The Relationships Between the Ruminal Digestibility Kinetics of Fiber, Total-Tract Nutrient Digestibility, and Methane Emissions from Lactating Dairy Cattle

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ABSTRACT

The efficiency of fiber utilization in dairy cattle plays a central role in optimizing production performance while minimizing environmental impacts, particularly enteric methane (**CH_4**) emissions. Ruminal degradation and passage kinetics of fiber fractions, potentially degradable (**pdNDF**) and undegraded (**uNDF**) neutral detergent fiber (**NDF**), are influenced by both forage quality and dietary composition. This dissertation examined how forage type, maturity, and dietary inclusion level affect nutrient digestibility, fiber passage dynamics, milk production, and CH_4 emissions in high-producing dairy cows. Chapter 2 evaluated the effects of alfalfa hay and orchardgrass hay on ruminal NDF degradation and passage kinetics. Diets were formulated to provide similar total NDF concentrations but differed in uNDF, with alfalfa contributing more uNDF relative to its total NDF content. Cows consuming alfalfa exhibited faster ruminal passage rate (**K_p**) and smaller ruminal pools of dry matter (**DM**), NDF, and pdNDF. Despite faster passage, cows fed alfalfa consumed more DM and uNDF and exhibited greater total-tract digestibilities of NDF and pdNDF. However, milk production and components were not affected by treatment. These findings suggest that the kinetics of ruminal degradation and passage influence NDF degradation in ways beyond uNDF concentration or forage quality. Chapters 3 and 4 evaluated the influence of triticale silage maturity [boot (**BT**) vs. soft-dough (**SFT**) stage] on production performance, nutrient digestibility, ruminal volatile fatty acid (**VFA**) profile, enteric CH_4

emissions, and fecal biomethane potential. Chapter 3 implemented a 2×2 factorial arrangement of treatments with high-forage (**HF**; 52% forage) or low-forage (**LF**; 37% forage) diets containing BT or SFT silages. Forage maturity did not affect DM intake (**DMI**) in LF diets, but cows fed HFBT consumed more DM than those fed HFSFT. While energy-corrected milk (**ECM**) production and feed efficiency did not differ by silage maturity, cows fed LF diets produced more ECM than those fed HF diets, though at the expense of digestibility. Cows consuming BT silage exhibited improved total-tract digestibilities of DM and NDF compared to SFT. Cows consuming HF diets also exhibited greater digestibilities of DM, NDF, and starch compared to LF. Cows consuming BT diets produced 23 g/d less enteric CH_4 than SFT, with no difference between HF and LF diets in absolute enteric CH_4 output. Cows consuming BT silage had lower enteric CH_4 yields on a DMI basis and DM-digested basis than SFT. Thus, harvesting triticale at the BT stage can enhance digestibility and milk yield while reducing enteric CH_4 intensity when fed to lactating dairy cattle, whereas LF diets boost milk yield and lower enteric CH_4 yield but compromise NDF digestibility. Chapter 4 further explored these effects using diets composed of either BT or SFT triticale silage, with additional evaluation of ruminal VFA profiles, fiber passage kinetics, and fecal biomethane potential. Although dietary K_p did not differ, marker-based estimates showed a faster K_p for the individual BT silage compared to the SFT silage. Cows fed BT silage had greater total VFA concentrations and a lower acetate-to-propionate ratio, reflecting more active fermentation. These cows also emitted less enteric CH_4 and exhibited reduced enteric CH_4 intensity. However, greater pdNDF intake in the BT diet resulted in greater fecal pdNDF output. While fecal biomethane potential did not statistically differ, cows consuming BT diets were estimated to emit 18 L/d more CH_4 from their fecal material, suggesting a potential trade-off between enteric and manure-derived CH_4 emissions. Collectively, these studies highlight the complex trade-offs

between forage quality, animal performance, and environmental outcomes. These findings challenge the assumption that faster ruminal K_p uniformly reduces enteric CH_4 emissions by limiting fermentation and emphasize the importance of integrated assessments of digestion kinetics, animal performance, and environmental outcomes in dairy nutrition.

The Relationships Between the Ruminal Digestibility Kinetics of Fiber, Total-Tract Nutrient Digestibility, and Methane Emissions from Lactating Dairy Cattle

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GENERAL AUDIENCE ABSTRACT

Efficient fiber utilization in dairy cows is essential for maximizing milk production while minimizing environmental impacts, particularly methane (**CH_4**) emissions. This dissertation investigated how different forages, their maturity at harvest, and their inclusion rates in the diet affect nutrient digestibility, ruminal function, milk yield, and methane emissions in high-producing dairy cows. The first study compared diets containing alfalfa or orchardgrass hay, formulated to provide similar total fiber content but differing in fiber degradability. Although milk yield was similar between treatments, cows fed alfalfa consumed more feed and digested more fiber. These cows also had faster ruminal passage rates and smaller fiber pools in the rumen, suggesting that fiber digestion is influenced not only by forage type or composition, but also by how quickly fiber moves and breaks down in the rumen. The next two studies focused on triticale silage harvested at either the boot (early) or soft-dough (late) maturity stages. These silages were fed in both high-forage and low-forage diets in the second study. Early-maturity silage improved dry matter intake, fiber digestibility, and milk production compared to late-harvested silage. It also reduced CH_4 emissions from the rumen per unit of feed and milk produced, making it a promising strategy for reducing the environmental footprint of dairy production. Low-forage diets increased milk yield and reduced methane intensity but lowered overall fiber digestibility, highlighting a performance–digestibility trade-off. The third study further examined the effects of triticale maturity on ruminal

fermentation, fiber passage rate, and fecal methane potential. Cows fed early-maturity triticale exhibited more active ruminal fermentation and emitted less CH_4 from the rumen. However, due to greater intake of digestible fiber, more potentially fermentable fiber was excreted in feces. While the methane-producing potential of the feces itself did not differ, the estimated daily CH_4 emissions from feces were numerically higher for cows fed early-maturity triticale, suggesting a shift in emissions from rumen to fecal sources. Collectively, these studies highlight the complex trade-offs between diet formulation, forage quality, animal performance, and environmental outcomes. Strategies that enhance fiber digestibility and milk production can reduce methane emissions from the rumen but may increase the risk of downstream emissions from feces, depending on the initial composition of the fiber. These findings highlight the importance of evaluating nutritional strategies within the broader context of the whole-animal system to ensure both productivity and sustainability in dairy operations.

CITATIONS

Material from this dissertation has been published in the following forms:

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“The greatest obstacle to discovery is not ignorance—it is the illusion of knowledge.”

-Daniel J. Boorstin

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LIST OF ABBREVIATIONS

The research chapters within this dissertation follow the format of the *Journal of Dairy Science*. Standard abbreviations from the journal are not explained within the text but are defined here. Non-standard abbreviations are defined here and within the text.

ADF = acid detergent fiber

ADL = acid detergent lignin

ALFA = alfalfa

BHB = beta-hydroxybutyrate

BT = boot

BW = body weight

CH_4 = methane

CP = crude protein

df = degrees of freedom

DIM = days in milk

DM = dry matter

DMI = dry matter intake

ECM = energy-corrected milk

FE = feed efficiency

HF = high-forage

iNDF = indigestible fiber

K_d = degradation rate

K_d1 = degradation rate of the rapidly fermenting fraction of potentially degradable neutral detergent fiber

K_d2 = degradation rate of the slowly fermenting fraction of potentially degradable neutral detergent fiber

K_p = passage rate

LF = low-forage

MRT = mean retention time

MUFA = monounsaturated fatty acid

MUN = milk urea nitrogen

NDF = neutral detergent fiber

NDS = neutral detergent solubles

OBCFA = odd-branched chain fatty acids

OM = organic matter

ORCH = orchardgrass

pdNDF = potentially degradable neutral detergent fiber

pdNDF1 = rapidly fermenting fraction of potentially degradable neutral detergent fiber

pdNDF2 = slowly fermenting fraction of potentially degradable neutral detergent fiber

peNDF = physically effective neutral detergent fiber

PUFA = polyunsaturated fatty acids

SD = standard deviation

SEM = standard error of the mean

SFA = saturated fatty acids

SFT = soft-dough

TMR = total mixed rations

TOR = turnover rate

uNDF = undegraded neutral detergent fiber

VFA = volatile fatty acid

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

High-quality forages are paramount in dairy cattle nutrition, as they supply the requisite long, fibrous particles that are critical for optimal ruminal function and digestive efficiency (NASEM, 2021). These fibrous components not only serve as fermentation substrates but also enhance mastication and rumination, stimulate saliva production, and contribute to the formation of a cohesive ruminal mat that supports effective ruminal motility and buffering capacity (Van Soest, 1994). The supply of physically effective fiber is essential, as it affects the dynamics of fiber degradation and passage through the gastrointestinal tract, thereby influencing nutrient utilization and overall cow performance.

In high-producing dairy systems, forages alone cannot meet the energy demands required for maximal milk yield, necessitating the inclusion of energy-dense concentrates. However, this introduces complexities into understanding digestive kinetics, as interactions between forage and concentrate ingredients can differentially affect fiber digestibility and passage rates. The quality of forages, determined by factors such as plant species, growth stage, and harvest conditions (Nelson and Moser, 1994), further complicates these dynamics. These relationships are critical not only for maximizing milk production but also for mitigating production costs and environmental impacts.

In the context of sustainable dairy production, there is a growing imperative to optimize cattle diets to achieve high production efficiency on reduced feed supply while minimizing environmental waste, such as enteric methane and fecal residues. A nuanced understanding of the balance between fiber degradation and passage is therefore essential, as it has direct implications for energy provision, ruminal turnover, dry matter intake, and overall digestive tract efficiency.

The ensuing literature review delves into these facets, examining how fiber characteristics and ruminal kinetics collectively shape digestive outcomes and environmental footprints in dairy systems.

1.2 Characterization of Fiber

In ruminant nutrition, fiber refers to the slowly fermentable or indigestible portion of feed that physically occupies space in the ruminant gastrointestinal tract and varies in its accessibility to ruminal microbes (Van Soest, 1994; Mertens, 1997). Despite being commonly discussed as a single nutritional entity, fiber is a complex and heterogenous component of plant biomass. A more accurate understanding considers fiber as a structurally organized macromolecule, rather than just a sum of its chemical components (Van Soest, 1994). Fiber is characterized by its structural organization within the plant cell wall, its chemical composition, and its variable susceptibility to ruminal degradation.

1.2.1 Structural Organization and Plant Cell Walls

The anatomical and cellular architecture of forages plays a foundational role in determining their nutritional value for ruminant animals. Structurally, forages are composed of a variety of tissue types, each differing in function and composition, which in turn influences their fiber concentration and potential degradability in the rumen. These plant tissues include epidermis, mesophyll, parenchyma bundle sheath, parenchyma, collenchyma, phloem fibers, sclerenchyma, and vascular tissues (Wilson, 1993). The distribution and proportion of these tissues vary by plant species, stage of maturity, and morphological components (i.e. leaf vs. stem), contributing to the heterogeneity in forage fiber characteristics (Akin, 1993). Importantly, their digestibility is closely tied to their specific cell wall structures and the degree of lignification.

In ruminant nutrition, fiber primarily refers to the structural polysaccharides and phenolic compounds located in the plant cell wall. The cell wall forms a complex and dynamic matrix that encases each plant cell and provides structural integrity. This matrix is also the primary site of interaction between plant material and the microbial community in the rumen, thereby governing the extent and rate of fiber degradation (Akin, 1993). During cell division and expansion, the primary cell wall is synthesized, consisting largely of cellulose microfibrils embedded in a network of hemicellulose and pectin (Wilson, 1993). The middle lamella, a layer rich in amorphous materials such as pectin and other gel-like polysaccharides, joins adjacent cells. In young or metabolically active tissues such as mesophyll or parenchyma, the primary cell wall and middle lamella are typically less lignified, making them particularly susceptible to microbial degradation (Wilson, 1993). However, in structural tissues and as cells mature, both the primary cell wall and middle lamella can become highly lignified, particularly in thick-walled cells, making them resistant to microbial degradation (Buxton and Redfearn, 1997). As cells complete expansion and differentiation, a secondary cell wall is often deposited interior to the primary cell wall. This secondary cell wall is typically composed of three distinct layers, each characterized by a specific orientation of cellulose microfibrils and increasing degrees of lignification (Wilson, 1993). As the plant matures and structural support becomes more critical, lignin deposition occurs in all three layers. In thick-walled cells, the middle lamella and primary wall can become especially lignified and resistant to microbial degradation, while lignified secondary walls may be partially digestible if microbes can access them from the cell interior (Buxton and Redfearn, 1997; Wilson, 1993). The extent of lignin accumulation and its cross-linking with hemicellulose and structural proteins impede the enzymatic accessibility of cellulose and reduce the fermentability of the fiber fraction. These structural features enhance the mechanical strength of the tissue but simultaneously reduce its digestibility.

The variation in cell wall construction not only differs among cell types within a single plant but also across anatomical components and plant species (Wilson, 1993). This dual variability, of cell wall composition among tissues and tissue distribution among forages, contributes to the inherent complexity in evaluating and predicting forage fiber digestibility. For instance, a forage high in stem content will contain a greater proportion of heavily lignified tissues such as sclerenchyma and vascular bundles, resulting in lower fiber digestibility. Conversely, leaf-dominant forages or less mature plant material are often richer in parenchyma and mesophyll tissues, with thinner, less lignified walls, supporting faster rates of ruminal degradation (Akin, 1993). Thus, the structural diversity of plant tissues and their associated cell wall layers play a critical role in modulating the digestibility of forage fiber and, by extension, the efficiency of nutrient utilization in dairy cattle. Although the research chapters presented in this dissertation do not directly investigate the anatomical or cellular architecture of forages, these structural attributes remain fundamental to understanding forage quality and are critical to interpreting nuanced differences in fiber composition and digestibility.

1.2.2 Chemical Characterization of Fiber

Major advancements in the understanding and assessment of fiber in ruminant diets occurred during the 1960s and 1970s, particularly through the work of Dr. Peter J. Van Soest (Mertens and Hall, 2021). He developed a detergent-based analytical system that enabled the partitioning of forage dry matter into fractions based on their bioavailability to ruminants (Van Soest, 1967). This approach provided a framework to isolate the fiber-containing cell wall from the soluble cell contents, such as sugars, starches, pectin, proteins, and lipids (Van Soest, 1967). The insoluble fraction, referred to as neutral detergent fiber (**NDF**), includes hemicellulose, cellulose, and lignin (Van Soest et al., 1994) and represents approximately 30 to 80% of plant matter (Buxton and Redfearn, 1997). The soluble fraction, or neutral detergent solubles (**NDS**), consists of non-structural and more digestible components, is rapidly fermentable, and contributes substantially to the dietary energy supply (Van Soest, 1994). Within the context of ruminant nutrition, NDF has become a foundational metric for estimating forage fiber and its functional implications in the rumen.

Among the cell wall components, cellulose is a linear polysaccharide composed exclusively of β(1→4)-linked glucose units. Despite being the most abundant carbohydrate, the digestibility of cellulose is highly variable and depends on its interaction with other structural components in the cell wall (Van Soest, 1994). Cellulose is often tightly embedded within a matrix of hemicellulose and lignin, which can constrict microbial access (Akin, 1993). Hemicellulose is a more heterogeneous group of polysaccharides, comprising a mixture of β(1→4)-linked sugars such as xylose, mannose, and arabinose, along with various side-chain substitutions (Van Soest, 1994). Its composition is highly variable across plant species, and its degradability is influenced both by its intrinsic structure and its degree of association with cellulose and lignin. In the secondary cell wall, hemicellulose is frequently cross-linked with lignin through ferulate bridges, which limits enzymatic degradation unless those linkages are chemically or physically disrupted (Van Soest, 1994). Particularly in grasses, where arabinoxylan is the dominant hemicellulose, the arabinose-to-xylose ratio has received particular attention as an indicator of hemicellulose branching and cross-linking potential. A higher arabinose-to-xylose ratio generally reflects a more branched structure, which may increase solubility and enzyme accessibility, but also provides more potential attachment sites for ferulate bridges, thereby enhancing cross-linking with lignin and potentially reducing fiber digestibility (Grabber et al., 1998; Hatfield et al., 1999).

Unlike cellulose and hemicellulose, lignin is not a carbohydrate but rather a complex polyphenolic polymer that provides structural rigidity to plants and resists microbial breakdown (Van Soest, 1994; Jung et al., 2012). The structural rigidity that lignin provides plays a vital role in the plant withstanding environmental and biological stresses (Buxton and Redfearn, 1997). However, from a nutritional standpoint, lignin is the most significant constraint to fiber digestibility, not only because it is indigestible itself (Akin, 1993; Van Soest, 1994), but also because it physically and chemically impedes enzymatic access to otherwise fermentable polysaccharides. Depending on the species and degree of plant maturity, the polysaccharides in the plant cell wall may or may not be directly linked to lignin. Such variability influences both the potential extent and the rate of fiber degradation (Van Soest, 1994).

The relative amounts of fiber components, such as cellulose or lignin, do not reliably predict the digestibility of the NDF fraction. This has historically posed a challenge for nutritionists attempting to accurately model the effects of insoluble fiber on digestion, intake, and animal performance (Hall and Mertens, 2017). While lignin is a well-established determinant of indigestibility, its impact must be understood in the context of its interactions with other cell wall constituents and the overall structural organization of the wall (Moore and Hatfield, 1994). Furthermore, our understanding is complicated by the fact that factors such as forage species, stage of maturity, and environmental growing conditions all influence the degradability of the NDF fraction (Galyean and Goetsch, 1993; Van Soest, 1994). Collectively, it is easier to think of fiber as a single nutritional entity. However, we must appreciate the complexity of the chemical nature of NDF.

Although NDF remains a practical and widely used metric for estimating feed bulk and predicting effects on rumination and fill (NASEM, 2021), it is not sufficient for predicting voluntary intake or productive responses with high precision (Mertens, 2015). A critical limitation is that NDF is chemically and functionally heterogeneous and contains fractions that differ in their fermentation characteristics (Raffrenato and Van Amburgh, 2010). Because tissue distribution and cell wall composition vary among forages, the digestibility of NDF must be evaluated on a forage-specific basis rather than assuming a uniform degradability across dietary fiber sources. Further sub-fractionation of NDF into potentially degradable and indigestible fractions has become essential for improving the precision of ration formulation and for more accurately predicting ruminal fermentation dynamics and animal responses to diets without determining the exact chemical makeup of fiber (Mertens, 2015).

1.2.3 Fractionation of Fiber into Digestibility Pools

Building upon the foundational characterization of fiber via the detergent system, NDF can be further partitioned into distinct pools based on their digestibility in the rumen. These subdivisions include a potentially degradable fraction (**pdNDF**) and an indigestible fraction (**iNDF**). The conceptual basis for this classification was first introduced by Waldo et al. (1972), who recognized that not all fiber components respond equally to microbial degradation. Later, Van Soest (1994) elaborated on this concept, defining iNDF as portions of hemicellulose and cellulose so tightly bound to lignin and other cell wall constituents that they remain refractory to fermentation, irrespective of ruminal retention time.

This binary or 2-pool framework of fiber degradability served as a critical foundation for kinetics models of fiber degradation. These models typically describe the degradation of pdNDF using first-order kinetics, assuming that the rate of degradation is proportional to the amount of fiber remaining, as described in a later section (Mertens, 2015). However, one of the inherent challenges with this approach is that iNDF is a theoretical construct and cannot be directly observed or chemically isolated as it is the portion of NDF that will never be fermented in the rumen even if retained indefinitely. As a result, researchers developed laboratory-based methods to empirically estimate the indigestible fraction. This led to the widespread adoption of undegraded NDF (**uNDF**) as a practical analog for iNDF in experimental and applied nutrition settings (Mertens, 2016; Raffrenato et al., 2018). The uNDF fraction is typically determined through in vitro or in situ fermentation techniques in which a forage or feed sample is incubated with ruminal fluid or within the rumen for an extended period, and the undigested NDF residue is measured. Raffrenato et al. (2018) showed that a 240-hour in vitro incubation period is sufficient to capture the truly undegradable portion of NDF, designated as uNDF240, as extending the fermentation beyond this point yields minimal additional fiber loss. Thus, uNDF240 has become a practical and standardized measure for quantifying the indigestible fiber pool in ruminant nutrition research. Nonetheless, some researchers use alternative endpoints for characterizing uNDF (Krämer et al., 2012; Krizsan et al., 2012), and, therefore, careful consideration must be taken when comparing uNDF estimates with the literature. While we commonly fractionate NDF, it is important to remember that pdNDF and uNDF are not distinct entities and are simplified constructs that provide an easier understanding of fiber kinetics as affected by different chemical compositions.

Mertens (1977) initially proposed that pdNDF can be further sub-fractionated into rapidly and slowly fermenting pools. This hypothesis was later supported by Raffrenato and Van Amburgh

(2010), who observed via curve-splitting that the "fast-degrading pool" of pdNDF is mostly degraded by 30 hours, and the "slow-degrading pool" of pdNDF can require up to 120 hours for significant degradation. Modeling these distinct degradation pools can offer a more nuanced approach to predicting fiber degradability, fermentation kinetics, and nutrient supply to the animal, and is further discussed in the next section.

1.3 Fiber Ruminal Degradation Rate

Although total-tract digestibility metrics offer a global perspective on diet utilization, they do not provide insight into the degradation of individual feed components within a mixed ration. Because the rumen is the principal site of fiber degradation in ruminants (Van Soest, 1994; NASEM, 2021), there is significant interest in assessing the ruminal degradation of specific feeds. This is often achieved using in situ techniques, wherein feed samples are incubated in nylon bags placed within the rumen of cannulated ruminants (Quinn, 1938; Mehrez and Ørskov, 1977), or through in vitro methods utilizing ruminal fluid extracted from ruminant animals (Goering and Van Soest, 1970).

1.3.1 Development of the Methodology

Pioneering work by Alexander et al. (1969a; 1969b) introduced a novel method to uniformly label alfalfa hay with radioactive carbon by cultivating the crop in a sealed plexiglass chamber infused with $^{14}CO_2$. The labeled forage was then fed to a rumen-cannulated Jersey cow, and ruminal contents were manually mixed prior to collecting samples at predetermined time points. By plotting the logarithm of the ^{14}C remaining against time, they obtained a linear relationship, indicating that the rate of disappearance followed a logarithmic decay function.

However, this disappearance rate reflected both microbial degradation and passage from the rumen, making it difficult to separate the two processes.

To address this limitation and distinguish between digestion and passage, Smith et al. (1971) investigated fiber degradation in vitro using ruminal fluid. Six different forages and their respective isolated cell walls were incubated for varying time intervals up to 72 hours. The use of isolated cell walls enabled the researchers to assess whether the presence of cellular solubles affected fiber degradation. Since previous attempts to model degradation rates based on total residue remaining were unsuccessful (Blaxter et al., 1956), Smith et al. (1971) instead focused on the disappearance of the potentially degradable fraction. Their findings supported a strong linear relationship between time and the logarithm of the remaining degradable fiber, consistent with first-order kinetics. Furthermore, degradation rates (**K_d**) did not differ between samples with or without cell solubles, suggesting that these components do not influence the K_d of fiber in vitro.

Building upon previous research, Waldo et al. (1972) proposed that fiber consists of two fractions: an indigestible portion that can only exit the rumen via passage and a potentially degradable portion that is subject to microbial degradation and passage. The authors identified lignin as a key factor limiting the extent of fiber degradation, though it does not influence the rate of degradation. Consistent with first-order kinetics, the degradation of fiber was modeled based on the amount of degradable material remaining (Waldo et al., 1972). The authors noted a decline in NDF degradability over time, which they attributed to the gradual loss of accessible substrate. As degradation progressed, the remaining fiber became increasingly lignin-rich and indigestible, rather than indicating a change in the actual pdNDF degradation rate.

Mertens (1973) later refined this model by introducing a lag phase, representing a delay prior to the onset of degradation. Lag time was defined as the intercept of the first-order model with the degradation curve at 100% of the degradable pool. Mertens attributed this delay to the time required for particles to hydrate or undergo necessary structural and chemical changes to permit microbial attachment and enzymatic activity. Additionally, Merten's analysis of log-transformed degradation curves suggested the presence of two pools with their own respective K_d within the pdNDF pool, evidence that laid the groundwork for what later became the 3-pool model discussed in subsequent sections (Raffrenato and Van Amburgh, 2010).

As computational tools advanced, Nocek and English (1986) evaluated four approaches for estimating fiber degradation rates: (1) simple log-linear regression without correction for undegradable residue, (2) log-linear regression corrected for undegradable residue, (3) curve peeling, which separates individual degradation pools, and (4) nonlinear iterative least squares regression, which simultaneously estimates lag time and the potential extent and rate of degradation. Their findings reinforced the necessity of basing K_d calculations on the pdNDF fractions. While nonlinear regression and logarithmic transformation were deemed suitable for substrates with a single degradable pool, curve peeling proved more appropriate when multiple pools were evident. These methods hold true today.

1.3.2 The 3-Pool Model and Its Assumptions

As discussed previously, the 2-pool fiber degradation model has long served as a practical framework in ruminant nutrition, partitioning NDF into pdNDF and uNDF fractions with a single K_d applied to pdNDF. However, increasing recognition of kinetic heterogeneity within the pdNDF fraction has motivated the development of more refined models. The 3-pool model extends the 2-pool framework by dividing pdNDF into two discrete pools, a rapidly fermenting pool (**pdNDF1**) and a slowly fermenting pool (**pdNDF2**) with their own K_d (**K_d1** and **K_d2**, respectively), alongside uNDF (Raffrenato and Van Amburgh, 2010). Each pool is described by first-order kinetics and subject to a discrete lag time. This was represented mathematically as:

$$NDF_{(t)} = pdNDF1 \times e^{-K_d 1(T-L)} + pdNDF2 \times e^{-K_d 2(T-L)} + uNDF,$$

where $NDF_{(t)}$ is the NDF residue remaining at time T and L is the discrete lag time before degradation. This approach, formalized by Raffrenato and Van Amburgh (2010), was designed to more accurately represent the structural and chemical diversity of fiber and its variable accessibility to microbial degradation. The model is typically parameterized using in vitro incubation data with multiple time points extending to 240 hours to estimate the relative pool sizes and their K_d using nonlinear regression. These extended incubations are intended to capture the full extent of fiber degradation, particularly for the slowly fermenting pool, which may require over 120 hours for substantial breakdown (Raffrenato and Van Amburgh, 2010).

While the 3-pool model captures the differences in pdNDF degradation of forages that may be affected by the inherent structural and chemical differences in plant tissues and cells, it may not be biologically relevant in an applied nutrition setting. A recent study by Barry and Hall (2025) provided a direct comparison of the 2-pool and 3-pool models using NDF degradation data from 12 forages incubated at 11 time points in two commercial laboratories. Model fits were evaluated using residual sums of squares and Akaike Information Criterion, and predictions of ruminal NDF degradation were calculated across a range of physiologically relevant passage rates (**K_p**; 2 to 7%/h; Seo et al., 2006). Ruminal NDF degradation was estimated based on an equation derived from Waldo et al. (1972), $\frac{K_d}{K_d+K_p}$. While the 3-pool model yielded marginally better statistical fits, the differences in predicted ruminal NDF degradation between models were minimal and typically less than 1.2 percentage units (Barry and Hall, 2025).

Despite its conceptual appeal, the 3-pool model presents several challenges when applied to in vivo systems. Notably, the model assumes that both pdNDF1 and pdNDF2 are exposed to ruminal degradation over an extended period of time. However, ruminal retention time for most fibrous particles is limited to approximately 24 to 48 hours (Van Soest, 1994; NASEM, 2021). Barry and Hall (2025) utilized K_p values of 2 to 7%/h, which correspond to mean retention times of 50 to 14 hours, respectively. As a result, the slow pool may not be degraded to the extent predicted by the model, potentially leading to overestimation of digestible fiber. Barry and Hall (2025) further observed that the degradation rate of pdNDF2 frequently approached zero in certain forages, functionally rendering portions of this pool equivalent to uNDF. In these cases, the 3-pool model may artificially assign fiber that truly escapes ruminal fermentation as being potentially degradable and subsequently underestimate the uNDF pool. Additionally, the 3-pool model showed greater variability in parameter estimates across incubation replicates and laboratories, particularly for K_d2 and lag time, reducing reproducibility compared to the simpler 2-pool model (Barry and Hall, 2025).

From an applied perspective, the 2-pool model offers advantages in terms of simplicity, stability, and feasibility for commercial feed evaluation. It requires fewer incubation time points, generates more consistent parameters, and yields ruminal NDF degradation estimates that are generally comparable to those of the 3-pool model under typical ruminal passage rates. While the 3-pool model offers more mechanistic detail and may prove valuable in research investigating tissue- and cell-level differences in forages, its assumptions about fermentation time and microbial access warrant caution in practical application. The utility of either model should be judged in context, balancing model stability and the relevance of predictions within the physiological limits of the ruminant digestive system. For the context of the work presented in the following research chapters, the 2-pool model of fiber degradation with a lag phase was utilized following a simpler equation:

$$NDF_{(t)} = pdNDF \times e^{-K_d(T-L)} + uNDF.$$

1.3.3 *Integrating Fiber Fractionation and Degradation Kinetics*

The fractionation of NDF into pdNDF and uNDF provides valuable insight into the structural characteristics and fermentability of forages. These fractions, typically determined through long-term in vitro or in situ incubations, offer a means of evaluating the extent to which fiber may be accessible to microbial degradation within the rumen. Forages with less uNDF and greater pdNDF are generally considered more digestible as more material appears to be potentially degradable. However, while pdNDF and uNDF describe the theoretical degradability of fiber, they do not capture the dynamic nature of fiber utilization in the rumen. To better characterize this dynamic, K_d constants have been incorporated into fiber evaluation for characterizing forage quality, offering a temporal dimension to the otherwise static measures of fiber pool sizes. Yet, neither the relative digestible pool size nor K_d alone can reliably predict the nutritional value of a forage.

For example, forages with large pools of pdNDF may exhibit slow rates of degradation that may limit their utilization within the rumen. Alternatively, forages with fast rates of degradation may have large pools of uNDF, similarly limiting their utilization within the rumen. This complex relationship was portrayed by Varga and Hoover (1983), who used the model of Mertens and Loften (1980) to compare oat and barley NDF. Although the pdNDF of oats exhibited a faster K_d (27.0%/h) than barley (14.5%/h), its pdNDF fraction was significantly smaller (26.4 vs. 58.0 %NDF, respectively). Initially, it seemed oats were more digestible than barley via rates alone.

However, when both the rate and extent of NDF degradation were considered, barley provided more digestible NDF per hour (8.4%) than oats (7.1%).

Furthermore, these relationships are critical when comparing grasses and legumes. Legumes generally contain more uNDF than grasses due to the overall greater concentration of lignin within the cell walls (Galyean and Goetsch, 1993), and from this perspective, may appear to be less digestible than grasses. However, legumes also generally have faster rates of cell wall degradation (Galyean and Goetsch, 1993). It has been suggested that this phenomenon is due to the restricted localization of lignin and the greater proportion of core versus non-core lignin, minimizing the physical restriction of cell wall digestion in legumes (Moore and Cherney, 1986; Buxton and Russell, 1988; Jung, 1989). Perhaps total NDF utilization is limited, but the pdNDF may be utilized more effectively in legumes than grasses, depending on the specific forage and observed tissue. Discrepancies in pdNDF, uNDF, and K_d can differentially influence NDF utilization within the rumen and must be evaluated on an individual forage basis.

Of course, fiber utilization in vivo is further influenced by how long fiber particles remain in the rumen, a factor that is not captured by degradation kinetics alone. The next sections will explore ruminal passage rate and its role in determining whether pdNDF can be effectively fermented within the limits of ruminal residence time.

1.4 Fiber Passage Rate

It has been well-established in the literature that ruminal NDF degradation, and consequently total-tract degradation, is the result of the competing processes of degradation in and passage from the rumen (Waldo et al., 1972; Ellis et al., 1994; Van Soest, 1994; Jung and Allen, 1995). While the potential extent and rate of degradation of NDF are frequently evaluated, passage rate (**K_p**)

remains less commonly characterized than degradation kinetics, in part due to the methodological complexity and variability inherent in its estimation. This section briefly discusses the qualitative aspects of general digesta flow through the gastrointestinal tract, along with specific considerations for fiber passage from the rumen. It also addresses the challenges involved in quantitatively estimating fibrous digesta flow from the rumen using two primary methods.

1.4.1 *Qualitative Aspects of Digesta Passage*

Following ingestion, feed undergoes a complex and highly coordinated journey through the gastrointestinal tract, initiated by multiple, intermittent feeding bouts that can span up to 5 to 10 hours per day (Van Soest, 1994; Grant and Albright, 2000). In addition to ingestion, ruminants dedicate a significant portion of their time to rumination, often exceeding 8 hours per day (Van Soest, 1994; Grant and Albright, 2000). This process involves the regurgitation, remastication, and reswallowing of digesta, contributing to the physical breakdown of fibrous material and enhancing microbial access.

The primary site of microbial fermentation is in the reticulorumen, where consumed forages are mixed, stratified, and degraded (Sutherland, 1988; Van Soest, 1994). Digesta exit the reticulum through coordinated biphasic contractions, which occur roughly once per minute and drive partially digested material toward the omasum (Schalk and Amadon, 1928). Following transit from the omasum, some material flows directly to the abomasum via the reticular groove in the omasum, while other portions are temporarily retained as they move slowly through the omasal laminae. The passage of digesta into the abomasum itself has been characterized as occurring in irregular, pulsatile flows, described as “slow blobs and fast trickles” (Phillipson, 1939).

Within the abomasum, the acidic environment facilitates enzymatic degradation but also leads to differential retention of digesta components. There is evidence that solid particles are retained longer than solutes, resulting in a greater dry matter (**DM**) concentration of abomasal digesta (12 to 15%) compared to the chyme entering the small intestine (Boyne et al., 1956; Faichney, 1980). Rhythmic contractions of the abomasum propel digesta into the proximal duodenum, though reverse flow into the abomasum has been observed, particularly in the early segments of the intestine, due to muscular constriction and reflux (Ridges and Singleton, 1962).

