ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DOTTORATO DI RICERCA IN SCIENZE

VETERINARIE

Ciclo 38

Settore Concorsuale: 07/G1 - SCIENZE E TECNOLOGIE ANIMALI

Settore Scientifico Disciplinare: AGR/18 - NUTRIZIONE E ALIMENTAZIONE ANIMALE

WHAT ABOUT PRECISION FEEDING? AN INVESTIGATION OF OPPORTUNITIES TO IMPROVE THE EFFICIENCY IN THE DAIRY INDUSTRY

Presentata da: Simone Silvestrelli

Coordinatore Dottorato
Carolina Castagnetti

Supervisore
Andrea Formigoni

Co-supervisore

Ludovica Maria Eugenia Mammi
Fausto Toni

Esame finale anno 2026

Abstract of the Thesis

This thesis investigates how Precision Feeding (PF) can enhance efficiency, sustainability, and animal performance in dairy farming by combining nutritional science, process control, and digital decision support. The introductory chapters describe the technological and managerial foundations of PF, focusing on feed ingredient variability, Total Mixed Ration (TMR) execution accuracy, and the enabling role of automated systems such as Automatic Milking Systems (AMS) and Automatic Milk Feeders (AMF).

Five applied studies progressively explore PF from raw material characterization to dietary design and process control. Study 1 analyzes the compositional and digestibility variability of common feedstuffs in the Italian feed industry, demonstrating large inconsistencies and emphasizing the value of routine NIR/XRF analyses to improve formulation accuracy and nutrient-use efficiency. Study 2 audits TMR preparation using *Progeo Dairy Manager (Algomilk)*, revealing systematic deviations in ingredient loading and mixing. Operator training, calibration, and standardized workflows reduced execution errors, aligning fed and formulated rations.

Study 3 tests four isoenergetic diets in a Latin-square design with multiparous Holstein cows, comparing crude protein levels and carbohydrate sources. Diets with reduced protein and rumen-protected amino acids improved nitrogen efficiency (+5.6%) and decreased water intake (–21 L/day) but slightly compromised milk yield, indicating potential for sustainable dietary refinement. Study 4 evaluates a molasses-based liquid feed with tannins under heat stress, reporting higher milking frequency, increased milk yield (+2.15 kg/day), and lower methane intensity (–0.75 g/kg ECM) without altering milk composition. Study 5 assesses AMF operation, showing that temperature and solids variability in milk replacer preparation negatively affect calf growth, identifying calibration and system maintenance as critical control points.

Overall, the thesis concludes that PF represents a multiscale management paradigm where data integration, analytics, and process precision reduce the gap between formulated and delivered diets, achieving measurable gains in feed efficiency, nutrient utilization, profitability, and environmental performance.

Index

List of Tables

Acronyms and Abbreviations

AA Rumen-Protected Amino Acids

AA-HC Rumen-Protected Amino Acids and High Cereals

AA-HF Rumen-Protected Amino Acids and High Fibrous By-products

ACF Automatic Calf Feeder

ADF Acid Detergent Fiber

ADG Average Daily Gain

ADIP Acid Detergent Insoluble Protein

ADL Acid Detergent Lignin

AF As Feed

AI Artificial Intelligence

AMF Automatic Milk Feeder

AMS Automatic Milking System

aNDFom ash corrected Neutral Detergent Fiber with α-amylase and sodium sulfite

ANOVA Analysis of Variance

AOAC Association of Official Analytical Chemists

AUC Area Under Curve

BCS Body Condition Score

BW Body weight

bypassCP By-pass protein (RUP, %CP)

CNCPS Cornell Net Carbohydrate and Protein System

CP Crude Protein

CV Coefficient of Variation

DF Degree of Freedom

DIM Days In Milk

DM Dry Matter

DMI Dry Matter Intake

dNDFD Fibre Degradability

dNDFom Fiber digestibility (aNDFom)

