

Indigenous lactic acid bacteria from bovine feces as dual-purpose inoculants: A review of isolation, probiotic characterization, and napier grass silage quality under tropical conditions

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Abstract

Securing enough roughage during Thailand's dry season largely depends on Napier grass (*Pennisetum purpureum*) silage. However, ensiling this forage is quite challenging. Its naturally low water-soluble carbohydrates and high buffering capacity, along with intense tropical heat, make the silage vulnerable to rapid aerobic spoilage. To make things worse, standard commercial lactic acid bacteria (LAB) inoculants, which are usually taken from temperate regions, often do not work well in such hot and humid conditions. This review looks at the potential of using indigenous LAB sourced directly from ruminant feces as a local solution. These microbial strains have co-evolved in the harsh environment of the gastrointestinal tract, so they naturally show strong tolerance to acid and bile, heat resistance, and unique antimicrobial properties. While existing studies from other tropical areas show that ruminant-adapted LAB can improve silage fermentation and stability after opening, there is almost no empirical data on Thai bovine-fecal LAB. There is also growing interest in the "dual-purpose" role of these indigenous strains, which could serve as both silage inoculants and direct-fed rumen probiotics. However, recent in vitro tests are merely promising, and this dual function is still just a hypothesis that requires thorough in vivo testing. To clarify a path forward, this paper highlights critical research gaps. It argues that systematic strain-level characterization and field-based comparisons with commercial additives are urgently needed. Building a solid evidence base is essential before promoting these strains as a reliable strategy for tropical feed security.

Keywords: Lactic acid bacteria; Napier grass silage; Aerobic stability; Ruminant probiotic; Bovine feces; Tropical forage

Introduction

Supporting nearly 10 million beef cattle and dairy cows, Thailand's livestock industry has an annual roughage need of over 20 million tonnes (Department of Livestock Development, 2024). A major issue in this production system is the long dry season from November to April. During these months, the typical monsoon climate changes, causing a significant drop in both the yield and nutritional value of fresh pastures. As a result, herds experience lower dry matter intake and digestibility, which negatively impacts meat and milk production. This situation forces farmers to depend heavily on costly commercial concentrates (Jirawattanasomkul et al., 2026). To address this feed gap, ensiling freshly harvested forage is an effective conservation method. It uses anaerobic lactic acid fermentation to preserve vital nutrients (McDonald et al., 1991; Muck et al., 2018). Across the country, Napier grass (*Cenchrus purpureus* (Schumach.) Morrone; syn. *Pennisetum purpureum* (Schumach.) cv. Pak Chong 1} has become the preferred forage due to its fast regrowth and high biomass yields, often exceeding 60 tonnes per hectare yearly.

However, ensiling this tropical grass comes with specific biochemical challenges. Its naturally low water-soluble carbohydrate (WSC) levels and strong buffering capacity slow down the rapid acidification needed to stop the growth of harmful microbes (Li and Nishino, 2013). This problem worsens in the tropical heat. Ambient temperatures regularly above 30 °C speed up the growth of heat-tolerant yeasts as soon as a silo is opened, leading to significant aerobic spoilage and dry matter losses (Dunière et al., 2017; Muck et al., 2018). Without focused biological solutions, producing high-quality Napier silage in Thailand remains a tough challenge.

In agricultural practice worldwide, lactic acid bacteria (LAB) serve as the gold standard for silage additives. Homofermentative strains, particularly *Lactobacillus plantarum*, excel at swiftly converting available WSC into lactic acid to drive down pH. Conversely, heterofermentative counterparts like *Lactobacillus buchneri* generate acetic acid alongside lactic acid, a mechanism explicitly known to inhibit yeast growth and drastically extend shelf life during feed-out (Kung et al., 2003; Muck et al., 2018; Jin et al., 2024). Yet, a major disconnect exists in tropical applications. Many commercial LAB preparations are derived from temperate crops. When deployed in the intense thermal environment and unique microbial ecology of Thai forages, these imported strains often lack the resilience to dominate the fermentation process effectively (Bureenok et al., 2012; Khota et al., 2016). Looking inward for a localized solution, the ruminant gastrointestinal tract and consequently, bovine feces provide an untapped reservoir of robust, indigenous LAB. Having co-evolved within the host's gut, these

microbial populations are biologically pre-conditioned to survive extreme acidity, high bile concentrations, and strict anaerobiosis. These stressors closely mirror the hostile microenvironment of a fermenting silo (Conway et al., 1987; Fuller, 1989).

Harnessing these gut-derived strains introduces an exciting prospect: dual-purpose functionality. Theoretically, they could excel not only as silage fermenters but also carry over as direct-fed probiotics to optimize rumen microflora and bolster host immunity (Kechagia et al., 2013; Sornplang and Piyadeatsoontorn, 2016). Despite such biological potential, literature severely lacks empirical validation. Rigorous characterization of Thai bovine-fecal LAB, coupled with head-to-head field trials against standard commercial inoculants in Pak Chong 1 silage at high ambient temperatures, remains completely unexplored.