As digesta progresses through the small intestine, it is mixed with bile, pancreatic secretions, and other endogenous fluids. This section of the tract serves as the principal site for enzymatic digestion and nutrient absorption, including water, amino acids, fatty acids, and carbohydrates that escaped microbial fermentation (Van Soest, 1994). Transit through the proximal three-fifths of the small intestine occurs relatively rapidly, while digesta tends to remain longer in the distal portion, likely due to increased absorptive interaction and reduced peristaltic intensity (Grovum and Williams, 1973).

Entry into the cecum and large intestine occurs in intermittent pulses, governed by peristaltic activity. Within the cecum, digesta are subject to extensive mixing in both forward and retrograde directions. Although digesta continuously flows into the colon, large evacuations from the cecum can occur several times per day, influencing overall flow patterns (Van Soest, 1994). As digesta advances through the colon, water is progressively reabsorbed, and undigested material consolidates into fecal matter. The frequency of defecation varies widely depending on intake level and overall kinetics of gastrointestinal flow, ranging from infrequent events to multiple eliminations per day (Van Soest, 1994).

While this section outlines the general flow of digesta through the ruminant gastrointestinal tract, it is important to recognize that the majority of fiber degradation occurs in the rumen, where microbial fermentation is most active (Archimède et al., 1997; Huhtanen et al., 2010). As such, the retention and passage dynamics of fiber within the rumen are particularly important for understanding its nutritional contribution. The physical and chemical properties of fiber, along with ruminal stratification and selective retention mechanisms, introduce unique complexities that warrant focused discussion. The following section explores these specific considerations governing passage from the rumen.

1.4.2 *Forage-Specific and Dietary-Level Factors Influencing Ruminal Fiber Passage*

When a forage particle enters the rumen, its fate is influenced by many parameters such as its initial size, structure, and chemical composition. During ingestion and rumination, mastication reduces particle size and increases surface area, which promotes microbial colonization and enzymatic attack (McAllister et al., 1994). As microbial degradation progresses at designated rates influenced by the particle's chemical composition and structure, its structural integrity weakens, making it more fragile and prone to further size reduction. These changes are also associated with alterations in buoyancy and density. As gas is released from interstitial spaces and moisture is absorbed, the particle becomes denser and less buoyant (Sutherland, 1988). Simultaneously, changes in hydration and density influence the particle’s location within the rumen, and as such, particles are not uniformly distributed. Larger, more buoyant particles tend to accumulate in the dorsal sac, forming the ruminal mat, where they are less likely to encounter the reticulo-omasal orifice. Over time, physical disruption and microbial degradation reduce particle size and increase density, facilitating movement toward the ventral sac where escape becomes more likely (Allen and Mertens, 1988; Sutherland, 1988). Once particles become a critical size, often between 1.18 and 2.36 mm, they are small enough to pass through the omasal orifice and exit the rumen when positioned appropriately (Poppi et al., 1981; Mertens, 1997). This buoyancy and size-selective retention mechanism allows extended fermentation time for large particles, particularly those from more lignified or slowly degradable forages. These processes are summarized in **Figure 1.1**, as adapted by Seo et al. (2009).

Forage type, plant maturity, and processing methods, such as chopping or pelleting, can affect both the rate and extent of forage fiber breakdown and consequently passage from the rumen at the individual forage level. These effects arise from alterations in physical structure, such as particle size and fragility, as well as chemical composition, such as lignin, both of which shape the interactions between fiber particles and ruminal stratification, microbial attachment, and escape potential. More fragile forages, such as legumes and immature grasses, tend to fragment rapidly and pass sooner. In contrast, mature grasses and fibrous crop residues exhibit greater lignification and structural rigidity, making them more resistant to breakdown and subject to prolonged retention in the rumen (Allen and Mertens, 1988). For example, Nelson and Satter (1992) compared early-bud and early-flower maturities of alfalfa hays and silages and found that advanced maturity increased ruminal retention time. Furthermore, grass silages generally have slower K_p and longer retention times than legume silages, even when matched for particle size, due to differences in cell wall composition and fragility (Krizsan et al., 2010; Kammes and Allen, 2012). Legumes such as alfalfa tend to shatter during mastication, producing smaller particles with faster passage, while grasses maintain structural integrity longer in the rumen (Akin, 1993).

In addition to forage characteristics, dietary context plays a critical role in modulating fiber passage. The forage-to-concentrate ratio influences the ruminal environment and digesta flow.

High-concentrate diets typically increase feed intake, perhaps due to lower ruminal fill and depressed stimulation of ruminal stretch receptors signaling satiety centers due to decreased physically effective fiber (Colucci et al., 1990; Van Soest, 1994; Dado and Allen, 1995). The increased feed intake leads to greater turnover of feed in the rumen and, by mass action, increases the K_p of all components within the rumen, potentially reducing the time available for microbial degradation (Ulyatt et al., 1986; Colucci et al., 1990).

These complex interactions between forage characteristics and dietary formulation highlight the necessity of evaluating passage kinetics not only at the whole-diet level but also for individual feed ingredients, as their interrelated effects can influence ruminal degradation and ultimately affect total-tract NDF degradation. This complexity also highlights the value of methods that enable forage-specific assessments, which are better suited to capture the biological nuances discussed here and which are further explored in the next section.

1.4.2 Methods of Estimating Ruminal Passage Rate

Estimates of ruminal K_p vary considerably due to differences in methodology, marker selection, model structure, and sampling location. These methodological disparities have contributed to challenges in interpreting results biologically, as it is often difficult to separate the true effects of animal and dietary factors from artifacts introduced by the experimental approach.

One primary method for estimating ruminal K_p is the marker dilution method. The underlying principle involves labelling a known fraction of feed with an inert external marker and tracking its dilution over time using a time series of samples to estimate its escape. The reliability of marker-based K_p estimation depends heavily on the physicochemical behavior of the marker and the method of its application. Ideally, external markers should be indigestible, remain associated with the undigested feed particles, mimic the flow characteristics of the labeled feed, and stay bound throughout the gastrointestinal tract (Ellis et al., 1994).

Early studies employed visible stains (Balch, 1950), where marked particles were manually recovered from feces by sieving and counted. However, this approach was highly labor-intensive and susceptible to subjective bias. Furthermore, subsequent research indicated that the size of the sieve used significantly affected estimates of retention time (Ellis and Huston, 1967). Advancements in marker chemistry have since introduced a range of externally applied and chemically quantifiable markers. Chromium-mordanted fiber, for example, emerged as one of the first widely adopted markers following its introduction by Udén et al. (1980). Chromium-mordanted fiber is prepared using chromium(III) salts that form coordination bonds with functional groups on plant cell wall polymers, resulting in a relatively stable complex that persists through both the rumen and acidic abomasum. However, mordanting renders the fiber more indigestible, preventing fermentation-induced alterations to particle size and buoyancy that can alter passage kinetics and effectively overestimate ruminal retention time (Ellis et al., 1982).

Rare-earth elements, such as ytterbium, lanthanum, dysprosium, and cerium, have become the preferred particulate-phase markers because of their flexibility, analytical traceability, and the ability to differentially mark different particulates simultaneously (Allen, 1982). These elements can be applied by spraying or soaking. Spraying offers simplicity, but several studies documented significant marker migration between larger and smaller particles and between marked and unmarked particles after ingestion (Dixon et al., 1983; Teeter et al., 1984). In contrast, soaking results in more stable binding and significantly reduces marker migration (Hartnell and Satter, 1979b; Mader et al., 1984), making it the preferred method of externally marking forages with rare-earth elements. Furthermore, removing solubles from forages prior to soaking enhances marker binding (Allen, 1982; Bernard and Doreau, 2000), which is why researchers commonly utilize extracted NDF residues in the marker dilution method. It is important to note that if NDF residues are used to track ruminal K_p, interpreting the resulting K_p as DM passage, as more recently done by Oberson et al. (2019), Lascano et al. (2016), and Castro et al. (2010), for example, may be incorrect. The neutral detergent procedure potentially alters DM fragility, buoyancy, and degradation. Because NDF is specifically marked, the resulting K_p may be more correctly identified as NDF passage. However, all undigested nutrients within a particle should, in theory, pass together, and this distinction may not be necessary.

Even with improved binding techniques, some solubilization of rare-earth markers may occur that could overestimate K_p, especially under low-pH conditions found in the abomasum (Allen, 1982; Combs et al., 1984; Turnbull and Thomas, 1987). Differences in the cation-exchange capacity of various forages may also affect marker binding, further influencing K_p estimates (Allen et al., 1985; Owens and Hanson, 1992). However, the likelihood of marker dissociation is reduced by rinsing externally marked particles in an acetic acid solution to remove loosely bound marker (Allen, 1982; Owens and Hanson, 1992; Bernard and Doreau, 2000). Moreover, studies indicate that the dissociation rate of rare-earth elements from fiber particles in the rumen is less than 1%/h (Hartnell and Satter, 1979a; Beauchemin and Buchanan-Smith, 1989; Bernard and Doreau, 2000), suggesting that marker loss plays only a minor role in estimated ruminal K_p when samples are taken proximal to the abomasum.

Sampling for estimating marker disappearance can be performed at various locations within the gastrointestinal tract after pulse-dosing externally marked feed particles, including the rumen, omasum, abomasum, duodenum, ileum, or feces, depending on the cannulated status of the animal.

However, due to potential marker dissociation under post-ruminal acidic conditions, this review focuses on modeling K_p from ruminal samples.

Early models of particulate passage assumed the rumen to behave as a single, homogenous compartment where all particles are equally available for outflow and follow an exponential distribution of retention times. This approach is mathematically straightforward, typically relying on a first-order exponential decay function to describe the disappearance of marker:

$$C_T = C_0 e^{-K_p T},$$

where C_T is the concentration of marker at time T and C_0 is the initial concentration of marker in the rumen (Ellis et al., 1994). Similarly, the slope of the linear relationship between log-transformed concentration and time yields the fractional K_p, and the reciprocal of fractional K_p defines the mean ruminal retention time (Ellis et al., 1994). This single-compartment model may oversimplify the physiological complexity of the rumen, which exhibits stratification and selective retention of particles based on size, density, and buoyancy. It also does not account for potential delays associated with particle degradation and reduction or the existence of multiple particle pools, if they are indeed relevant to modeling passage.

To address these limitations, more sophisticated models have been developed. In modeling marker disappearance from feces samples, the plot of log-transformed marker concentration versus time often exhibits a curvilinear line pattern, suggesting the presence of two pools or compartments. Grovum and Williams (1973) first introduced a two-compartment model via curve-splicing, interpreting the faster K_p as colonic and cecal passage and the slower K_p as ruminal passage. Ellis (1979) later provided a more nuanced interpretation, proposing that the first K_p reflects the entry of newly ingested particles into the large-particle pool, while the second K_p reflects movement into the small-particle pool within the rumen. Although this interpretation has not been widely adopted, it supports the possibility of multiple, size-dependent passage pools within the rumen, which has biological importance. Visual inspection of the plot of the log-transformed marker concentration over time can guide researchers in choosing between a single- and multi-compartment model.

Additionally, models incorporating gamma distributions of retention time within single and multi-compartmental models have been developed, as summarized by Pond et al. (1988). The equations used for modelling the concentration of marker remaining in a single compartment with various gamma distributions are summarized in **Table 1.1**. The use of gamma distributions is relevant when there is a lag in particle escape depending on the age of the particle, and again, can be assessed visually by plotting the data before model selection. An overall lag term can be introduced into the model by replacing t with t–L, similar to fiber degradation models. Ultimately, model selection should be based on statistical goodness-of-fit and the specific sampling location, as these factors influence the biological interpretation of K_p.

The second major approach for estimating K_p is the flux/compartmental pool method (Robinson et al., 1987; Ellis et al., 1994). Based on mass balance, this method assumes that all resident particles, regardless of age, have an equal chance of escape and that the rumen operates under steady-state conditions where the influx of indigestible material equals its outflux from the rumen. Under these conditions, the turnover rate (**TOR**) of indigestible entities, which exit the rumen solely by passage, is representative of K_p (Ellis et al., 1994). As such, the fractional K_p of indigestible entities is calculated either as the intake rate divided by the ruminal pool or, equivalently, as the outflux rate divided by the ruminal pool (Ellis et al., 1994). A key assumption is that ruminal evacuations to estimate ruminal pool sizes do not disturb normal ruminal function.

A limitation of this method is that it yields an aggregate K_p value for the total diet without differentiating among individual feed components.

Although different feed ingredients within the rumen may exhibit variable K_p values within the same rumen due to their chemical and physical properties, the various nutrients, i.e. pdNDF, uNDF, starch, and protein, within the same particle should theoretically share the same K_p. Despite this, the flux/compartmental pool method has been adapted to provide differential K_p for individual dietary nutrients. For example, Oba and Allen (2000) estimated the K_p (%/h) of NDF, pdNDF, uNDF, and starch using the following equation:

$$K_p = \frac{duodenal\ flow}{ruminal\ pool} / 24 \times 100$$

where the individual duodenal flow (kg/d) of a nutrient is divided by its ruminal pool size (kg) to extrapolate passage from the rumen. In a low NDF diet based on brown midrib corn silage, they reported that the K_p of NDF, pdNDF, uNDF, and starch were 3.55, 3.42, 3.73, and 15.70 %/h, respectively (Oba and Allen, 2000). Furthermore, Kammes et al. (2012) obtained negative K_p values for pdNDF utilizing this same method, a biologically implausible result that likely reflects model misapplication.

Essentially, this adapted flux/compartmental pool method estimates the overall escape of nutrients from the rumen, a process influenced by both degradation and passage. Therefore, describing this metric as passage rate is misleading in the context of the literature. Moreover, sampling from the duodenum may introduce errors because the omasum and abomasum differentially retain solids of various particle sizes before the digesta reaches the duodenum, as previously discussed. Therefore, the flux/compartmental pool method is best applied to indigestible entities, such as uNDF, where passage is the sole mechanism of disappearance (Ellis et al., 1994). Turnover rate is an appropriate metric to observe potentially digestible entities using this method by evaluating intake rates relative to ruminal pool sizes. However, it is crucial to acknowledge that the measured TOR reflects the combined effects of degradation and passage.

No methodology for estimating ruminal K_p is without its limitations, and there is currently no standardized approach. Meta-analyses have demonstrated that K_p estimated from the marker dilution method for individual ingredients tends to be faster than K_p estimated for the comprehensive diet using the flux/compartmental pool method (Seo et al., 2006; Krizsan et al., 2010; **Table 1.2**). This highlights the need to interpret K_p differentially based on the method utilized and brings into question the validity of each method. The variability in methods and their interpretations has led to inconsistencies within the literature. Future research should work toward standardizing K_p estimation methods to improve forage quality databases and to advance our understanding of fiber utilization in the rumen.

1.5 The Balance Between Fiber Degradation and Passage Rates

Collectively, the delicate balance between the K_d of the pdNDF within the rumen and the K_p of undegraded NDF from the rumen, in congruence with the balance of pdNDF and uNDF within the NDF fraction, will influence ruminal NDF degradation and effectively total-tract digestibility. Subsequently, these relationships can influence ruminal turnover, dry matter intake (**DMI**), enteric fermentation, and fecal waste products, which have implications for dairy cattle productivity and the environment.

1.5.1 Ruminal Degradability

Ruminal digestibility of fiber is largely dictated by the synchronization between degradation and passage rates. While K_d can be assessed via in situ or in vitro techniques, it is only meaningful when contextualized by ruminal retention time. Waldo et al. (1972) proposed a model to estimate the amount of cellulose digested in the rumen, incorporating both the digestion rate of potentially digestible cellulose (K_d) and the rate of passage from the rumen (K_p). Extrapolating this model to the more modern NDF fraction, the model is expressed as:

$$rNDFD = pdNDF \times \frac{K_d}{K_d + K_p}$$

where *rNDFD* is the percentage of NDF digested in the rumen and *pdNDF* is the percentage of NDF that is potentially degradable. This can be applied at the dietary level or the individual forage level when all three metrics are specifically known.

Mathematically, if pdNDF and K_d are held constant, then increasing K_p will decrease the ruminal digestibility of NDF. However, pdNDF and K_d are metrics that are highly variable. Comprehensively, all three of these metrics must be evaluated for forages to truly compare forage quality and understand their NDF utilization within the rumen. These relationships are further complicated at the individual forage level, as the K_p of individual forages can be influenced by the diet formulation, as previously discussed. From this standpoint, it is likely inappropriate to laterally compare the ruminal NDF degradation of individual forages with this method when their K_p is estimated in different diets, particularly when they differ in their forage-to-concentrate ratio. This additionally brings a nuanced discussion into developing a standardized methodology.

Even highly digestible forages can yield poor ruminal digestibility if K_p is elevated beyond the point where microbial fermentation can occur effectively (Allen and Mertens, 1988). For example, Teimouri Yansari et al. (2004) evaluated the influence of alfalfa hay particle size on K_p and digestibility. They fed midlactation dairy cattle diets containing 20% alfalfa hay (DM basis) chopped or ground to a theoretical length of 19, 10, or 2 mm. While K_d theoretically stayed the same, K_p increased from 2.93%/h for the 19-mm diet to 3.28%/h for the 2-mm diet, and the digestibility of NDF decreased from 61.3% for the 19-mm diet to 56.6% for the 2-mm diet (Teimouri Yansari et al., 2004).

However, increasing K_p does not always induce reduced NDF digestibility, particularly when K_d is simultaneously faster. Oba and Allen (2000) fed high-producing dairy cattle diets containing either a brown midrib variety of corn silage or a conventional corn silage at two concentrations of dietary NDF. At the dietary level, K_p was faster for the brown midrib corn silage diet compared to the conventional corn silage diet (3.64 vs. 3.2%/h) when evaluated with the flux/compartmental pool method, and ruminal NDF digestibility was not significantly affected (Oba and Allen, 2000). While they observed that the K_d of the pdNDF of the diets containing brown midrib corn silage was slower than that of the conventional corn diets, the method by which they estimated K_d was likely inaccurate. The flux/compartmental pool method was inappropriately used to differentially estimate the K_p of pdNDF, and this was subtracted from the TOR of pdNDF to extrapolate the K_d. Alternatively, several in vitro and in situ analyses of individual forages have consistently demonstrated that brown midrib forage varieties exhibit faster K_d than their conventional counterparts (Mustafa et al., 2004; Hassanat et al., 2007; Ferreira et al., 2022), and, therefore, diets largely composed of brown midrib corn silage likely demonstrate faster K_d compared to similar diets with conventional corn silage. Both the dietary K_d and K_p were likely faster for the brown midrib diet, in addition to a slightly greater pdNDF concentration (NDF basis; 63 vs. 59%), to maintain ruminal NDF digestibility.

To evaluate how diet formulation influences these relationships, Poore et al. (1990) fed increasing amounts of dietary concentrate and observed faster K_p and reduced ruminal NDF digestibility of forages with increasing concentrate. They fed diets containing 30, 60, or 90% of a concentrate based on flaked sorghum grain and a 50:50 mixture of wheat straw and alfalfa hay to cannulated steers and estimated the K_p of individual ingredients using external markers. Poore et al. (1990) observed that the K_p of wheat straw increased by 28% and alfalfa hay increased by 14% when the inclusion of concentrate increased from 30 to 90%. In addition to changes in K_p, the more acidic ruminal environment provided by the high-concentrate diet slowed K_d. The K_d of wheat straw slowed from 6.8 to 3.9%/h, and the K_d of alfalfa hay slowed from 4.1 to 2.8%/h (Poore et al., 1990). Subsequently, the estimated ruminal digestibilities of wheat straw and alfalfa hay were significantly reduced by 72% and 57%, respectively (Poore et al.,1990). Collectively, it is important to consider changes to ruminal kinetics at the dietary forage level and the impact of the ration composition.

1.5.2 Voluntary Dry Matter Intake

Neutral detergent fiber plays a vital role in ruminal function by forming the ruminal digesta mat. This mat is essential for stimulating rumination and saliva production, both which are essential for buffering ruminal pH and supporting microbial activity (Van Soest, 1994). However, high dietary NDF concentrations are typically associated with lower DMI, largely due to slower ruminal turnover. Ruminal turnover time is a function of both K_p and total digesta mass and is often inversely associated with DMI in forage-rich diets (Ellis et al., 1994; Allen, 1996). As summarized by Allen (2000), numerous studies have reported an inverse relationship between ruminal retention time, affected by either forage type or forage-to-concentrate ratio, and voluntary DMI.

For instance, Oba and Allen (1999) found that cows fed brown midrib corn silage, which contains reduced lignin and exhibits faster pdNDF degradation, had shorter ruminal turnover times and greater DMI compared to cows fed conventional corn silage. Similarly, Dado and Allen (1995) observed that increasing the dietary proportion of forage reduced K_p, extended ruminal retention, and ultimately limited DMI. In another study, Teimouri Yansari et al. (2004) showed that reducing alfalfa particle size increased K_p, decreased retention time, and enhanced DMI. Collectively, these findings highlight the influence of fiber degradation and passage kinetics on feed intake, likely mediated through changes in ruminal fill.

The effect of digesta turnover on intake is closely linked to the size of the ruminal solids pool. Faster turnover – either through faster K_p, faster K_d, or their collective action – can reduce the bulk of indigestible material retained in the rumen, thereby mitigating fill limitation (Kammes and Allen, 2012; Krämer et al., 2013; Huhtanen et al., 2016). This concept has been demonstrated experimentally through the artificial manipulation of ruminal fill. Campling and Balch (1961) inserted rubber bladders containing 23, 34, or 45 kg of water into the rumen of cannulated dry cows. As inert fill increased, voluntary hay intake declined linearly, at a rate of approximately 52 g/L of added volume. This effect is attributed to mechanoreceptors in the reticulorumen that detect distension and send inhibitory signals to the hypothalamus to suppress intake (Allen, 1996; Allen, 2000; Van Soest, 1994).

Further support for the ruminal fill hypothesis came from Dado and Allen (1995), who filled the rumen of cannulated cows with polyethylene containers filled with water equating to 25% of their pre-trial ruminal volume. When cows were fed a low-NDF diet (25% DM), DMI was unaffected by the additional inert fill. However, when fed a high-NDF diet (35% DM), DMI declined with inert fill (Dado and Allen, 1995). These findings suggest that the impact of ruminal fill on intake may additionally depend on physiological factors, such as the cow's energy demands and lactation stage (Allen and Piantoni, 2013), which are out of the scope of this review.

Consequently, the interaction between fiber degradation and passage also has important implications for milk production through its influence on DMI. Faster turnover of ruminal fiber can increase feed intake and energy supply, supporting greater milk yield (Van Soest, 1994). However, milk composition, particularly protein and fat, depends on the balance of nutrient utilization and the specific provision of amino acids and fatty acids absorbed post-ruminally (Van Soest, 1994). Therefore, optimizing both the quantity and quality of nutrients derived from ruminal fiber requires an integrated understanding of degradation kinetics, passage rates, and dietary formulation to support both milk volume and component yield.

1.5.3 Enteric Methane

Fiber degradation in the rumen is a major contributor to enteric methane (**CH_4**) emissions, as the fermentation of structural carbohydrates generates H_2 and CO_2, which serve as substrates for methanogenesis by archaea (Van Soest, 1994; Moss et al., 2000). The extent of enteric CH_4 production is thus closely tied to the degree of fermentation, particularly of NDF. A common hypothesis in the literature is that faster ruminal K_p in dairy cattle will result in reduced enteric CH_4 emissions (Moss et al., 2000; Brask et al., 2013; Wenner et al., 2017; Hristov et al., 2025). The ideology behind this postulate is that faster ruminal K_p reduces the mean ruminal retention time and therefore limits ruminal degradation of NDF. However, as previously discussed, ruminal degradation of NDF is not governed by K_p alone. It also depends on the concentration of pdNDF and its K_d. The interaction among these variables ultimately determines the amount of fermentable substrate available for fermentation and subsequent methanogenesis of its byproducts.

To experimentally assess the impact of ruminal K_p on CH_4 emissions, Wenner et al. (2017) used continuous culture fermenters to stimulate ruminal conditions and manually increased the solids K_p from 2.5 to 5.0%/h. This intervention indeed resulted in a 33% reduction in daily in vitro CH_4 production. While this finding supports the theoretical relationship between faster K_p and enteric CH_4, it is important to note that K_p cannot be manipulated independently in vivo. Changes in K_p achieved through diet formulation, forage selection, or physical processing will likely affect K_d as well. Therefore, depending on the relationship between K_d and K_p, NDF fermentation in the rumen may not necessarily be reduced to decrease enteric CH_4 production. Moreover, these interventions can alter total VFA production, shift the molar proportions of individual VFA, and influence microbial community structure, all of which affect hydrogen availability and methanogenic activity (NASEM, 2021; Terranova et al., 2024).

Another key consideration is that faster ruminal K_p generally promotes greater DMI by alleviating ruminal fill constraints, as previously discussed. Dry matter intake is one of the strongest predictors of enteric CH_4 emissions in dairy cattle, exhibiting a positive linear relationship that accounts for 60 to 80% of the variation observed among animals (NASEM, 2021). Therefore, even if increased K_p reduces enteric CH_4 emissions per unit of DMI, it may concurrently elevate total enteric CH_4 emissions through increased feed intake.

To date, no studies have directly evaluated the relationship between ruminal K_p and enteric CH_4 emissions in dairy cattle to support this postulate. Eslamizad et al. (2025) evaluated the partial replacement of high-fiber forages with corn silage and evaluated total gastrointestinal mean retention time and enteric CH_4 emissions. They observed that increasing corn silage from 31% DM to 38% DM in the diet reduced the total gastrointestinal residence time and decreased CH_4 yield (g/kg DMI) by 8% (Eslamizad et al., 2025). However, the study did not specifically evaluate ruminal residency time or K_p, nor NDF digestibility to support the idea that reduced ruminal fermentation as affected by fast K_p is the mechanism by which enteric CH_4 production is reduced by feeding high-quality forages. The study additionally observed that increasing corn silage decreased the acetate-to-propionate ratio due to greater starch provision and reduced NDF that likely explained the reduced enteric CH_4 per unit DMI. The overall production of enteric CH_4 per day, however, was not affected by increasing corn silage in the diet due to the resulting increased DMI (Eslamizad et al., 2025).

Compared to the extensive literature on chemical inhibitors such as 3-nitrooxypropanol and alternative feed additives like macroalgae, the interaction between ruminal kinetics and enteric CH_4 production remains relatively underexplored. This represents a significant knowledge gap, particularly given the emphasis placed on this proposed mechanism in CH_4 mitigation discussions surrounding forage quality (Hristov et al., 2013). Further research is needed to clarify the biological relevance of this postulate under practical feeding conditions and to better integrate it into strategies aimed at reducing greenhouse gas emissions from ruminant livestock.

1.5.4 Fecal Excretion of Potentially Digestible Material and Fecal Methane Emissions

Although fiber fermentation occurs predominantly in the rumen, a portion of pdNDF may escape and be fermented in the hindgut (Van Soest, 1994; NASEM, 2021). However, hindgut fermentation is typically less efficient and is associated with increased fecal energy loss (Van Soest, 1994). Therefore, the majority of pdNDF that escapes ruminal fermentation is excreted in the feces. Consequently, when K_p increases beyond the fermentative capacity of the rumen, the total-tract digestibility of NDF may decline. Huhtanen et al. (2009) and Nousiainen et al. (2009) extrapolated a negative association between ruminal K_p and total-tract NDF digestibility from meta-analyses, particularly in diets with high forage concentration and limited fermentability. However, K_p data was not specifically evaluated, leaving the causative relationship largely inferential.

When unfermented pdNDF is excreted, it remains a viable substrate for microbial activity during manure storage. Under anaerobic conditions, this undigested pdNDF can contribute substantially to CH_4 (Chadwick et al., 2000). Boadi and Wittenberg (2002) showed that manure from cows fed low-digestibility forages produced more CH_4 during storage, reinforcing the importance of maximizing fiber degradation within the rumen to mitigate downstream emissions. Furthermore, Darabighane et al. (2024) observed a 4%-unit reduction in pdNDF digestibility by supplementing rapeseed oil at 5% DM inclusion in high-forage (65% DM) and low-forage diets (35% DM) to dairy cattle. As a result, rapeseed oil supplementation decreased enteric CH_4 emissions by 15% but increased fecal CH_4 emissions by 15%, supporting the links between reduced ruminal NDF degradation, reduced enteric CH_4, but consequently increased CH_4 from feces due to undigested feed (Darabighane et al., 2024).

However, evaluating only digestibility percentages may obscure the absolute quantity of undigested pdNDF that enters manure systems. Even when ruminal digestibility improves, elevated DMI and high dietary pdNDF concentrations can lead to considerable excretion of fermentable NDF. For instance, Benchaar and Hassanat (2019) fed lactating dairy cattle diets containing either a brown midrib variety of corn silage, which has previously been shown to improve NDF digestibility, or conventional corn silage at 59% of the diet (DM basis). Despite the enhanced digestibility associated with brown midrib corn silage, cows excreted 2 kg more OM per day. Although pdNDF was not specifically quantified, the lower lignin concentration of the fecal NDF suggests a greater proportion of pdNDF. Subsequent anaerobic incubation of this manure with psychrophilic residual sludge resulted in greater NDF degradation and daily CH_4 production per unit of OM compared to the manure from cows fed the conventional corn silage (Bencharr and Hassanat, 2019).

The escape of pdNDF from ruminal fermentation and its subsequent microbial degradation during manure storage poses both energetic inefficiencies and environmental concerns. While strategies that enhance ruminal fiber digestibility are generally beneficial, high intake levels and diets rich in pdNDF can still result in considerable quantities of fermentable fiber entering manure systems. This highlights the importance of viewing fiber utilization not only in terms of ruminal or total-tract digestibility percentages, but also in terms of absolute mass flow. There may or may not be a trade-off in utilizing dietary strategies that increase K_p to reduce enteric CH_4. While faster K_p may reduce ruminal fiber fermentation and thus lower enteric CH_4 production, this same shift can increase the amount of pdNDF entering the manure stream, where it remains a substrate for methanogenesis under anaerobic conditions. As such, emissions that are avoided in the rumen may simply be displaced to manure storage systems. To accurately assess the net environmental impact of such interventions, future research must adopt more integrated approaches that simultaneously quantify both enteric and manure-derived CH_4 emissions. The lack of consistent relationships in the literature reflects the complex nature of fiber degradation and utilization, with multiple interacting factors operating at the level of individual forages and whole diets. A systems-level perspective is therefore essential for the development of effective CH_4 mitigation strategies in ruminant production.

1.6 Summary and Research Objectives

Collectively, ruminal digestibility of fiber is a complex system with many interrelating factors that cannot be completely isolated from one another. Generally, the ruminal digestibility and consequent total-tract digestibility of fiber rely on the intrinsic ratios of potentially degradable and undegradable material, the rate at which fiber degrades within the rumen, and the rate at which undigested particles escape the rumen. These relationships can have critical implications on nutrient utilization, dairy cattle productivity, and the environment (**Figure 1.2**).

The objectives of the following research studies were to evaluate the relationships between forage quality as affected by forage type and forage maturity, ruminal fiber degradation and passage rates, ruminal fermentation, and subsequent total-tract fiber degradation and their influence on CH_4 emissions from lactating dairy cattle. These results support the development of nutritional strategies in dairy production systems that enhance productivity while addressing environmental sustainability.

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1.8 Tables

Table 1.1. Modelling the concentration of marker remaining in one-compartment models with various gamma residence-time distributions for estimating ruminal passage rate as adapted by Pond et al. (1988)

Model	Residence-time distribution	Model expression[1]
G1→	Gamma-1 or Exponential	Ce^{-K_pT}
G2→	Gamma-2	$Ce^{-\lambda T}[1 + \lambda T]$
G3→	Gamma-3	$Ce^{-\lambda T}[1 + \lambda T + \frac{(\lambda T)^2}{2}]$
Gn→	Gamma-n	$Ce^{-\lambda T}\sum_{i=0}^{n-1}\frac{(\lambda T)^i}{i!}$

[1] C = initial concentration of marker in the compartment, K_p = rate parameter for exponentially distributed residence times, T = time after dose of marker, λ = rate parameter for gamma distributed residence time times, n = integer value of gamma distribution

Table 1.2. Typical ruminal passage rate (%/h) estimates based on either the marker dilution method or the flux/compartmental pool method determined from meta-analyses

Method	Mean	SD	Range
Marker dilution[1]			
Forages	4.00	1.40	1.30 – 7.40
Concentrates	6.20	2.00	2.40 – 10.20
Flux/compartmental pool[2]			
Dietary uNDF[3]	2.56	0.67	1.10 – 5.11

[1] Seo et al., 2006; evaluated 88 and 37 studies that used chromium-mordanted or rare earth element-marked forages and concentrates, respectively, to assess individual ingredient passage rate in different dietary contexts

[2] Krizsan et al., 2010; evaluated 172 studies that utilized undegraded neutral detergent fiber to assess dietary passage rate

[3] Undegraded neutral detergent fiber

1.9 Figures

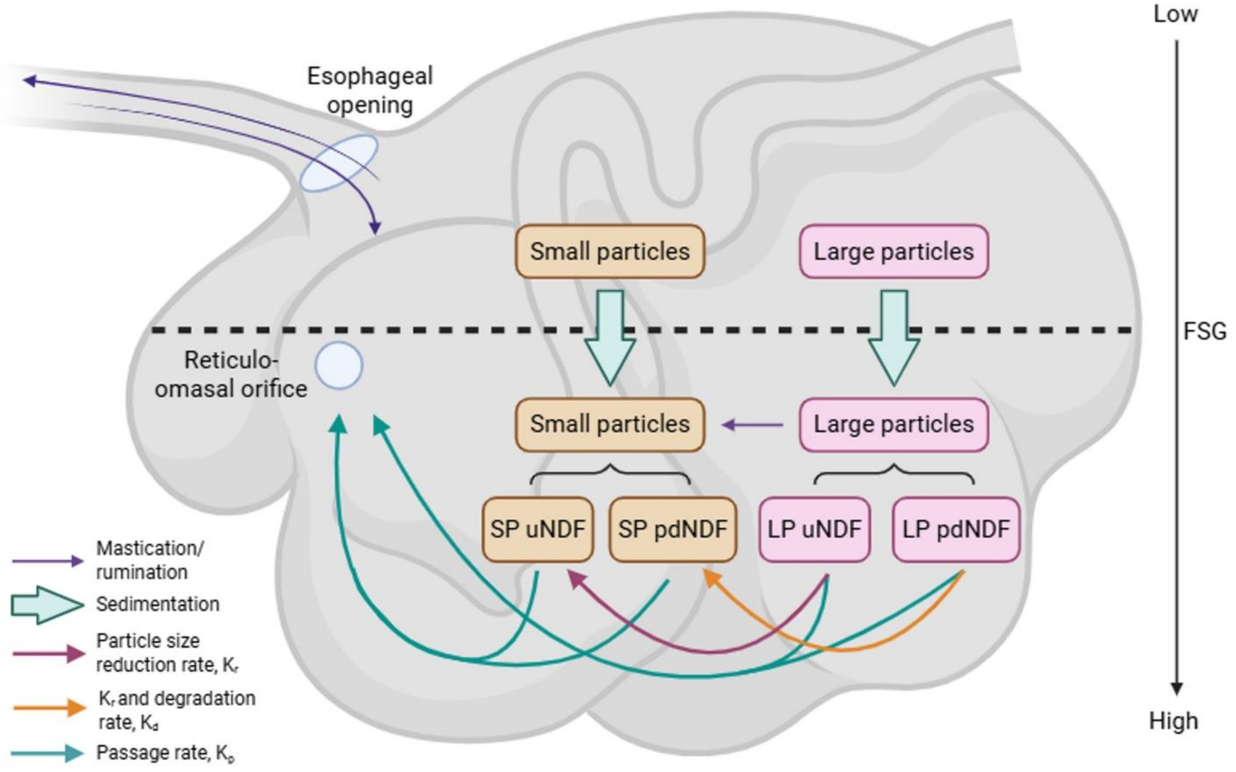

Figure 1.1. Conceptual pools of potentially degradable fiber (pdNDF) and undegradable fiber (uNDF) within small (SP) and large particles (LP) and the complex dynamics governing their passage from the reticulorumen, as partially adapted from Seo et al. (2009). The dorsal rumen (above the dotted line) is defined as the inescapable pool, and the ventral and cranial rumen and reticulum (below the dotted line) are defined as the escapable pool. The functional specific gravity (FSG) of a particle determines its location. Mastication and rumination, particle size reduction rate (K_r), and fiber degradation rate (K_d) describe functions that occur between conceptual pools, and passage rate (K_p) describes the function of passage from the reticulorumen.