ECM Energy Corrected Milk

EE Ether Extract

FCM Fat Corrected Milk

HC High Cereals

HF High Fibrous By-products

HP High Protein

HP-HC High Protein and High Cereals

HP-HF High Protein and High Fibrous By-products

HPLC High Performance Liquid Chromatography

INRA Institut National de la Recherche Agronomique

IntDig/bypass Intestinal digestibility

IntDigCP Intestinal digestibility (% of CP entering intestine)

IoT Internet of Things

IVNDFD In Vitro NDF Degradability

LCA Life Cycle Assessment

LF Liquid Feed

MR Milk Replacer

MUN Milk Urea Nitrogen

MY Milk Yield

NASEM National Academies of Sciences, Engineering, and Medicine

NDF Neutral Detergent Fiber

NDIP Neutral Detergent Insoluble Protein

NIRS Near Infrared Reflectance Spectroscopy

OM Organic Matter

PDM Progeo Dairy Manager

pdNDFom Potentially digestible aNDFom

PDO Protected Designation of Origin

peNDF Phisically Effective NDF

peNDF Physically Effective Neutral Detergent Fiber

peuNDF Phisically Effective uNDF240.

PF Precison Feeding

PMR Partial Mixed Ration

PR Parmigiano Reggiano

PSPS Penn State Particle Separator

Q Quantile

RDP Rumen Degradable Protein

RFID Radio Frequency Identification

RP Rumen Protected

RumDigCP Ruminal degradability (%CP)

RUP Rumen Undegraded Protein

SARA Sub-Acute Ruminal Acidosis

SD Standard Deviation

SE Standard Error

SOP Standard Operating Procedure

Starch Dig Starch digestibility

TD *Triticum durum*

TMR Total Mixed Ration

TotDigCP Total digestibility (%CP)

TS *Triticum aestivum*

TTNDF240D Total Tract NDFom Digestibility

TTpdNDFom Total Tract potentially digestible NDFom

uNDF Undigested NDF

uNDF24 Undigested NDF via long-term 24 h in vitro fermentation

uNDF240 Undigested NDF via long-term 240 h in vitro fermentation

VFA Volatile fatty Acid

XRF X-ray Fluorescence

INTRODUCTION

The global dairy industry stands at a critical juncture where traditional production paradigms must evolve to meet unprecedented challenges spanning economic viability, environmental sustainability, and animal welfare optimization (FAO, 2018; Gerber et al., 2013). As world population growth, it drives dairy product demand toward an estimated 70% increase by 2050. The sector faces mounting pressure to intensify production efficiency while simultaneously reducing its environmental footprint and maintaining high standards of animal care (Alexandratos & Bruinsma, 2012). This complex optimization challenge has catalysed the emergence of Precision feeding (PF) technologies as a transformative approach to dairy cattle management (Berckmans, 2017).

PF represents a fundamental shift from conventional group based nutritional strategies, embracing instead an individualized approach that try to match nutrient supply with the specific requirements of each animal or homogeneous group within the herd. This paradigm change is enabled by converging technological advances in sensors, artificial intelligence, and automated feeding systems that collectively generate unprecedented volumes of real time data about animal performance, health status, and behavioural patterns (Eastwood et al., 2019; Wolfert et al., 2017).

The technological infrastructure supporting modern PF systems extends far beyond simple feed delivery mechanisms to encompass sophisticated data integration platforms capable of processing heterogeneous information from milk meters, body weight scales, rumination monitors, and environmental sensors (Cabrera et al., 2020; Halachmi et al., 2019). These systems employ machine learning algorithms to identify patterns and relationships that would be impossible to detect through traditional management approaches, enabling predictive rather than reactive nutritional interventions.

Central to the successful implementation of PF is the recognition that feed ingredient variability represents both a fundamental challenge and a significant opportunity for optimization (Van Amburgh et al., 2015). The compositional variation inherent in raw materials, ranging from relatively stable processed concentrates to highly variable forages, necessitates feeding systems capable of maintaining nutritional consistency despite input heterogeneity (Lapierre et al., 2018). This challenge has driven the refinement of TMR preparation technologies and the integration of real time analytical capabilities that enable adaptive formulation adjustments.