This review looks closely at the existing knowledge gaps regarding ruminant-derived LAB and how they can be used in tropical forage conservation. It breaks down the biodiversity and probiotic characteristics of fecal isolates. The discussion connects basic fermentation biochemistry to the challenges of aerobic stability in Napier grass. It also evaluates new findings that suggest local strains perform better than commercial imports in hotter climates. By identifying these research gaps, this paper seeks to encourage the development of an effective inoculant designed specifically for Thailand's agricultural environment.

Characteristics of Tropical Forages and the Challenge of Silage Fermentation

Napier Grass: Chemical Composition and Fermentation Constraints

The successful preservation of any forage fundamentally depends on the biochemical interaction between its water-soluble carbohydrate (WSC) pool and buffering capacity (BC). While available WSC serves as the primary substrate for lactic acid bacteria, endogenous buffering agents such as plant proteins, organic acids, and minerals actively resist the acidification process (McDonald et al., 1991). This biochemical antagonism is especially pronounced in Napier grass. At standard harvest intervals of 45 to 60 days, the crop offers a notoriously limited WSC pool typically ranging between 60 and 100 g/kg on a dry matter basis while presenting a buffering resistance that far exceeds that of conventional temperate crops like whole-plant maize (Li and Nishino, 2013; Khota et al., 2016). Because these values are expressed on different bases, direct comparison should be made cautiously. Moreover, the minimum WSC requirement for successful fermentation varies with forage buffering capacity and

dry matter concentration. To ensure rapid, homolactic-dominant fermentation, fresh biomass must theoretically supply a baseline of at least 30 g of WSC per kg of fresh weight (McDonald et al., 1991). Tropical C4 grasses frequently fail to meet this metabolic requirement, particularly as plant maturity advances or during periods of low solar radiation. Beyond carbohydrate limitations, the inherently high cellular water content of freshly harvested tropical pastures often surpasses 75% presents a major physiological hurdle. High moisture environments not only dilute the already sparse sugar supply but also drastically lower the osmotic pressure within the silo. This condition may create favorable conditions for *Clostridium* proliferation if the initial pH fails to drop rapidly below 4.8, although actual growth also depends on other factors such as moisture content and substrate availability. A clostridial shift deteriorates the structural and nutritional integrity of the silage matrix; these microbes metabolize lactic acid into pungent butyric acid and trigger extensive proteolysis, yielding high levels of ammonia (Muck et al., 2018). Consequently, the ensiled material suffers severe dry matter losses and a sharp decline in palatability. While field wilting is the standard preventative measure in temperate climates to manage moisture, achieving an effective dry-down under the oppressive relative humidity typical of Thailand is largely impractical. This climatic constraint dictates that relying solely on natural fermentation is highly risky, making the application of exogenous biological additives an operational necessity rather than an option (Bureenok et al., 2012).

Temperature Effects on Silage Fermentation in Thailand

The ambient thermal environment exerts dictatorial control over silage population dynamics. In temperate climates, where storage temperatures naturally range between 20 and 25 °C, lactic acid bacteria face minimal competition, allowing them to rapidly acidify the forage mass during early fermentation. Conversely, the standard daytime temperatures in Thailand frequently exceeding 30 °C during warmer periods. This extreme heat perfectly aligns with the physiological growth optima of aggressive, heat-tolerant yeast species, including *Candida*, *Pichia*, and *Saccharomyces*. When silos are eventually breached for feed-out, the sudden influx of oxygen triggers a massive proliferation of these resident epiphytic yeasts. As Li and Nishino (2013) clearly demonstrated, forage conserved at 30 °C suffers a drastic reduction in aerobic stability compared to cooler storage conditions. This thermal stress initiates a predictable and highly destructive microbial succession. Initially, lactate-assimilating yeasts rapidly metabolize the accumulated lactic acid, effectively stripping the silage of its primary chemical preservative and causing a sharp spike in pH. Once this acidic shield is compromised, a secondary wave of

opportunistic, heat-generating aerobic bacteria and filamentous molds aggressively colonizes the structural matrix (Dunière et al., 2017; Muck et al., 2018). This cascade is not merely a superficial spoilage issue; it results in severe dry matter losses and frequently culminates in the synthesis of harmful mycotoxins. For many small- and medium-scale cattle operations in Thailand, temperature-controlled storage is economically unfeasible. Consequently, their bunker or bagged silage is left entirely at the mercy of the tropical climate. Under these relentless thermal conditions, the transition from pristine conserved forage to degraded, unpalatable feed can occur in a matter of hours, severely jeopardizing both herd health and farm profitability (Chen et al., 2020).