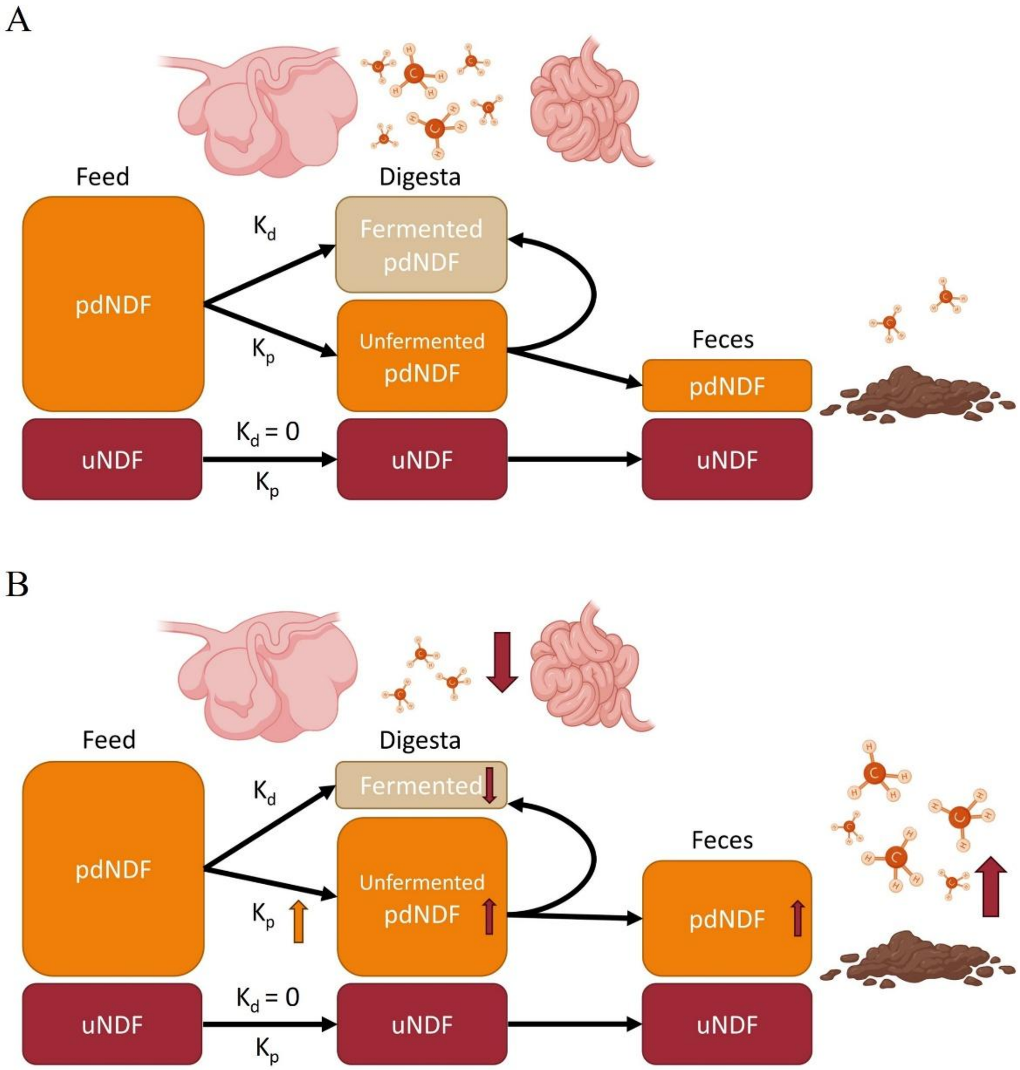

Figure 1.2. The interrelationships between potentially degradable fiber (pdNDF), undegradable fiber (uNDF), fiber degradation rate (K_d), and passage rate (K_p) within the rumen. Under "standard" conditions (A), ruminal fermentation of pdNDF yields substantive methane, little pdNDF is excreted, and there is minimal methane from feces. Under fast K_p conditions (B), it is often hypothesized that ruminal degradation of pdNDF is reduced, and subsequently enteric methane is reduced. However, it is hypothesized that more pdNDF is excreted in the feces resulting in greater methane emissions from fecal material. Yet, the exact result of increased K_p will be dependent on the initial pdNDF concentration and the K_d of pdNDF.

CHAPTER 2: Ruminal Passage Rate and Digestibility of Fiber from Dairy Cows Consuming Diets Containing Alfalfa and Orchardgrass Hays with Different Concentrations of Undegradable Neutral Detergent Fiber

2.1. Abstract

This study aimed to evaluate the effect of feeding diets with different fractions of undegraded NDF (**uNDF**) and potentially degradable (**pdNDF**) on ruminal NDF degradation and passage kinetics of lactating dairy cows. Six rumen-cannulated (533 ± 43 kg BW and 122 ± 15 DIM) and 6 non-cannulated (558 ± 62 kg BW and 126 ± 16 DIM) primiparous Holstein dairy cows were randomly assigned to 1 of 2 experimental diets in a crossover design with two 28-d periods. The experimental diets were formulated to include either alfalfa hay (**ALFA**) or orchardgrass hay (**ORCH**) in addition to corn silage. Rations were formulated to contain 30% NDF (DM basis), where the concentrate, corn silage, and each of the hays provided one third of the dietary NDF. The marker dilution technique was used to measure the passage rate using a pulse dose of marked corn silage fiber. On d 17 and 24 of each period, ruminal contents were evacuated to determine ruminal pool size. Following the return of the ruminal contents containing the pulse dose of marked corn silage to the rumen, ruminal grab samples were collected at 0, 3, 6, 9, 12, 24, 36, 48, 60, and 72 h. Samples from each time point were separated into solids and liquid, and the solids were analyzed for NDF, uNDF, and marker concentration. Alfalfa hay had a higher concentration of CP (16.4% vs. 10.7%) and a lower concentration of NDF (38.0% vs. 63.2%) than orchardgrass hay. Alfalfa hay had a greater concentration of uNDF than orchardgrass hay (36.5% vs. 32.8% uNDF; NDF basis). Cows consuming the ALFA diet had similar milk yield (39.1 kg/d) and similar milk fat and protein concentrations (3.72% fat and 3.24% protein, respectively) than cows consuming

the ORCH diet. Cows consuming the ALFA diet consumed more DM (26.7 vs. 24.6 kg/d) and uNDF (2.7 vs. 2.3 kg/d), than cows consuming the ORCH diet. Cows consuming the ALFA diet digested more NDF and pdNDF than cows consuming the ORCH diet (3.3 vs. 2.8 kg/d). Even though cows consuming the ALFA diet had a smaller pool size of NDF than cows consuming the ORCH diet (5.4 vs. 6.7 kg), the pool size of uNDF did not differ between groups (2.4 kg). Cows consuming the ALFA diet had a faster rate of passage than cows consuming the ORCH diet (5.02 vs. 4.03%/h). This translated into a shorter mean retention time for cows consuming the ALFA diet relative to cows consuming the ORCH diet (21.0 vs. 26.2 h). Overall, cows consuming diets containing alfalfa hay had a faster ruminal passage rate and a shorter mean retention time than cows consuming diets containing orchardgrass hay, and this occurred despite the greater concentrations of dietary uNDF in the alfalfa-based diet. These findings suggest that the kinetics of ruminal digestion and passage influence NDF degradation in ways beyond uNDF concentration or forage quality.

2.2. Introduction

Including sufficient but not excessive NDF in diets for high-producing dairy cows is paramount to ensuring ruminal health and maximizing DMI and milk fat secretion (Mertens, 1997; Allen, 2000; NASEM, 2021). In the 8th edition of *Nutrient Requirements of Dairy Cattle* (NASEM, 2021), the committee placed more emphasis on forage NDF than on dietary NDF and recommended including at least 15% to 19% forage NDF in the diets of lactating dairy cows (DM basis). However, no distinction exists concerning the quality of NDF (Kahyani et al., 2019; NASEM, 2021).

Neutral detergent fiber is composed of an undegradable NDF (**uNDF**) fraction and a potentially degradable NDF (**pdNDF**) fraction, such that NDF = pdNDF + uNDF. The uNDF fraction represents the residual NDF remaining after a 240 or 288 h of ruminal fermentation. According to the Lucas test (Van Soest, 1994; Raffrenato et al., 2018), uNDF has zero true digestibility and thus provides no nutrients to the host animal. In addition, uNDF only escapes the rumen through passage (Ellis et al., 1994; NASEM 2021), and the ruminal passage rate (**K_p**) of uNDF represents the rate of ruminal escape of uNDF. In contrast, pdNDF has the potential to provide nutrients to the host animal depending on the balance between the ruminal rates of degradation (**K_d**) and passage, meaning that most pdNDF not utilized by the animal due to a fast escape from the rumen ends up in the feces with the uNDF, as marginal amounts are fermented in the intestines (Huhtanen et al., 2006).

Without addressing NDF composition, different forages could be used indistinctly in dairy rations to fulfill the recommendations for dietary forage NDF. For example, providing 15% NDF from alfalfa hay would be indistinct from providing 15% NDF from grass hay (in both cases as a percentage of dietary DM). However, the NDF of grasses typically has a lower concentration of lignin and a lower concentration of uNDF than the NDF of alfalfa (NDF basis). This means that, relative to grasses, alfalfa has a lower concentration of pdNDF (Merchen and Bourquin, 1994; Buxton and Redfearn, 1997; Ferreira and Teets, 2020), which would provide less energy to the host animal per unit of NDF. Considering NDF composition, therefore, alfalfa seems to have an inferior quality to grasses.

Beyond the proportions of pdNDF and uNDF in NDF, the interaction between the rates of degradation and passage determines NDF digestibility, implying that the rate of degradation must not lag behind the rate of passage for optimal NDF degradation (Ellis et al., 1994). Kammes and Allen (2012b) reported that alfalfa has a faster passage rate and a shorter ruminal mean retention time (**MRT**) than orchardgrass (3.27%/h and 2.52%/h, respectively). Despite the greater uNDF concentration and shorter ruminal MRT (Kammes and Allen, 2012b), alfalfa also has a fast K_d compared with grasses (Ferreira and Teets, 2020), which means that ruminal microbes degrade the pdNDF of alfalfa more quickly than that of grasses (Kammes and Allen, 2012b). In summary, factors beyond cell wall lignification and uNDF concentration, such as NDF degradation rate and NDF passage rate, should be considered when including different forage classes in a ration.

Under the premise that alfalfa forages have a faster rate of degradation than grasses, we hypothesized that NDF digestion is greater for alfalfa-based diets than for orchardgrass-based diets despite the faster rate of passage of alfalfa. Therefore, this study aimed to evaluate the effect of feeding diets with different fractions of uNDF and pdNDF (i.e., alfalfa- and orchardgrass-based diets) on ruminal NDF degradation and the passage kinetics of lactating dairy cows. Under the premise that ruminal metabolism is affected by forage type, this study also aimed to evaluate the effect of forage type on milk fatty acid profile.

2.3. Materials and Methods

2.3.1. Animals, Housing, and Diets

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Virginia Tech (Protocol 22-167). We randomly assigned 6 rumen-cannulated (533 ± 43 kg BW and 122 ± 15 DIM) and 6 non-cannulated (558 ± 62 kg BW and 126 ± 16 DIM) Holstein dairy cows (all primiparous) to 1 of 2 experimental diets in a crossover design (Cochran and Cox, 1992) with two 28-d periods. All cows were housed in a 12-stall pen within a freestall barn. Cows were fed once daily (0830 h) via a Calan gate system (American Calan Inc., Northwood, NH). Cows were trained to find their specific door for a 7-d period before the beginning of the study. However, due to an inadequate adaptation to the Calan gate system, 2 non-cannulated cows were removed from the study before the completion of the first period.

The experimental diets were formulated to include either alfalfa hay (**ALFA**) or orchardgrass hay (**ORCH**) in addition to corn silage (**Table 2.1**). The alfalfa hay was purchased from a broker and came from Kansas, whereas the orchardgrass hay was purchased from a local farmer in Virginia. Upon arrival, grab samples of corn silage and core samples of both alfalfa hay and orchardgrass hay were collected and submitted for forage analyses to a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA). The outputs of these analyses were used to formulate the experimental diets (**Table 2.1**). The large square bales of alfalfa hay were chopped using a vertical mixer (FS600, NDEco, Sioux Falls, SD), whereas the round bales of orchardgrass hay were chopped using a hay chopper (Roto Grind 760, Burrows Enterprises, Greeley, CO).

Rations were formulated to meet the requirements of first-parity dairy cows (544 kg BW and 120 DIM) producing 38 kg of milk per day containing 3.80% fat and 3.05% protein using version

3.0.8.1 of CPM Dairy (CAHP Software Information, Philadelphia, PA). Both diets were formulated to contain 30% NDF (DM basis), where the concentrate, corn silage, and each of the hays provided one third of the dietary NDF. Concentrates were ordered from a commercial feed mill (Exchange Milling Company Inc., Rocky Mount, VA). Concentrates were mixed with the corn silage and their respective hays using a Calan Data Ranger (American Calan Inc.), and the TMR were delivered to feed ad libitum and to obtain ~5% of feed refusals. The amounts of feed offered and feed refused were measured daily. Cows were milked twice daily (0100 and 1200 h), and individual milk weights were automatically recorded at each milking. For statistical analyses, the average daily milk yield and DMI were determined from d 10 to 16 of each period.

2.3.2. Marked Fiber Preparation for Passage Rate

To measure the passage rate, we used the marker dilution technique (Cochran et al., 1987; Ellis et al., 1994). For this, a pulse dose of marked corn silage fiber was prepared following unpublished laboratory protocols from Dave Mertens' laboratory at the Dairy Forage Research Center (Madison, WI) with modifications. In brief, corn silage in its original form (i.e., non-ground) was dried at 55°C on mesh wire trays lined with cheesecloth and then sieved using the Penn State Particle Separator (Kononoff and Heinrichs, 2003) to collect grain-free fibrous material retained by the 8-mm and upper sieves. Next, ~750 g of corn silage fibrous material was subjected to a macro NDF procedure that consisted of boiling the material for 90 min in 20 L of a neutral detergent (FND20C, Ankom Technology, Macedon, NY) containing 200 g of sodium sulfite and 40 mL of α-amylase (FAA, Ankom Technology). To maximize the solubilization of nonfibrous components, the system was stirred every 10 min during boiling. The resulting fiber was rinsed twice with 20 L of boiling water containing 40 mL of α-amylase for 20 min and twice with 20 L of boiling water for 20 min. The resulting fibrous residue (hereafter referred to as macro-fiber) was dried in a forced-air oven at 55°C. Three batches of macro-fiber were then soaked overnight in 20 L of either lanthanum chloride ($LaCl_3$) for fiber allocated to ruminal evacuations on d 17 per period, or ytterbium chloride ($YbCl_3$) for fiber allocated to ruminal evacuations on d 24 per period. The $LaCl_3$ solution was prepared by La_2O_3 with HCl as described by Yang et al. (2017). The $YbCl_3$ solution was prepared in the same manner except that Yb_2O_3 was reacted with HCl under heat. After the overnight soak, the macro-fiber was removed and soaked in 20 L of 0.2 *M* acetic acid for 8 h to remove any loosely bound marker (Allen, 1982; Ellis et al., 1994). The resulting marked fiber was air-dried and stored until passage rate measurements. The marking procedure was repeated as necessary to provide enough marked fiber for 6 cows in each evacuation. The dosage (g/cow) of marked fiber allocated to each cow was 3% of the daily NDF intake, which was estimated as the individual average DMI from d 13 to 15 multiplied by 30% NDF. A 100% NDF concentration was assumed for the marked fiber.

2.3.3. Ruminal Pool Size and Passage Rate

On d 17 of each period, ruminal contents from 3 rumen-cannulated cows were evacuated at 2 h before feeding (0630 h), and ruminal contents from the other 3 rumen-cannulated cows were evacuated at 2 h after feeding (1030 h) to determine ruminal pool size. On d 24 of each period, cows were evacuated again at the opposite evacuation times to account for varying ruminal pool sizes respective to feeding time. During the ruminal evacuation, the entire contents of the rumen and reticulum were removed by hand into a 200-L drum and weighed. The contents were then separated into solid and liquid pools using a 20-L hydro press (EJWOX, Wilmington, CA), and the individual pools were weighed. A 200-g sample of the pressed solids was taken to determine the ruminal pool sizes of DM, NDF, and uNDF. The DM concentration of the liquid contents was considered minimal (<5% DM) and therefore ignored. To determine the DM pool size, the mass of pressed solids was multiplied by the DM concentration of the pressed solids. The ruminal pool sizes of NDF and uNDF were determined by the multiplying DM pool size by the respective concentration of NDF and uNDF of the pressed solids. Within each period, the pool sizes from d 17 and 24 were averaged per cow.

Immediately after collecting weights, the solid and liquid contents and a pulse dose of marked fiber were thoroughly mixed within the 200-L drum, and the reconstituted ruminal contents were manually returned to the rumen. Following the return of the ruminal contents to the rumen, grab samples (~400 g total) were collected from 5 locations within the rumen at 0, 3, 6, 9, 12, 24, 36, 48, 60, and 72 h. Samples from each time point were separated into solids and liquid using a 3-L fruit press (EJWOX). The solids were then dried in a forced-air oven at 55°C until reaching constant weight, then ground to pass a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ), and stored until NDF, uNDF, and La or Yb analyses.

Passage rate was determined as the dilution rate of the marker per cow in each evacuation using the NLIN procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) according to Equation 1:

$$Marker = Initial \times e^{(-K_p \times T)}, [1]$$

where *Marker* is the ratio of the marker relative to uNDF (mg/g uNDF) at time *T*, and *Initial* is the ratio of the marker relative to uNDF at *T* = 0 (mg/g uNDF). As a first step, the ratio of marker to uNDF over time was plotted in Microsoft Excel (Microsoft Corporation, Redmond, WA), and an exponential line was fitted. In several cases, the ratio of marker relative to uNDF at T = 0 seemed to be out of the fitted line, which leveraged down the intercept. This has been previously addressed by Ellis et al. (1994). In a follow-up study (unpublished data), we have concluded that an off ratio of marker relative to uNDF at T = 0 is attributed to the difficulty of properly mixing the pulse dose of marker fiber in the ruminal contents. When the ratio of marker relative to uNDF at T = 0 was suspiciously low from the visual assessment, an outlier test was performed following statistical procedures (Barnett and Lewis, 1994; University of Wisconsin, 2001). Briefly, an intercept (Y_{Pred}) and its standard deviation (S_{YPred}) were predicted including all the time points but without the suspected outlier (Y^*) at T = 0, and a *t*-test was performed to test the null hypothesis H_0: $Y^* = not\ outlier$, according to Equation 2:

$$t = \frac{(Y^* - Y_{pred})}{SE_{Y_{pred}}}. \ [2]$$

A 2-tailed *t*-test with an α equal to 0.05 and 8 degrees of freedom was used to determine a critical value equal to 2.306. When the *t* value was greater than 2.306, the null hypothesis was rejected, the observation Y^* was removed as an outlier, and the predicted intercept Y_{Pred} was used to determine the passage rate. The estimates of passage rate from d 17 and 24 were averaged per cow in each period. Ruminal MRT was calculated as MRT = $1/K_p$.

2.3.4. Nutrient Digestibility

Apparent total-tract nutrient digestibility was estimated using dietary uNDF as an internal marker. In each period, individual fecal grab samples were collected every 6 h (skipping 2 h every 4 samples) from d 13 to 16. Fecal samples were dried at 55°C in a forced-air oven for at least 96 h and pooled per cow. The pooled sample was ground to pass through a 1-mm screen of a Wiley mill and stored until analysis. Apparent DM digestibility (*DMD; %*) and nutrient digestibilities (*NutrD; %*) were calculated according to Equation 3 and Equation 4, respectively:

$$DMD = 100 - \frac{Dietary\ [uNDF]}{Fecal\ [uNDF]} \times 100, \ [3]$$

$$NutrD = 100 - \frac{Dietary[uNDF]}{Fecal[uNDF]} \times \frac{Fecal[Nutr]}{Dietary[Nutr]} \times 100, [4]$$

where [*uNDF*] is the concentration of uNDF (% DM) in the TMR or feces and [*Nutr*] is the concentration of either CP, NDF, pdNDF, or starch (% DM) in the TMR or feces.

2.3.5. Ruminal In Situ NDF Degradability

The rates of degradation of alfalfa hay, orchardgrass hay, and corn silage were determined using an in situ ruminal degradation study according to Ferreira et al. (2022). A 0.25-g sample of each forage was placed in a filter bag (F57, Ankom Technology) previously rinsed with acetone. Duplicate samples were incubated for 0, 3, 6, 12, 24, 48, 96, and 240 h in the rumen of 2 rumen-cannulated lactating dairy cows consuming a ration containing 40% corn silage, 10% grass hay, and 50% concentrate, resulting in 4 replicates per sample for each time point. All bags were inserted simultaneously at feeding time (0900 h) into the rumen and extracted at designated time points. After extraction, the bags were rinsed with tap water in three 5-min cycles using a washing machine (SKY2767, Best Choice Products, Irvine, CA), then dried in a forced-air oven at 55°C. The ash-free NDF residue was then weighed.

The K_d of pdNDF (%/h) was determined using the NLIN procedure of SAS according to Equation 5:

$$ISNDFD = pdNDF \times \{1 - e^{[-K_d \times (T - Lag)]}\}, [5]$$

where *ISNDFD* is the degraded NDF (% NDF) at time *T* when *T* is greater than *Lag*, *Lag* is the discrete lag time of NDF degradation (h) (Mertens, 1973), and *pdNDF* is the potentially degradable NDF (% NDF) during a 240-h ruminal fermentation. The discrete lag time was estimated using simple linear regression as $ISNDFU = a + b \times ln_{(T)}$, where *ISNDFU* is the undegraded NDF (% NDF) at time T, *a* represents the intercept, and *b* is the predicted slope. The discrete lag time was derived according to Equation 6:

$$Lag = e^{\left[\frac{(a-100)}{-b}\right]}. [6]$$

2.3.6. Sample Collection and Analysis

Samples of feed ingredients and TMR were collected weekly and composited per period. Composited samples of feed ingredients and TMR were dried in a forced-air oven at 55°C until constant weight and ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific). The concentration of ash was determined after combusting samples in a furnace (Thermolyne 30400, Barnstead International) for 3 h at 500°C (method 942.05; AOAC International, 2019). The concentration of CP was calculated as percent N × 6.25 after combustion analysis of N (method 990.03; AOAC International, 2019) using a Vario El Cube CN analyzer (Elementar Americas Inc.). The concentration of ash-free NDF was determined using the Ankom200 Fiber Analyzer (Ankom Technology) with sodium sulfite and α-amylase (Ferreira and Mertens, 2007). Forage samples were additionally analyzed for ADF and ADL, which were determined sequentially. Concentrations of ADF were determined using the Ankom200 Fiber Analyzer. After determining ADF residue weights, residues were incubated for 3 h in 72% sulfuric acid within a 4-L jar that was placed in a DaisyII Incubator (Ankom Technology). Starch concentration was determined using the acetate buffer method (Hall, 2009) with α-amylase from *Bacillus licheniformis* (FAA, Ankom Technology) and amyloglucosidase from *Aspergillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland). The concentration of uNDF in TMR, feces, and ruminal samples was determined by placing 0.25 g of samples in filter bags (F57, Ankom Technology) previously rinsed in acetone. Duplicate bags were incubated for 240 h in the rumen of 2 lactating rumen-cannulated Holsteins consuming a ration containing 40% corn silage, 10% grass hay, and 50% concentrate.

Milk samples (a.m. and p.m. milkings) were collected on d 21 and 22 of each period. Concentrations of fat, true protein, lactose, and MUN were analyzed by Lancaster DHIA (Mannheim, PA). Energy-corrected milk yield (kg) was estimated by deriving the equation of Tyrrell and Reid (1965) as described in Equation 7:

$$ECM = 0.327 \times MY + 12.95 \times FY + 7.65 \times PY, [7]$$

where MY is the daily milk yield (kg), FY is the daily milk fat yield (kg), and PY is the daily protein yield (kg). Additional milk samples (a.m. and p.m. milkings) were collected on d 21 to determine the milk fatty acid profile. Milk fatty acids were extracted and methylated according to Chouinard et al. (1999) and analyzed as described by Yang et al. (2017).

The concentrations of lanthanum (La) and ytterbium (Yb) in ruminal contents were determined by inductively coupled plasma optical emission spectrometry (Arcos II ICP-AES, Spectro Analytical Instruments GmbH). Briefly, 2.0 g of the sample was placed in a 50-mL glass beaker and combusted at 500°C for 5 h. The resulting ash was dissolved in two 12.5-mL aliquots of HCl. After 1 h, the dissolved ash was transferred to a tared 50-mL plastic tube, while rinsing the beaker with 41.7 m*M* LiOH until the combined solution weighed 50 g. Following overnight sedimentation, an aliquot of the solution was diluted (1:10 dilution) with deionized water and analyzed for La or Yb.

2.3.7. Statistical Analysis

Before designing the experiment, a statistical power analysis was performed using the POWER procedure of SAS (SAS version 9.4, SAS Institute Inc.). Considering a statistical power equal to 0.80, a probability of committing a type I error (α) equal to 0.10, and an SD equal to 0.45%/h (Kammes and Allen, 2012b), 8 cows per treatment within a main effect were deemed sufficient to detect a 1.00%/h-difference in passage rate between the 2 treatments. Considering the availability of labor and resources (i.e., rumen-cannulated cows), a sample size equal to 6 was finally decided.

All variables were analyzed using the MIXED procedure of SAS (SAS version 9.4, SAS Institute Inc., Cary, NC). The statistical model for the variables of interest included the fixed effect of diet (df = 1) and the random effects of cow (df = 5 or 9), period (df = 1), and the random residual error. For ruminal fill and passage rate variables, a significant difference was declared at $P < 0.10$, and a tendency to significantly differ was declared at $P < 0.15$. For the remaining variables, a significant difference was declared at $P < 0.05$, and a tendency to significantly differ was declared at $P < 0.10$.

2.4. Results and Discussion

In general, alfalfa hay contains greater concentrations of CP and substantially lower concentrations of NDF than cool-season grasses (NASEM, 2021). In this study, the alfalfa hay had a higher concentration of CP (16.4 ± 4.5% vs. 10.7 ± 1.0% CP) and a lower concentration of NDF (38.0 ± 1.0% vs. 63.2 ± 0.3% NDF) than orchardgrass hay (**Table 2.2**). Despite having a lower concentration of NDF, alfalfa hay had a slightly higher concentration of ADL (7.0 ± 1.2% vs. 5.8 ± 0.1% ADL; DM basis). Relative to NDF, alfalfa hay had a substantially greater concentration of ADL than orchardgrass hay (18.4 ± 3.6% vs. 9.2 ± 0.2% ADL), which indicates a greater degree of lignification of the cell wall in alfalfa hay. Coherently, alfalfa hay had a greater concentration of uNDF than orchardgrass hay (36.5 ± 6.6% vs. 32.8 ± 1.8% uNDF; NDF basis), although this difference was lower than in other studies (Kammes and Allen, 2012b; Ferreira and Teets, 2020). Alfalfa hay had a substantially shorter lag time (0.7 ± 0.7 vs. 6.0 ± 1.2 h) and a faster K_d (7.46 ± 1.2 vs. 4.95 ± 1.2%/h) than orchardgrass hay. Both alfalfa hay and orchardgrass hay had marginal concentrations of starch (3.0 ± 0.3% and 3.1 ± 0.2% starch, respectively).

Cows consuming the ALFA diet produced similar amounts of milk (39.1 kg/d, $P = 0.26$) to cows consuming the ORCH diet (**Table 2.3**). The concentrations of fat (3.72%, $P = 0.63$), true protein (3.24%, $P = 0.73$), and lactose (4.85%, $P = 0.71$) in milk did not differ between cows consuming the ALFA diet and cows consuming the ORCH diet. Similarly, the yields of milk fat (1.43 kg/d, $P = 0.42$), milk protein (1.26 kg/d, $P = 0.34$), and milk lactose (1.90 kg/d, $P = 0.32$) did not differ between cows consuming the ALFA and ORCH diets. All these results converged into similar energy-corrected milk yields (40.9 kg/d, $P = 0.26$) between both groups of cows. From a ration formulation perspective, we expected similar production performances among treatments given the similar nutritional composition of the diets (**Table 2.1**). However, cows consuming the ALFA diet had lower concentrations of MUN than cows consuming the ORCH diet (14.1 vs. 15.5 mg/dL, $P = 0.02$). A similar observation was reported by Ferreira and Teets (2020), although it was not observed by Kammes and Allen (2012b). We attribute the lower MUN concentration to a faster passage rate, a greater NDF digestibility (Miller et al., 2021), or a combination of these.

Cows consuming the ALFA diet consumed more DM than cows consuming the ORCH diet (26.7 vs. 24.6 kg/d, $P < 0.01$; **Table 2.4**). However, cows consuming the ALFA diet consumed similar amounts of NDF (8.0 kg/d, $P = 0.26$), consumed greater amounts of uNDF (2.7 vs. 2.3 kg, $P < 0.01$), and tended to consume smaller amounts of pdNDF (5.3 vs. 5.6 kg/d, $P = 0.06$) than cows consuming the ORCH diet. We attribute the similar intake of NDF to the slightly lower NDF concentration in the ALFA diet (30.3 ± 0.6% vs. 32.0 ± 0.4% NDF; **Table 2.1**), which may have allowed some additional DMI (Allen, 2000). A combination of a greater DMI and a greater concentration of uNDF (32.4 ± 1.1% vs. 25.8 ± 1.4% uNDF; **Table 2.1**) explains the greater intake of uNDF from cows consuming the ALFA diet (**Table 2.4**). The tendency to consume less pdNDF by cows consuming the ALFA diet is surprising, as a greater DMI should have compensated for the lower concentration of pdNDF. Cows consuming the ORCH diet had a greater feed efficiency than cows consuming the ALFA diet (1.65 vs. 1.56 kg ECM/kg DM, $P < 0.01$). A higher feed efficiency on grass-based diets, relative to alfalfa-based diets, was also reported in previous studies (Kammes and Allen, 2012b; Ferreira and Teets, 2020), although the reasons for these observations are not clear. The partition of nutrients to adipose tissue might explain these differences. It is worth highlighting that the partition toward tissue accretion cannot be determined in this study and the cited short-term studies (Kammes and Allen, 2012b; Ferreira and Teets, 2020).

Despite the lower concentration of pdNDF of the ALFA diet, cows consuming the ALFA diet digested more NDF than cows consuming the ORCH diet (3.3 vs. 2.8 kg/d, $P = 0.02$; **Table 2.4**). The greater total-tract NDF digestibility (40.4% vs. 35.9%, $P = 0.04$) and total-tract pdNDF digestibility (61.3% vs. 50.4%, $P < 0.01$) of the ALFA diet explain these observations. To compensate for the lower pdNDF intake, the dietary pdNDF must have degraded at much faster rates for the ALFA diet relative to the ORCH diet, and this is confirmed by the greater total-tract digestibilities of NDF and pdNDF of the ALFA diet relative to the ORCH diet.

