023	261,866	g	MUY[1]	0.27
Stoop et al., 2007	1,953	mg/100ml	MU[1]	0.14
Stoop et al., 2007	1,953	g	MUY[1]	0.28
Bahithige et al., 2021	634	mg/dL	MUN[1]	0.22
Wood et al., 2003	6,704	mg/dL	MUN[1]	0.44-0.59

[1]MU= Milk urea, MUY= milk urea yield, BUN= blood urea nitrogen, MUN= Milk urea nitrogen.

SECTION 12: GENETICS AS A SOLUTION

Genetics presents a long-term, sustainable solution for reducing CH_4 emissions and N excretion in cattle. Unlike nutritional or microbiome-based interventions, genetic improvements are cumulative and permanent, making them a highly effective strategy for mitigation (Garnsworthy et al., 2019). While direct CH_4 and N measurements provide valuable insight, predicted values serve as critical tools for estimating emissions when direct measurement is impractical. Understanding the genetic basis of these predicted traits is essential for integrating CH_4 and N reduction strategies into breeding programs.

High N excretion not only represents a loss in efficiency but also poses environmental risks, particularly to groundwater quality. Unlike CH_4 emissions, the traits correlated with N excretion are poorly understood. With further research, we can explore whether direct selection for N efficiency is feasible or if an alternative approach would be more effective. As Lakamp et al. (2022) suggested for CH_4, incorporating N efficiency into an economical selection index may be the most practical strategy for genetic improvement.

There is no universal agreement on which traits should be prioritized in breeding objectives to mitigate CH_4 or N. However, existing research indicates that CH_4 production is influenced by additive genetic variation, suggesting that meaningful genetic gains can be achieved if these traits are included in selection programs (Brito et al., 2018). As research continues to refine heritability estimates and genetic correlations, integrating CH_4 and N traits into breeding indices will be crucial for developing cattle that are both economically efficient and environmentally sustainable.

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CHAPTER 3: GENETIC PARAMETERS FOR BLOOD UREA NITROGEN, METHANE EMISSIONS, AND FEED INTAKE IN HEREFORD BEEF CATTLE

INTRODUCTION

The livestock sector plays a critical role in global food production. Cattle convert forages and by-products that are inedible to humans into high-quality protein (Gillespie & van den Bold, 2017). However, they also contribute to environmental challenges, especially through greenhouse gas (GHG) emissions and nitrogen losses. In the United States, livestock agriculture accounts for approximately 3.8% of total GHG emissions, with enteric methane (CH_4) from ruminants making up nearly 27% of the country's overall CH_4 output (U.S. EPA, 2025). Methane is a potent GHG produced during enteric fermentation that traps heat in the atmosphere and contributes to climate change. In addition to CH_4, nitrogen excretion from cattle presents environmental concerns. Reactive nitrogen compounds, primarily ammonia and nitrate, contribute to water contamination, eutrophication, and atmospheric nitrogen deposition, which can harm sensitive ecosystems through polluted precipitation events (Sutton et al., 2011). Blood urea nitrogen (BUN) is a measurable indicator of protein metabolism that reflects the overall nitrogen status in the animal. Higher BUN levels are associated with greater nitrogen excretion in the urine (Eggleston et al., 2006; Broderick, 2003). Therefore, BUN can be used as a proxy trait to identify and select cattle that emit less nitrogen.

Historically, most mitigation efforts targeting greenhouse gas and nitrogen excretion from cattle have focused on nutritional or management interventions (Beauchemin et al., 2020; Kebreab et al., 2023). While these strategies can be effective in the short term, their impacts often diminish once interventions cease, and consistent implementation can be challenging

across diverse production systems. Genetic selection offers a complementary, long-term approach by exploiting natural variation in traits such as CH_4 production, dry matter intake (DMI), and blood urea nitrogen (BUN). Dry matter intake is included in the study because it influences both CH_4 emissions and nitrogen excretion; the efficiency with which animals convert feed into product—rather than simply how much they eat—is key to reducing environmental waste as well. Understanding the heritability of these traits and their genetic relationships between another is essential for developing sustainable breeding programs aimed at reducing the environmental footprint of beef cattle.