Lactic Acid Bacteria: Classification, Fermentation Biochemistry, and Role as Silage Inoculants

Homofermentative vs. Heterofermentative Metabolism

Biologically, lactic acid bacteria (LAB) represent a diverse clade of Gram-positive, non-spore-forming, and catalase-negative anaerobes. Their unifying biochemical trait is the excretion of lactic acid as the primary, if not exclusive, terminal metabolite of carbohydrate degradation. Taxonomically and functionally, these organisms are bifurcated into distinct metabolic pathways that dictate their utility in forage conservation (Axelsson, 2004). Obligate homofermenters such as *Lactobacillus delbrueckii* and *Lactococcus lactis* catabolize hexose sugars strictly through the Embden-Meyerhof-Parnas (EMP) glycolytic cascade. This highly efficient route theoretically converts a single mole of glucose into two moles of lactic acid, achieving up to 90% substrate-to-product recovery. In contrast, facultative heterofermenters (e.g., *Lactobacillus plantarum* and *Pediococcus pentosaceus*) rely on the EMP pathway for hexoses but possess the enzymatic plasticity to degrade pentose sugars via the phosphoketolase pathway, generating a mix of lactic acid, acetic acid, or ethanol alongside carbon dioxide. Finally, obligate heterofermenters, notably *Lentilactobacillus buchneri* (formerly *Lactobacillus buchneri*) and *L. fermentum*, rely entirely on the phosphoketolase route for both sugar types, typically yielding equimolar proportions of lactic and acetic acids (Axelsson, 2004). When applied to silo management, this metabolic divergence is strategically exploited. Homofermentative inoculants are conventionally deployed to drive a precipitous drop in pH, thereby minimizing dry matter losses and arresting early proteolytic degradation (Kung et al., 2003). However, a rapid pH decline alone offers little protection against aerobic deterioration during the feed-out phase. To combat this, obligate heterofermenters have revolutionized inoculant technology. As the ensiling period

progresses under strict anaerobiosis, strains like *L. buchneri* actively degrade accumulated lactic acid into fungicidal acetic acid and 1,2-propanediol. This shift profoundly enhances the shelf-life of the open face, as acetic acid is a potent inhibitor of lactate-assimilating yeasts (Muck et al., 2018). Recent empirical work elegantly corroborates this protective mechanism. For instance, Jin et al. (2024) demonstrated that inoculating *Silphium perfoliatum* silage with *L. buchneri* fundamentally altered the fermentation profile. Compared to untreated controls, the heterofermentative treatment yielded significantly higher acetic acid concentrations, which systematically suppressed yeast populations and dramatically extended aerobic stability. Crucially, this delayed spoilage was achieved alongside notable reductions in ammonia-nitrogen and preserved *in vitro* digestibility, underscoring the protective power of controlled heterofermentative metabolism in challenging forage matrices (Jin et al., 2024).

LAB Inoculants for Tropical Forage Silage

The application of exogenous LAB has become an indispensable strategy to overcome the inherent ensiling bottlenecks of difficult-to-preserve crops. Recent evaluations confirm that inoculating forage biomass with either homofermentative or heterofermentative strains substantially upgrades the overall fermentation profile. For instance, Jin et al. (2024) clearly illustrated that treating mixed-forage matrices with specific *Lactobacillus* species heavily suppresses undesirable proteolysis reflected in sharply reduced ammonia-nitrogen fractions while simultaneously boosting dry matter recovery compared to untreated controls.

However, transferring this biotechnology directly to tropical agricultural systems introduces a critical ecological mismatch. Standard commercial inoculants are overwhelmingly manufactured using microbial isolates sourced from cool, temperate climates. When these imported strains are subjected to the aggressive thermal stress and distinct chemical substrates of tropical forages, their metabolic vigor frequently plummets (Bureenok et al., 2012). Recognizing this fundamental limitation, researchers have increasingly pivoted toward bioprospecting indigenous LAB populations that are naturally adapted to warmer, harsher climates. Empirical evidence robustly supports this localized approach. Khota et al. (2016) demonstrated that natural epiphytic LAB recovered from tropical grasses exhibit unique fermentative resilience, facilitating a more rapid pH drop and superior nutrient preservation than temperate alternatives. Similarly, Nkosi et al. (2009) documented that locally sourced inoculants tailored for tropical whole-plant maize significantly outperformed generic commercial blends under high-temperature regimes. This performance advantage is largely attributed to the

indigenous strains elevated physiological growth optima and robust thermal tolerance, allowing them to rapidly dominate the silo microbiome before spoilage organisms can establish. Table 1 synthesizes the overarching chemical and microbiological shifts driven by control, homofermentative, and heterofermentative treatments, drawing on the foundational frameworks established by Muck et al. (2018) and corroborated by recent empirical data from Jin et al. (2024).

Table 1 Comparative effects of lactic acid bacteria inoculant type on Napier grass silage quality parameters

Parameter	Control (No inoculant)	Homofermentative LAB	Heterofermentative LAB
Silage pH	Higher (>4.5 in tropical forage)	Lower; rapid acidification	Moderately low; slower decline
Lactic acid (% DM)	Low	High	Moderate
Acetic acid (% DM)	Very low	Low	High (key for aerobic stability)
NH ₃ -N (% TN)	High (proteolysis)	Reduced	Further reduced
DM loss (%)	High	Reduced	Reduced
Aerobic stability (h)	Low (<24 h tropical)	Slightly improved	Markedly improved (often >120 h)
Yeast/mold inhibition	Poor	Moderate (acid pH)	Strong (acetic acid mediated)