Even though cows consuming the ALFA diet had a smaller ruminal pool size of NDF than cows consuming the ORCH diet (5.4 vs. 6.7 kg, $P = 0.03$), the pool size of uNDF did not differ between groups (2.4 kg, $P = 0.99$; **Table 2.5**). Ruminal pool sizes in this study agree with those of lactating dairy cows consuming total-mixed rations estimated by others. Kammes and Allen (2012c) reported pools of DM being 11.4 kg and 12.8 kg, NDF being 6.0 kg and 7.3 kg, and uNDF being 2.8 kg and 4.2 kg when feeding diets containing early and late harvest orchardgrass silage, respectively, and when they averaged pools before and after feeding. The same group found in feeding orchardgrass silage of different chop lengths that the pools of DM, NDF, and uNDF were 12.3 kg, 5.6 kg, and 3.2 kg on average, respectively (Kammes and Allen, 2012a). Because uNDF can only escape the rumen through passage to the intestine (Ellis et al., 1994; NASEM 2021), only a faster passage rate could explain a similar pool size in both groups of cows, especially given the greater uNDF intake of cows consuming ALFA. In agreement with this concept, cows consuming the ALFA diet had a faster rate of passage than cows consuming the ORCH diet (5.02%/h vs. 4.03%/h, $P = 0.06$). This translated into a shorter MRT for cows consuming the ALFA diet relative to cows consuming the ORCH diet (21.0 vs. 26.2 h, $P = 0.09$). Similar to our study, Kammes and Allen (2012b) also reported faster passage rates for cows consuming alfalfa-based diets relative to grass-based diets, even when the former contained greater concentrations of uNDF.

Traditionally, diets are formulated for NDF concentration and ignore the uNDF of the diet (NRC, 2001). The *8th edition of Nutrient Requirements of Dairy Cattle* (NASEM, 2021) placed more emphasis on forage NDF than on total NDF, although no distinction exists concerning the quality of NDF. In either case, diets formulated for similar NDF or forage NDF concentrations may contain more uNDF when using alfalfa forages compared with alternative grass forages (Kammes and Allen, 2012b; Ferreira and Teets, 2020). In this study, we expected that cows consuming diets with greater concentrations of uNDF in the NDF fraction (i.e., ALFA diet) would have a greater ruminal pool size of uNDF than cows consuming diets with lower concentrations of uNDF in the NDF fraction (i.e., ORCH diet). Contrarily, the pool size of uNDF did not differ between diets, which indicates that ruminal degradation of NDF depends on degradation and passage kinetics rather than just the proportions of pdNDF and uNDF within NDF. In this study, even though it contained more uNDF on an NDF basis, the alfalfa hay had a shorter discrete degradation lag and a faster pdNDF degradation rate than the orchardgrass hay (**Table 2.2**). Collectively, all these results highlight that the balance between the rates of degradation and passage may be as relevant as NDF composition in determining ruminal NDF utilization.

Despite the similar NDF intake, we observed a larger ruminal NDF pool size for cows consuming the ORCH diet than for cows consuming the ALFA diet. The filling effect of NDF due to a longer lag of degradation of pdNDF, a slow rate of degradation of pdNDF, and a slow passage rate in cows fed the ORCH diet can explain the difference in DMI observed between treatment groups. Alternatively, the rapid turnover due to a short lag of degradation of pdNDF, a fast rate of degradation of pdNDF, and a fast rate of passage in the ALFA diet can also explain the difference in DMI observed between treatment groups. Under both perspectives, the kinetics of ruminal degradation and passage seem to play a role that goes beyond the concentration of uNDF in NDF. Measuring the particle size distributions in the forages would have helped our interpretations. As we have not determined these, we recognized this limitation in this study.

The National Academy of Science, Engineering, and Medicine (NASEM, 2021) recommends using forage NDF as a means to provide adequate physically effective fiber to ensure ruminal health. In this study, we formulated the diets to contain equivalent forage NDF, with hay and corn silage each contributing one third to the dietary NDF. Because the alfalfa hay contained 38.0% NDF and the orchardgrass hay contained 63.2% NDF, we avoided providing equivalent hay levels on a DM basis and subsequently needing to significantly alter the concentrate composition to accommodate differences in forage NDF (Ferreira and Teets, 2020). Thus, the forage-to-concentrate ratios of the diets inherently differed, with the ALFA diet containing 54% forage and 46% concentrate and the ORCH diet containing 46% forage and 54% concentrate. Although we recognize that the difference in forage-to-concentrate ratio may have affected ruminal degradation and passage kinetics of NDF, we also highlight that this is inevitable when formulating solely based on NDF or forage NDF. Despite the different forage-to-concentrate ratios, the concentrates contributed to 23% and 17% of the dietary uNDF (DM basis) in the ALFA and ORCH diets, respectively, which can be considered a minor difference from the perspective of NDF digestion and passage kinetics.

To interpret the kinetics of ruminal degradation and passage, it seems important to highlight the structure of the forage plant. Ellis et al. (1994) suggested that pdNDF and uNDF of a same forage fragment can be assumed to have similar rates of passage. Leaves from alfalfa and leaf blades from cool-season grasses have 9.4% and 8.6% uNDF (NDF basis), respectively, whereas stems from alfalfa and cool-season grasses have 60.2% and 45.7% uNDF, respectively (unpublished data from Ferreira's laboratory). Although pdNDF and uNDF in a specific tissue might have similar rates of passage, the fact that most uNDF is concentrated in the stem rather than in the leaves challenges the assumption that pdNDF and uNDF have similar rates of passage at the whole plant or ration level. Given the pdNDF/uNDF ratio is lower in alfalfa stems than in grass stems, the degradation of pdNDF of the whole plant is less dependent on uNDF in alfalfa than in grasses. Therefore, differences in the structure of the whole plant can also explain the faster degradation of pdNDF in alfalfa.

Van Soest (1994) suggested that faster ruminal passage rates could result in increased excretion of potentially digestible solids such as pdNDF in feces. From an environmental perspective, an elevated excretion of fermentable substrate may increase methane emissions from manure (Benchaar and Hassanat, 2019). In this study, the feces from cows consuming the ALFA diet contained lower concentrations of NDF than the feces from cows consuming the ORCH diet

(46.1% vs. 52.2%, P < 0.01; **Table 2.6**). In addition, the fecal NDF from cows consuming the ALFA diet contained less pdNDF than the fecal NDF from cows consuming the ORCH diet (42.9% vs. 54.7%, P < 0.01). This last observation suggests that there is less potentially degradable substrate in feces from cows consuming the ALFA diet compared with those from cows consuming the ORCH diet. It is important to highlight that the cows consuming the ALFA diet also consumed more DM than the cows consuming the ORCH diet. Assuming that uNDF intake equals the amount of uNDF excreted daily, the amounts of NDF and pdNDF excreted by cows consuming the ALFA diet would be 4.7 kg and 2.0 kg, respectively, whereas the amounts of NDF and pdNDF excreted by cows consuming the ORCH diet would be 5.1 kg and 2.8 kg, respectively. Therefore, despite exhibiting a faster passage rate and consuming more DM, cows consuming the ALFA diet released less pdNDF in the feces than cows consuming ORCH diet, which deserves further investigation from an environmental perspective.

As mentioned previously, cows consuming the ALFA diet had lower concentrations of MUN and digestibility of CP than cows consuming the ORCH diet. Ferreira and Teets (2020) also reported lower MUN concentrations for cows consuming diets containing alfalfa hay relative to cows consuming diets containing grass hay. By contrast, Kammes and Allen (2012b) reported similar MUN concentrations for cows consuming alfalfa-based and grass-based diets. A greater flux of nitrogen toward the small intestine due to a faster passage rate may reduce the recycling of nitrogen within the rumen, therefore linking the ruminal metabolism of nitrogen to ruminal passage rate. In this study, a greater flux of nitrogen to the small intestine could also explain the lower CP digestibility (**Table 2.4**) and the lower concentration of fecal NDF (**Table 2.6**) for cows consuming the ALFA diet.

In this study, we also evaluated the effect of the diets on milk fatty acid profile (**Table 2.7**) to relate forage quality to ruminal metabolism of fatty acids and fatty acid synthesis in the mammary gland. The diets in this study contained low concentrations of fatty acids (3.7 ± 0.5% and 4.6 ± 0.8% fatty acids for ALFA and ORCH, respectively; **Table 2.1**). The low dietary concentration of fatty acids and adequate provision of forage NDF translated into a low risk of milk fat depression. Both groups of cows had similar changes in the ratios between saturated, monounsaturated, and polyunsaturated fatty acids between the diet and the milk fatty acid profile. Comprehensively, these observations provide little evidence linking ruminal fatty acid metabolism and ruminal degradation and passage kinetics.

2.5. Conclusions

In conclusion, cows consuming diets containing alfalfa hay had a faster ruminal passage rate and a shorter MRT than cows consuming diets containing orchardgrass hay, and this occurred despite the greater concentrations of dietary uNDF in the alfalfa-based diet. The faster passage rate for the alfalfa-based diet seems to be related to the fast degradation rate of the pdNDF in alfalfa-based diets. Overall, these findings suggest that the kinetics of ruminal degradation and passage influence NDF degradation in ways beyond uNDF concentration or forage quality.

2.6. Notes

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2.8. Tables

Table 2.1. Ingredients and nutritional composition of diets containing either alfalfa hay (ALFA) or orchardgrass hay (ORCH)

Item	ALFA	ORCH
Ingredient, %DM		
Corn silage	29.4	30.9
Alfalfa hay	24.1	-
Orchardgrass hay	-	15.2
Corn grain	17.0	14.5
Soybean meal	8.4	9.9
Canola meal	10.3	-
Soybean hulls	5.1	0.8
Corn distillers grains with solubles	-	23.6
Supplemental fat	2.2	1.8
Calcium carbonate	0.7	0.7
Salt	0.5	0.5
Sodium bicarbonate	1.0	1.0
Magnesium oxide	0.2	0.2
Trace mineral premix[1]	0.5	0.5
Sodium selenite (0.06% Se)	0.5	0.5
Vitamin ADE[2]	0.05	0.05
Ionophore[3]	0.01	0.01
Composition[4], %DM		
OM	97.7 (0.1)	97.9 (0.6)
CP[5]	15.6 (0.6)	17.4 (0.1)
NDF	30.3 (0.6)	32.0 (0.4)
uNDF, %NDF	32.4 (1.1)	25.8 (1.4)
Starch	20.6 (0.6)	21.8 (0.6)
SFA	1.86 (0.2)	1.80 (0.1)
MUFA	0.65 (0.1)	0.89 (0.1)
PUFA	1.19 (0.2)	1.91 (0.6)

[1] Contained 30.7% calcium; 6.3% sulfur; 35,800 mg/kg zinc; 27,400 mg/kg manganese; 6,269 mg/kg copper; 1,548 mg/kg iron; 1,190 mg/kg iodine; 524 mg/kg cobalt.

[2] Contained 24.3% calcium; 9,900 KIU/kg vitamin A; 2,200 KIU/kg vitamin D3; 3,300 IU/g vitamin E.

[3] Rumensin 90 (200g monensin/kg).

[4] Values represent the mean (and SD) of 2 composited samples (one for each period).

[5] Estimated RUP: 37% and 46% CP for ALFA and ORCH, respectively.

Table 2.2. Nutritional composition and parameters of fiber degradation kinetics of forages

	Alfalfa hay	Orchardgrass hay	Corn silage
OM, %	97.4 (0.1)	97.1 (0.1)	97.3 (0.1)
CP, %	16.4 (4.5)	10.7 (1.0)	8.9 (0.8)
NDF, %	38.0 (1.0)	63.2 (0.3)	29.4 (1.2)
ADF, %	36.8 (1.4)	43.5 (0.8)	20.0 (0.6)
ADL, %	7.0 (1.2)	5.8 (0.1)	2.6 (0.4)
ADL, %NDF	18.4 (3.6)	9.2 (0.2)	8.8 (1.1)
uNDF, %	13.9 (3.1)	20.7 (1.4)	10.4 (0.2)
uNDF, %NDF	36.5 (6.6)	32.8 (1.8)	36.0 (1.8)
pdNDF, %NDF	63.5 (6.6)	67.2 (1.8)	64.0 (1.8)
Discrete lag, h	0.7 (0.7)	6.0 (1.2)	10.9 (5.9)
K_d of pdNDF, %/h	7.46 (1.2)	4.95 (1.2)	2.87 (0.8)
Starch, %	3.0 (0.3)	3.1 (0.2)	35.8 (2.9)

All values are on a DM basis unless stated differently.

Values represent the mean (and SD) of 2 composited samples (one for each period).

Table 2.3. Production performance of primiparous lactating dairy cows consuming diets containing alfalfa hay (ALFA) or orchardgrass hay (ORCH)

Item	ALFA	ORCH	SEM	*P*-value
Milk yield, kg/d	39.6	38.5	1.43	0.26
Milk fat, %	3.69	3.75	0.24	0.63
Milk protein, %	3.23	3.25	0.06	0.73
Milk lactose, %	4.85	4.85	0.03	0.71
Milk fat yield, kg/d	1.44	1.41	0.07	0.42
Milk protein yield, kg/d	1.27	1.25	0.05	0.34
Milk lactose yield, kg/d	1.92	1.87	0.07	0.32
ECM yield, kg/d	41.4	40.4	0.96	0.26
Feed efficiency, kg ECM/kg DMI	1.56	1.65	0.05	0.01
MUN, mg/dL	14.1	15.5	0.85	0.02

Values represent the mean of 10 cows per each treatment.

Table 2.4. Dry matter intake, nutrient intake, and nutrient digestion of primiparous lactating dairy cows consuming diets containing alfalfa hay (ALFA) or orchardgrass hay (ORCH)

Item	ALFA	ORCH	SEM	*P*-value
Intake, kg/d				
DM	26.7	24.6	0.71	<0.01
CP	4.2	4.3	0.13	0.26
NDF	8.1	7.9	0.22	0.26
uNDF	2.7	2.3	0.09	<0.01
pdNDF	5.3	5.6	0.20	0.06
Starch	5.5	5.4	0.20	0.36
Digestibility, %				
DM	60.8	60.5	1.35	0.85
CP	54.3	62.2	1.83	<0.01
NDF	40.4	35.9	2.06	0.04
pdNDF	61.3	50.4	2.37	<0.01
Starch	95.5	95.6	0.36	0.63
Digested, kg/d				
DM	16.3	14.9	0.69	0.04
CP	2.3	2.7	0.13	<0.01
NDF	3.3	2.8	0.23	0.02
pdNDF	3.3	2.8	0.23	0.02
Starch	5.3	5.1	0.20	0.30

Values represent the mean of 10 cows per each treatment.

Table 2.5. Ruminal pool sizes and kinetics of primiparous lactating dairy cows consuming diets containing alfalfa hay (ALFA) or orchardgrass hay (ORCH)

Item	ALFA	ORCH	SEM	*P*-value
Pool size, kg				
Total ruminal contents[1]	65.8	75.4	4.7	0.02
DM	8.8	10.0	0.6	0.04
NDF	5.4	6.7	0.3	0.03
uNDF	2.4	2.4	0.2	0.99
pdNDF	3.0	4.3	0.1	0.01
Kinetics				
K_p, %/h	5.02	4.03	0.45	0.06
Mean retention time, h	21.0	26.2	2.6	0.09

Values represent the mean of 6 cows per each treatment.

[1] Mix of ruminal solids and liquid.

Table 2.6. Fecal composition of primiparous lactating dairy cows consuming diets containing alfalfa hay (ALFA) or orchardgrass hay (ORCH)

Item	ALFA	ORCH	SEM	*P*-value
NDF, %DM	46.1	52.2	0.9	<0.01
pdNDF, %NDF	42.9	54.7	1.1	<0.01
pdNDF, %DM	19.8	28.6	0.9	<0.01

Values represent the mean of 6 cows per each treatment.

Table 2.7. Milk fatty acid profile (g/100 g fatty acid) of primiparous lactating dairy cows consuming diets containing alfalfa hay (ALFA) or orchardgrass hay (ORCH)

Item	ALFA	ORCH	SEM	*P*-value
4:0	2.22	2.05	0.28	0.14
6:0	1.47	1.32	0.17	0.07
8:0	1.02	0.92	0.09	0.07
10:0	2.72	1.44	0.18	0.03
11:0	0.09	0.09	0.02	0.96
12:0	3.43	3.06	0.17	0.01
13:0	0.14	0.14	0.02	0.77
14:0	11.09	9.69	0.24	<0.01
cis-9 14:1	1.23	0.93	0.13	0.05
2OH 10:0	0.05	0.04	0.01	0.25
15:0	1.19	0.99	0.12	0.08
iso 15:0	0.17	0.17	0.01	0.94
anteiso 15:0	0.39	0.36	0.01	<0.01
16:0	31.99	28.2	1.07	<0.01
iso 16:0	0.18	0.20	0.02	0.14
cis-9 16:1	1.45	1.10	0.14	0.05
17:0	0.56	0.46	0.02	<0.01
iso 17:0	0.27	0.26	0.01	0.47
18:0	8.28	11.91	0.70	<0.01
trans-6,8 18:1	0.38	0.33	0.05	0.14
trans-9 18:1	0.40	0.31	0.04	<0.01
trans-10 18:1	1.38	1.16	0.45	0.41
trans-11 18:1	1.25	1.11	0.12	0.29
trans-12 18:1	0.51	0.38	0.02	<0.01
cis-9 18:1	21.02	25.53	0.98	<0.01
cis-11 18:1	1.20	0.81	0.07	<0.01
cis-9,*cis*-12 18:2	3.28	4.11	0.20	<0.01
cis-9,*cis*-12,*cis*-15 18:3	0.56	0.31	0.03	<0.01
20:0	0.14	0.16	0.01	<0.01
cis-9,*trans*-11 CLA	0.64	0.59	0.05	0.47
trans-10,*cis*-12 CLA	0.02	0.01	<0.01	0.33
Other	1.21	0.85	0.09	<0.01

Values represent the mean of 10 cows per each treatment.

CHAPTER 3: Production Performance, Nutrient Digestibility, and Enteric Methane Emissions of Lactating Holsteins Fed Triticale Silage of Different Maturities in Different Dietary Forage Inclusions

3.1. Abstract

This study evaluated the production performance, milk fatty acid profile, nutrient digestibility, and enteric methane (**CH_4**) emissions of high-producing dairy cows fed high-forage (**HF**; 52% forage) or low-forage (**LF**; 37% forage) diets containing triticale silages harvested at either the boot (**BT**) or soft-dough (**SFT**) stage. The BT stage silage contained 16.7% CP, 51.1% NDF, 35.0% ADF, 3.7% ADL, and 2.2% starch, while the SFT stage silage contained 8.7% CP, 62.6% NDF, 46.1% ADF, 6.4% ADL, and 4.6% starch. The experiment followed a replicated 4×4 Latin Square design with 21-day periods using 8 primiparous and 16 multiparous Holstein cows. Treatments followed a 2×2 factorial arrangement of forage inclusion level and triticale maturity stage. Forage maturity did not affect DMI in cows fed LF diets, but cows fed the HFBT diet consumed more DM than those fed the HFSFT diet (26.3 vs. 23.4 kg/d). Cows fed BT stage diets produced more milk than those on SFT stage diets (46.9 vs. 43.4 kg/d), while cows on LF diets produced more milk than those on HF diets (47.1 vs. 43.2 kg/d). The production of ECM did not differ between maturity stages but was greater for cows consuming LF diets compared to HF diets (49.1 vs. 45.6 kg/d). Cows consuming BT stage diets exhibited greater apparent total-tract digestibility of DM (69.3 vs. 67.3%) and NDF (59.4 vs. 54.5%) than SFT stage diets. Cows consuming HF diets exhibited greater digestibilities of DM (70.2 vs. 66.4%), NDF (60.3 vs. 55.8%), and starch (95.5 vs. 94.8%) than LF diets. Cows fed BT stage diets produced 23 g/d less enteric CH_4 than cows on SFT stage diets (368 vs. 391 g/d), with no difference between HF and LF diets in absolute enteric

CH_4 output. However, enteric CH_4 intensity was lower in cows consuming BT stage diets compared to SFT stage diets on a DMI basis (14 vs. 15 g/kg DMI) and DM-digested basis (19 vs. 22 g/kg). Cows consuming LF diets exhibited reduced enteric CH_4 yields compared to HF diets (13 vs. 16 g/kg DMI and 13 vs. 16 g/kg DM digested). Milk from cows fed BT stage diets contained greater concentrations of *de novo* fatty acids (23.13 vs. 22.08 g/100 g fatty acid), PUFA (4.53 vs. 4.22 g/100 g fatty acid), *trans* fatty acids (4.50 vs. 3.64 g/100 g fatty acid), and OBCFA (2.43 vs. 2.27 g/100 g fatty acid) than cows on SFT stage diets. Cows on LF diets produced milk containing lower SFA (64.22 vs. 65.89 g/100 g fatty acid) but higher PUFA (4.85 vs. 3.90 g/100 g fatty acid), *trans* fatty acids (4.38 vs. 3.76 g/100 g fatty acid), and OBCFA (2.39 vs. 2.31 g/100 g fatty acid) concentrations than those on HF diets. In conclusion, dairy production appears relatively resilient to differences in triticale silage maturity. However, harvesting at the boot stage offers an enteric methane mitigation strategy by reducing CH_4 yield while enhancing DMI, digestibility, and milk production. Low-forage diets improved milk yield and reduced enteric CH_4 emissions at the expense of nutrient digestibility.

3.2. Introduction

Including forages in the rations of high-producing dairy cattle is essential for maintaining ruminal health by supplying physically effective NDF (**peNDF**; Mertens, 1997) and providing energy through the fermentation of structural carbohydrates. In dairy farming systems where small-grain grasses are harvested for feed (Harper et al., 2017; Brown et al., 2018; Ferreira et al., 2025), rations for high-producing cattle may include triticale silage, or silages of other small-grain grasses, as a source of peNDF. The maturity stage at which farmers harvest these small-grain grasses is a pre-harvest decision affecting the nutritional composition and digestibility of the resulting silage (Coblentz et al., 2018a; Ferreira et al., 2025).

Deciding when to harvest small-grain grasses for silage can be controversial (Ferreira et al. 2025). The existing forage inventories, crop rotation, and nutrient management plan are some of the many factors determining harvesting time. From a nutritional perspective, Coblentz et al. (2018a; 2018b) and Ferreira et al. (2025) reported that harvesting small-grain grasses at the boot stage of maturity provides a forage with a greater concentration of CP, a lower concentration of NDF, and a higher NDF digestibility than harvesting small-grain grasses at the soft-dough stage of maturity. From a forage yield perspective, however, Coblentz et al. (2018a; 2018b) and Ferreira et al. (2025) reported that harvesting small-grain grasses at the boot stage of maturity yields substantially less forage than harvesting small-grain grasses at the soft-dough stage of maturity. When factoring in DM yield, estimated silage costs, and forage quality, a least-cost ration formulation approach suggested that the maturity of small-grain grasses at harvest has minimal impact on dairy cattle feeding costs (Ferreira et al., 2025). Ferreira et al. (2025) suggested that the optimal harvesting time of triticale for silage may depend on how sensitive dairy cattle performance is to the nutritional differences between boot and soft-dough stage silages.

Schultz et al. (2025) reported no differences in DMI, ECM yield, NDF digestibility or de novo fatty acid concentration in milk fat between cows fed diets containing boot or soft-dough stage triticale silages, suggesting that dairy cattle performance is not sensitive to triticale silage maturity. However, that study only achieved modest differences in silage nutrient composition, despite a 26-d gap between harvests. Specifically, Schultz et al. (2025) reported variations of 1.3% units in CP, 2.0% units in NDF, 2.0% units in acid ADF, 1.3% units in ADL, and 2.1% units in starch on a DM basis, which were less than expected (Coblentz et al., 2018a; Ferreira et al., 2025). Because the study of Schultz et al. (2025) may have been limited by its harvesting methods prior to feeding triticale silages to dairy cattle, the sensitivity of dairy cattle performance to triticale silage maturity deserves further evaluation.

Moreover, no study to date has examined the influence of harvesting maturity of triticale for silage on enteric methane (**CH_4**) emissions when included in rations of lactating dairy cows. Enteric CH_4 emissions from ruminants are heavily modulated by DMI and the digestibility of DM and NDF, such that increases in DMI and digestibility are associated with increased ruminal fermentation (Hristov et al., 2013; Marumo et al., 2023). Therefore, disparities in forage quality of triticale at different maturities likely influence CH_4 emissions. To determine the feasibility of using triticale as forage for dairy cattle and to identify its optimal harvesting maturity, further research must evaluate its influence on dairy cattle performance, digestive efficiency, and CH_4 emissions as affected by maturity.

In the present study, we hypothesized that cows fed diets containing triticale silage harvested at the boot stage of maturity would outperform cows fed diets containing triticale silage harvested at the soft-dough stage of maturity due to differences in forage quality. However, we hypothesized that boot stage-harvested silage diets might lead to greater enteric CH_4 emissions due to greater fermentation of structural carbohydrates. Additionally, we anticipated that the disparities in performance and enteric CH_4 emissions would be more pronounced in diets with greater forage inclusion. Accordingly, the objective of this study was to evaluate the production performance, milk fatty acid profile, nutrient digestibility, and enteric CH_4 emissions of high-producing dairy cows consuming high- or low-forage diets containing triticale silages harvested at either the boot stage or soft-dough stage of maturity.

3.3. Materials and Methods

3.3.1 Triticale Silages

Triticale was cultivated at Kentland Farm (Blacksburg, VA) as described by Schultz et al. (2025). A single 10-ha field was seeded on October 18, 2022, with 105 kg/ha of triticale (TriCal Grainer 154; Butte, MT). Corn for silage was harvested from the field on September 22, 2022. On November 18, 2022, 45 kg N/ha from dairy manure was applied, and on March 18, 2023, an additional 45 kg N/ha was broadcast as urea and ammonium nitrate. Starting on April 18, 2023, the growth stage of the crop was monitored weekly to assess maturity following the method outlined by Ferreira et al. (2025) and using the Zadoks scale (Zadoks et al., 1974) to determine the appropriate maturity stage for harvest.

The field was divided into 4 quadrants, and alternate quadrants were harvested for each maturity stage to reduce field effects on the nutritional qualities of the resulting crops (Schultz et al., 2025). The crop was harvested at two distinct stages of maturity: the boot (**BT**) stage on April 29, 2023, and the soft-dough (**SFT**) stage on May 28, 2023. At the BT stage, half of the crop was cut using a mower with conditioner rolls (John Deere 946 MoCo; Moline, IL), wilted for 2 days, tedded, and chopped with a forage chopper (John Deere 7400) set to a theoretical length of cut of

19 mm. A microbial inoculant (Early Sile Plus; Micron Bio-Systems Inc.; Buena Vista, VA) was applied to the biomass during chopping at a rate of 10 mL/kg (on a fresh-weight basis). At the SFT stage, the other half of the crop was similarly cut, wilted for less than 12 h, tedded, chopped with the same theoretical length of cut, and ensiled similarly. Immediately after chopping, the biomass was ensiled in separate concrete-walled bunker silos, each lined with a plastic sheath on the floor and walls. The ensiled material was then covered with a plastic sheet and a green mesh tarp (Secure Cover; Bag Man, LLC), which were secured with silo tire sides. The ensiling period lasted for a minimum of 42 days before the first sampling and 82 days before the inclusion in dairy rations.

Multiple subsamples were collected and composited on July 10, 2023, to assess the chemical composition of each silage. The composite samples were dried in a forced-air oven at 55°C until they reached constant weight, then ground to pass through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Ground samples were sent to a commercial laboratory (Cumberland Valley Analytical Services, Lancaster, PA) for preliminary nutritional analysis using near-infrared reflectance spectroscopy (NIR 2 Package).

3.3.2 Animals, Housing, and Diets

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Virginia Tech. The experiment was designed as a replicated 4×4 Latin Square design with 21-day periods and a 2×2 factorial arrangement of treatments, where forage maturity (BT vs. SFT) and dietary forage inclusion [high forage (**HF**) vs. low forage (**LF**)] were the main factors. The study included 16 multiparous (664 ± 53 kg BW, 111 ± 16 DIM) and 8 primiparous Holstein dairy cows (559 ± 45 kg BW, 84 ± 19 DIM), which were allocated to Latin squares based on parity

(1, 2, or ≥3), milk yield, and DIM. Within each square, cows were randomly assigned to 1 of 4 diets balancing for carry-over effects.

The HF diets were formulated to contain 52% forage, and the LF diets were formulated to contain 37% forage. A triticale silage with a nutrient profile reflecting the average of the two triticale silages was used to initially formulate the HF and LF diets with corn silage as a secondary forage to meet the requirements of second-parity dairy cows (605 kg BW and 82 DIM) consuming 24 kg of DM/d and producing 44 kg of milk/d containing 3.75% fat and 3.05% protein using version 3.0.8.1 of CPM Dairy (CAHP Software Information, Philadelphia, PA). Then, the individual silages (BT and SFT) replaced the average to create the final 4 rations (**Table 3.1**). Concentrates were ordered from a commercial feed mill (Exchange Milling Company Inc., Rocky Mount, VA).

All cows were housed in a 24-stall pen within a free-stall barn. Cows were fed once daily (1100 h) via a Calan gate system (American Calan Inc., Northwood, NH). Cows were trained to find their specific door for a 7-d period before the beginning of the study. The TMR were delivered to feed ad libitum and to obtain ~5% of feed refusals. The amounts of feed offered and feed refused were measured daily. Cows were milked twice daily (0100 h and 1200 h), and individual milk weights were automatically recorded at each milking. For statistical analyses, the average daily milk yield and DMI were determined from d 15 to 21 of each experimental period. Average DMI included DMI from the bait pellet used for measuring enteric CH_4 emissions. The average DMI of the bait pellet was estimated from the number of cow visits to the GreenFeed system (C-Lock Inc., Rapid City, SD) and the average pellet DM deployment per drop.

3.3.3 Sample Collection and Analysis

Samples of feed ingredients and TMR were collected twice weekly and composited by period. A subsample of the period-composited triticale and corn silages was submitted to Cumberland Valley Analytical Services for fermentation profile analysis. All samples were dried in a forced-air oven at 55°C until constant weight and ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific). The concentration of ash was determined after combusting samples in a furnace (Thermolyne 30400, Barnstead International) for 3 h at 600°C (method 942.05; AOAC International, 2019). The concentration of CP was calculated as percent N × 6.25 after combustion analysis of N (method 990.03; AOAC International, 2019) using a Vario El Cube CN analyzer (Elementar Americas Inc.). The concentration of ash-free NDF was determined using the Ankom200 Fiber Analyzer (Ankom Technology) with sodium sulfite and α-amylase (Ferreira and Mertens, 2007). Forage samples were additionally analyzed for ADF and ADL, which were determined sequentially. Concentrations of ADF were determined using the Ankom200 Fiber Analyzer. After determining ADF residue weights, residues were incubated for 3 h in 72% sulfuric acid within a 4-L jar that was placed in a DaisyII Incubator (Ankom Technology). Starch concentration was determined using the acetate buffer method (Hall, 2009) with α-amylase from Bacillus licheniformis (FAA, Ankom Technology) and amyloglucosidase from Aspergillus niger (E-AMGDF, Megazyme International, Wicklow, Ireland).

Milk samples (a.m. and p.m. milkings) were collected on d 17 and 18 of each period. Concentrations of milk fat, true protein, lactose, and MUN were analyzed by Lancaster DHIA (Mannheim, PA). Weighted average component yields were calculated according to the milk yields of each sampling. Energy-corrected milk yield (kg) was estimated by deriving the equation of Tyrrell and Reid (1965) as described in Equation 1:

$$ECM = 0.327 \times MY + 12.95 \times FY + 7.65 \times PY, \; [1]$$

where *MY* is the daily milk yield (kg), *FY* is the daily milk fat yield (kg), and *PY* is the daily protein yield (kg). Additional milk samples (a.m. and p.m. milkings) were collected on d 18 to determine the milk fatty acid profile. Milk fatty acids were extracted and methylated according to Chouinard et al. (1999) and analyzed as described by Yang et al. (2017).

3.3.4 Enteric Methane Emissions

Enteric CH_4 emissions were measured using a single GreenFeed unit (hereafter unit) accessible to all cows within the pen with 24 stalls. The unit was maintained and calibrated following the manufacturer's recommendations. The CO_2 recoveries averaged 103.9 ± 6.6% (n = 4). Cows were trained to the unit for a 7-d period before the beginning of the study. Enteric CH_4 production was estimated from voluntary visits of cows to the unit to receive a palatable bait pellet containing 17% CP, 33% NDF, and 26% starch on a DM basis. Cows visited the unit at least twice daily to collect adequate data per cow. Cow visitation to the unit was monitored daily, and cows were guided to the unit when needed to ensure the criteria were met. For statistical analyses, the average daily enteric CH_4 production was determined from d 15 to 21 of each experimental period. Average DMI, DM digested, and ECM yield from d 15 to 21 for the experimental periods were used to calculate enteric CH_4 yield (g of CH_4/kg DMI and g of CH_4/kg DM digested) and CH_4 intensity (g CH_4/kg ECM), respectively.

3.3.5 Nutrient Digestibility

Apparent total-tract nutrient digestibility was estimated using lanthanum chloride ($LaCl_3$) as an external marker as described by Yang et al. (2017). In each period, individual fecal grab samples were collected every 6 h (skipping 2 h every 4 samples) from d 17 to 21. A total of 12 samples were collected per cow per period, dried at 55°C until constant weight, composited, ground to pass through a 1-mm screen of a Wiley mill, and stored until analysis. The concentrations of La in the TMR and feces were determined by inductively coupled plasma optical emissions spectrometry (Arcos II ICP-AES, Spectro Analytical Instruments GmbH) as described by Galyon et al. (2024). The concentrations of CP, NDF, and starch were determined as previously described.

Apparent DM digestibility (*DMD*; %) and nutrient digestibilities (*NutrD*; %) were calculated according to Equation 2 and Equation 3, respectively:

$$DMD = 100 - \frac{Dietary\ [La]}{Fecal\ [La]} \times 100\ , [2]$$

$$NutrD = 100 - \frac{Dietary\ [La]}{Fecal\ [La]} \times \frac{Fecal\ [Nutr]}{Dietary\ [Nutr]} \times 100\ , [3]$$

where *[La]* is the concentration of La (g/kg DM) in the TMR or feces and *[Nutr]* is the concentration of either CP, NDF, or starch (% DM) in the TMR or feces. To account for the intake of the bait pellet, a weighted average TMR composition for each cow was calculated based on the nutrient profiles of the TMR and the bait pellet and the average intake of each cow.