Given the increasing regulatory scrutiny aimed at reducing CH_4 emissions from major sources such as the oil and gas sector—which accounts for approximately 27% of total U.S. CH_4 emissions—and the cattle industry, contributing roughly 25%, it is likely that livestock production will face heightened environmental regulations in the future (EPA, 2024). As government policies tighten on high-emission industries, the beef sector must proactively develop mitigation strategies. This study contributes to that effort by evaluating the potential for genetic improvement of traits associated with both CH_4 and nitrogen emissions in a population of Hereford cattle.

The objective of this study was to estimate heritability for DMI, CH_4, and BUN, and to evaluate genetic and phenotypic correlations among these traits in Hereford cattle in southeast Nebraska. By characterizing the genetic components of CH_4 emissions and reactive nitrogen excretion, this research provides foundational insight for incorporating environmental efficiency traits into existing programs. Long term, selection for cattle with reduced CH_4 emissions and improved nitrogen utilization could help the beef industry mitigate environmental impacts while maintaining its ability to produce high-quality protein from forage-based systems.

MATERIALS AND METHODS

Environment and Cattle Management

This study was conducted at Olsen Ranches, located approximately 64 kilometers south of Scottsbluff, Nebraska. Olsen Ranches participates in the American Hereford Association's (AHA) National Reference Sire Program, which facilitates the evaluation of young Hereford sires under commercial production conditions. The dataset included 766 animals, comprising 83 bulls and 683 steers. The study spanned six trials, each consisting of four pens. A trial was defined as the period during which animals remained in the pens while CH_4, BUN, and DMI were measured. Each pen was equipped with both Vytelle (Vytelle, Greeley, CO) feed intake monitoring systems and automated GreenFeed head-chamber systems (C-Lock Inc., Rapid City, SD) to collect individual intake and gas emission data. At the start of their respective trial periods, animals had an average age of 431 days.

Measurements and Laboratory Analyses

Blood Urea Nitrogen

Blood samples were collected via jugular venipuncture from 393 animals' midway through the trial period. Blood urea nitrogen data were not obtained for 373 animals due to failed restraint in the head catch or sample mislabeling caused by ear-tube tag mis-matches. Whole blood was transported to the laboratory and centrifuged within 24 hours of collection to separate serum. Samples were centrifuged at 5,000 × g for 20 minutes using a Beckman TJ-6 centrifuge (Beckman Instruments, Inc., Fullerton, CA). Serum was aliquoted and stored at –20 °C until analysis.

Serum BUN concentrations were determined using the Invitrogen™ Urea Nitrogen BUN Colorimetric Detection Kit (Thermo Fisher Scientific, Waltham, MA), following the

manufacturer's protocol. Each sample was assayed in duplicate. Fourteen, 96-well plates were used in total. Each plate included two blanks and eight urea nitrogen standards (run in duplicate) to generate a standard curve.

Following assay completion, mean absorbance values were calculated for each sample and standard. Standard curves were constructed by plotting the mean blank-corrected absorbance of each standard against the corresponding known urea nitrogen concentrations. For each plate, a linear regression equation was generated to interpolate sample BUN concentrations from their corrected absorbance values. The regression equation took the form:

$$\{BUN\} = m \times \{Absorbance\} + b$$

where *m* represents the slope and b the intercept of the standard curve. Blood urea nitrogen concentrations were calculated by solving the inverse of this equation for each sample's absorbance. All curve fitting was performed in Microsoft Excel, and the coefficient of determination (R^2) was used to evaluate the quality of each plate's standard curve. All R^2 values were greater than 0.97. Additionally, if BUN values fell outside the previously reported range of 4–24 mg/dL (Kohn et al., 2005), the samples were reanalyzed to ensure accuracy.