Ruminant Feces as a Source of Indigenous LAB

Microbial Ecology of the Ruminant Gastrointestinal Tract and Feces

The gastrointestinal tract of ruminants is widely recognized as one of the most formidable and densely populated microbial ecosystems in nature. With microbial loads routinely reaching 10^{10} to 10^{11} cells per milliliter in the rumen alone, this dynamic consortium comprises highly competitive bacteria, archaea, and anaerobic fungi (Bergman, 1990). Within this dense biological matrix, lactic acid bacteria particularly robust genera such as *Lactobacillus*, *Enterococcus*, and *Pediococcus* firmly establish themselves across the intestinal mucosa and are subsequently shed in the feces (Fuller, 1989; Kechagia et al., 2013). To survive the perilous journey through the host, these epiphytic populations must constantly navigate severe physiological bottlenecks. They face the fluctuating pH of the reticulorumen, the extreme acidity

of the abomasum (frequently plummeting below pH 3.0), and the highly concentrated bile salts bathing the proximal intestine. Strikingly, these precise physiological stressors perfectly mimic the harsh, progressively acidic, and oxygen-depleted environment inside a sealed silo. Consequently, isolating LAB directly from ruminant feces essentially provides researchers with a biologically pre-screened library of elite, stress-tolerant strains (Conway et al., 1987). This profound ecological pre-conditioning translates directly into functional superiority. As thoroughly reviewed by Sornplang and Piyadeatsoontorn (2016), bioprospecting unconventional reservoirs such as ruminant feces consistently yield robust isolates equipped with potent antagonistic capabilities against enteric pathogens. These indigenous strains survive severe osmotic shock and actively synthesize an arsenal of organic acids, hydrogen peroxide, and specific bacteriocins. When strategically repurposed as silage inoculants, this inherent antimicrobial weaponry becomes a massive asset. It allows the fecal-derived LAB to aggressively outcompete putrefactive epiphytic flora, establish rapid dominance within the forage matrix, and drastically accelerate the initial pH drop. Groundbreaking empirical work in Thailand has already begun to map this functional potential. Thamacharoensuk et al. (2013) extensively profiled LAB diversity from the feces of large domestic herbivores, recovering highly resilient strains from both Thai buffaloes and elephants. Their taxonomic screening identified key functional species, notably *Enterococcus hirae*, *Pediococcus pentosaceus*, and *Lactobacillus pentosus*. From an applied perspective, the buffalo-derived isolates are particularly compelling. Sharing a nearly identical digestive physiology with dairy and beef cattle, these buffalo strains demonstrated remarkable phenotypic plasticity. They produced lactic acid across a wide volumetric gradient (ranging from 21.68 to over 112 g/L) while comfortably surviving elevated bile salt concentrations (0.1 to 1.0%). Such rigorous combined acid and bile tolerance firmly validates ruminant feces as a premier biological reservoir for isolating next-generation inoculants specifically adapted for tropical silage systems (Thamacharoensuk et al., 2013).

Probiotic Screening Criteria in Ruminant-Origin LAB

The foundational paradigm of probiotic therapy, originally articulated by Fuller (1989), hinges on administering live microorganisms to positively modulate the host's intestinal microbiome. In modern screening protocols, candidate strains must clear several rigorous *in vitro* hurdles. Kechagia et al. (2013) stipulate that these clinical benchmarks include surviving extreme gastric acidity (pH 2.0 to 3.0) for at least three hours, proliferating in the presence of 0.3 to 0.5% bile salts, and actively antagonizing key indicator pathogens. When evaluating LAB

sourced from ruminants, these selection criteria are remarkably translatable to forage conservation. The bovine hindgut and a sealed silo share certain common stress conditions, such as acidification and low-oxygen environments, although the two systems differ substantially in their overall physicochemical characteristics. This evolutionary cross-tolerance is well-documented in the literature. An extensive review by Sornplang and Piyadeatsoontorn (2016) highlighted that *Lactobacillus* and *Enterococcus* species recovered from Southeast Asian cattle and buffalo exhibit striking physiological resilience. Driven by continuous exposure to the rumen's fluctuating pH and the intestine's bile-laden environment, these indigenous isolates consistently outlast standard commercial probiotic strains under extreme *in vitro* challenges. Beyond mere survival, these gut-adapted strains particularly *L. plantarum* variants demonstrate aggressive competitive exclusion against zoonotic threats, notably suppressing *Salmonella* species and *Escherichia coli* O157:H7. Exploiting this innate antimicrobial capacity offers a compelling biological rationale for developing a uniquely Thai silage inoculant that delivers secondary prophylactic health benefits to the herd. Consequently, the concept of a "dual-purpose" biological additive emerges as a highly pragmatic strategy. Rather than functioning solely as fermentation enhancers, these ruminant-derived LAB theoretically act as direct-fed microbials upon ingestion. Unlike inoculants isolated from plant epiphytes, gut-adapted strains possess the specific surface binding attributes necessary for mucosal adhesion, allowing them to survive transit and temporarily colonize the gastrointestinal tract (Conway et al., 1987). Once established, they can favorably alter rumen fermentation dynamics, potentially shifting volatile fatty acid profiles to maximize host energy yield (Bergman, 1990). However, to successfully bridge the gap between silo preservation and host nutrition, the inoculant must first endure the ensiling process itself. The biological prerequisites of acid and bile resistance perfectly equip these strains to maintain the critical viability threshold of 10^6 CFU per gram of fresh matter throughout prolonged anaerobic storage, a fundamental requirement for ensuring both preservation efficacy and subsequent probiotic delivery (Muck et al., 2018).