3.3.6 Ruminal in Situ NDF Degradability

The rates of NDF degradation of the forages were determined using an in situ ruminal degradation study according to Ferreira et al. (2022). A 0.25-g sample of each forage was placed in filter bags (F57, Ankom Technology) previously rinsed with acetone. Duplicate samples were incubated for 0, 3, 6, 12, 24, 48, 96, and 240 h in the rumen of two rumen-cannulated lactating dairy cows consuming a ration containing 42% corn silage, 5% triticale silage, 3% mixed grass hay, and 50% concentrate mix, resulting in four replicates per sample for each time point. All bags were inserted simultaneously at feeding time (0900 h) into the rumen and extracted at the designated time points. After extraction, the bags were rinsed with tap water in three 5-min cycles using a portable washing machine (SKY2767, Best Choice Products, Irvine, CA), then dried in a forced-air oven at 55°C. Subsequently, the NDF residue was determined.

The degradation rate (**K$_d$**) of pdNDF was determined using the NLIN procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) according to Equation 4:

$$ISNDFD = pdNDF \times \left(1 - e^{(-K_d \times (T - Lag))}\right), [4]$$

where *ISNDFD* is the degraded NDF (% NDF) at time *T* when *T* is greater than *Lag*, *Lag* (h) is the discrete lag time of NDF degradation (Mertens, 1973), and *pdNDF* is the potentially degradable NDF (% NDF) during a 240-h ruminal fermentation estimated by subtracting the remaining undegraded NDF at 240 h (**uNDF**) on a NDF basis from 100. The discrete lag time was estimated using simple linear regression as $ISNDFU = a + b \times ln_{(T)}$, where *ISNDFU* is the remaining undegraded NDF residue (% NDF) at time *T*, *a* represents the intercept, *b* is the predicted slope, the $ln_{(T)}$ is the natural log of fermentation time. *Lag* was derived according to Equation 5:

$$Lag = e^{\left(\frac{(a-100)}{-b}\right)}. [5]$$

3.3.7 Statistical Analysis

Before designing the experiment, a statistical power analysis was performed using the POWER procedure of SAS. Considering a statistical power equal to 0.80, a probability of committing a type I error (α) equal to 0.05, and a SD equal to 2.0 kg/d for ECM, 34 experimental units per main effect was determined to be sufficient to detect a 2.0-kg/d difference in ECM production.

All variables were analyzed using the MIXED procedure of SAS. The statistical model for the variables of interest included the fixed effects of square (df = 5) and diet (df = 3), and the random effects of period (df = 3), cow within square (df = 18), and the random residual error. Orthogonal contrasts were used to test the main effects of forage maturity (BT vs. SFT), dietary forage (HF vs. LF), and their interaction. When an interaction existed, Tukey's pairwise comparisons contrasted combinations of forage maturity and dietary forage. Statistical significance was declared at $P < 0.05$ and a tendency to statistically differ was declared at $P < 0.10$.

3.4. Results and Discussion

This study aimed to determine dairy cattle performance, nutrient digestibility, and enteric CH_4 emissions when fed silage of triticale harvested at different maturities. Schultz et al. (2025) previously evaluated dairy cattle performance when fed silage of triticale harvested at either the boot or soft-dough stage and found no significant differences in DMI, ECM yield, *de novo* fatty acid concentrations, or NDF digestibility. However, the harvested crops and resulting silages were nutritionally similar. The approach of harvesting one side of the field at the boot stage and the other at the soft-dough stage may have undermined the intended differences in forage quality due to variations in growing conditions across the field (Schultz et al., 2025). This study alternatively took the approach of dividing the field seeded with triticale into 4 quadrants and harvested alternate quadrants to reduce field effects while achieving the two harvesting maturities. As such, we obtained substantially different forages with the same variety of triticale and in the same field as reported by Schultz et al. (2025).

The silage of triticale harvested in the BT stage contained 16.7% CP, 51.1% NDF, 35.0% ADF, 3.7% ADL, and 2.2% starch, whereas the silage of triticale harvested in the SFT stage contained 8.7% CP, 62.6% NDF, 46.1% ADF, 6.4% ADL, and 4.6% starch (**Table 3.2**). As such, harvesting triticale for silage in the BT stage resulted in 8.0% units more CP and 11.5% units, 11.1% units, and 2.7% units less NDF, ADF, and ADL, respectively, than harvesting in the SFT stage. Across two years, Coblentz et al. (2018a) reported that triticale harvested in the BT stage contained 8.0% units more CP and 8.5% units, 8.3% units, and 2.4% units less NDF, ADF, and ADL, respectively, relative to harvesting in the SFT stage. Similarly, Ferreira et al. (2025) found that triticale harvested in the BT stage contained 4.5% units more CP and 9.1% units, 8.4% units and 2.5% units less NDF, ADF, and ADL, respectively, relative to harvesting in the SFT stage.

The NDF degradation kinetics parameters of the BT and SFT stage triticale silages differed substantially (**Table 3.2**). Although the pdNDF concentration of the BT and SFT stage silages was similar on a DM basis (44.2 vs 40.2%), the forages differed substantially in their uNDF concentration (6.8 vs. 22.4%, DM basis). On a NDF basis, the BT stage silage contained 13.4% uNDF and 86.6% pdNDF, whereas the SFT stage silage contained 35.8% uNDF and 64.2% pdNDF. Furthermore, the BT stage silage had a shorter NDF degradation lag time (3.7 vs. 5.0 h) and a faster K_d of the pdNDF (2.33 vs. 1.53%/h) than the SFT stage silage. On a NDF basis, the uNDF concentration of triticale harvested at the BT stage versus the SFT stage seems to be consistent (Coblentz et al., 2018b; Ferreira et al., 2025; Schultz et al., 2025). Triticale harvested in the BT stage also consistently demonstrates faster K_d of the pdNDF than triticale harvested in the SFT stage (Ferreira et al., 2025; Schultz et al., 2025). While K_d reported by Coblentz et al. (2018b) were overall faster (5.90%/h for BT vs. 3.10%/h for SFT), these differences may be attributed to variations in triticale varieties or estimation methods. Collectively, harvesting triticale for silage in the BT stage of maturity yields a higher-quality crop with greater fiber digestibility relative to the SFT stage of maturity.

Two primary approaches are commonly used to formulate experimental diets for forage evaluation. The first approach aligns diets nutritionally by accounting for the nutritional composition of the forages (Schultz et al., 2025). While this method mirrors field conditions, it often results in diets with significantly different ingredient compositions. For example, in our study, the silage of triticale harvested at the SFT stage contained less CP and NDF compared to its BT stage counterpart. Consequently, if diets were formulated based on the nutrient composition of the silages within each forage level, the SFT stage diets would necessitate more corn silage and less triticale silage (on a DM basis) to balance for differences in CP and NDF concentrations. This approach could also necessitate additional or alternative ingredients in the concentrate of the SFT stage diets to balance the nutrient disparities and achieve a similar TMR nutrient composition as the BT stage diets. Such discrepancies can confound experimental outcomes by altering both the triticale-to-corn silage ratio and ingredient composition. The second approach involves replacing experimental forages on a one-to-one ratio (DM basis). While this ensures a consistent triticale-to-corn silage ratio within forage level, this method results in variations in the diet nutrient composition when the experimental forages substantially vary in their nutrient composition. This also poses potential confounding issues. To reduce confounding effects related to the inclusion of alternative ingredients or differing triticale-to-corn silage ratios within forage levels, we adopted the second formulation method. As a result, the diets containing BT stage triticale silage had slightly more CP and less NDF than those with SFT stage triticale silage (**Table 3.1**), reflecting the inherent differences in the nutritional composition of the individual silages (**Table 3.2**). However, differences were marginal, and the diets containing BT stage triticale silage contained 1.8% units more CP, 2.5% units less NDF, and 0.5% unit less starch than the diets containing SFT stage triticale silage. Therefore, observed differences in dairy cattle production are likely attributed to variations in ruminal fermentation and digestibility influenced by forage maturity.

Cows consuming diets containing BT stage triticale produced 3.5 kg/d more milk than cows consuming SFT stage diets (46.9 vs. 43.4 kg/d; $P < 0.01$; **Table 3.3**), while cows consuming LF diets produced 3.9 kg/d more milk than cows consuming HF diets (47.1 vs. 43.2 kg/d; $P < 0.01$). These differences are likely linked to DMI. While milk fat concentration did not differ between cows consuming HF and LF diets ($P = 0.20$), cows consuming diets containing SFT stage triticale had greater milk fat concentration than cows consuming diets containing BT stage triticale (4.13 vs. 3.71%; $P < 0.01$). Additionally, milk fat yields did not differ across dietary treatments and averaged 1.75 kg/d. Cows consuming BT stage diets tended to produce milk containing a slightly greater concentration of protein compared to cows consuming SFT stage diets, although this difference was marginal (2.89 vs. 2.85%; $P = 0.06$). Cows consuming LF diets produced milk containing a greater concentration of milk protein than cows consuming HF diets (2.90 vs. 2.83%; $P = 0.01$). This may be associated with the increased amount of CP digested by cows consuming diets containing BT stage silage compared to cows consuming SFT stage silage (2.3 vs. 1.7 kg/d; $P < 0.01$; **Table 3.4**) and by cows consuming LF diets compared to HF diets (2.1 vs. 1.9 kg/d; $P < 0.01$). Cows consuming diets containing BT triticale silage had a greater milk protein yield than cows consuming diets containing SFT triticale (1.36 vs. 1.24 kg/d; $P < 0.01$). Similarly, cows consuming LF diets had a greater milk protein yield compared to cows consuming HF diets (1.37 vs. 1.22 kg/d; $P < 0.01$). Comprehensively, cows consuming BT stage diets tended to produce more ECM than cows consuming SFT stage diets (48.0 vs. 46.6 kg/d; $P = 0.09$) and cows consuming LF diets produced 3.5 kg/d more ECM than cows consuming HF diets (49.1 vs. 45.6 kg/d; $P = 0.01$).

Despite differences in DM yield, estimated silage costs, and forage quality, Ferreira et al. (2025) determined that the harvesting maturity of triticale silage has minimal impact on feeding costs for dairy cattle. From this ration formulation approach, one may determine that triticale silage can be fed to lactating dairy cattle indiscriminately regardless of maturity. However, the production response of lactating dairy cattle to the differences in the nutritional composition of the resulting silages must be evaluated as income-over-feeding costs may be influenced if dairy cattle are sensitive to the nutritional differences of the silages. To evaluate this, Schultz et al. (2025) evaluated dairy cattle production response to triticale silage harvested at either the BT or SFT stages of maturity at low- and high-dietary forage inclusion levels. While they did not observe a significant effect of triticale silage maturity on DMI or ECM yield, Schultz et al. (2025) did not achieve substantially different forages by harvesting at the BT and SFT stage of maturity, and therefore their conclusions were limited. We repeated the study with slight modifications to the harvesting of the field to achieve substantially different forages as observed by Ferreira et al. (2025) and Coblentz et al. (2018a; 2018b). We observed that feed efficiency (1.81 kg ECM/kg DMI; $P = 0.63$; **Table 3.3**) and ECM production were not particularly affected by feeding triticale silage of different maturities. Presently, neither silage cost, ration costs (Ferreira et al., 2025), nor dairy cattle production performance seem important in determining the harvesting time of triticale for silage.

While forage maturity did not affect the DMI of cows consuming LF diets (28.5 kg/d; $P = 0.76$), cows consuming the HF diet with BT stage triticale consumed 2.9 kg/d more DM than those consuming the HF diet with SFT stage triticale (26.3 vs. 23.4 kg/d; $P = 0.01$; **Table 3.4**). We hypothesized that DMI would be affected by forage maturity regardless of forage inclusion level. However, in LF diets, where triticale silage only constituted 18% DM, the effects of maturity may have been diluted by including corn silage on an equivalent basis. Digestibility data, however, contradicts this explanation. Cows consuming diets containing BT triticale silage had greater apparent total-tract digestibilities of DM (69.3 vs. 67.3%; $P < 0.01$) and NDF (59.4 vs. 54.5%; $P < 0.01$) than cows consuming diets containing SFT triticale silage, regardless of forage inclusion level (**Table 3.4**). Assuming that the ruminal digestibilities of DM and NDF are greater, it may be possible that the lower inclusion of triticale silage in the LF diets was not sufficient to alter the ruminal passage rate. Therefore, the differences in ruminal DM and NDF digestibility were not great enough to elicit a cohesive influence on ruminal emptying and DMI (Ellis et al., 1994).

Cows consuming HF diets exhibited greater digestibilities of DM (70.2 vs. 66.4%; $P = 0.03$), NDF (60.3 vs. 55.8%; $P < 0.01$), and starch (95.5 vs. 94.8%; $P < 0.01$) than their LF counterparts. An interaction between forage maturity and forage inclusion level existed for the CP digestibility ($P = 0.04$), which was driven by the reduced digestibility of the LFSFT diet compared to the other three diets (51.3 vs. 59.3%). We anticipated that total-tract digestibility would be improved in LF diets due to the reduced forage-to-concentrate ratio and increase in fermentable carbohydrates. To balance for the reduced forage NDF in the LF diets, other fibrous ingredients in the concentrate, primarily soybean hulls, were included in greater proportions than the HF diets (**Table 3.1**). Low-forage diets likely had a reduced mean particle size and less physically effective fiber. As a result, the ruminal passage rate could have been faster in LF diets compared to HF diets. A faster ruminal rate of passage could decrease the effective ruminal degradation of LF diets and explain the observed decrease in apparent total-tract digestibility. Likewise, faster ruminal passage rates of LF diets could explain the increased DMI ($P < 0.01$) of cows consuming these diets due to faster ruminal emptying and greater turnover.

Feed intake explains 60 to 80% of the variation in enteric CH_4 production (NASEM, 2021). However, despite the greater DM ingestion and digestion, cows consuming BT stage triticale silage produced 23 g CH_4/d less than those consuming SFT stage silage (368 vs. 391 g CH_4/d; $P = 0.01$; **Figure 3.1**). Curiously, CH_4 production did not differ between cows consuming HF and LF diets ($P = 0.28$) even though the cows consuming LF diets ingested and digested significantly more

DM. On a DMI basis, cows consuming diets containing BT stage triticale yielded less CH_4 than cows consuming diets containing SFT stage triticale (14 vs. 15 g CH_4/kg DMI; $P = 0.01$), and cows consuming LF diets yielded less CH_4 than cows consuming HF diets (16 vs. 13 g CH_4/kg DMI; $P = 0.01$). Similarly, on a DM digested basis, cows consuming diets containing BT stage triticale yielded less CH_4 than cows consuming diets containing SFT stage triticale (19 vs. 22 g CH_4/kg DM digested; $P = 0.01$), and cows consuming LF diets yielded less CH_4 than cows consuming HF diets (16 vs. 13 g CH_4/kg DM digested; $P = 0.01$).

There is a clear drive in the literature for using additives such as macroalgae and 3-nitrooxypropanol in rations of dairy cattle to reduce enteric CH_4 emissions, especially in the recent special issue on feed additives for methane mitigation published by the *Journal of Dairy Science* in January of 2025. While feed additives can significantly reduce enteric CH_4 emissions by 30 to 50% (Purba and Sangsawad, 2025), DMI is often reduced along with milk production (Fouts et al., 2022). Here we uniquely show that reduced enteric CH_4 emissions can be achieved while sustaining DMI and increasing milk production by feeding triticale silage of an earlier maturity compared to a later maturity. Achieving similar DMI and greater milk yield while reducing enteric CH_4 emissions makes forage maturity a more attractive CH_4 mitigation strategy for producers to implement than paying for costly feed additives that provide no economic return (Hristov et al., 2022; Martins et al., 2025). Understanding the mechanisms behind how forage maturity influences enteric CH_4 emissions will allow producers to make informed decisions on optimal harvesting maturity for their crops while being environmentally conscientious. However, the paucity in the literature regarding the influence of forage maturity on enteric CH_4 emissions presently makes conclusions difficult.

The differences in CH_4 production and CH_4 yields between forage maturity and dietary forage inclusion level suggest alterations in ruminal fermentation, either by hydrogen dynamics or digestion kinetics within the rumen. Adams et al. (1987) evaluated the effects of advancing forage maturity of mixed-grass pasture on ruminal fermentation of cannulated steers over 5 months and determined that advanced maturity increases the molar proportion of acetate in ruminal fluid while propionate molar proportion decreases and butyrate molar proportion stays relatively constant. We additionally observed that the BT stage triticale silage contained less acetate on a DM basis than the SFT stage triticale silage (**Table 3.2**). Diets with higher forage-to-concentrate ratios similarly demonstrate greater ruminal acetate-to-propionate ratios (Penner et al., 2009; Shi et al., 2018). These ruminal fermentation responses are likely linked to increased microbial cellulolytic activity due to the increased presence of cellulose and hemicellulose (Van Soest, 1994). Additionally in the case of LF versus HF diets, LF diets may lead to proliferation of propionogenic microbes due to the increased presence of non-forage fiber carbohydrates (Bauman et al., 1971; Wang et al., 2018). Thus, we might expect that cows consuming the LF diets and those consuming diets containing BT stage triticale silage exhibited reduced acetate-to-propionate ratios. This potential shift in VFA proportions lends itself to decreased CH_4 emissions as less hydrogen is produced with propionate than acetate (Van Soest, 1994), thereby decreasing available hydrogen for the synthesis of CH_4 (NASEM, 2021). This could additionally explain the lower milk fat concentrations observed for cows consuming BT stage diets as acetate and BHB are necessary precursors for *de novo* fatty acid synthesis (Mu et al., 2021). However, differences in molar percentages do not necessarily reflect the pools of acetate, propionate, and butyrate in the rumen. If ruminal fermentation activity was increased in cows consuming LF diets and those containing BT stage triticale, the relative pools of acetate may have been greater compared to cows consuming HF diets and those containing SFT stage triticale silage.

Regarding ruminal digestion kinetics, high-quality forages with faster K_d are often associated with faster passage rates (Ellis et al., 1994; Galyon et al., 2024). Faster passage rates could reduce ruminal fermentation of carbohydrates, which could reduce CH_4 emissions (Beauchemin et al., 2022; Hristov et al., 2022). However, if the NDF passage rates of the BT stage diets were faster than the SFT stage diets, the balance between the NDF degradation and passage rates was not so different that NDF digestibility was impaired in cows consuming BT stage diets. If we assume that hindgut digestion of NDF is less than 10% of total NDF digestion (Huhtanen et al., 2006; Lopes et al., 2015), then ruminal fermentation of NDF was greater in cows consuming BT triticale silage than SFT triticale silage based on the total-tract digestibility results. Perhaps ruminal passage rate plays an alternative role in enteric CH_4 reductions than reduced ruminal fermentation. Conversely, faster ruminal passage rates in cows fed the LF diets could explain the reduced enteric CH_4 emissions via reduced DM and NDF digestibility relative to cows fed HF diets. This may additionally explain the reduced feed efficiency of cows consuming LF diets compared to HF diets (1.75 vs. 1.88 kg ECM/kg DMI; $P < 0.01$; **Table 3.4**). Discussion at this point is theoretical without simultaneous measurements of the ruminal VFA profile and ruminal passage rate. Studies that specifically evaluate the relationships between forage maturity, ruminal passage rate, ruminal VFA production, and enteric CH_4 emissions are needed to disseminate these findings.

While the milk fat yield of cows in this study was not affected by forage maturity or inclusion level, the milk fatty acid profile (**Table 3.5**) further indicates alterations in ruminal fermentation. Cows consuming diets containing BT stage triticale silage produced milk fat containing slightly greater concentrations of *de novo* fatty acids (23.13 vs. 22.08 g/100 g fatty acid; $P = 0.01$), PUFA

(4.53 vs. 4.22 g/100 g fatty acid; $P = 0.01$), *trans* fatty acids (4.50 vs. 3.64 g/100g fatty acid; $P = 0.01$), and OBCFA (2.43 vs. 2.27 g/100 g fatty acid; $P = 0.01$). Additionally, cows consuming BT stage triticale produced milk fat with a greater *trans*-10 18:1 to *trans*-11 18:1 ratio (1.74 vs 0.98; $P = 0.01$) and concentration of *trans*-10, *cis*-12 CLA ($P < 0.01$) compared to cows consuming SFT stage. Milk fat from cows consuming LF diets contained lower concentrations of SFA (64.22 vs. 65.89 g/100 g fatty acid; $P = 0.01$) but greater concentrations of PUFA (4.85 vs. 3.90 g/100 g fatty acid; $P = 0.01$), *trans* fatty acids (4.38 vs. 3.76 g/100 g fatty acid; $P = 0.01$), and OBCFA (2.39 vs. 2.31 g/100 g fatty acid; $P = 0.01$). Additionally, cows consuming LF diets produced milk fat with a greater ratio of *trans*-10 18:1 to *trans*-11 18:1 (1.77 vs 0.95; $P = 0.01$) and concentration of *trans*-10, *cis*-12 CLA ($P < 0.01$) relative to the milk fat produced by cows consuming HF diets. These milk fatty acid profile observations indicate a potential shift in the biohydrogenation pathways or incomplete biohydrogenation of unsaturated FA provided by the LF diets and diets containing BT stage triticale. Typically, the *trans*-11 18:1 isomer is the favored intermediate over the *trans*-10 18:1 isomer in the biohydrogenation process (Dewanckele et al., 2020b). Dewanckele et al. (2020a) suggested that specific microorganisms, such as *Cutibacterium acnes*, produce the *trans*-10 18:1 intermediate in the rumen. The ruminal environment provided by the LF diets and diets containing BT stage triticale may have allowed for greater proliferation of these bacteria to result in the *trans*-10 shift observed. Faster ruminal passage rates and shorter ruminal retention times may have additionally allowed *trans* 18:1 intermediates to escape the rumen before complete biohydrogenation, resulting in the observed increase in *trans* fatty acids. *Trans* 18:1 intermediates (particularly *trans*-10 18:1) and *trans*-10, *cis*-12 CLA fatty acids are associated with milk fat depression (Bauman and Griinari, 2003), and this may at least partially explain the marginally reduced milk fat concentration observed in cows fed diets containing BT stage triticale. At this point, biohydrogenation activity in the rumen cannot be evaluated without knowing the relative intake and outflow of fatty acids from the rumen, and it is difficult to speculate on the relative relationship between biohydrogenation of unsaturated fatty acids, the availability of hydrogen, and methanogenic activity in the rumen. However, fatty acid synthesis was not impaired in cows consuming diets containing BT stage triticale as evidenced by the increased concentration of *de novo* fatty acids in the milk, and milk fat yield was not influenced by forage maturity or inclusion level. Furthermore, milk fat concentration was not different between LF and HF diets despite the observed shifts in milk fatty acid profile. With the lowest milk fat concentration at 3.60%, we are confident that cows in this study were not milk fat depressed.

3.5. Conclusion

In conclusion, dairy cattle production performance does not seem overly sensitive to the harvesting maturity of triticale silage. We initially hypothesized that cows consuming BT stage triticale silage would produce more enteric CH_4 due to the improved digestibility of the diet. Contrary to our hypothesis, cows consuming BT stage triticale produced less enteric CH_4, likely due to shifts in ruminal fermentation and passage kinetics. Feeding low-forage diets with these forages seems to improve dairy cow performance and reduce CH_4 emissions at the expense of digestive efficiency. From a production standpoint, it seems there is not a substantive production benefit of feeding triticale silage in the BT stage of maturity relative to the SFT stage. From an environmental standpoint, feeding triticale silage of the BT stage maturity seems to be a sustainable CH_4 mitigation strategy that simultaneously increases DMI and milk yield. However, the mechanism behind the reduction in enteric CH_4 in response to earlier forage maturity is speculative at this point. Studies that comprehensively evaluate ruminal passage rate, ruminal VFA production, and enteric CH_4 emissions are needed to disseminate these relationships regarding the environmental implications of feeding triticale silage of differing nutritional qualities as affected by harvesting maturity.

3.6. Notes

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3.8. Tables

Table 3.1. Ingredient and nutritional composition of high-forage (HF) or low-forage (LF) diets containing silage of triticale harvested in the boot (BT) or soft-dough (SFT) stage of maturity

	HF		LF	
	BT	SFT	BT	SFT
Ingredient, % DM				
Corn silage	26.3	26.3	18.8	18.8
Triticale silage, BT	25.3	-	18.3	-
Triticale silage, SFT	-	25.3	-	18.3
Corn grain	19.5	19.5	23.0	23.0
Soybean meal expeller	18.0	18.0	13.5	13.5
Soybean hulls	5.25	5.25	12.3	12.3
Corn distiller's grains	-	-	4.18	4.18
Whole-roasted soybeans	-	-	4.01	4.01
Supplemental fat[1]	1.82	1.82	1.88	1.88
Bentonite	0.81	0.81	0.84	0.84
Calcium carbonate	0.81	0.81	0.84	0.84
Salt	0.51	0.51	0.52	0.52
Sodium bicarbonate	1.01	1.01	1.04	1.04
Magnesium oxide	0.16	0.16	0.17	0.17
Trace mineral premix[2]	0.04	0.04	0.04	0.04
Vitamin ADE[3]	0.04	0.04	0.05	0.05
Vitamin E (44 IU/g)	0.06	0.06	0.06	0.06
Larvicide[4]	0.04	0.04	0.04	0.04
Ionophore[5]	0.41	0.41	0.43	0.43
Composition, % DM				
CP	13.5 (1.1)	11.4 (1.0)	14.1 (1.1)	12.6 (1.1)
NDF	32.2 (2.1)	35.1 (1.8)	33.1 (1.7)	35.2 (1.5)
Starch	25.7 (1.2)	26.3 (1.2)	24.8 (2.0)	25.2 (2.1)

Values represent the mean (SD) of 4 composite samples by period per diet.

[1] Energy Booster 100 (Hubbard Feeds; Mankato, MN).

[2] Contained 30.7% calcium; 6.3% sulfur; 35,800 mg/kg zinc; 27,400 mg/kg manganese; 6,269 mg/kg copper; 1,548 mg/kg iron; 1,190 mg/kg iodine; 524 mg/kg cobalt.

[3] Contained 24.3% calcium; 9,900 KIU/kg vitamin A; 2,200 KIU/kg vitamin D_3; 3,300 IU/g vitamin E.

[4] Clarify (0.67%).

[5] Rumensin 90 (200 g monensin/kg).

Table 3.2. Nutritional composition and parameters of fiber degradation kinetics of forages[1] included in the experimental diets

	Corn silage	Triticale BT	Triticale SFT
Nutrient composition[2]			
CP, %	7.2 (0.8)	16.7 (1.4)	8.7 (0.8)
NDF, %	39.3 (5.4)	51.1 (1.4)	62.6 (2.0)
ADF, %	24.6 (3.0)	35.0 (0.7)	46.1 (1.7)
ADL, %	3.0 (0.4)	3.7 (0.3)	6.4 (0.3)
uNDF, %	11.4 (0.4)	6.8 (0.9)	22.4 (1.6)
uNDF, % NDF	29.4 (4.3)	13.4 (1.5)	35.8 (2.1)
pdNDF, %	27.9 (5.4)	44.2 (0.5)	40.2 (1.6)
pdNDF, %NDF	70.6 (4.3)	86.6 (1.5)	64.3 (2.1)
Starch, %	39.2 (4.8)	2.2 (0.4)	4.6 (1.0)
Degradation kinetics			
Discrete lag, h	6.3 (0.4)	3.7 (0.3)	5.0 (0.9)
Degradation rate of pdNDF, %/h	1.14 (0.14)	2.33 (0.24)	1.53 (0.23)
pH	3.82 (0.15)	4.31 (0.22)	4.05 (0.09)
Fermentation profile, %DM			
Ammonia	0.99 (0.25)	2.48 (0.44)	1.33 (0.09)
Lactic acid	4.78 (1.27)	8.10 (0.89)	6.25 (0.61)
Acetic acid	1.84 (0.72)	1.52 (0.56)	2.49 (0.69)
Propionic acid	0.00 (0.00)	0.09 (0.11)	0.07 (0.10)
Butyric acid	0.00 (0.00)	0.10 (0.20)	0.00 (0.00)
1,2 Propanediol	0.04 (0.08)	0.18 (0.13)	0.12 (0.24)
Total VFA	6.61 (1.98)	9.81 (1.59)	8.82 (1.29)

Values represent the mean (SD) of 4 composited samples by period per forage.

[1] BT = boot stage of maturity; SFT = soft-dough stage of maturity.

[2] DM basis unless otherwise stated.

Table 3.3. Production performance of lactating dairy cows consuming high-forage (HF) or low-forage (LF) diets containing silage of triticale harvested in the boot (BT) or soft-dough (SFT) stage of maturity

	HF		LF			*P*-value[1]		
	BT	SFT	BT	SFT	SEM	M	F	MxF
Milk yield, kg/d	44.9	41.4	48.9	45.3	1.2	0.01	0.01	0.98
Milk fat, %	3.81	4.19	3.60	4.07	0.17	0.01	0.20	0.73
Milk protein, %	2.85	2.81	2.92	2.88	0.06	0.06	0.01	0.97
Milk lactose, %	4.82	4.80	4.83	4.82	0.02	0.26	0.30	0.42
Milk fat yield, kg/d	1.69	1.73	1.75	1.83	0.08	0.27	0.12	0.68
Milk protein yield, kg/d	1.28	1.16	1.43	1.31	0.04	0.01	0.01	0.96
Milk lactose yield, kg/d	2.17	1.98	2.37	2.19	0.07	0.01	0.01	0.96
ECM yield, kg/d	46.4	44.7	49.6	48.5	1.4	0.09	0.01	0.74
Feed efficiency, kg ECM/kg DMI	1.82^{ab}	1.93^{a}	1.78^{ab}	1.71^{b}	0.11	0.63	0.01	0.04
MUN, mg/dL	14.4	14.0	13.8	14.7	0.9	0.51	0.82	0.08

Values represent the mean of 24 cows per treatment.

[1] M = effect of forage maturity (BT vs. SFT); F = effect of forage inclusion level (HF vs. LF); MxF = interaction between forage maturity and forage inclusion level.

[ab] Means with different letter superscripts within row are different ($P < 0.05$).

Table 3.4. Dry matter intake, nutrient intake, and nutrient digestion of lactating dairy cows consuming high-forage (HF) or low-forage (LF) diets containing silage of triticale harvested in the boot (BT) or soft-dough (SFT) stage of maturity

	HF		LF			*P*-value[1]		
	BT	SFT	BT	SFT	SEM	M	F	MxF
Intake, kg/d								
DM	26.3^{b}	23.4^{c}	28.2^{a}	28.7^{a}	1.45	0.01	0.01	0.01
CP	3.4	2.8	3.9	3.4	0.28	0.01	0.01	0.85
NDF	10.3^{b}	9.6^{b}	11.1^{a}	11.3^{a}	0.59	0.24	0.01	0.02
Starch	6.5^{bc}	6.1^{c}	6.9^{b}	7.5^{a}	0.36	0.49	0.01	0.01
Digestibility, %								
DM	71.1	69.2	67.5	65.3	1.28	0.03	0.01	0.92
CP	60.5^{a}	57.2^{a}	60.3^{a}	51.3^{b}	2.74	0.01	0.04	0.04
NDF	62.4	58.1	56.3	50.8	2.57	0.01	0.01	0.70
Starch	95.2	95.7	94.7	94.9	0.81	0.09	0.01	0.49
Digested, kg/d								
DM	18.9^{a}	16.3^{b}	19.1^{a}	18.9^{a}	1.00	0.01	0.01	0.01
CP	2.1	1.6	2.4	1.8	0.19	0.01	0.01	0.52
NDF	6.5	5.6	6.2	5.8	0.40	0.01	0.85	0.28
Starch	6.3^{bc}	5.9^{c}	6.6^{b}	7.2^{a}	0.33	0.44	0.01	0.01

Values represent the mean of 24 cows per treatment.

[1] M = effect of forage maturity (BT vs. SFT); F = effect of forage inclusion level (HF vs. LF); MxF = interaction between forage maturity and forage inclusion level.

[abc] Means with different letter superscripts within row are different ($P < 0.05$).

Table 3.5. Milk fatty acid profile (g/100 g fatty acid) of lactating dairy cows consuming high-forage (HF) or low-forage (LF) diets containing silage of triticale harvested in the boot (BT) or soft-dough (SFT) stage of maturity

	HF		LF			*P*-value[1]		
	BT	SFT	BT	SFT	SEM	M	F	MxF
4:0	3.87	4.00	3.69	3.85	0.79	0.02	0.01	0.83
6:0	2.23	2.22	2.16	2.20	0.07	0.78	0.24	0.50
8:0	1.25	1.19	1.25	1.23	0.05	0.13	0.52	0.49
10:0	2.69	2.42	2.75	2.60	0.13	0.01	0.11	0.42
11:0	0.06	0.04	0.07	0.05	<0.01	0.01	0.01	0.87
12:0	2.95	2.59	3.04	2.80	0.15	0.01	0.11	0.53
13:0	0.09	0.07	0.11	0.09	0.01	0.01	0.01	0.90
14:0	10.05	9.37	9.96	9.44	0.27	0.01	0.97	0.63
cis-9 14:1	1.06	0.95	1.08	0.91	0.07	0.01	0.80	0.50
15:0	0.82	0.68	0.88	0.74	0.03	0.01	0.03	0.97
iso 15:0	0.18	0.18	0.16	0.18	0.01	0.04	0.01	0.11
anteiso 15:0	0.35	0.34	0.35	0.35	0.01	0.17	0.28	0.36
16:0	30.39	30.25	27.96	28.56	0.48	0.51	0.01	0.31
iso 16:0	0.15	0.17	0.15	0.16	<0.01	0.04	0.51	0.85
cis-9 16:1	1.24	1.27	1.19	1.09	0.05	0.30	0.01	0.06
17:0	0.41	0.40	0.40	0.40	0.01	0.28	0.76	0.99
iso 17:0	0.34	0.34	0.33	0.33	0.01	0.24	0.65	0.65
18:0	10.05	11.07	10.07	11.47	0.37	0.01	0.43	0.46
trans-6,8 18:1	0.35	0.30	0.47	0.36	0.03	0.01	0.01	0.14
trans-9 18:1	0.34	0.31	0.42	0.33	0.02	0.01	0.01	0.11
trans-10 18:1	1.28	0.82	2.03	1.21	0.23	0.01	0.01	0.21
trans-11 18:1	1.10	1.12	0.99	1.02	0.05	0.47	0.01	0.88
trans-12 18:1	0.49	0.41	0.55	0.47	0.02	0.01	0.01	0.98
cis-9 18:1	22.15	23.82	22.55	23.57	0.58	0.01	0.88	0.50
cis-11 18:1	0.84	0.77	0.95	0.81	0.04	0.01	0.01	0.06
cis-9,*cis*-12 18:2	3.04	2.94	4.09	3.73	0.13	0.01	0.01	0.14
cis-9,*cis*-12,*cis*-15 18:3	0.43	0.39	0.51	0.44	0.02	0.01	0.01	0.10
19:0	0.18	0.11	0.21	0.12	0.02	0.01	0.02	0.50
20:0	0.15	0.15	0.14	0.16	<0.01	0.01	0.84	0.17
cis-9,*trans*-11 CLA	0.50	0.48	0.46	0.45	0.02	0.28	0.01	0.95
trans-10,*cis*-12 CLA	0.008	0.005	0.014	0.007	0.002	0.01	0.01	0.35
Other	0.98	0.86	1.04	0.88	0.03	0.01	0.17	0.47
Σ De Novo[2]	23.20	21.90	23.05	22.25	0.65	0.01	0.79	0.53
Σ SFA	66.20	65.58	63.69	64.74	0.87	0.73	0.01	0.16
Σ MUFA	28.84	29.77	30.22	29.78	0.74	0.66	0.22	0.22
Σ PUFA	3.98	3.82	5.07	4.62	0.16	0.01	0.01	0.15
Σ Trans fatty acids	4.07	3.44	4.92	3.83	0.33	0.01	0.01	0.22
Σ OBCFA[3]	2.40	2.22	2.46	2.31	0.05	0.01	0.04	0.78
Biohydrogenation[4]	1.17	0.73	2.31	1.23	0.25	0.01	0.01	0.09

Values represent the mean of 24 cows per treatment.