Methane Emissions

Methane emissions were quantified using an automated head-chamber system (AHCS; GreenFeed, C-Lock Inc., Rapid City, SD) installed in each pen. Prior to data collection, all animals were fitted with radio frequency identification (RFID) ear tags (Allflex, Merck & Co., Rahway, NJ) using a manual applicator to enable individual identification and tracking. Animals underwent a 20-day acclimation period to familiarize themselves with the AHCS units.

During the measurement period, pen configurations were designed to ensure that only one animal could access the AHCS at a time. Cattle had voluntary access to the units throughout the day. When an animal inserted its head into the AHCS, approximately 32 grams of alfalfa pellets were dispensed, encouraging the animal to remain in position for a longer duration of gas measurement. This feeding mechanism facilitated the collection of reliable CH_4 emission data during each visit. To ensure data integrity, system performance was monitored through monthly carbon dioxide recovery tests. The onboard calibration system performed zero and span calibrations for CH_4, CO_2, and O_2 gas analyzers every three days. Raw gas flux data were validated by C-Lock Inc. based on head proximity, visit duration, and airflow consistency, with wind corrections applied when necessary.

Data from individual visits were excluded if the visit duration was less than two minutes or greater than 8 minutes, airflow was below 26 L/s, and pipe temperature was below –10 °C, consistent with established protocols (Arthur et al., 2017). Only animals with at least 40 valid AHCS visits per trial phase were included in the final analysis, as recommended by Arthur et al. (2017). After parameters were applied, 320 animals had valid CH_4 measurements.

Dry Matter Intake

Individual DMI data were collected on 766 animals using Vytelle feed intake monitoring systems (Vytelle, Greeley, CO) installed in each pen. Data collection followed Beef Improvement Federation (BIF) recommended guidelines to ensure standardization and quality. The AHA provided all processed feed intake data and associated body weights collected as part of their routine data collection and management protocols. No additional calculations or processing of raw intake data were performed by the authors for the purposes of this study. **Data Summary**

Data was collected from 2021 to 2024. A three-generation pedigree was constructed using animals with records for at least one of the measured traits. The resulting pedigree contained 3242 animals with 459 unique sires and 2002 unique dams.

Contemporary groups (CG) for each trait were designed to account for differences in management and environment. Blood urea nitrogen CG was defined as assay plate, sex, and trial number. This resulted in 19 CG for BUN, with an average size of 21 animals and a standard deviation (SD) of 12 animals. Pen was excluded from the BUN contemporary group model due to a lack of statistical significance during model testing. Methane CG was defined by AHCS identification number—which accounts for pen number—along with sex and trial number. Sex was the difference in castrated and uncastrated male bovine. Trial number was considered the start date of each trial. This resulted in 16 CG, with the average size of 21 animals and a SD of 9 animals. Dry matter intake CG was defined as birth CG, weaning weight CG, trial number, and sex. This is the CG constructed and provided by AHA. This resulted in 12 CG, with the average size of 60 animals and a SD of 67 animals.

Statistics Analysis

Heritabilities and genetic, phenotypic and residual correlations were obtained using the software package ASReml 3.0 (Gilmore et al., 2009). Single-trait and 2-trait analyses were conducted to obtain genetic and residual variances to serve as starting values for a 3-trait analysis that is reported on in this study. The 3-trait analysis included BUN, CH_4, DMI for estimation of heritabilities and genetic, phenotypic and residual correlations. The 3-trait animal model were expressed as:

$$\begin{bmatrix} Y_1 \\ Y_2 \\ Y_3 \end{bmatrix} = \begin{bmatrix} X_1 & 0 & 0 \\ 0 & X_2 & 0 \\ 0 & 0 & X_3 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \\ \beta_3 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 & 0 \\ 0 & Z_2 & 0 \\ 0 & 0 & Z_3 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \\ u_3 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ e_3 \end{bmatrix}$$