LAB Diversity in Cattle and Buffalo Rumen: Implications for Strain Selection

Despite obvious morphological differences, cattle and buffalo consuming similar roughage-based diets in tropical environments share a remarkably conserved gastrointestinal microbiome. Within this shared microbial ecology, core lactic acid bacteria genera specifically *Lactobacillus*, *Enterococcus*, *Pediococcus*, and *Leuconostoc* are ubiquitous and persistently

isolated from both species (Thamacharoensuk et al., 2013; Sornplang and Piyadeatsoontorn, 2016). However, merely identifying a promising species is scientifically insufficient for inoculant development. As comparative genomic analyses clearly reveal, crucial fermentative capabilities such as the velocity of acidification, terminal lactic acid yield, and the induction of heterofermentative pathways are highly strain-specific traits rather than universal species characteristics (Axelsson, 2004; Sun et al., 2015). Because of this profound intraspecific variation, relying on generalized taxonomic assumptions often leads to inoculant failure in the field. Consequently, rigorous phenotypic characterization and comparative ensiling trials become mandatory. When researchers actively screen and select strains adapted to local conditions, the empirical results are striking. Investigations utilizing tropical forage substrates demonstrate that indigenous LAB recovered from native ecosystems vastly outperform imported temperate strains. These highly adapted local isolates drive down the pH much faster, secure significantly higher lactic acid concentrations, and robustly inhibit opportunistic spoilage organisms (Nkosi et al., 2009; Khota et al., 2016). Ultimately, navigating this microbial diversity to pinpoint elite, region-specific strains offer a direct economic advantage. By minimizing dry matter losses and securing post-opening stability, these tailored biological additives ensure that local farmers can effectively preserve the nutritional integrity of their feed, reducing their vulnerability to seasonal roughage shortages.

Isolation and Identification Methods

Isolating elite lactic acid bacteria from the fiercely competitive matrix of ruminant feces demands rigorous microbiological curation. Typically, this initial recovery relies on the classic cultivation framework established by De Man et al. (1960). When fecal suspensions are subjected to progressive serial dilutions and cultured on MRS agar enriched with 0.3% calcium carbonate, scientists gain a rapid, visual diagnostic tool to identify aggressive acidifiers. The biochemistry driving this step is elegant yet simple: as organic acids accumulate, they dissolve the suspended calcium carbonate, carving out a distinct, transparent halo around the most metabolically active colonies. After these high-potential candidates are visually flagged, they must undergo exhaustive, repetitive streak-plating. This stringent purification is critical to eliminate the persistent coliforms and opportunistic enterobacteria that naturally co-inhabit the lower gut. Given the target application in tropical agriculture, these newly purified, Gram-positive, and catalase-negative isolates should ideally undergo an intermediate physiological screen. Evaluating their growth kinetics under elevated thermal stress (e.g., 35–40 °C) and severe acidic

challenge ensures they can endure the brutal realities of a field-scale silo before researchers invest in costly molecular identification. Ultimately, classical bacteriological profiling only scratches the surface. In the modern era of additive development, securing precise taxonomic classification is a non-negotiable prerequisite. This workflow routinely commences with 16S rRNA gene amplification, where the resulting sequences are benchmarked against curated international databases (like NCBI GenBank) utilizing a strict $\geq 99\%$ similarity cutoff (Thamacharoensuk et al., 2013). Nevertheless, seasoned microbial ecologists are acutely aware of a glaring methodological blind spot: the 16S locus is notoriously hyper-conserved. Within the *Lactobacillus sensu lato* group, this marker frequently lacks the resolution to differentiate between closely related sister species. In the search for superior silage inoculants, this genetic ambiguity is a significant liability. The crucial technological traits that dictate field success such as explosive lactic acid synthesis, robust competitive exclusion, and secondary probiotic functions are strictly governed by strain-specific genetic architecture, not broad species-level taxonomy. Consequently, to definitively validate an isolate's commercial and biological merit, researchers must upgrade to high-resolution genomic platforms. By deploying multilocus sequence typing (MLST) or comprehensive whole-genome sequencing (WGS), scientists can finally bypass taxonomic guesswork and directly map the functional blueprint of these prospective inoculants (Sun et al., 2015).