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[1] M = effect of forage maturity (BT vs. SFT); F = effect of forage inclusion level (HF vs. LF); MxF = interaction between forage maturity and forage inclusion level.

[2] The sum of 4:0 through 14:0.

[3] The sum of 11:0, 13:0, *iso* 15:0, *anteiso* 15:0, 15:0, *iso* 16:0, *iso* 17:0, and 17:0.

[4] The ratio of *trans*-10 18:1 to *trans*-11 18:1.

3.9 Figures

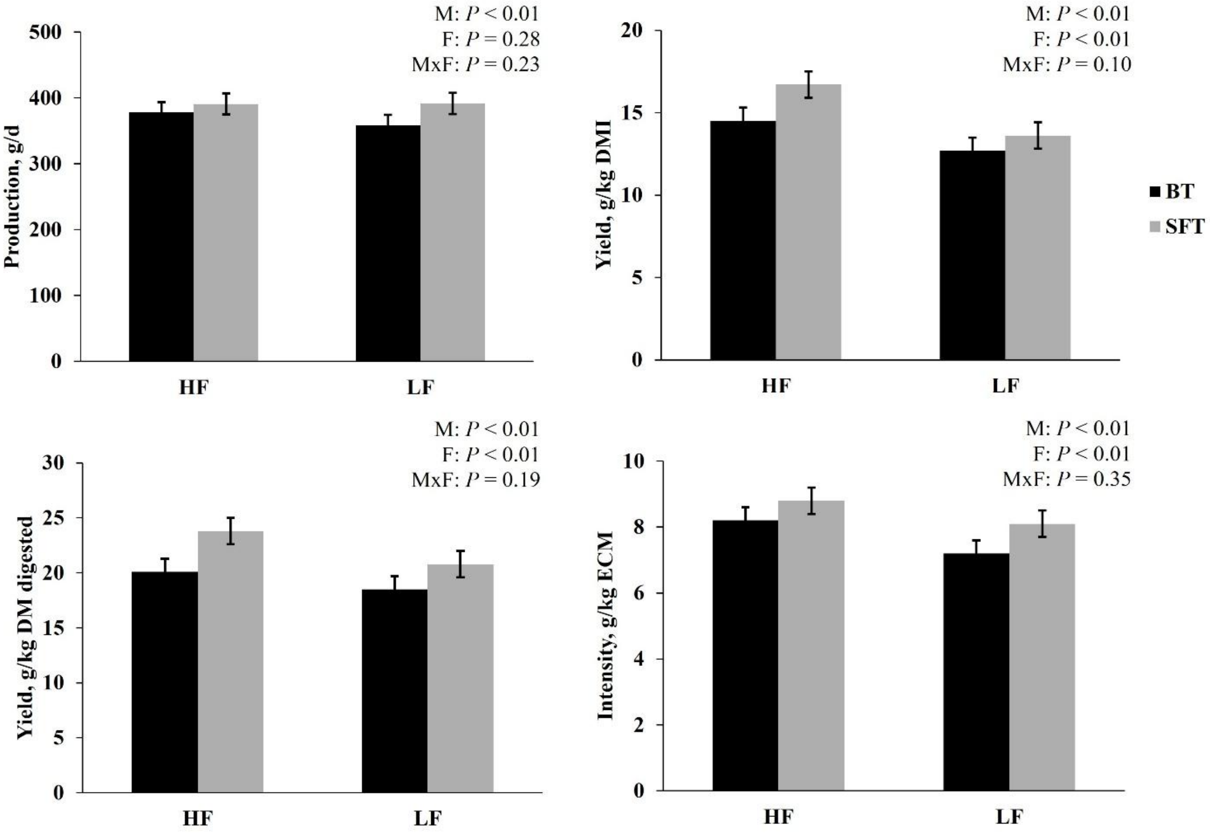

Figure 3.1. Daily enteric methane production (g/d), yield (g/kg DMI and g/kg DM digested), and intensity (g/kg ECM) of lactating dairy cows (n = 24) consuming high-forage (HF) and low-forage (LF) diets containing triticale silage harvested either at the boot (BT) or soft-dough (SFT) stage of maturity. M = effect of forage maturity (BT vs. SFT); F = effect of forage inclusion level (HF vs. LF); MxF = interaction between forage maturity and forage inclusion level.

CHAPTER 4: Ruminal Passage Rate, Fiber Digestibility, and Methane Emissions from Lactating Holsteins Fed Triticale Silage of Different Maturities

4.1. Abstract

This study evaluated the production performance, nutrient digestibility, ruminal VFA, enteric methane (**CH_4**) emissions, fecal biomethane potential, and milk fatty acid profile of high-producing dairy cows fed diets containing triticale silages harvested at either the boot (**BT**) or soft-dough (**SFT**) stage. The BT silage contained 16.4% CP, 53.1% NDF, 32.9% ADF, 2.5% ADL, and 0.7% starch, while the SFT silage contained 9.3% CP, 63.2% NDF, 42.2% ADF, 5.1% ADL, and 3.2% starch. Experimental diets included either BT or SFT triticale silage in equivalent proportions (DM basis). The experiment followed a randomized crossover design with 28-d periods and a 7-d covariate period using 16 primiparous and 8 multiparous Holstein cows, half of which were rumen-cannulated. All cows were assessed for production performance, nutrient digestibility, and milk fatty acid profile. Rumen-cannulated cows were evaluated for ruminal VFA and passage kinetics and non-cannulated cows for CH_4 emissions via GreenFeed and fecal biomethane potential. Cows fed BT triticale were more feed efficient (1.81 vs. 1.70 kg ECM/kg DMI) and produced more milk (39.6 vs. 36.3 kg/d) with similar fat, protein, and lactose concentrations as cows fed SFT triticale, resulting in more ECM (45.6 vs. 42.2 kg/d). Cows consuming BT triticale showed improved total-tract digestibilities of DM (64.1 vs. 57.8%), CP (52.2 vs. 44.3%), NDF (51.8 vs. 43.7%), and pdNDF (65.4 vs. 60.4%). Marker-based estimates indicated a faster K_p of BT triticale NDF than SFT (6.38 vs. 4.75 %/h). However, dietary K_p did not differ (3.20 %/h). Ruminal fermentation was more active and altered in cows fed BT triticale, evidenced by greater total VFA concentration (166.4 vs. 151.5 mM) and a lower acetate-to-propionate ratio (3.8 vs. 4.0) compared to SFT

triticale. Cows consuming BT triticale emitted less enteric CH_4 (332 vs. 348 g/d) and showed reduced CH_4 intensity (7.8 vs. 8.7 g/kg ECM). Despite improved digestibility, feces from cows consuming BT triticale contained more pdNDF (27.3 vs. 24.8% DM) due to their greater pdNDF intake (7.1 vs. 6.5 kg/d). The biomethane potential of feces did not differ (22.1 mL CH_4/g OM). However, cows consuming the BT diet were estimated to emit 18 L/d more CH_4 from their feces compared to cows consuming the SFT diet (195 vs 177 L/d). Although marginal, the milk fatty acid profile reflected altered fermentation dynamics. Milk fat from cows consuming BT triticale contained more *de novo* fatty acids (25.21 vs. 24.70 g/100 g fatty acid), *trans* fatty acids (3.05 vs. 2.87 g/100 g fatty acid), and OBCFA (2.71 vs. 2.55 g/100 g fatty acid), consistent with elevated VFA production and ruminal cellulolytic activity. In conclusion, feeding BT triticale silage enhanced nutrient digestibility and milk production, reduced enteric CH_4 emissions, and altered ruminal fermentation patterns compared to SFT. However, the increased intake of pdNDF in the BT diet led to greater fecal pdNDF excretion, potentially offsetting reductions in enteric CH_4 emissions. These findings highlight the complex trade-offs between forage quality, animal performance, and environmental outcomes, and challenge the assumption that faster ruminal K_p uniformly reduces enteric CH_4 emissions by limiting fermentation.

4.2. Introduction

Enteric methane (**CH_4**) is the largest source of CH_4 emissions in the dairy industry. In the United States, enteric fermentation from livestock contributed 3.1% of the 6,340 MMT of CO_2-equivalent emissions emitted to the atmosphere in 2021 (EPA, 2023). With the global population growing and food demand projected to increase by 35% to 56% by 2050 (van Dijk et al., 2021), enteric CH_4 emissions from livestock are expected to significantly influence global greenhouse gas emissions. As such, sustainable and applied methods to reduce cattle enteric CH_4 emissions without sacrificing cattle productivity and feed efficiency must be determined.

Current recommended enteric CH_4 mitigation strategies for dairy cattle include selective breeding and dietary interventions, such as increasing dietary fat and incorporating feed additives that alter the volatile fatty acid profile or the population of CH_4-producing microbes (Beauchemin et al., 2022; Hristov et al., 2022). Particularly, a meta-analysis showed that supplementation of microalgae and 3-nitrooxypropanol are the most effective at mitigating enteric CH_4 emissions, reducing emissions by 50% and 30%, respectively, compared to supplementation of nitrate, oils, phytochemicals, and ionophores (Purba and Sangsawad, 2025). However, microalgae are not readily available in the United States. While 3-nitrooxypropanol has recently been approved for use in the United States in commercial herds, it is an added cost to the ration without a consistent proven benefit in milk production (Hristov et al., 2022; Martins et al., 2025). Furthermore, a meta-analysis by Pupo et al. (2025) showed that supplementing dairy rations with 3-nitrooxypropanol reduces average income over feed costs by \$0.35 per cow/d for cows producing 39 kg milk/d and requires additional compensation of \$128,320/yr for an operation with 1,000 milking cows. Profitability frequently serves as the primary factor influencing the adoption of mitigation practices in production systems. Livestock industries are likely to reject practices that demand extra investment without guaranteed economic benefit or those that might reduce animal productivity or raise production costs. Therefore, researchers need to further evaluate producer-friendly methods for mitigating enteric CH_4 emissions.

Forage management presents a more sustainable and practical method to reduce enteric CH_4 emissions, yet it remains underexplored. Extensive research evaluates microalgae's bromoform-mediated inhibition of methanogen activity and 3-nitrooxypropanol's direct inhibition of methyl-coenzyme M reductase in modulating enteric CH_4 emissions (Purba and Sangsawad, 2025). Yet, the role of forage management in modulating enteric CH_4 production is less understood. Nonetheless, forage quality is outlined as a factor that can influence enteric CH_4 emissions (Hristov et al., 2013).

Forage quality is mostly defined by its NDF concentration, a feed component that is digested slowly and incompletely according to the Lucas test principle (Van Soest, 1994; Galyon et al., 2024). Neutral detergent fiber is further categorized into the potentially degradable fraction (**pdNDF**) and the undegradable fraction (**uNDF**). While pdNDF may be fermented by rumen microbes, uNDF cannot be degraded. The balance between the rate at which pdNDF is degraded (**K_d**) and the rate at which it exits the rumen (**K_p**) determines NDF utilization by dairy cattle (Ellis et al., 1994). Since CH_4 emissions are a byproduct of fiber degradation in the rumen, CH_4 emissions are likely influenced by pdNDF and uNDF concentrations and the delicate balance between K_d and K_p. Forages with greater uNDF concentration, faster K_p, or their combination may reduce pdNDF fermentation in the rumen, potentially decreasing enteric CH_4 emissions.

Triticale (x *Triticosecale* Wittmack) silage offers an opportunity to explore these dynamics. Prior research indicates that feeding triticale silage harvested in the boot stage reduces enteric CH_4 emissions by 10% compared to feeding triticale silage harvested in the soft-dough stage of maturity, while also increasing DM intake by 1.2 kg and ECM yield by 1.4 kg (Galyon et al., 2025). When harvested at the boot stage, triticale contains a greater pdNDF concentration (83.0% vs. 61.9% NDF) and a lower uNDF concentration (17.0% vs. 38.1% NDF) on a NDF basis compared to the soft-dough stage (Ferreira et al., 2025). Additionally, boot stage triticale has a pdNDF K_d of 3.87%/h, while soft-dough stage triticale has a pdNDF K_d of 2.13%/h (Ferreira et al., 2025). Preliminarily, it seems that enteric CH_4 emissions should be greater for cows consuming boot stage triticale due to greater ruminal degradation. However, feeding high-quality forages has been suggested to reduce enteric CH_4 emissions by increasing the rate of passage from the rumen (Brask et al., 2013; Wenner et al., 2017; Hristov et al., 2025). Assuming that higher forage quality corresponds to faster K_p (Van Soest, 1994; Galyon et al., 2024), boot stage triticale likely has a faster K_p compared to soft-dough stage triticale. Therefore, this may be the mechanism through which enteric CH_4 emissions are reduced by feeding forages of differing nutritional qualities. To our knowledge, no in vivo studies have simultaneously evaluated ruminal K_p and enteric CH_4 emissions from dairy cattle to confirm this postulate.

Additional questions are raised about the role of K_p in CH_4 emissions from fecal material. If faster K_p reduces ruminal fermentation, increased excretion of unfermented pdNDF may become a substrate for CH_4 production in feces. To date, no in vivo studies have directly explored the connection between ruminal K_p and fecal CH_4 emissions. Investigating this holistic concept is essential to determine whether forage quality-driven variations in K_p influence total CH_4 emissions. Connecting forage quality to passage rate and CH_4 emissions could encourage dairy producers and nutritionists to adopt better forage management practices targeting reduced CH_4 emissions.

We hypothesized that feeding boot stage triticale silage would result in a faster ruminal K_p and decreased enteric CH_4 emissions, albeit with increased fecal CH_4 emissions, compared to feeding soft-dough stage triticale silage. The objectives of the present study were to elucidate the relationship between enteric CH_4 emissions and ruminal K_p and to examine the effects of forage quality and ruminal K_p on fecal CH_4 emissions. Furthermore, under the premise that forage quality affects ruminal metabolism, this study aimed to evaluate the influence of forage maturity on ruminal VFA, dairy cattle performance, and milk fatty acid profiles.

4.3. Materials and Methods

4.3.1 Triticale Silages

Triticale was cultivated at Kentland Farm (Blacksburg, VA) similarly to Schultz et al (2025) and Galyon et al. (2025). A single 10-ha field was seeded on October 19, 2023, with 105 kg/ha of triticale (TriCal Grainer 154; Butte, MT). Corn for silage was harvested from the field on September 17, 2023. On November 19, 2023, 45 kg N/ha from dairy manure was applied, and on March 19, 2024, an additional 45 kg N/ha was broadcast as urea and ammonium nitrate. Starting on April 19, 2024, the growth stage of the crop was monitored weekly to assess maturity following the method outlined by Ferreira et al. (2025) and using the Zadoks scale (Zadoks et al., 1974) to determine the appropriate maturity stage for harvest.

The field was divided into 4 quadrants, and alternate quadrants were harvested for each maturity stage to reduce field effects on the nutritional qualities of the resulting crops. The crop was harvested at two distinct stages of maturity: the boot **(BT)** stage of maturity on April 20, 2024, and the soft-dough **(SFT)** stage of maturity on May 25, 2024. At the BT stage, half of the crop was cut using a mower with conditioner rolls (John Deere C250 MoCo; Moline, IL), wilted for 2 days, tedded, and chopped with a forage chopper (John Deere 7400) set to a theoretical length of cut of 19 mm. A microbial inoculant (Early Sile Plus; Micron Bio-Systems Inc.; Buena Vista, VA) was applied to the biomass during chopping at a rate of 10 mL/kg (on a fresh-weight basis). At the SFT stage, the other half of the crop was similarly cut, wilted for less than 12 h, tedded, chopped with the same theoretical length of cut, and ensiled similarly. Immediately after chopping, the biomass was ensiled in separate concrete-walled bunker silos, each lined with a plastic sheath on the floor and walls. The ensiled material was then covered with a plastic sheet and a green mesh tarp (Secure Cover; Bag Man, LLC), which were secured with silo tire sides. The ensiling period lasted for a minimum of 52 days before the first sampling and 137 days before inclusion in dairy rations. Multiple subsamples were collected and composited on July 16, 2024, to assess the chemical composition of each silage. The composite samples were immediately frozen and sent to a commercial laboratory (Cumberland Valley Analytical Services, Lancaster, PA) for preliminary nutritional analysis using near-infrared reflectance spectroscopy (NIR 2 package).

4.3.2 Animals, Housing, and Diets

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Virginia Tech. Sixteen primiparous (609 ± 45 kg BW, 156 ± 27 DIM) and 8 multiparous (673 ± 52 kg BW, 169 ± 65 DIM) Holstein dairy cows, half of which were rumen-cannulated, were randomly assigned to one of two experimental diets in a crossover design (Cochran, 1992) with two 28-d periods and a 7-d preceding covariate period. A random number generator was used to equally distribute treatments among parity and rumen-cannulated and non-cannulated cows. All cows were housed in a 24-stall pen within a freestall barn. Cows were fed once daily (0830 h) via a Calan gate system (American Calan Inc., Northwood, NH). Cows were trained to find their specific door for a 7-d period before the beginning of the study. For reasons unrelated to the study, one non-cannulated cow was removed from the study before completion of the covariate period.

The experimental diets were formulated to include either triticale silage harvested in the BT stage or the SFT stage of maturity in addition to corn silage (**Table 4.1**). Subsamples of corn silage were collected, composited, and analyzed in the same manner as the triticale silages. Using the preliminary composition of corn and triticale silages, rations were formulated to meet the requirements of second-parity dairy cows (645 kg BW and 160 DIM) consuming 24 kg of DM/d and producing 40 kg of milk/d containing 3.75% fat and 3.10% protein using version 3.0.8.1 of CPM Dairy (CAHP Software Information, Philadelphia, PA). A triticale silage with a nutrient profile reflecting the average of the two triticale silages was used to formulate a base ration containing 33% NDF (DM basis), where the concentrate, the corn silage, and the triticale silages each provided one-third of the dietary NDF. The final rations were developed by replacing the average silage with either the BT triticale silage or the SFT triticale silage. Concentrates were ordered from a commercial feed mill (Exchange Milling Company Inc., Rocky Mount, VA). The TMR were delivered to feed ad libitum and to obtain ~5% of feed refusals. The amounts of feed offered and feed refused were measured daily. Cows were milked twice daily (0100 h and 1200 h), and individual milk weights were automatically recorded at each milking. For statistical analyses, the average daily milk yield and DMI were determined from d 4 to d 7 during the covariate period and from d 10 to 16 of each experimental period. The average DMI included DMI from the bait pellet used for measuring enteric CH_4 emissions. Weekly, 5 drops of bait pellet were manually deployed from the GreenFeed system and individually weighed. The average DMI of the bait pellet was estimated from the number of cow visits to the GreenFeed system (C-Lock Inc., Rapid City, SD) and the average pellet DM deployment per drop.

4.3.3 Enteric Methane Emissions

Enteric CH_4 emissions were measured using a single GreenFeed unit (hereafter unit) accessible to all cows within the pen with 24 stalls. The unit was maintained and calibrated following the recommendations of the manufacturer. The CO_2 recoveries averaged 100.9 ± 3.5% (n = 5). Cows were trained to the unit for a 7-d period before the beginning of the covariate period. Methane production was estimated from voluntary visits of cows to the unit to receive a palatable bait pellet containing 17% CP, 33% NDF, and 26% starch on a DM basis. Cows visited the unit at least twice daily to collect adequate data per cow. To capture diurnal variations in CH_4 production, cows visited the system at least once at 0000 – 0359 h, 0400 – 0759 h, 0800 – 1159 h, 1200 – 1559 h, 1600 – 1959 h, and 2000 – 2359 h during the experimental data collection periods. Cow visitation to the unit was monitored daily, and cows were guided to the system when needed to ensure the criteria were met.

For statistical analyses, the average daily enteric CH_4 production was determined from d 4 to d 7 during the covariate period and from d 10 to 16 of each experimental period. Average DMI and ECM yield from d 4 to 7 for the covariate period and average DMI, DM digested, and ECM yield from d 10 to 16 for the experimental periods were used to calculate CH_4 yield (g of CH_4/kg DMI and g of CH_4/kg DM digested) and CH_4 intensity (g CH_4/kg ECM), respectively. Due to the potential leakage of gases from ruminal cannulas (Hristov et al., 2015), data were only utilized from non-cannulated cows.

4.3.4 Marked Fiber Preparation for Passage Rate

We utilized the marker dilution technique (Cochran et al., 1987; Ellis et al., 1994) with modifications to measure K_p (Galyon et al., 2024). Corn silage in its original form (i.e., non-ground) was dried at 55°C on mesh wire trays lined with cheesecloth and then sieved using the Penn State Particle Separator (Kononoff and Heinrichs, 2003) to collect mostly grain-free fibrous material retained by the 8-mm and upper sieves. Approximately 750 g of corn silage fibrous material was subjected to a macro NDF procedure (Galyon et al., 2024). The resulting fibrous residue (hereafter named macro-fiber) was dried in a forced-air oven at 55°C. Three batches of macro-fiber were then soaked overnight in 20 L of 0.2 M lanthanum chloride ($LaCl_3$). The $LaCl_3$ solution was prepared by reacting La_2O_3 with HCl as described by Yang et al. (2017). After the overnight soak, the macro-fiber was removed and soaked in 20 L of 1 M acetic acid for 8 h to remove any loosely bound marker (Allen, 1982; Ellis et al., 1994). The resulting marked fiber was air-dried and stored until K_p measurements.

To evaluate if the marked forage utilized significantly affects the estimated K_p, the same procedure was followed to produce marked BT triticale silage and SFT triticale silage except the resulting macro-fiber residues were soaked in ytterbium chloride ($YbCl_3$). The 0.2 M $YbCl_3$ solution was prepared in the same manner as $LaCl_3$ except that Yb_2O_3 was reacted with HCl under heat (Galyon et al., 2024). To compensate for the greater NDF concentration of the triticale silages, the amount of dry triticale silage macro-fiber soaked in marker was equivalent to that of the dry corn silage macro-fiber.

The preparation of marked fibers was repeated as necessary to produce enough for the entire trial, and marked fibers of the same type were pooled per period. For each ruminal evacuation, the total dosage (g DM/cow) of marked fiber allocated to each cow was 3% of their daily NDF intake during the covariate period. Each cow received $LaCl_3$-marked corn silage DM equating to 1.5% of their daily NDF intake during the covariate period. Each cow additionally received an equal portion of $YbCl_3$-marked triticale silage DM of the same maturity in their assigned diet.

4.3.5 Ruminal Pool Size and Passage Rate

On d 17 of each period, ruminal contents from 6 rumen-cannulated cows were evacuated at 2 h before feeding (0630 h), and ruminal contents from the other 6 rumen-cannulated cows were evacuated at 2 h after feeding (1030 h) to determine ruminal pool size such that diets were balanced among evacuation times. On d 24 of each period, cows were evacuated again at the opposite evacuation times to account for varying ruminal pool sizes respective to feeding time. During the ruminal evacuation, the entire contents of the rumen and reticulum were removed by hand into a 200-L drum and weighed. The contents were then separated into solid and liquid pools using a 20-L hydro press (EJWOX, Wilmington, CA), and the liquid pool was weighed. The solid pool was estimated as the difference between the total pool of ruminal contents minus the liquid pool. A 200-g sample of the pressed solids was taken to determine the ruminal pool sizes of DM, NDF, uNDF, and pdNDF (100 − uNDF, NDF basis). The DM concentration of the liquid contents was considered minimal (<5% DM, Galyon et al., 2024) and, therefore, unaccounted. To determine the DM pool size, the mass of pressed solids was multiplied by the DM concentration of the pressed solids. The ruminal pool sizes of NDF, uNDF, and pdNDF were determined by multiplying the DM pool size by the respective concentration of NDF, uNDF, and pdNDF (DM basis) of the pressed solids. Within each period, the pool sizes from d 17 and 24 were averaged per cow.

Immediately after collecting weights, the solid and liquid contents and a pulse dose of marked fiber were thoroughly mixed within the 200-L drum, and the reconstituted ruminal contents were manually returned to the rumen. Following the return of the ruminal contents to the rumen, grab samples (~400 g total) were collected from five locations within the rumen at 0, 2, 4, 6, 9, 12, 24, 36, 48, 60, 72, and 96 h. Samples from each time point were separated into solids and liquid using a 3-L fruit press (EJWOX). The solids were then dried in a forced-air oven at 55°C until reaching constant weight, then ground to pass a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ), and stored until uNDF, La, and Yb analyses.

Passage rate was determined as the dilution rate of the marker among uNDF over time per cow in each evacuation using the NLIN procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC) according to Equation 1:

$$Marker = Initial \times e^{(-Kp \times T)}, [1]$$

where *Marker* is the ratio of the marker relative to uNDF at time T (mg/g uNDF), and *Initial* is the ratio of the marker relative to uNDF at $T = 0$ (mg/g uNDF) (Galyon et al., 2024). As a first step, the ratio of marker to uNDF over time was plotted in Microsoft Excel (Microsoft Corporation, Redmond, WA), and an exponential line was fitted. When the ratio of marker relative to uNDF at T = 0 was suspiciously low from the visual assessment, an outlier test was performed following statistical procedures (Galyon et al., 2024). An intercept (Y_{Pred}) and its standard deviation (SE_{YPred}) were predicted including all the time points except the suspected outlier (Y^*) at T = 0, and a *t*-test was performed to test the null hypothesis *"H_0: Y^* = not outlier"* according to Equation 2.

$$t = \frac{(Y^* - Y_{Pred})}{SE_{YPred}}, [2]$$

A two-tailed *t*-test with an α equal to 0.05 and 10 degrees of freedom was used to determine a critical value equal to 2.228. When the absolute *t* value was greater than 2.228, the null hypothesis was rejected, the observation Y^* was removed as an outlier, and the predicted intercept Y_{Pred} was utilized to determine the K_p. The estimates of K_p from d 17 and 24 evacuations were averaged per cow in each period. Ruminal mean retention time (**MRT**) was calculated as MRT = $1/K_p$.

The turnover rate (**TOR**) of uNDF (TOR_{uNDF}; %/h) was estimated using a flux/compartmental pool method (Ellis et al., 1994) following Equation 3 and Equation 4:

$$Turnover_{uNDF} = \frac{uNDFI}{uNDF\ pool\ size}, [3]$$

$$TOR_{uNDF} = \frac{Turnover_{uNDF}}{24} \times 100, [4]$$

where $Turnover_{uNDF}$ is the turnover of uNDF in the rumen (/d), *uNDFI* is the average daily uNDF intake (kg/d) from d 10 to d 16, and *uNDF pool size* is the average pool size (kg) of uNDF determined from d 17 and d 24 ruminal evacuations.

4.3.6 Ruminal Fermentation

Samples of ruminal fluid were collected from rumen-cannulated cows -0.5, 4, 8, 14, and 19 h after meal delivery on d 13, coinciding with other sampling times to limit animal handling events. Grab samples (~400 g total) of ruminal contents were collected from five locations within the rumen. Samples were separated into solids and liquid using a 3-L fruit press (EJWOX) and filtered through two layers of cheesecloth. Aliquots of filtered ruminal fluid were immediately frozen at -20° C and later analyzed for VFA (Amirault et al., 2024) and ammonia-N (Weatherburn, 1967) concentrations.

4.3.7 Nutrient Digestibility

Apparent total-tract nutrient digestibility was estimated using dietary uNDF as an internal marker. In each period, individual fecal grab samples were collected every 6 h (skipping 2 h every

4 samples) from d 11 to 14. Fecal grab samples were immediately frozen at -20° C in a composite container per cow. At the end of the collection period, composite samples were thawed and mixed via an electric hand mixer (Hamilton Beach Brands, Inc., Glen Allen, VA) at a speed of 2 for 5 min. A 200-g subsample of mixed feces was dried at 55°C in a forced-air oven for at least 96 h per cow. Samples were ground to pass through a 1-mm screen of a Wiley mill and stored until analysis of DM, CP, NDF, uNDF, pdNDF, and starch. The remaining composited feces were frozen until analysis for the fecal biomethane potential assay.

Apparent DM digestibility (*DMD*; %) and nutrient digestibilities (*NutrD*; %) were calculated according to Equation 5 and Equation 6, respectively:

$$DMD = 100 - \frac{Dietary\ [uNDF]}{Fecal\ [uNDF]} \times 100, [5]$$

$$NutrD = 100 - \frac{Dietary\ [uNDF]}{Fecal\ [uNDF]} \times \frac{Fecal\ [Nutr]}{Dietary\ [Nutr]} \times 100, [6]$$

where *[uNDF]* is the concentration of uNDF (% DM) in the TMR or feces and *[Nutr]* is the concentration of either CP, NDF, pdNDF, or starch (% DM) in the TMR or feces. To account for the intake of the bait pellet, a weighted average TMR composition was calculated based on the nutrient profiles of the TMR and the bait pellet and the average intake of each for each cow.

4.3.8 Ruminal In Situ NDF Degradability

The rates of NDF degradation of BT triticale silage, SFT triticale silage, corn silage, and TMR samples were determined using an in situ ruminal degradation study according to Ferreira et al. (2022). A 0.25-g sample of each forage and TMR was placed in filter bags (F57, Ankom Technology) previously rinsed with acetone. Duplicate samples were incubated for 0, 3, 6, 12, 24, 48, 96, and 240 h in the rumen of two rumen-cannulated lactating dairy cows consuming a ration containing 40% corn silage, 10% grass hay, and 50% concentrate (DM basis), resulting in four replicates per sample for each time point. All bags were inserted simultaneously at feeding time (0900 h) into the rumen and extracted at the designated time points. After extraction, the bags were rinsed with tap water in three 5-min cycles using a washing machine (SKY2767, Best Choice Products, Irvine, CA), then dried in a forced-air oven at 55°C. Subsequently, the NDF residue was determined.

The K_d of pdNDF was determined using the NLIN procedure of SAS according to Equation 7:

$$ISNDFD = pdNDF \times \left(1 - e^{(-K_d \times (T - Lag))}\right), [7]$$

where *ISNDFD* is the degraded NDF (% NDF) at time *T* when *T* is greater than *Lag*, *Lag* is the discrete lag time of NDF degradation (h) (Mertens, 1973), and *pdNDF* is the potentially degradable NDF (% NDF) during a 240-h ruminal fermentation, which is estimated as 100 – uNDF (NDF basis) where uNDF is the NDF residue remaining after 240 h. The discrete lag time was estimated using simple linear regression as $ISNDFU = a + b \times ln_{(T)}$, where *ISNDFU* is the remaining undegraded NDF at time *T* on a NDF basis, *a* represents the intercept, *b* is the predicted slope, and the $ln_{(T)}$ is the natural log of fermentation time. *Lag* was derived according to Equation 8.

$$Lag = e^{\left(\frac{(a-100)}{-b}\right)}, [8]$$

4.3.9 Fecal Biogas Emissions Potential

Fecal CH_4 emissions potential was evaluated using a batch in vitro biomethane potential assay (Filer et al., 2019). Only feces from non-cannulated cows were analyzed to coincide with enteric CH_4 emissions data. A composite inoculum was prepared with ruminal fluid and solids collected before the morning feeding from 2 rumen-cannulated lactating dairy cows that were fed a diet containing 40% corn silage, 10% grass hay, and 50% concentrate (DM basis) as described by Ferreira and Mertens (2005). Frozen fecal material was thawed to room temperature and remixed per cow per period. Wet fecal material (10 g containing approximately 1.4 g OM on a DM basis) was mixed with 28.5 mL of in vitro media, 1.5 mL of reducing media, and 20.0 mL of inoculum and placed in 250-mL serum bottles under purging with nitrogen gas to maintain an anaerobic environment during preparation. The total inoculum volume and mass of fecal material were selected to achieve an approximate headspace volume of 180 mL and an organic loading rate of approximately 20 g OM/L based on a fecal DM concentration of 16% and OM concentration of 89%, DM basis. Serum bottles were immediately sealed with aluminum crimp seals with rubber septa and placed in a water bath at 39°C, the standard temperature for in vitro incubations with ruminal fluid (Goering and Van Soest, 1970; Ferreira and Mertens, 2005). Serum bottles remained in the water bath for the duration of the in vitro assessment and were swirled every 12 h. Due to space limitations in the water bath, one bottle per cow per period was analyzed with two replicated blanks containing only in vitro media, reducing media, and inoculum to account for background CH_4 emissions.