Above, $Y_{\$}$ was a vector of observations for trait 1, trait 2, and trait 3, $X_{\$}$ was an incidence matrix relating observations in $Y_{\$}$ to the fixed effects in $\beta_{\$}$. $\beta_{\$}$ was a vector of known fixed effects for CG and age, $Z_{\$}$was an incidence matrix relating observations to additive genetic effects, $u_{\$}$ was a vector of additive genetic effects and $e_{\$}$ was a vector of random residuals.

Above, random effects were assumed to have means of 0 and variances represented below.

The (co)variance structure for the random effects in the 3-trait analysis were expressed as follows:

$$var = \begin{matrix} u \\ e \\ a \end{matrix} = \begin{matrix} G \otimes A & 0 \\ 0 & R \otimes I_{n} \end{matrix}$$

In the above equation, *G* was the additive genetic (co)variance matrix and *R* was the residual (co)variance matrix among each of the traits, and I_n was an identity matrix whose order was equal to the number of observations for each of the traits, Y_1 to Y_3.

Wald F statistics were utilized to test the significance of the fixed effects included in the analysis. Single-trait models were first fit to evaluate the contribution of each fixed effect, and results guided the specification of the fixed effects used in the final three-trait model. Type III Wald F tests were used to assess the fixed effects individually. All tests were conducted at the $\alpha = 0.05$ level, with trends or tendencies considered for P-values between 0.05 and 0.10.

In the DMI model, CG was significant ($P<0.001$), yearling weight (YWT) was significant ($P<0.001$), and age also showed a significant effect ($P<0.001$). For BUN, CG ($P<0.001$), age ($P=0.048$), and YWT ($P<0.001$) were significant. However, in the reduced model excluding YWT, both CG and age were highly significant ($P<0.001$) for BUN. A similar pattern was observed for CH_4: in the full model, age and YWT were highly significant and CG neared

significance (P=0.062), but in the reduced model without WWT, both CG and age (P<0.001) were strongly significant. Based on these findings, CG and age were retained as fixed effects in the final three-trait model to control for known sources of variation while preserving biological interpretability. Due to its strong correlation with age and high model complexity, WWT was excluded from the final multi-trait models. Despite being statistically significant in the ANOVA ($p < 0.001$), inclusion of WWT did not improve model parsimony; AIC and BIC values were substantially higher in the full model compared to the reduced model. Additionally, variance inflation factors (VIF) indicated collinearity between age and WWT, with age exhibiting a high GVIF-adjusted value (>10), supporting the decision to avoid redundancy in fixed effects. From a biological perspective, WWT is a highly heritable trait, and its inclusion as a fixed effect risks removing genetic variability that the model aims to capture. Because our objective was to account for environmental sources of variation rather than adjust for genetic potential, WWT was excluded to avoid overfitting and to preserve the genetic signal relevant to the traits of interest.

RESUTLS AND DISCUSSION

Due to logistical constraints across trials, not all animals were phenotyped for every trait, leading to different sample sizes for BUN, CH_4, and DMI. Table 3.1 provides descriptive statistics for each, including the number of records, means, standard deviations, and observed ranges. Although the recommended physiological range for BUN concentrations in cattle is 4-25 mg/dL (Kohn et al., 2005), 94% of observations in this dataset fell within that range. Despite this, all BUN data were retained because they met multiple quality control criteria, such as high assay reliability (all plate standard curves had $R^2 > 0.97$), inclusion of sample duplicates, and repeat assays for values outside the recommended range. Excluding these out-of-range samples would have substantially reduced the sample size.