Key Silage Quality Parameters and Analytical Methods

Fermentation Quality Indices

Evaluating the success of a preservation profile traditionally begins with an aqueous extraction, typically utilizing a 1:10 (w/v) dilution of fresh ensiled biomass. The most immediate and practical diagnostic tool derived from this matrix is the terminal pH. For high-moisture tropical grasses, driving the pH rapidly below 4.2 indicates a robust, homolactic-driven fermentation. Conversely, a pH remaining above approximately 4.5 following fermentation may indicate insufficient acid production and may increase the risk of proliferation of undesirable microorganisms such as *Clostridium* species (Kung et al., 2003). To decipher the precise microbial pathways dictating the fermentation quality, researchers depend on high-performance liquid chromatography (HPLC) or gas chromatography (GC) to accurately map the organic acid profile (Muck et al., 2018). Within these chromatograms, a butyric acid concentration exceeding 3 g/kg of dry matter acts as the undeniable fingerprint of secondary clostridial degradation. This putrefactive shift invariably triggers aggressive proteolysis, which is quantified clinically as

ammonia-nitrogen ($\text{NH}_3\text{-N}$). When $\text{NH}_3\text{-N}$ surpasses 10% of the total nitrogen pool, it exposes a massive breakdown of true plant proteins into non-protein nitrogen, severely compromising the nutritional integrity of the roughage (McDonald et al., 1991). Finally, the overall dry matter (DM) loss calculated via the strict mass balance between the initial and recovered forage captures the costly, invisible attrition from microbial gas emissions and nutrient-rich effluent seepage, directly reflecting the ultimate economic efficiency of the silo system.

Nutritive Value

Accurately determining the feeding value of ensiled biomass demands a strictly standardized analytical approach. The foundational proximate composition capturing true dry matter, crude protein, ether extract, and total inorganic ash is routinely quantified following the official protocols detailed by AOAC International (2000). However, traditional proximate analysis alone cannot predict how a ruminant will physically process the feed. Because the plant's structural carbohydrate matrix actively limits voluntary intake and dictates ruminal degradation, characterizing the cell wall architecture is biologically imperative. Researchers achieve this by isolating neutral detergent fiber (NDF) and acid detergent fiber (ADF) via the sequential detergent extraction method (Van Soest et al., 1991). In practical diet formulation, these specific fractions are indispensable: NDF directly predicts the physical gut-fill effect that caps maximum forage consumption, whereas ADF serves as a highly reliable inverse indicator of apparent digestibility. Alongside these critical fiber metrics, monitoring the transition of water-soluble carbohydrates (WSC) from the freshly cut crop to the mature silage offers a precise window into the efficiency of microbial substrate utilization (McDonald et al., 1991). Ultimately, this comprehensive analytical profile does far more than merely catalogue chemical shifts in a laboratory. It supplies animal scientists with the hard data needed to verify whether a specific biological additive successfully preserves the highly digestible fractions and metabolizable energy required to drive profitable livestock production.

Aerobic Stability Assessment

Fundamentally, aerobic stability dictates the resilience of conserved forage against oxygen-induced deterioration during the feed-out phase. Operationally, researchers define this metric as the exact duration required for the internal mass of exposed silage to spike more than 2 °C above the surrounding ambient baseline (Muck et al., 2018). To accurately capture these spoilage kinetics in a controlled laboratory setting, standardized protocols dictate packing 1 to

2 kg aliquots of silage into aerated matrices. High-precision thermocouple sensors are then driven directly into the geometric center of the forage, logging thermal shifts at strict 30-minute intervals across a 7 to 10-day evaluation period. This continuous monitoring yields highly detailed temperature-time curves, allowing scientists to extract nuanced variables like the heating onset lag and the cumulative temperature sum above ambient (CTAS). Yet, temperature elevation is merely the physical symptom of underlying biological degradation. To comprehensively unravel the spoilage cascade, thermal data must be meticulously paired with temporal microbiological tracking. Monitoring the simultaneous rebound in pH, the explosive proliferation of yeasts and molds on specific selective substrates, and total carbon dioxide efflux paints a complete picture of the deteriorating matrix (Dunière et al., 2017). Applying these rigorous, multi-dimensional parameters is especially critical when simulating the unforgiving thermal realities of Southeast Asia. Under typical Thai farm conditions where daytime ambient temperatures easily sustain 30 to 35 °C the inherent stability of untreated Napier grass routinely collapses in less than 24 hours. Without effective biological intervention, this rapid aerobic failure unleashes massive dry matter losses and nutritional hemorrhaging long before the roughage ever reaches the feed bunk (Bureenok et al., 2012).

Evidence for Indigenous Ruminant-Adapted LAB Superiority in Napier Grass Silage Systems under Tropical Conditions

Before the case for indigenous inoculants can be made, the target substrate must be stated without ambiguity: this review is directed specifically at Napier grass (*Cenchrus purpureus* (Schumach.) Morrone; syn. *Pennisetum purpureum* (Schumach.), including the Thai hybrid cultivar “Pakchong 1”, which underpins the forage base of the Thai cattle sector. Napier grass is singled out not for convenience but because it concentrates every ensiling risk factor into one crop dry matter frequently below 20%, a shallow and inconsistent water-soluble carbohydrate (WSC) reserve, and a sparse epiphytic lactic acid bacteria (LAB) population a combination that renders it dependent on exogenous inoculation in a way that other tropical crops are not (Bureenok et al., 2012; Khota et al., 2016; Kaewpila et al., 2020). Against this substrate-specific backdrop, the scientific consensus increasingly dictates that deploying imported, temperate-climate inoculants in tropical agricultural systems and most acutely in high-moisture, low-WSC grasses such as Napier are fundamentally flawed. Instead, harnessing indigenous, ruminant-adapted LAB isolates offers a distinct and measurable biological advantage. Empirical evidence strongly supports this localized approach.