After feces, inoculum, and media were placed into serum bottles, the total effluent volume was determined to estimate the remaining headspace volume (V_{HS}). Headspace volume was estimated by the mass difference of adding water with a density of 1 g/mL to a tared serum bottle past the effluent volume to the top of the serum bottle. The headspace volume averaged 179.0 ± 6.4 mL. Separate 50-g subsamples of feces were dried at 100°C for 48 h to determine the DM concentration of the wet fecal material. Based on the total volume of inoculant and feces and the OM within the feces, the average organic loading rate achieved was 20.5 ± 1.7 g OM/L.

Biogas production and CH_4 concentration were determined daily through d 3, once every 3 d through d 12, and once every 5 d through d 22 for a total of 8 measurements. Before measurements were taken on each sampling day, ambient pressure (P_{amb}) was determined using the Barometer Plus phone application (version 3.7.5; PVDApps), and the actual water bath temperature was recorded. Internal headspace pressure (P_{HS}) was determined using a digital manometer (MG-9V, 0 – 15 psi ± 0.25%; SSI Technologies, LLC., Janesville, WI) attached to a luer lock needle. The concentration of CH_4 in the produced biogas was determined using gas chromatography as described by Gras et al. (2007). Using a 1-mL gas-tight syringe (Agilent Technologies, Santa Clara, CA), 0.5 mL of headspace gas was manually injected into a 6890 N Network GC System Gas Chromatograph (Agilent Technologies) equipped with a GS-CarbonPLOT column (30 m x 0.32 mm x 3 µm; Agilent Technologies). A lower pressure drop, ultra inert liner was used (Agilent Technologies). Hydrogen was used as the carrier gas with a flow rate of 1.9 mL/min in constant flow mode. The inlet was set to 250°C and the sample was split 3:1. The oven temperature was set to be isothermal at 150°C and held for 2 min per run. A flame ionization detector was set to 320°C and used with hydrogen at 30 mL/min, air at 350 mL/min, and nitrogen at 20 mL/min. Three 0.5-mL aliquots of an external gas standard containing 0.04% H_2, 3.5% CH_4, and 11.5% CO_2 balanced with N_2 (Airgas, Inc., Radnor, PA) were injected to relate the average peak area response to CH_4, and this relationship was used to quantify CH_4 concentration in the headspace of the sample bottles. The serum bottle was then vented until the internal pressure decreased to P_{amb}. Serum bottles were analyzed in the same order every sampling day.

Because water vapor is produced from fermentation and heat within the bottles, headspace pressure must be adjusted to account for the pressure contributed by water vapor. Water vapor pressure (P_w) was determined using the Magnus Form equation (Equation 9), assuming complete saturation of biogas with water vapor:

$$P_w = 0.61094 \times e^{\frac{17.625 \times (T_{HS} - 273.15)}{243.04 + (T_{HS} - 273.15)}}, [9]$$

where T_{HS} is the temperature of the headspace in °C assumed to be equivalent to the water bath temperature at the time of sampling. The dry headspace pressure (P_{DHS}) was then determined following Equation 10.

$$P_{DHS} = P_{HS} - P_W, [10]$$

The standardized dry biogas volume within each sample bottle was determined using a derivation of the Combined Gas Law following Equation 11:

$$V_{ST} = \frac{V_{HS} \times P_{DHS} \times T_{ST}}{P_{ST} \times T_{HS}}, [11]$$

where V_{ST} is the standardized volume in mL of dry biogas at standard pressure (P_{ST}; 101.325 Kpa) and standard temperature (T_{ST}; 273.15 K) and V_{HS} is the headspace volume.

Similarly, the CH_4 concentration in the headspace is saturated in water vapor. Therefore, the CH_4 headspace concentration must be adjusted for water vapor following Equation 12 (Valero et al., 2016):

$$[CH_4]_{dry} = [CH_4]_{wet} \times (1 - \frac{P_W}{P_{amb} + P_{HS}}), [12]$$

where $[CH_4]_{dry}$ is the concentration of CH_4 in dry conditions in ppm and $[CH_4]_{wet}$ is the original measured concentration of CH_4 in ppm. Per sampling day, CH_4 production was determined as $V_{ST} \times [CH_4]_{dry} \times 10^{-6}$, minus the average CH_4 produced from blank bottles. Cumulative fecal CH_4 production over the 8 sampling days was then standardized based on substrate OM as mL/g OM. The total daily fecal CH_4 emittance potential of individual cows was calculated by multiplying the standardized cumulative fecal CH_4 production by the estimated daily OM excretion of individual cows per period. The daily OM excretion of individual cows was estimated as the difference between the daily DMI and amount of DM digested multiplied by the OM concentration of the feces on a DM basis.

4.3.10 Sample Collection and Analysis

Samples of feed ingredients were collected weekly. Samples of TMR were collected twice weekly and composited by week. Samples of feed ingredients and TMR were dried in a forced-air oven at 55°C until constant weight. Weekly forage and TMR sample particle size was determined by dry sieving using a shaker (model RX-29, Tyler, Mentor, OH) containing sieves with 19-mm, 13.2-mm, 9.5-mm, 4.75-mm, 2.36-mm, 1.18-mm, 600-µm, and 300-µm apertures plus a bottom pan. The geometric mean particle size was estimated by regressing the percent cumulative undersized particles against the diagonal screen length (ASAE, 1992). The slope and intercept of the regression were used to estimate the mean particle size as $\frac{50-Intercept}{Slope}$ and the standard deviation as $\frac{84-Intercept}{50-Intercept}$ (ASAE, 1992). Samples were then ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific). The concentration of ash was determined after combusting samples in a furnace (Thermolyne 30400, Barnstead International) for 3 h at 600°C (method 942.05; AOAC International, 2019). The concentration of CP was calculated as percent N × 6.25 after combustion analysis of N (method 990.03; AOAC International, 2019) using a Vario El Cube CN analyzer (Elementar Americas Inc.). The concentration of ash-free NDF was determined using the Ankom200 Fiber Analyzer (Ankom Technology) with sodium sulfite and α-amylase (Ferreira and Mertens, 2007). Forage samples were additionally analyzed for ADF and ADL, which were determined sequentially. Concentrations of ADF were determined using the Ankom200 Fiber Analyzer. After determining ADF residue weights, residues were incubated for 3 h in 72% sulfuric acid within a 4-L jar that was placed in a DaisyII Incubator (Ankom Technology). Starch concentration was determined using the acetate buffer method (Hall, 2009) with α-amylase from *Bacillus licheniformis* (FAA, Ankom Technology) and amyloglucosidase from *Aspergillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland). The concentration of uNDF in TMR, feces, and ruminal samples was determined by placing 0.25 g of samples in filter bags (F57, Ankom Technology) previously rinsed in acetone. Duplicate bags were incubated for 240 h in the rumen of two lactating rumen-cannulated Holsteins consuming a ration containing 40% corn silage, 10% grass hay, and 50% concentrate. Secondary weekly samples of forages were collected, immediately frozen, and then submitted to a commercial laboratory (Cumberland Valley Analytical Services) at the end of the study for determination of the fermentation profile.

Milk samples (a.m. and p.m. milkings) were collected on d 12 and 13 of each period. Concentrations of fat, true protein, lactose, and MUN were analyzed by Lancaster DHIA (Mannheim, PA). Weighted average component yields were calculated according to the milk yields of each sampling. Energy-corrected milk yield (kg/d) was estimated by deriving the equation of Tyrrell and Reid (1965) as described in Equation 13:

$$ECM = 0.327 \times MY + 12.95 \times FY + 7.65 \times PY, \quad [13]$$

where MY is the daily milk yield (kg), FY is the daily milk fat yield (kg), and PY is the daily protein yield (kg). Additional milk samples (a.m. and p.m. milkings) were collected on d 12 to determine the milk fatty acid profile. Milk fatty acids were extracted and methylated according to Chouinard et al. (1999) and analyzed as described by Yang et al. (2017).

The concentrations of lanthanum (La) and ytterbium (Yb) in ruminal contents were determined by inductively coupled plasma optical emission spectrometry (Arcos II ICP-AES, Spectro Analytical Instruments GmbH) and as described by Galyon et al. (2024). A 2.0-g sample was placed in 50-mL Pyrex beakers and combusted at 500°C for 5 h. The resulting ash was dissolved in two 12.5-mL aliquots of HCl. After 1 h, the dissolved ash was transferred to a tared 50-mL Falcon tube, while rinsing the beaker with 41.7 m*M* LiOH until the combined solution weighed 50 g. Following overnight sedimentation, a 1:100 dilution of the sample with deionized water was analyzed for La and Yb.

4.3.11 Statistical Analysis

Before designing the experiment, a statistical power analysis was performed using the POWER procedure of SAS. Considering a statistical power equal to 0.80, a probability of committing a type I error (α) equal to 0.10, and a SD equal to 0.94 %/h for K_p (Galyon et al., 2024), 12 cows per treatment were deemed sufficient to detect a 1.00%/h-difference in passage rate between the two treatments. A total of 24 animals (12 cannulated and 12 non-cannulated) were included in the study. One of the non-cannulated cows was removed before the completion of the covariate period due to reasons outside of the study. Therefore, 12 cannulated and 11 non-cannulated animals were utilized for data analysis.

All variables were analyzed using the MIXED procedure of SAS. The statistical model for the total VFA and ammonia-N concentrations in the ruminal fluid over time included the fixed effects of diet (df = 1), time (df = 4), and their interaction (df = 4) and the random effects of cow (df = 11), period (df = 1), and the random residual error. Repeated measures over time were modeled using a compound symmetry covariance structure, with the subject defined as cow within period. Degrees of freedom were adjusted using the Satterthwaite approximation. The statistical model for the remaining variables of interest included the fixed effect of diet (df = 1) and the random effects of cow (df = 10, 11, or 22), period (df = 1), and the random residual error. Covariates were included in the model for DMI, nutrient intake, milk production, milk components, and enteric CH_4

production (g/d), yield (g/kg DMI), and intensity (g/kg ECM) data. For ruminal fill and passage rate variables, a significant difference was declared at $P < 0.10$, and a tendency to significantly differ was declared at $P < 0.15$. For the remaining variables, a significant difference was declared at $P < 0.05$, and a tendency to significantly differ was declared at $P < 0.10$.

4.4.Results and Discussion

The objective of this study was to evaluate the relationship between ruminal fiber K_p and enteric and fecal CH_4 emissions as affected by differing forage nutritional compositions. The triticale silage harvested in the BT stage contained 91.6% OM, 16.4% CP, 53.1% NDF, 32.9% ADF, 2.5% ADL, and 0.7% starch, whereas the triticale silage harvested in the SFT stage contained 93.1% OM, 9.3% CP, 63.2% NDF, 42.2% ADF, 5.1% ADL, and 3.2% starch (**Table 4.2**). The differences in CP, NDF, ADF, and ADL are coherent with previous reports, with harvesting in the BT stage resulting in substantially more CP and less NDF, ADF, and ADL (Coblentz et al., 2018; Ferreira et al., 2025; Galyon et al., 2025).

In addition to improved nutritional composition, harvesting triticale silage in the BT stage yielded improved NDF degradation kinetics (**Table 4.2**). The BT triticale, on an NDF basis, contained 80.7% pdNDF and 19.3% uNDF, whereas the SFT triticale contained 57.6% pdNDF and 42.4% uNDF. While the lag time did not differ (2.5 and 2.2 h for BT and SFT, respectively), the pdNDF of the BT silage had a faster K_d than the pdNDF of the SFT silage (3.31 and 2.67%/h, respectively). Schultz et al. (2025) tested the same variety of triticale which was seeded in the same field in 2021. They observed that triticale silage harvested in the BT stage contained 77.1% pdNDF and 22.9% uNDF on an NDF basis and exhibited a degradation lag of 4.0 h and a K_d of 2.30%/h while triticale silage harvested in the SFT stage contained 60.8% pdNDF and 39.2%

uNDF on an NDF basis and exhibited a degradation lag of 5.2 h and a K_d of 1.43%/h. Galyon et al. (2025) similarly seeded the same variety of triticale in the same field in 2022 and observed that triticale silage harvested in the BT stage contained 86.6% pdNDF and 13.4% uNDF on an NDF basis and exhibited a degradation lag of 3.7 h and a K_d of 2.33%/h while triticale silage harvested in the SFT stage contained 64.3% pdNDF and 35.8% uNDF on an NDF basis and exhibited a degradation lag of 5.0 h and a K_d of 1.53%/h. Slight differences in the NDF profile and degradation kinetics between these studies highlight year-to-year harvest variations and the importance of repetitions across years when evaluating forage quality.

Given that our main objective was to evaluate K_p, we wanted to minimize any extrinsic factors that may additionally influence K_p beyond differences in forage quality driven by harvesting maturity. Therefore, we formulated diets such that the only difference was the selection of one forage maturity over the other (**Table 4.1**). Despite the differences in the nutritional composition of the two triticale silages, the diets had similar nutritional profiles. The diet containing BT triticale contained slightly more CP (14.2 vs. 12.5%), slightly less NDF (35.1 vs. 37.5%), and less uNDF (20.5 vs. 32.1% NDF) compared to the diet containing SFT triticale. Additionally, even though they had similar lag times (1.9 and 1.6 h, respectively), the BT diet had a slightly faster K_d than the SFT diet (2.82 vs. 2.54%/h). As such, differences in ruminal kinetics and fermentation due to the inclusion of one triticale silage over the other can explain performance differences in dairy cattle fed these two diets.

Based on the differences in the NDF profile between the two triticale silages, we anticipated that dairy cows consuming the BT stage triticale would perform better than cows consuming the SFT stage triticale. Cows consuming BT triticale produced 3.3 kg/d more milk than cows consuming SFT triticale (39.6 vs. 36.3 kg/d; $P < 0.01$; **Table 4.3**). Forage maturity did not affect the concentration of milk fat (4.47%; $P = 0.43$) or milk protein (3.22%; $P = 0.38$). However, cows consuming BT triticale produced milk containing marginally more milk lactose than cows consuming SFT triticale (4.85 vs. 4.81%; $P < 0.01$). While few studies have evaluated dairy cattle performance when fed triticale silage, those that did agree that including BT stage triticale silage in dairy rations improves milk yield with negligible differences in concentrations of milk fat, protein, and lactose compared to SFT triticale (Schultz et al., 2025; Galyon et al., 2025). As a result, cows consuming BT triticale yielded more milk fat (1.76 vs. 1.65 kg/d; $P = 0.03$), milk protein (1.29 vs. 1.17 kg/d; $P < 0.01$), and milk lactose (1.94 vs. 1.78 kg/d; $P < 0.01$) than cows consuming SFT triticale. Consequently, cows consuming BT triticale produced 3.4 kg more ECM than cows consuming SFT triticale (45.6 vs. 42.2 kg/d; $P < 0.01$).

Similar to Schultz et al. (2025) but contrary to Galyon et al. (2025), we did not observe a difference in DMI (24.9 kg/d; $P = 0.22$; **Table 4.4**) between cows fed diets containing BT triticale silage and SFT triticale silage. Due to the compositional differences in the diets, cows consuming BT triticale consumed more CP (3.5 vs. 3.1 kg/d, $P < 0.01$) and less starch (5.5 vs. 5.8 kg/d, $P < 0.01$) compared to those fed the SFT triticale. While NDF intake did not differ between diets (9.0 kg/d; $P = 0.99$), cows consuming BT triticale consumed 0.6 kg/d less uNDF (1.9 vs. 2.5 kg/d; $P < 0.01$) and 0.6 kg/d more pdNDF (7.1 vs. 6.5 kg/d; $P < 0.01$) than cows consuming SFT triticale. Given that overall DMI did not differ between cows, cows consuming BT triticale likely performed better due to improved dietary digestibility, which increased feed efficiency (1.81 vs. 1.70 kg ECM/kg DMI; $P = 0.02$; **Table 4.3**). Similar to Galyon et al. (2025), cows consuming BT triticale showed improved total-tract digestibilities of DM (64.1 vs. 57.8%; $P < 0.01$), CP (52.2 vs. 44.3%; $P < 0.01$), NDF (51.8 vs. 43.7%; $P < 0.01$), and pdNDF (65.4 vs. 60.4%; $P < 0.01$) compared to cows consuming SFT triticale. Starch digestibility did not differ between diets and averaged

100.0% (*P* = 0.33). Collectively, cows consuming BT triticale digested more DM (16.1 vs. 14.2 kg/d; *P* < 0.01), CP (1.9 vs. 1.4 kg/d, *P* < 0.01), and NDF (4.7 vs. 3.9 kg/d; *P* < 0.01), but less starch (5.5 vs. 5.8 kg/d, *P* < 0.01) compared to cows consuming SFT triticale.

Cows consuming BT triticale had a 0.9-kg smaller ruminal pool of DM (7.9 vs. 8.8 kg; *P* = 0.07; **Table 4.5**), a 0.8-kg smaller ruminal pool of NDF (4.9 vs. 5.7 kg; *P* < 0.01), and a 0.9-kg smaller ruminal pool of uNDF (2.5 vs. 3.4 kg; *P* < 0.01) but a similar ruminal pool of pdNDF (2.4 kg; *P* = 0.55) compared to cows consuming SFT triticale. The smaller ruminal pool sizes of DM and NDF were despite similar intakes of DM and NDF, and the similar pool size of pdNDF was despite a greater intake of pdNDF by cows consuming the BT diet compared to the SFT diet. These relationships between nutrient intake and their respective pool size suggest greater turnover of DM, NDF, and pdNDF, potentially due to the faster K_d of the BT stage triticale silage. However, turnover is a function of both K_d and K_p, and, therefore, the observed differences between intakes and pool sizes may also reflect faster ruminal passage (Ellis et al., 1994; Galyon et al., 2024).

Because uNDF is indigestible and escapes the rumen solely by passage (Ellis et al., 1994; Van Soest, 1994; NASEM, 2021), its ruminal pool size is often used to infer K_p via TOR estimated by the flux/compartmental pool method. The TOR of uNDF did not differ between diets and averaged 3.20%/h (*P* = 0.89; **Table 4.5**). The similar outflow of material from the rumen likely explains the similar DMI between diets and may partially be explained by the similar mean particle size of the forages and resulting diets (**Tables 4.1** and **4.2**). With ruminal fractional digestibility of pdNDF being related to the balance between K_d and K_p, and ruminal digestibility accounting for 80 to 95% of total-tract NDF digestibility (Archimède et al., 1997; Huhtanen et al., 2010), it seems the improved total-tract pdNDF and NDF digestibilities of the BT diet were primarily due to the faster

K_d of the pdNDF and greater pdNDF concentration (NDF basis) of the diet due to inclusion of the BT triticale.

Although TOR derived from ruminal pool sizes can provide valuable insight into ruminal kinetics, interpreting them as direct estimates of dietary passage is problematic. The turnover of uNDF, calculated as influx over pool size, assumes a steady-state system in which uNDF intake equals uNDF outflow (Van Soest, 1994; Ellis et al., 1994). However, this approach may not reliably reflect K_p as uNDF is a heterogeneous fraction composed of particles varying in size, origin, and buoyancy, all of which influence passage and retention time independently of intake rate (Sutherland, 1988). Therefore, when diets differ in fiber source and digestibility, using static ruminal pools to infer K_p can obscure underlying differences in how individual feed components move through the rumen. To address this limitation, we evaluated K_p using a dynamic marker-based approach. The K_p assessed with La-disappearance profiles did not differ between the experimental diets and averaged 4.03%/h ($P = 0.47$), resulting in an average MRT of 25.7 h ($P = 0.27$). Alternatively, cows consuming BT triticale exhibited a faster ruminal K_p when assessed with Yb-disappearance profiles than cows consuming SFT triticale (6.38 vs. 4.75%/h; $P < 0.01$), which resulted in a shorter MRT (16.0 vs. 22.6 h; $P < 0.01$). The disparities between K_p estimated via the flux/compartmental pool method and the marker dilution method using three different forages deserve thorough discussion.

The flux/compartmental pool method measures the expected rate of escape of an indigestible component as the influx, or equivalent outflux, of the indigestible material into the rumen divided by the pool of the indigestible material present in the rumen, assuming that the rumen is in a steady state (Ellis et al., 1994). Since all indigestible particles, in this case uNDF, are evaluated, the estimated K_p of uNDF is reflective of the entire ration. On the other hand, K_p determined via the marker dilution method does not necessarily reflect the K_p of the entire ration when individual forages are externally marked. Krämer et al. (2013) concluded that K_p estimated by the marker dilution technique is affected more by the individual marked forage than the ration composition. Due to this possibility, for each cow during ruminal evacuations, we provided a pulse dose of Yb-marked triticale silage macro-fiber of the maturity designated in the diet in addition to a pulse dose of La-marked corn silage macro-fiber (Galyon et al., 2024). Although we assessed the ratio of marked particles relative to uNDF, which was provided by the entire ration and therefore was the same for the assessment of La and Yb passage within each cow, the markers themselves were associated with the NDF of the individual forages. Therefore, the estimated K_p from markers in this study can be interpreted as the escape of the NDF of the marked ingredient as influenced by the ruminal environment provided by the entire diet.

It is important to consider that external markers have their limitations for the interpretation of K_p estimates. Differences in Yb-derived K_p values between forages could be influenced by marker binding and dissociation from the fiber matrix. For external marker techniques to be valid, markers must remain bound to the particles with which they were originally associated. However, this assumption may not always hold true, particularly when comparing forages with markedly different structural or chemical properties. Triticale silages used in this study varied in maturity and in cell wall composition, with the BT triticale possessing more digestible cell wall components and less lignified tissue compared to the SFT triticale (**Table 4.2**). Lignification of plant tissues contributes to greater cation exchange capacity due to the increased availability of negatively charged carboxyl and phenolic groups associated with lignin, improving rare earth element binding affinity (Allen et al., 1985). Similarly, hemicellulose and cellulose bind external markers via their carboxyl groups. However, their binding may promote faster marker dissociation and escape through faster degradation and microbial activity (Allen et al., 1985). These factors may have contributed to rapid Yb disappearance and the inflated estimates of K_p observed in the BT diet due to the greater pdNDF concentration (NDF basis) of the BT triticale and the faster K_d of the pdNDF. This additionally changes the interpretation of K_p to be the escape of NDF instead of the passage of uNDF when utilizing external markers as utilized in this study as the marker does not distinguish between pdNDF and uNDF. The perceived disappearance of marked particles is the result of pdNDF degradation and the potential marker loss into the escapable pool, the escape of undigested pdNDF, and the passage of uNDF from the rumen. Collectively, these factors support the overall faster K_p estimates from both the La and Yb disappearance profiles compared to the K_p estimated via the TOR of uNDF.

Nonetheless, precautions were taken during marked fiber preparation to reduce the likelihood of dissociation by soaking marked macro-fiber in acetic acid to remove loosely bound marker, following protocols previously shown to reduce unbound marker presence (Allen, 1982; Bernard and Doreau, 2000). Moreover, studies show that the dissociation of rare earth markers such as Yb from fiber particles within the ruminal environment occurs at rates less than 1%/h (Hartnell and Satter, 1979; Beauchemin and Buchanan-Smith, 1989; Bernard and Doreau, 2000), suggesting that marker loss contributes minimally to the observed differences in K_p. While we cannot entirely rule out differential binding affinity or dissociation as a factor in the faster Yb-derived K_p of the BT stage triticale, the magnitude of the observed difference between the triticale silages (1.63%/h) and the congruence with faster K_d of the BT triticale supports a biological, rather than methodological, interpretation. Therefore, the observed differences in Yb-derived K_p reflect genuine differences in the forage degradation kinetics and particle escape from the rumen of the individual triticale silages.

As such, we may interpret that the K_p of the BT stage triticale silage NDF within the formulated BT diet was 6.38%/h, whereas the K_p of the SFT stage triticale silage NDF within the SFT diet was 4.75%/h in this study. However, at 19.3% DM inclusion in the diets, the differences in the K_p of these two forages were insufficient to meaningfully influence ruminal emptying and, consequently, DMI. Therefore, the K_p of the corn silage, included at 24.7% DM in the experimental rations, was not affected. Galyon et al. (2024) reported a faster K_p of externally marked corn silage when pulse-dosed to cows consuming a ration with alfalfa hay compared to those fed a ration with orchardgrass hay. Although the individual hays were not externally marked in that study, it is likely that the alfalfa hay had sufficiently faster K_d and K_p than the orchardgrass hay to sufficiently reduce ruminal fill and stimulate the observed increased DMI response while maintaining digestibility. This increased intake likely elicited a greater outflux of undigested corn silage NDF and resulted in the observed faster K_p of the externally marked corn silage by cows consuming the alfalfa diet (Galyon et al., 2024).

The K_p values observed in this study agree with the trend of the K_d of the forages, with K_p being the fastest for BT stage triticale, intermediate for SFT stage triticale, and the slowest for corn silage. We can estimate the ruminal digestibility of pdNDF of the triticale silages was 34.2% and 36.0% for BT and SFT, respectively, based on the equation $\frac{K_d}{K_d+K_p}$ (Ellis et al., 1994). Initially, it seems the ruminal digestibility of pdNDF was slightly impaired by the faster K_p of the BT stage triticale. However, if we consider the pdNDF concentration of the forages, the ruminal digestibility of NDF was 27.6% and 20.7% for BT and SFT triticale silages, respectively. Similarly, utilizing the K_d of the rations and K_p determined using the flux/compartmental pool method, we can estimate that the ruminal digestibility of the dietary NDF was greater for the BT diet than the SFT diet (37.2 vs. 30.1%). These values reflect the total-tract digestibility data we observed for NDF

(51.8% and 43.7% for the BT and SFT diet, respectively). The delicate balance between K_d and K_p drives the ruminant's ability to digest fiber and utilize the energy stored in structural carbohydrates. However, this information is insignificant when not placed in context with the relative balance between pdNDF and uNDF within the forage. These observations highlight the need to evaluate forage fiber quality rather than focusing solely on the provision of forage NDF regardless of origin when considering their inclusion in the rations of lactating dairy cattle (NASEM, 2021; Galyon et al., 2024).

Previous authors have proposed that reduced enteric CH_4 emissions associated with different forages result from reduced ruminal fermentation driven by faster ruminal K_p (Beauchemin et al., 2022; Hristov et al., 2022). However, this postulate is disproved under the current experimental conditions. While we did not observe a faster ruminal K_p at the dietary level, cows consuming BT stage triticale produced 5% less enteric CH_4 compared to those consuming SFT stage triticale (332 vs. 348 g/d; $P = 0.03$; **Figure 4.1**). Similarly, cows consuming BT stage triticale tended to yield 6% less CH_4 on a DMI basis (13.6 vs. 14.5 g/kg DMI; $P = 0.08$) and yielded 17% less CH_4 on a DM digested basis (21.3 vs. 25.6 g/kg DMI; $P < 0.01$). Furthermore, cows consuming BT stage triticale exhibited 10% lower CH_4 intensity than consuming SFT stage triticale (7.8 vs. 8.7 g/kg ECM; $P < 0.01$). These findings align with those of Galyon et al. (2025), who observed reduced enteric CH_4 emissions in high-producing dairy cattle fed triticale silage harvested in the BT compared to the SFT stage of maturity under high- and low-forage conditions (52 vs. 37% forage DM basis, respectively). In that study, feeding BT stage triticale reduced CH_4 production by 6%, CH_4 yield by 11% on a DMI basis and by 13% on a DM digested basis, and CH_4 intensity by 9% compared to feeding the SFT stage (Galyon et al., 2025).

Ruminal fermentation activity seemed elevated in cows consuming the BT diet and ruminal digestibility was apparently not impaired. Therefore, alterations in ruminal fermentation pathways could alternatively explain enteric CH_4 differences between diets. The ruminal fluid from cows consuming BT triticale contained 10% more total VFA (166.40 vs. 151.54 m*M*; *P* < 0.01; **Figure 4.2**) than that of cows consuming SFT triticale. The increase in total VFA present in the rumen of cows consuming the BT diet may be due to increased production of VFA from microbial fermentation of OM associated with the improved digestibility of the diet and availability of pdNDF. The increase in total ruminal VFA may also be, in part, due to the increased total VFA provided by the BT triticale silage compared to the SFT triticale silage (**Table 4.2**). While the molar percentages of isobutyrate (*P* = 0.37; **Table 4.6**), butyrate (*P* = 0.12), isovalerate (*P* = 0.11), and valerate (*P* = 0.25) did not differ between diets, cows consuming BT triticale had a smaller molar proportion of acetate (69.6 vs. 70.6% mol; *P* = 0.02) and a greater molar proportion of propionate (18.4 vs. 17.8% mol; *P* = 0.04) in the ruminal fluid compared to those consuming SFT triticale. Consequently, the acetate-to-propionate ratio was lower for cows consuming BT triticale compared to SFT triticale (3.8 vs. 4.0; *P* = 0.04). Adams et al. (1987) similarly observed that the acetate-to-propionate ratio increases when cattle consume forages of advancing maturity. This shift is likely due to the increase in cell wall components, particularly cellulose and hemicellulose, in the maturing crop as acetate is preferentially produced when these components are fermented in the rumen (Van Soest, 1994). Based on the difference in NDF and ADL concentrations of the forages (**Table 4.2**), the concentration of cellulose and hemicellulose seem to be decreased for the BT triticale silage compared to the SFT triticale silage (50.6 vs. 58.1% DM). It is suggested that decreasing the acetate-to-propionate ratio in the rumen can lead to reduced enteric CH_4 emissions due to a decrease in hydrogen availability for methanogenesis (NASEM, 2021). However, it is unclear if that is the case here as cows that consumed BT triticale produced less CH_4 while having overall increased concentrations of both acetate (109.8 vs. 101.5 m*M*; *P* = 0.02) and propionate (29.2 vs. 25.6 m*M*; $P < 0.01$) compared to cows that consumed SFT triticale.

The ruminal fluid from cows consuming BT triticale silage contained more propionate, and this may have contributed to the observed increase in milk lactose percent and yield as propionate is a glucose precursor (Van Soest, 1994). There tended to be an interaction between diet and time for the concentration of ammonia-N in the ruminal fluid. Overall, the ruminal fluid from cows consuming BT contained 19% more ammonia-N than that of cows consuming SFT (11.1 vs. 9.3 mg/dL; $P < 0.01$; **Figure 4.3**), which was driven by a greater peak ammonia-N production 4 h after meal delivery (16.4 vs. 12.0 mg/dL; $P < 0.01$) for cows consuming BT compared to SFT. This increase in ammonia-N is likely related to the slightly greater CP concentration and slightly lower starch concentration in the BT diet and the greater ammonia concentration in the BT triticale silage (**Table 4.2**). The greater peak of ammonia-N post-meal among cows consuming the BT diet likely exceeded the microbial capacity for synthesizing protein and thus resulted in the observed increased MUN in cows consuming the BT diet compared to those consuming the SFT diet (13.1 vs. 11.9 mg/dL; $P < 0.01$; **Table 4.3**).

The milk fatty acid profile (**Table 4.7**) reflected differences in VFA production between diets. The milk fat from cows consuming BT triticale tended to contain more *de novo* fatty acids (25.21 vs. 24.70 g/100 g fatty acid; *P* = 0.07) and contained more *trans* fatty acids (3.05 vs. 2.874 g/100 g fatty acid; *P* = 0.01) and OBCFA (2.71 vs. 2.55 g/ 100 g fatty acid; $P < 0.01$) compared to those consuming SFT triticale. Galyon et al. (2025) similarly observed that feeding BT triticale silage resulted in more *de novo* fatty acids, *trans* fatty acids, and OBCFA. Acetate and BHB are necessary precursors for *de novo* synthesis of fatty acids (Mu et al., 2021). Although the ruminal fluid from cows consuming BT triticale contained a decreased molar percentage of acetate and a similar molar percentage of butyrate compared to cows consuming SFT triticale, the ruminal fluid contained overall greater concentrations of acetate and butyrate. Thus, feeding BT triticale silage may have provided more substrate for *de novo* synthesis of fatty acids. However, the increase in *de novo* fatty acids was not so great as to increase the milk fat percentage. Galyon et al. (2025) reported that cows consuming BT triticale had a greater *trans*-10 to *trans*-11 18:1 ratio, indicating a shift or decline in biohydrogenation. In that study, it was hypothesized that cows fed BT triticale silage had accelerated ruminal K_p, and that potentially explained the increase in *trans* 18:1 fatty acid intermediates and incomplete biohydrogenation. Alternatively, we did not observe a difference in dietary ruminal K_p, and we did not observe any indication that biohydrogenation was impacted by triticale silage maturity in the present study through the ratio of *trans*-10 to *trans*-11 18:1 milk fatty acids or milk fat depression. We cannot disseminate the observed differences in *trans* fatty acids at this point. However, it is important to consider that we did not evaluate liquid passage from the rumen, and this may have a more considerable influence on fatty acid flushing from the rumen into the intestines than forage or dietary solids K_p. The concentration of OBCFA in milk has been correlated to acetate and the cellulolytic bacteria profile in the rumen (Kupczyński et al., 2024). Greater cellulolytic activity due to the presence of the more digestible BT triticale silage and greater concentrations of acetate in the ruminal fluid of cows consuming BT may have contributed to the observed increase in OBCFA for these cows.

We hypothesized that cows consuming BT triticale would have a faster K_p and this would result in more pdNDF being excreted in the feces if K_p was substantially faster than K_d. Therefore, we anticipated greater fecal CH_4 emissions from cows consuming BT triticale compared to those consuming SFT triticale. Despite the BT triticale silage exhibiting a faster K_p than the SFT triticale at the individual forage level within their respective diets, there was not a difference in K_p at the dietary level. The estimated ruminal NDF digestibility did not seem impaired for the BT stage triticale or the BT diet, and total tract digestibilities of pdNDF, NDF, and DM were improved for the BT diet compared to the SFT diet. However, feces excreted by cows consuming BT triticale contained marginally more OM than cows consuming SFT triticale (89.4 vs. 88.1%; $P < 0.01$; **Table 4.8**). While the NDF concentration in the feces excreted by cows in this study did not differ between diets (48.6%; $P = 0.17$), the feces from cows consuming BT triticale contained more pdNDF on an NDF basis (56.9 vs. 50.5% NDF; $P < 0.01$) and on a DM basis (27.3 vs. 24.8% DM; $P < 0.01$) than cows consuming SFT triticale. From this perspective, it seems that the increased total-tract digestibility of pdNDF was not enough to compensate for the greater intake of pdNDF among cows consuming BT triticale. Therefore, the feces excreted by cows consuming BT triticale may be more fermentable and have a greater environmental consequence than the feces excreted by cows consuming SFT triticale. However, the potential biogas production of cattle feces was not different between the two diets and averaged 116.8 mL/g OM ($P = 0.20$; **Table 4.8**). Similarly, the biomethane potential of cattle feces was not different between the two diets and averaged 22.1 mL CH_4/g OM ($P = 0.18$).