The average CH_4 emission in this study is comparable to values previously reported in the literature (e.g., Johnson and Johnson, 1995; Beauchemin et al., 2020), though the observed minimum and maximum values indicate a wider range than previously reported. These were retained based on data cleaning protocols described in Materials and Methods. Similarly, the mean DMI observed aligned closely with published reference values (e.g., NRC, 2000), supporting the overall validity of intake data. Collectively, these descriptive statistics highlight the biological variability observed in the dataset.

Table 3.1 Descriptive statistics for dry matter intake, CH_4 emissions, and blood urea nitrogen in beef cattle.

Trait (units)	No. of records	Average (SD)	Minimum	Maximum
BUN[1] (mg/dL)	393	12.2 (6.9)	3.5	36.4
CH_4 [1] (g/d)	320	234.5 (44.3)	141.3	369.1
DMI[1] (kg/d)	725	11.4 (1.5)	6.6	18.2

[1] DMI = dry matter intake (kg/day); BUN = blood urea nitrogen (mg/dL); CH_4 = methane emissions (g/day).

Table 3.2 illustrates variation in phenotype expression and sample availability across the six independent trials. Trial 1 was the only cohort composed of bulls, while all subsequent groups consisted entirely of steers. This distinction is important, as bulls are known to exhibit different protein metabolism and urea dynamics compared to steers (Hammond, 1997), which may partially explain the elevated BUN concentrations observed in that group. Substantial variation in average CH_4 and DMI values across trials is also apparent, likely reflecting differences in animal age, diet, and environmental conditions. Notably, CH_4 data were not available for Trial 1 due to the absence of an animal identification cross-reference required to link emissions data to individual animals. The number of observations for each trait also varied by trial, driven by data availability and quality control criteria described in the Materials and Methods. Regression

analysis indicated that for every 1-day increase in age, dry matter intake increased by 0.02 kg/day, CH_4 emissions increased by 0.57 g/day, and blood urea nitrogen decrease by 0.01 mg/dL.

Table 3.2 Descriptive statistics of each independent trial.

Trial Number	No. of animals	Sex	Average BUN[1]	No. BUN obs.[2]	Average CH_4[1]	No. CH_4 obs. [2]	Average DMI[1]	No. DMI obs.[2]
1	83	Bull	12.3	70 -	-	12.5	83	
2	70	Steer	17.8	63	218	67	10.3	68
3	193	Steer	6.0	64	239.0	53	11.8	193
4	84	Steer	7.0	36	284.3	61	12.0	57
5	211	Steer	9.8	78	208.0	92	10.7	206
6	125	Steer	16.9	82	251.5	47	11.3	118

[1] DMI = dry matter intake (kg/day); BUN = blood urea nitrogen (mg/dL); CH_4 = methane emissions (g/day); N = number of animals.

[2] The number of observations for the given trait in that specific trial.

Heritabilities

Heritability estimates for BUN, CH_4, and DMI are presented in Table 3.3 and indicate varying levels of genetic influence across traits. The estimate for BUN (0.17) was at the top end of what has been reported in dairy cattle, where values range from 0.07 to 0.17 (Peterson et al., 1982; Van Den Berg et al., 2021). Heritability for CH_4 emissions (0.14) fell within the range of published values of 0.11 to 0.45 (Arthur et al., 2016; Lassen et al., 2016; Pech et al., 2022), supporting the trait's potential for selection. The estimate for DMI (0.50) exceeded literature values, which typically range from 0.33 to 0.46 (Rolfe et al., 2011; Diaz et al., 2013), indicating strong genetic control. Importantly, all animals were of the same breed, from the same ranch, and managed under similar conditions for this study. Therefore, the observed variation is more likely to reflect underlying genetic differences. However, the relatively large standard errors warrant

caution in interpreting these results and underscore the need for larger, more uniformly sampled populations to improve estimate precision.

Table 3.3 Residual and genetic variances and heritabilities for BUN, CH4, and DMI.