That the requirement is crop-specific rather than generic has been demonstrated with unusual clarity in Thailand. Jaipolsaen et al. (2022) ensiled whole-plant corn and Napier grass in parallel under identical inoculation regimes using locally isolated *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) J39, *Levilactobacillus brevis* BCC42336 and *Pediococcus pentosaceus* TBRC7603, and reported that the starter cultures failed to depress the pH of corn silage yet proved decisive for Napier grass, where inoculation particularly with *L. plantarum* or the three-strain consortium measurably improved fermentation quality relative to the uninoculated control. Wu et al. (2026) reinforced this from the thermal angle, showing that a self-screened thermostable *Lactobacillus rhamnosus* applied to Napier grass ensiled at 40 °C for 75 days achieved higher lactic and acetic acid concentrations, lower pH (3.70), lower butyric acid and lower ammonia-nitrogen than both the uninoculated control and a commercial *L. plantarum* product, while driving a directed microbial succession toward *Lentilactobacillus buchneri*. Taken together, these results establish Napier grass as the substrate in which inoculant sourcing is simultaneously rate-limiting and demonstrably solvable the precise justification for the focus adopted here.

For instance, Nkosi et al. (2009) explicitly demonstrated that inoculating a low-dry-matter tropical ensiling substrate with a rumen-derived *Lactobacillus plantarum* strain dramatically accelerated the acidification process, dropping the final pH to 3.91 compared to 4.52 in the untreated control. Crucially, this treatment nearly halved the ammonia-nitrogen pool (7.8 vs. 14.6% TN), proving that rumen-origin LAB can aggressively curtail proteolytic spoilage, the exact failure mode that dominates Napier grass ensiled at high moisture. Furthermore, Khota et al. (2016) highlighted how naturally adapted, indigenous LAB possesses the requisite thermal resilience to rapidly dominate the tropical silage microbiome, a competitive feat that commercial imports frequently fail to achieve under severe heat stress, a conclusion derived directly from Guinea grass and Napier grass, in which the authors mapped the dominant epiphytic LAB flora and showed that Napier grass carrying adequate WSC (2.38% DM) ensiled satisfactorily, whereas fermentation collapsed once that reserve proved insufficient. Pholsen et al. (2016) extended this work by characterising thermotolerant Thai isolates TH14 (*Lacticaseibacillus casei*), TH21 and TH64 (*L. plantarum*) and validating them as functional inoculants for tropical silage, while Pitiwittayakul et al. (2021) confirmed that selectively screened thermotolerant Thai strains outperform mesophilic reference cultures at elevated ensiling temperatures. At the applied end of the same continuum, Bureenok et al. (2012) showed that additive-treated Napier grass silage improved not only fermentation profile but

also feed intake, nutrient digestibility and rumen fermentation *in vivo*, and Kaewpila et al. (2020) confirmed that Napier grass harvested at late maturity remains dependent on strategic additive intervention to secure both fermentation quality and aerobic stability.

The underlying rationale for repurposing these gut-derived microbes rests on a fascinating evolutionary parallel. Conway et al. (1987) elucidated that resident intestinal bacteria are naturally equipped to survive the severe physiological bottlenecks of the mammalian digestive tract, specifically extreme gastric acidity and bile exposure. These inherent survival mechanisms perfectly pre-adapt them to thrive in the hostile, oxygen-depleted, and rapidly acidifying environment of a fermenting silo. The ecological premise is not speculative: Guo et al. (2020) recovered 104 LAB isolates from the rumen fluid and faeces of dairy cows and demonstrated that selected strains improved fermentation quality and *in vitro* digestibility of ensiled forage relative to a commercial forage-derived inoculant, while Han et al. (2014) recovered silage-borne LAB alive from the rumen and faeces of cattle fed that same silage evidence that the gut and the silo constitute a continuous, mutually colonisable corridor. By deliberately mining this ruminant reservoir, researchers gain access to a highly curated microbial library encompassing both homofermentative and heterofermentative phenotypes (Axelsson, 2004; Sun et al., 2015). This functional diversity is critical, as it allows for the strategic selection of strain consortia capable of delivering the dual targets essential for Napier grass silage: explosive initial acidification indispensable in a crop whose WSC reserve is exhausted within the first days of ensiling and prolonged aerobic stability during feed-out, which remains the dominant field-level failure mode for Napier silage stored in bunkers and bag silos at Thai ambient temperatures.