The biogas and biomethane production estimates in this study are lower than those reported by others. For example, Fincham (2024) reported that manure from dairy cattle fed various diets containing dried distiller's grains produced 444 mL biogas/g OM and 280 mL CH_4/g OM on average when incubated with inoculum from anaerobic manure digesters. Sutaryo et al. (2012) reported that various treatments of dairy cattle manure co-digested with inoculum from an active commercial biogas digester produced 222 mL CH_4/g OM on average. Therefore, the amount of biogas and biomethane produced may have been limited by using ruminal fluid as the inoculum in this study. It is additionally important to highlight that feces alone were utilized as the substrate in this study and not the composited manure, i.e. dairy cattle feces and urine. Urine additionally contains OM and VFA that could contribute to the CH_4 emissions from dairy cattle manure. Therefore, the results of the present study offer a relative comparison of the biomethane potential of feces excreted by cows consuming BT triticale or SFT triticale, but it does not directly reflect CH_4 emissions from manure.

For evaluating the environmental consequence of dairy cattle feces when cattle are fed BT vs. SFT stage triticale, however, it is important to consider that cows consuming BT triticale were estimated to excrete less feces per day due to the improved digestibility of the diet and similar DMI. Considering the estimated average daily intakes and the amount of nutrients digested per day, we can estimate that cows consuming BT triticale excreted 9.0 kg DM/d, 8.0 kg OM/d, 4.3 kg NDF/d, 2.4 kg pdNDF/d, and 1.9 kg uNDF/d while cows consuming SFT excreted 10.4 kg DM/d, 9.2 kg OM/d, 5.1 kg NDF/d, 2.6 kg pdNDF/d, and 2.5 kg uNDF/d on average. Despite the apparent differences in feces excretion, we did not observe a difference in the total biomethane potential of daily excreted OM (186.5 L CH_4/d/cow; $P = 0.62$). Numerically, however, the daily excreted OM by cows consuming the BT diet was estimated to potentially emit nearly 18 L more CH_4 relative to the OM excreted by cows consuming the SFT diet despite excreting less OM/d.

This study was underpowered to detect a significant difference in the total biomethane potential. According to a post hoc power analysis via the POWER procedure of SAS, this study achieved a power of only 8% for the total biomethane potential variable. This study placed emphasis on the detection of a significant difference in ruminal K_p. Therefore, repeated studies specifically evaluating the total biomethane potential of dairy cattle manure when cows are fed diets composed of either BT stage triticale or SFT stage triticale should be conducted in similar methodologies as previous biomethane potential studies to more accurately estimate the environmental consequences of feeding these forages (Filer et al., 2019). Nonetheless, it seems that there may be contradictory influences of forage quality on methane emissions from lactating dairy cattle.

In a previous study, we evaluated the effects of forage source on fiber digestion and fecal characteristics in dairy cows fed diets containing equal concentrations of NDF, derived either from alfalfa hay or orchardgrass hay (Galyon et al., 2024). Despite the standardized NDF concentrations, the alfalfa-based diet contained more uNDF on a NDF basis due to inherent compositional differences between the forages. The alfalfa diet exhibited a faster ruminal K_p, which is typically associated with reduced NDF digestibility and hypothesized to increase fecal output of pdNDF, thereby potentially elevating methane emissions from manure. However, we also observed a faster K_d of pdNDF in the alfalfa diet relative to orchardgrass, which contributed to improved total-tract digestibility of NDF and pdNDF and reduced fecal excretion of both (Galyon et al., 2024). In contrast, the present study evaluated triticale silages harvested at either the BT stage or SFT stage, where BT silage contributed a higher dietary proportion of pdNDF and lower uNDF than SFT. However, dietary K_p remained unchanged, and although total-tract pdNDF digestibility was indeed greater, the increased pdNDF intake associated with the BT diet resulted in greater fecal pdNDF excretion. This suggests that the enhanced digestibility was insufficient to compensate for the greater intake load. Collectively, the results of these two studies highlight the complexity of fiber utilization dynamics and indicate that neither uNDF nor pdNDF should be interpreted in isolation when assessing forage quality.

The interrelationships between K_p and K_d of pdNDF are fundamental to understanding fiber utilization and its environmental impacts. Generally, a faster K_p can limit the time available for microbial degradation of pdNDF, potentially increasing its expulsion in feces and contributing to methane emissions. Conversely, a faster K_d can enhance pdNDF digestion, reducing fecal fiber losses and mitigating manure methane output. However, the balance between these rates is not uniform across forages, necessitating a more integrated approach to diet formulation. The variability in fiber composition, especially the ratio of pdNDF to uNDF, should be considered alongside these kinetic factors to optimize fiber digestion efficiency while minimizing environmental consequences. This perspective challenges simplistic assumptions about forage digestibility and highlights the need to evaluate fiber fractions collectively, rather than treating NDF as a singular, homogenous entity. Future research should refine methodologies for assessing pdNDF degradation dynamics and uNDF passage rates across different forages and dietary formulations to improve predictive models for dairy nutrition and methane mitigation strategies.

4.5. Conclusions

Under the conditions of this study, we observed that feeding higher-quality BT triticale reduced enteric CH_4 emissions compared to SFT triticale while maintaining dietary ruminal K_p, enhancing ruminal and total-tract NDF digestibility, and improving feed efficiency and ECM production. These findings challenge the assumption that higher-quality forages reduce methane emissions via faster passage rate and reduced ruminal fermentation and highlight the need to consider fiber composition alongside ruminal kinetics when evaluating forage quality. Feeding forages of improved quality, as affected by earlier harvesting maturity, may be a more appealing enteric CH_4 mitigation strategy than synthetic feed additives, as performance was enhanced while enteric CH_4 emissions were reduced. However, there is a potential offset by increased methane emissions from excreted feces by cows consuming BT vs SFT stage triticale due to the increased presence of pdNDF within the forage. Future research should refine methodologies for predicting fiber digestibility and methane emissions to optimize dairy nutrition strategies that enhance efficiency while minimizing environmental consequences.

4.6. Notes

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4.8. Tables

Table 4.1. Ingredient and nutritional composition and particle size distribution of diets containing triticale silage harvested either at the boot (BT) stage or soft-dough (SFT) stage of maturity

	BT	SFT
Ingredient, % DM		
Triticale silage, BT	19.3	-
Triticale silage, SFT	-	19.3
Corn silage	24.7	24.7
Corn grain	24.7	24.7
Canola meal	12.4	12.4
Soybean meal expeller	5.4	5.4
Soybean hulls	4.5	4.5
Corn distillers grains with solubles	4.9	4.9
Supplemental fat	1.6	1.6
Calcium carbonate	0.6	0.6
Salt	0.5	0.5
Sodium bicarbonate	1.0	1.0
Magnesium oxide	0.2	0.2
Trace mineral premix[1]	0.04	0.04
Sodium selenite (0.06% Se)	0.04	0.04
Vitamin ADE[2]	0.04	0.04
Vitamin E (44 IU/g)	0.06	0.06
Ionophore[3]	0.01	0.01
Composition, % DM		
OM	93.5 (0.7)	93.9 (0.7)
CP	14.2 (0.6)	12.6 (0.6)
NDF	35.1 (1.3)	37.5 (1.7)
uNDF	7.2 (1.0)	12.1 (1.8)
uNDF, %NDF	20.5 (2.4)	32.1 (4.1)
Starch	22.3 (1.2)	22.5 (1.1)
Discrete lag, h	1.9 (0.3)	1.6 (0.3)
K_d of pdNDF, %/h	2.82 (0.16)	2.54 (0.18)
Particle size distribution[4], % DM		
19 mm	0.2 (0.1)	0.4 (0.6)
13.2 mm	0.3 (0.3)	0.7 (0.4)
9.5 mm	1.1 (0.3)	1.4 (0.3)
4.75 mm	12.5 (1.6)	14.2 (2.7)
2.36 mm	26.5 (1.1)	23.0 (1.0)
1.18 mm	23.7 (1.3)	22.6 (1.3)
600 µm	18.5 (1.1)	18.1 (1.6)
300 µm	10.8 (0.6)	11.4 (1.0)
<300 µm	6.5 (0.7)	8.3 (1.2)
Mean particle size, mm	3.6 (0.2)	3.4 (0.4)

Values represent the mean (SD) of 8 weekly samples per diet.

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[1] Contained 30.7% calcium; 6.3% sulfur; 35,800 mg/kg zinc; 27,400 mg/kg manganese; 6,269 mg/kg copper; 1,548 mg/kg iron; 1,190 mg/kg iodine; 524 mg/kg cobalt.

[2] Contained 24.3% calcium; 9,900 KIU/kg vitamin A; 2,200 KIU/kg vitamin D_3; 3,300 IU/g vitamin E.

[3] Rumensin 90 (200g monensin/kg).

[4] Amount of material retained on the sieve.

Table 4.2. Nutritional composition, pH, fermentation profile, particle size distribution, and parameters of fiber degradation kinetics of forages[1] included in the experimental diets

	Corn silage	Triticale BT	Triticale SFT
Nutrient composition[2]			
OM, %	98.0 (0.7)	91.6 (1.4)	93.1 (1.5)
CP, %	7.4 (0.5)	16.4 (0.7)	9.3 (0.4)
NDF, %	32.9 (2.4)	53.1 (0.3)	63.2 (2.8)
ADF, %	19.3 (1.2)	32.9 (0.5)	42.2 (2.5)
ADL, %	1.8 (0.2)	2.5 (0.2)	5.1 (0.6)
uNDF, %	9.1 (1.1)	10.0 (0.9)	25.5 (1.3)
uNDF, % NDF	28.5 (2.6)	19.3 (1.8)	42.4 (1.7)
pdNDF, %	22.9 (2.1)	41.7 (1.2)	34.7 (2.1)
pdNDF, %NDF	71.5 (2.6)	80.7 (1.8)	57.6 (1.7)
Starch, %	38.2 (3.5)	0.7 (0.1)	3.2 (1.0)
Degradation kinetics			
Discrete lag, h	2.1 (0.8)	2.5 (0.2)	2.2 (0.4)
K_d of pdNDF, %/h	2.10 (0.23)	3.31 (0.11)	2.67 (0.2)
pH	4.01 (0.09)	4.90 (0.46)	4.46 (0.27)
Fermentation profile, % DM			
Ammonia	0.5 (0.2)	1.5 (0.3)	0.6 (0.3)
Lactic acid	4.5 (1.4)	7.7 (1.5)	3.3 (0.9)
Acetic acid	3.1 (0.8)	1.6 (0.4)	2.6 (0.9)
Propionic acid	0.11 (0.14)	0.01 (0.02)	0.02 (0.03)
Butyric acid	0.00 (0.00)	0.02 (0.07)	0.00 (0.00)
1,2 Propanediol	0.7 (0.4)	0.3 (0.1)	0.7 (0.3)
Total VFA	7.7 (2.1)	9.4 (1.6)	5.8 (1.7)
Particle size distribution[3], % DM			
19 mm	0.5 (0.7)	1.5 (0.4)	1.2 (0.8)
13.2 mm	1.0 (0.6)	2.3 (0.5)	2.5 (1.3)
9.5 mm	4.9 (1.4)	3.0 (0.5)	4.7 (1.4)
4.75 mm	31.7 (4.2)	18.7 (1.8)	24.2 (1.8)
2.36 mm	34.3 (2.5)	47.1 (1.5)	39.2 (2.2)
1.18 mm	15.4 (1.4)	21.2 (2.3)	18.1 (2.1)
600 µm	6.4 (0.8)	4.3 (0.4)	5.4 (0.6)
300 µm	3.2 (0.4)	1.4 (0.2)	3.1 (0.5)
<300 µm	2.7 (0.5)	0.5 (0.1)	1.5 (0.3)
Mean particle size, mm	6.3 (0.3)	6.4 (0.1)	6.4 (0.3)

Values represent the mean (SD) of 8 weekly samples per forage.

[1] BT = boot stage; SFT = soft-dough.

[2] DM basis unless otherwise stated.

[3] Amount of material retained on the sieve.

Table 4.3. Production performance of lactating dairy cows consuming diets containing triticale silage harvested either at the boot (BT) stage or soft-dough (SFT) stage of maturity

	BT	SFT	SEM	*P*-value
Milk yield, kg/d	39.6	36.3	1.3	<0.01
Milk fat, %	4.44	4.50	0.11	0.43
Milk protein, %	3.23	3.20	0.07	0.38
Milk lactose, %	4.85	4.81	0.02	<0.01
Milk fat yield, kg/d	1.76	1.65	0.05	0.03
Milk protein yield, kg/d	1.29	1.17	0.05	<0.01
Milk lactose yield, kg/d	1.94	1.78	0.08	<0.01
ECM yield, kg/d	45.9	42.2	1.2	<0.01
Feed efficiency, kg ECM/kg DMI	1.81	1.70	0.07	0.02
MUN, mg/dL	13.1	11.9	1.68	<0.01

Values represent the mean of 23 cows per treatment.

Table 4.4. Dry matter intake, nutrient intake, and nutrient digestion of lactating dairy cows consuming diets containing triticale silage harvested either at the boot (BT) stage or soft-dough (SFT) stage of maturity

	BT	SFT	SEM	*P*-value
Intake, kg/d				
DM	25.1	24.6	0.55	0.22
CP	3.5	3.1	0.05	<0.01
NDF	9.0	9.0	0.31	0.99
uNDF	1.9	2.5	0.15	<0.01
pdNDF	7.1	6.5	0.16	<0.01
Starch	5.5	5.8	0.13	<0.01
Digestibility, %				
DM	64.1	57.8	1.86	<0.01
CP	54.1	44.5	4.39	<0.01
NDF	51.8	43.7	1.29	<0.01
pdNDF	65.4	60.4	1.19	<0.01
Starch	100.0	100.0	<0.01	0.33
Digested, kg/d				
DM	16.1	14.2	0.37	<0.01
CP	1.9	1.4	0.15	<0.01
NDF	4.7	3.9	0.13	<0.01
pdNDF	4.7	3.9	0.13	<0.01
Starch	5.5	5.8	0.16	<0.01

Values represent the mean of 23 cows per treatment.

Table 4.5. Ruminal pool sizes and passage kinetics of lactating dairy cows consuming diets containing triticale silage harvested either at the boot (BT) stage or soft-dough (SFT) stage of maturity

	BT	SFT	SEM	*P*-value
Ruminal content fiber composition				
NDF, %DM	62.5	65.5	1.97	0.03
uNDF, %DM	32.7	39.8	3.45	<0.01
uNDF, %NDF	52.1	60.4	3.61	<0.01
pdNDF, %DM	29.9	25.7	1.59	<0.01
pdNDF, %NDF	47.9	39.6	3.6	<0.01
Pool size, kg				
Total ruminal contents[1]	72.4	78.1	3.13	0.06
DM	7.9	8.8	0.45	0.07
NDF	4.9	5.7	0.23	<0.01
uNDF	2.5	3.4	0.17	<0.01
pdNDF	2.4	2.3	0.23	0.55
Kinetics				
TOR uNDF, %/h	3.21	3.18	0.35	0.89
La K_p, %/h	4.15	3.91	0.24	0.47
Yb K_p, %/h	6.38	4.75	0.45	<0.01
La MRT, h	24.6	26.8	1.44	0.27
Yb MRT, h	16.0	22.6	1.76	<0.01

Values represent the mean of 12 cows per treatment.

[1] Mix of ruminal solids and liquid.

Table 4.6. Average VFA concentration of ruminal fluid from lactating dairy cattle consuming diets containing triticale silage harvested at either the boot (BT) stage or soft-dough (SFT) stage of maturity

	BT	SFT	SEM	*P*-value
VFA concentration, m*M*				
Acetate	109.8	101.5	7.18	0.02
Propionate	29.2	25.6	1.93	<0.01
Butyrate	15.4	13.7	1.11	0.02
Isobutyrate	0.98	0.87	0.058	0.03
Valerate	2.0	1.8	0.21	0.02
Isovalerate	0.54	0.50	0.044	0.19
VFA molar percentage, % mol				
Acetate	69.6	70.6	0.26	0.02
Propionate	18.4	17.8	0.23	0.05
Butyrate	9.7	9.5	0.17	0.12
Isobutyrate	0.6	0.6	0.03	0.37
Valerate	1.3	1.2	0.05	0.25
Isovalerate	0.3	0.4	0.02	0.11
Acetate:Propionate	3.8	4.0	0.07	0.04

Values represent the mean of 12 cows per treatment.

Table 4.7. Milk fatty acid profile (g/100 g fatty acid) of lactating dairy cows consuming diets containing triticale silage harvested either at the boot (BT) stage or soft-dough (SFT) stage of maturity

	BT	SFT	SEM	*P*-value
4:0	3.44	3.45	0.09	0.80
6:0	2.16	2.25	0.06	0.17
8:0	1.31	1.27	0.05	0.02
10:0	3.02	2.88	0.18	<0.01
11:0	0.08	0.07	<0.01	<0.01
12:0	3.45	3.26	0.23	0.01
13:0	0.12	0.10	0.01	<0.01
14:0	10.53	10.34	0.33	0.13
cis-9 14:1	1.09	1.09	0.08	0.95
15:0	0.97	0.87	0.03	<0.01
iso 15:0	0.21	0.20	<0.01	<0.01
anteiso 15:0	0.40	0.40	0.01	0.51
16:0	28.90	30.25	0.94	<0.01
iso 16:0	0.21	0.21	0.01	0.25
cis-9 16:1	1.12	1.19	0.07	0.05
17:0	0.49	0.45	0.02	<0.01
iso 17:0	0.36	0.35	0.01	<0.01
18:0	10.61	10.44	0.72	0.40
trans-6,8 18:1	0.23	0.22	0.05	0.66
trans-9 18:1	0.28	0.28	<0.01	0.11
trans-10 18:1	0.69	0.59	0.14	0.04
trans-11 18:1	0.96	0.96	0.05	0.97
trans-12 18:1	0.41	0.36	0.01	<0.01
cis-9 18:1	23.36	23.28	0.85	0.84
cis-11 18:1	1.01	0.91	0.05	<0.01
cis-9,*cis*-12 18:2	2.63	2.60	0.12	0.61
cis-9,*cis*-12,*cis*-15 18:3	0.32	0.26	0.02	<0.01
20:0	0.17	0.17	0.01	0.01
cis-9,*trans*-11 CLA	0.47	0.47	0.03	0.76
trans-10,*cis*-12 CLA	0.002	0.003	0.002	0.88
Other	0.94	0.79	0.06	<0.01
Σ De Novo[1]	25.21	24.70	0.81	0.07
Σ SFA	66.43	66.95	1.01	0.30
Σ MUFA	29.16	28.88	0.95	0.54
Σ PUFA	3.42	3.33	0.12	0.15
Σ Trans fatty acids	3.05	2.87	0.16	0.01
Σ OBCFA[2]	2.71	2.55	0.03	<0.01
Biohydrogenation[3]	0.74	0.65	0.19	0.16

Values represent the mean of 23 cows per treatment.

[1] The sum of 4:0 through 14:0.

[2] The sum of 11:0, *iso* 15:0, *anteiso* 15:0, 15:0, *iso* 16:0, *iso* 17:0, and 17:0.

[3] The ratio of *trans*-10 18:1 to *trans*-11 18:1.

Table 4.8. Composition[1] and fermentation potential[2] of feces excreted by lactating dairy cows consuming diets containing triticale silage harvested either at the boot (BT) stage or soft-dough (SFT) stage of maturity

	BT	SFT	SEM	*P*-value
Fecal composition				
OM, % DM	89.4	88.1	0.28	<0.01
NDF, % DM	48.0	49.2	0.80	0.17
uNDF, %NDF	43.1	49.5	0.68	<0.01
uNDF, %DM	20.7	24.3	0.31	<0.01
pdNDF, % NDF	56.9	50.5	0.68	<0.01
pdNDF, % DM	27.3	24.8	0.72	<0.01
Fecal fermentation				
Biogas potential, mL/g OM	121	113	4.3	0.20
CH_4 potential, mL/g OM	25	19	2.9	0.18
Total CH_4 potential, L/d/cow	195	178	25.0	0.62

[1] Values represent the mean of 23 cows per treatment.

[2] Values represent the mean of 11 cows per treatment.

4.9. Figures

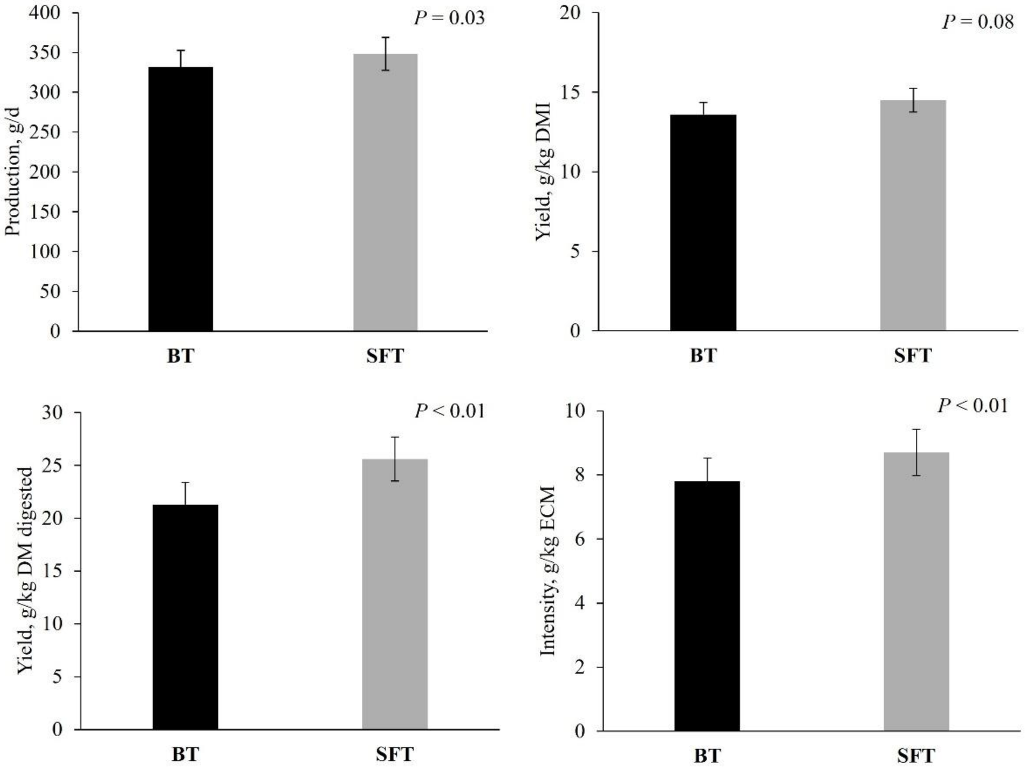

Figure 4.1. Daily enteric methane production (g/d), yield (g/kg DMI and g/kg DM digested), and intensity (g/kg ECM) of lactating dairy cows (n=11) consuming diets containing triticale silage harvested either at the boot (BT) stage or soft-dough (SFT) stage of maturity

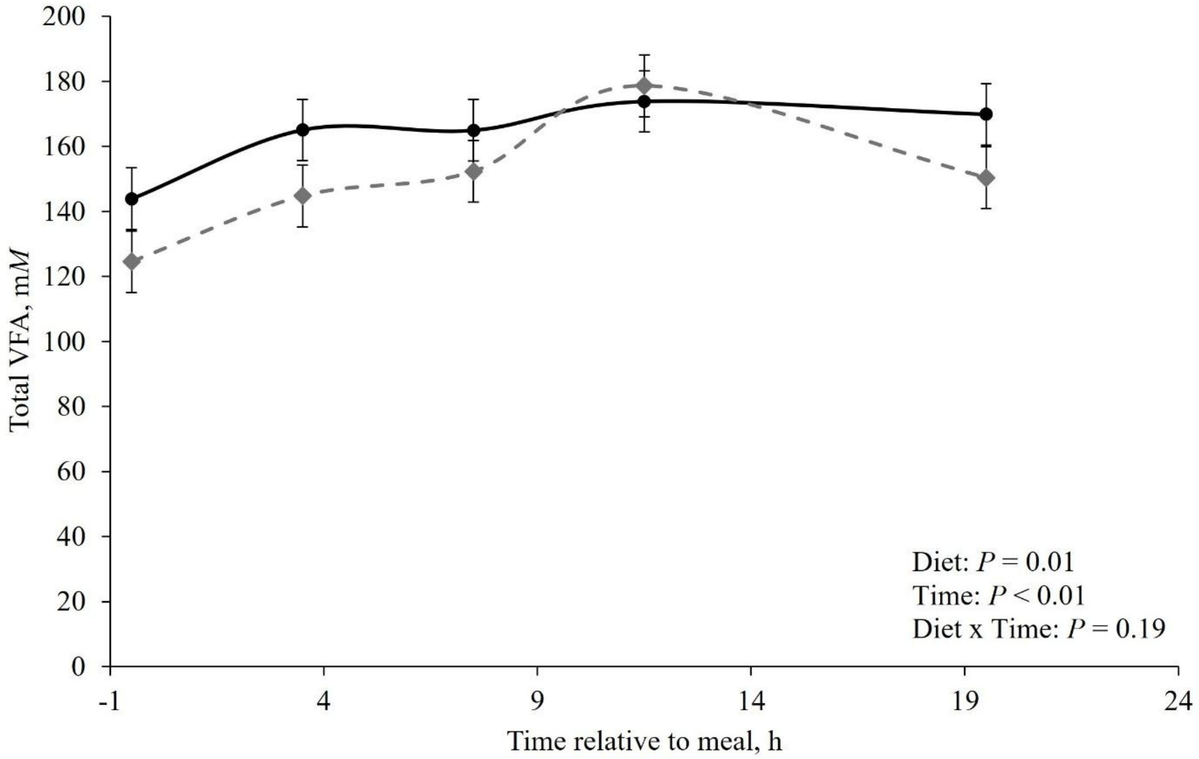

Figure 4.2. Total VFA concentration in the ruminal fluid of lactating dairy cows (n=12) consuming diets containing triticale silage harvested either at the boot (BT; solid black line) stage or soft-dough (SFT; dashed gray line) stage of maturity

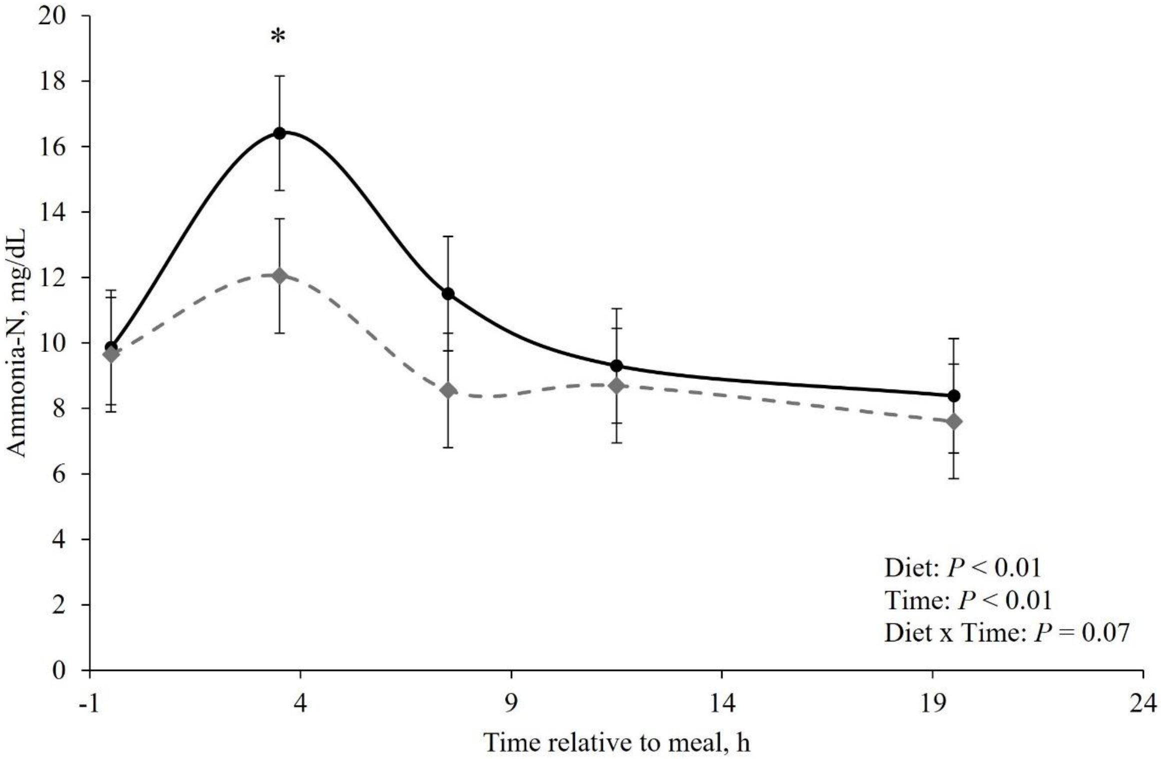

Figure 4.3. Ammonia-N concentration in the ruminal fluid of lactating dairy cows (n=12) consuming diets containing triticale silage harvested either at the boot (BT; solid black line) stage or soft-dough (SFT; dashed gray line) stage of maturity

CHAPTER 5: Conclusions and Implications

5.1. Integrative Summary

This dissertation explored the dynamic relationships between fiber digestibility, ruminal passage kinetics, and CH_4 emissions in lactating dairy cows, focusing on how forage type, maturity, and dietary composition influence both animal performance and environmental outcomes. Collectively, the findings highlight that ruminal fiber degradation and CH_4 emissions are not solely determined by static indicators of forage quality, such as fiber fractionation, but are substantially shaped by the dynamic behavior of fiber in the rumen and the balance between digestion, passage, and microbial fermentation.

In the first study, a diet containing alfalfa hay, despite having a greater concentration of uNDF, resulted in a faster K_p and shorter mean retention time than the orchardgrass hay diet, as determined using externally marked corn silage. This outcome appeared to be driven by the more rapidly degradable pdNDF in alfalfa, and the total-tract digestibilities of DM, NDF, and pdNDF were not compromised. These findings challenge the overextrapolation that high uNDF limits ruminal turnover and emphasize that K_d and K_p are critical determinants of fiber utilization beyond what fiber fractionation alone can explain.

The second study evaluated triticale silage harvested at either the BT or SFT stage, fed in diets with high or low forage inclusion. Boot silage supported greater DMI, nutrient digestibility, and milk production while reducing enteric CH_4 yield compared to SFT silage. Contrary to initial hypotheses, the improved digestibility of the BT silage did not increase CH_4 emissions but instead appeared to shift ruminal fermentation patterns in a way that reduced methanogenesis. Although low-forage diets additionally improved milk production and reduced CH_4 emissions, these benefits were accompanied by reductions in NDF digestibility, which may have been induced by an overall faster dietary K_p that limited residency time of forages in the rumen.

The third study explored the underlying mechanisms of the observations made in the second study by measuring ruminal kinetics and fermentation responses. Using externally marked forages, the BT triticale exhibited faster K_p than the SFT triticale within their respective diets. However, the K_p of corn silage, the overall dietary K_p, and DMI were not affected by triticale maturity, suggesting that the 19% DM inclusion level of triticale silage was not enough to elicit a collective effect on ruminal turnover of the complete digesta. Feeding BT triticale increased ruminal VFA production and favored propionate formation, reducing enteric CH_4 emissions and improving feed efficiency. However, the greater intake of pdNDF with BT silage resulted in increased fecal excretion of pdNDF. Although the statistical power of the study limited the interpretation of the biomethane potential of feces, estimated daily fecal CH_4 emissions were numerically greater for cows consuming BT silage despite excreting less feces per day. This highlights an important trade-off between enteric and manure-derived CH_4 emissions and the complexity of optimizing both production and environmental goals.

Collectively, these studies show that forage quality must be assessed not only in terms of chemical composition, but also through its behavior in the rumen and its downstream effects on nutrient flows and greenhouse gas emissions. Faster K_p seems to coincide with faster K_d at the individual forage level. This is likely due to more rapid particle size reduction and increased specific gravity, both of which facilitate ruminal clearance. Thus, at the individual forage level, fast K_p does not seem to inherently reduce ruminal digestibility. However, the overall forage-to-concentrate ratio and the inclusion level of individual forages with their respective digestibilities may significantly alter overall dietary turnover and potentially lead to the premature loss of pdNDF from the rumen and limit ruminal digestibility. Although not always statistically significant, chapters in this dissertation provided evidence that shifts in degradation and passage kinetics influence the amount of fermentable material excreted in the feces. Therefore, while enteric CH_4 can be reduced by forage management and ration formulation strategies, these techniques may inadvertently increase the fermentability of manure, shifting emissions downstream.

5.2. Future Directions

These findings call into question simplistic assumptions and instead advocate for a systems-based approach to dairy nutrition. A more comprehensive approach to evaluating dietary strategies is needed, one that considers effects on both animal productivity and emissions sources across the entire feeding and manure management system. Further research should aim to refine predictive models that integrate ruminal degradation and passage kinetics, VFA profiles, and manure characteristics to more accurately predict net CH_4 emissions from lactating dairy cattle. Longitudinal studies that measure both enteric and manure-derived CH_4 in tandem will be essential for evaluating true mitigation outcomes. Furthermore, enhanced digestibility prediction tools that incorporate fiber heterogeneity and kinetics behavior will support more precise ration formulation aimed at improving efficiency while minimizing environmental wastes. Additional work is needed to determine how individual forage digestibilities, as affected by fiber fractionation, K_d, and K_p, and their inclusion rates interact to influence overall digestion, productivity, and CH_4 emissions. Identifying threshold inclusion levels of individual forages to support optimal digestion and production without exacerbating CH_4 emissions may be key to advancing more sustainable and efficient dairy production systems.