Trait	Residual Variance (SE)	Genetic Variance (SE)	h^2 (SE)[1]
BUN[1]	10.5 (12.2)	2.2 (2.2)	0.17 (0.17)
CH_4[1]	808.2 (161.0)	126.3 (154.8)	0.14 (0.16)
DMI[1]	5.1 (1.3)	5.0 (1.7)	0.50 (0.14)

[1] DMI = dry matter intake (kg/day); BUN = blood urea nitrogen (mg/dL); CH_4 = methane emissions (g/day); h^2 = heritability; SE = standard error.

Correlations

Table 3.4 highlights genetic and phenotypic correlations among BUN, CH_4, and DMI. As expected, phenotypic correlations tended to lie between the genetic and residual (environmental) correlations. The genetic correlation between BUN and DMI (0.71) was notably stronger than the corresponding phenotypic correlation (0.13), suggesting a meaningful shared genetic basis that is masked by weaker or opposing environmental influences. This finding is consistent with previously reported phenotypic correlations in other species. For example, milk urea nitrogen (MUN) in dairy cattle has been phenotypically correlated with DMI at 0.16 (Villalobos et al., 2018), while a correlation of 0.24 has been reported in pigs (Schmid et al., 2024). Although these species and biological matrices differ from BUN in beef cattle, they are referenced here due to the limited availability of comparable literature in beef. Stronger associations have also been reported between DMI and urea-related traits such as urinary and fecal nitrogen excretion in beef cattle (0.59 and 0.69, respectively; Schmid et al., 2024). This finding adds to the limited body of research examining genetic relationships between BUN and DMI in beef cattle.

In contrast, the relationship between DMI and CH_4 showed a positive phenotypic correlation (0.39), in line with previous reports averaging 0.54 (Bird-Gardiner et al., 2017; Donoghue et al., 2016; Fitzsimons et al., 2013). The estimated genetic correlation (-0.29) diverged in both direction and magnitude from literature averages, which are typically positive and centered around 0.83 (Donoghue et al., 2016; Manzanilla-Pech et al., 2016). This discrepancy may be attributed to differences in breed composition—our study focused exclusively on Hereford cattle, whereas many published estimates are based on multi-breed populations. Additionally, variation in diet composition, trial duration, and methane measurement protocols across studies may contribute to the observed differences in genetic estimates. Finally, the genetic (-0.26) and phenotypic (0.08) correlations between BUN and CH_4 were moderate and low, respectively, and to our knowledge, no published studies have reported estimates for this relationship. These findings highlight the complexity of genetic and phenotypic relationships among nitrogen metabolism and CH_4 emissions in beef cattle.

Table 3.4 Genetic (top diagonal) and phenotypic (bottom diagonal) correlations for BUN, CH4, and DMI.

Trait	BUN[1] (SE)	CH_4[1] (SE)	DMI[1] (SE)
BUN [1]	-	-0.26 (0.85)	0.71 (0.45)
CH_4[1]	0.08 (0.08)	-	-0.29 (0.57)
DMI[1]	0.13 (0.06)	0.39 (0.06)	-

[1] DMI = dry matter intake (kg/day); BUN = blood urea nitrogen (mg/dL); CH_4 = methane emissions (g/day) ; SE = standard error.

Residual correlations among BUN, CH_4, and DMI reveal the extent to which non-genetic, non-fixed sources of variation contribute to observed trait relationships, such as environmental effects. The strongest residual association was observed between CH_4 and DMI (0.71), suggesting that unaccounted environmental factors may be contributing to variation in both traits. These factors could include feeding behavior, timing of gas measurement relative to intake, or shared dietary influences. In contrast, BUN showed weaker and more inconsistent residual correlations with CH_4 (0.14) and DMI (-0.13), suggesting environment and management do not influence performance in a related direction. The negative residual association between BUN and DMI indicates negative short-term connection between nitrogen excretion and feed intake, consistent with transient variation in metabolic demands or protein utilization efficiency (Lavery & Ferris, 2021). Collectively, these findings underscore that while genetic and fixed effects account for much of the structured trait variation, residual factors can still shape observed phenotypic patterns, particularly for traits tied to metabolism and intake behavior.