Within the specific context of Thailand, however, the translational research pipeline for bovine-fecal LAB remains strictly in its infancy. While seminal *in vitro* work by Thamacharoensuk et al. (2013) successfully isolated highly resilient, probiotic-grade LAB from domestic buffalo feces, and Sornplang and Piyadeatsoontorn (2016) rigorously confirmed the potent antimicrobial properties of regional ruminant isolates against enteric pathogens, these biological assets have yet to be applied to forage conservation and have never been evaluated in Napier grass, notwithstanding its status as the forage on which the national dairy and beef industries most depend. Even the strongest faecal proof-of-concept available internationally (Guo et al., 2020) was executed in temperate alfalfa, precisely the ecological setting from which performance cannot safely be extrapolated to a tropical, low-WSC grass. The glaring absence of controlled, field-scale ensiling trials utilizing Thai bovine-fecal inoculants in Napier grass constitutes a

profound research void. Addressing this precise knowledge gap is the primary catalyst for the proposed experimental framework, aiming to systematically develop a locally sourced, climate-resilient biological additive for Napier grass silage in the nation's cattle sector.

Research Gaps and Future Directions

A critical review of the existing literature reveals four distinct research voids regarding tropical forage conservation. First, the microbial reservoir within Thai bovine feces remains largely uncharacterized at the genomic level. Because metabolic efficacy is highly strain-dependent, employing whole-genome sequencing (WGS) is essential to accurately pinpoint viable inoculant candidates (Sun et al., 2015). Second, empirical head-to-head comparisons are urgently needed. Indigenous isolates must be evaluated against standard commercial inoculants under severe tropical heat (30–37 °C) to definitively prove their presumed competitive advantage (Nkosi et al., 2009; Khota et al., 2016). Third, specific aerobic stability data for Napier grass (*Pennisetum purpureum* cv. Pak Chong 1) under Thai climatic conditions is virtually non-existent, leaving local farmers without evidence-based management protocols. Finally, the "dual-purpose" hypothesis positing that these adapted strains can function sequentially as silo preservatives and direct-fed rumen probiotics require rigorous experimental validation (Conway et al., 1987; Sornplang and Piyadeatsoontorn, 2016). To definitively bridge these gaps, future trials should adopt a factorial completely randomized design (CRD). The experimental model must evaluate Napier grass treated with varying inoculants (uninoculated control, commercial homofermentative/heterofermentative strains, and indigenous strains individually or combined) alongside molasses supplementation (0 vs. 3–5% fresh matter) to correct inherent carbohydrate deficits. Silos should be evaluated after 21 days of fermentation, followed by a strict 7- to 10-day aerobic stability challenge at 30 ± 2 °C. Statistical rigor, utilizing ANOVA under a General Linear Model with mean separation via Duncan's Multiple Range Test ($P < 0.05$), will be crucial for validating treatment efficacies (Steel et al., 1997).

Conclusion

The successful conservation of Napier grass in tropical climates is uniquely constrained by its low dry matter, inconsistent carbohydrate reserves, and susceptibility to rapid proteolytic spoilage under extreme ambient temperatures (Bureenok et al., 2012; Kaewpila et al., 2020). While imported, temperate-climate inoculants consistently underperform in this substrate

(Jaipolsaen et al., 2022), the present review identifies a compelling biological alternative: the exploitation of indigenous, ruminant-adapted lactic acid bacteria (LAB).

Resolving this bottleneck requires a fundamental shift in biological sourcing. The ruminant hindgut presents a highly curated reservoir of indigenous lactic acid bacteria. Having evolved under intense physiological constraints, these gut-adapted strains inherently possess the acid, bile, and thermal resilience required to survive both the ensiling process and gastrointestinal transit (Conway et al., 1987; Sornplang and Piyadeatsoontorn, 2016). Evidence of this gut-silo synergy is robust; not only do ruminant-derived LAB systematically outperform generic commercial blends in acidification and aerobic stability (Nkosi et al., 2009; Khota et al., 2016; Wu et al., 2026), but the gut and the silo have been shown to function as a continuous, mutually colonisable ecological corridor (Han et al., 2014; Guo et al., 2020). Furthermore, the successful isolation of potent, probiotic-grade strains from Thai domestic buffaloes proves that this biological asset is locally abundant and primed for application (Thamacharoensuk et al., 2013). and validated as thermotolerant functional additives for tropical silage (Pholsen et al., 2016; Pitiwittayakul et al., 2021).

The immediate scientific mandate is to translate these in vitro successes into field-scale realities. Future research must execute statistically rigorous, factorial trials (Steel et al., 1997) that directly benchmark Thai bovine-fecal strains against industry standards specifically in Napier grass silage, integrating substrate correctors like molasses to offset natural carbohydrate deficits as required for this specific crop (Bureenok et al., 2012; Kaewpila et al., 2020). By meticulously tracking fermentation kinetics and aerobic stability under field conditions, investigators can unequivocally validate the dual-purpose efficacy of these inoculants. Ultimately, developing a tailored, Thai-origin LAB additive will sever the sector's reliance on imported biotechnology. This transition from basic bioprospecting to applied biotechnology is not merely an academic exercise; it is a vital step toward safeguarding national feed security and elevating the profitability of cattle production systems across Southeast Asia through the systematic integration of bovine-fecal LAB and Napier grass-based silage technology.

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