Table 3.5 Residual (top diagonal) correlations for BUN, CH4, and DMI.

Trait	BUN[1] (SE)	CH_4[1] (SE)	DMI[1] (SE)
BUN [1]	-	0.14 (0.16)	-0.13 (0.12)
CH_4[1]	-	-	0.71 (0.19)
DMI[1]	-	-	-

[1] DMI = dry matter intake (kg/day); BUN = blood urea nitrogen (mg/dL); CH_4 = methane emissions (g/day) ; SE = standard error.

CONCLUSION

This study leveraged phenotypic data from a structured population of Hereford cattle to investigate the biological and statistical relationships among BUN, CH_4, and DMI. Heritability estimates revealed low h^2 for BUN, low genetic control for CH_4, and strong heritability for DMI, highlighting the potential for selective breeding to improve these traits. Genetic correlations showed a meaningful shared genetic basis between BUN and DMI, while the relationship between CH_4 and DMI differed from previous reports, suggesting population-specific dynamics. Residual correlations emphasized the role of environmental factors influencing CH_4 and DMI simultaneously, and possible short-term metabolic trade-offs affecting BUN and intake. Taken together, these results underscore the complex and partially decoupled nature of genetic versus environmental contributions to key production and environmental traits in beef cattle. While genetic selection for reduced CH_4 and optimized DMI appears feasible, further investigation in larger and more uniform populations will be necessary to confirm these relationships—especially regarding BUN's genetic architecture and its potential role as an indirect selection criterion for nitrogen efficiency.

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CONCLUSION

This research represents one of the first efforts to quantify the genetic relationships among dry matter intake (DMI), methane (CH_4) emissions, and blood urea nitrogen (BUN) in beef cattle simultaneously, and especially for the Hereford breed. Together, these traits capture major components of the beef production environmental footprint—feed intake influencing resource use, CH_4 representing carbon emissions, and BUN serving as a proxy for nitrogen utilization and excretion. The findings contribute to a growing body of work aimed at integrating environmental efficiency into genetic improvement programs.

Heritability estimates across traits revealed varying degrees of genetic control, suggesting that these environmental impact traits can be effectively improved through selection but likely will require longer-term or indirect strategies given the nature of these data collections. In particular, the moderate genetic basis observed for nitrogen-related traits like BUN supports its continued evaluation as a cost-effective indicator trait for improving nitrogen use efficiency and the high heritability observed for feed intake further reinforces the potential for incorporating intake-related phenotypes into breeding goals, both for improving efficiency and mitigating environmental outputs. All environmental traits evaluated in this study have the potential to be improved through selection and genetic improvement.

The genetic associations identified among traits highlight the biological interconnectedness of feed utilization, methane production, and nitrogen metabolism. The positive genetic relationship between BUN and DMI suggests shared physiological mechanisms linking nutrient intake and nitrogen turnover, while weaker or inconsistent relationships between

CH_4 and the other traits indicate that reducing methane through selection may be possible without compromising intake or nitrogen metabolism. Collectively, these results suggest opportunities for balanced genetic progress toward improved environmental sustainability in beef production systems.

Importantly, this study also underscores the continued need for large-scale, multi-breed evaluations to validate these findings and refine genetic parameter estimates for inclusion in national evaluation systems. As phenotyping technologies advance and multi-trait genomic tools become more accessible, traits such as CH_4 and BUN could become integrated components of selection indices targeting environmental efficiency.

Ultimately, this research provides foundational evidence supporting the feasibility of selecting beef cattle for reduced environmental impact without sacrificing productivity. By advancing understanding of the genetic architecture and relationships among key metabolic traits, this work contributes to the broader goal of developing sustainable, data-driven breeding programs that align animal performance with environmental stewardship.