The evolution toward AMS has created additional opportunities for PF implementation, as these platforms provide continuous access to individual animal performance data while offering controlled environments for targeted nutritional interventions (King et al., 2018). Similarly, automatic calf feeding systems extend precision management principles to the earliest stages of animal development, establishing feeding protocols that optimize growth trajectories while minimizing labor requirements (Medrano-Galarza et al., 2018).

However, the adoption of PF technologies is not only a technical challenge but represents a complex socio technical transition requiring substantial investments in human capital, infrastructure, and management systems (Rose et al., 2021; Wolfert et al., 2017). The economic justification for these investments depends upon demonstrated improvements in feed conversion efficiency, production performance, and environmental sustainability metrics that collectively enhance the long term viability of dairy operations (Bewley et al., 2015).

This thesis investigates the multifaceted opportunities for improving efficiency within the dairy industry through PF approaches, examining both the technological capabilities and practical implementation challenges that define this emerging field (Chase & Fortina, 2023). Through comprehensive analysis of feed ingredient variability, TMR optimization strategies, and automated feeding system integration, the research provides evidence based insights for enhancing the sustainability and profitability of modern dairy production systems.

The investigation encompasses four interconnected dimensions: first, the conceptual foundations and technological infrastructure of PF systems, including modern software for PF and data integration platforms; second, the characterization and management of feed and forage ingredients variability as a critical determinant of nutritional program effectiveness; third, the role of TMR systems in translating variable inputs into consistent nutritional delivery; and fourth, the integration of automated milking and feeding technologies in creating comprehensive precision management ecosystems.

The significance of this research extends beyond immediate production optimization to address broader sustainability imperatives facing the global dairy sector. By demonstrating pathways for simultaneously improving economic performance and environmental control through technology enabled precision management, this work contributes to the development of sustainable strategies essential for meeting future food production challenges while maintaining environmental integrity.

The findings presented in this thesis are based on comprehensive literature review, industry data analysis, experimental trials and examination of emerging technologies that collectively illuminate the current state and future prospects of PF in dairy cattle production. The research provides practical guidance for industry stakeholders while identifying priority areas for continued technological development and adoption support initiatives.

CHAPTER 1: WHAT ABOUT PRECISION FEEDING?

1.1 Overview on Precision Feeding, automatization and artificial intelligence in dairy farm

As stated in the introductory chapter, the dairy sector faces the dual challenge of meeting rising global demand for nutritious products while reducing environmental impact and improving resource efficiency. PF has emerged as a key strategy within precision livestock farming to address these challenges by matching nutrient supply more closely to animal requirements, thereby reducing feed costs, minimizing nutrient excretion, and enhancing overall production sustainability. This approach has particular relevance for dairy cattle systems, where feed represents up to sixty percent of production costs, and where small improvements in feed efficiency translate into substantial economic and environmental gains (Y. Chen et al., 2022). Traditional feeding systems are built on herd average nutrient requirements, with rations formulated to meet or slightly exceed the needs of a "typical" cow. While this approach ensures that most animals are not underfed, it inevitably results in overfeeding of others, particularly when individual variability in stage of lactation, body condition, or health status is high. The result is reduced nutrient efficiency, higher production costs, and excess nitrogen and phosphorus excretion into the environment. PF aims to address this inefficiency by tailoring nutrient supply more closely to demand, ideally at the level of the individual cow.

The conceptual pillars of PF were articulated in early reviews (Empel et al., 2016). Three interlinked components are emphasized:

- Accurate characterization of nutrient requirements. Requirement models must be sensitive to dynamic changes in lactation stage, body weight, and health.
- Optimization of feed nutrient supply. This involves not only ration formulation but also consideration of feed conservation, processing and delivery technologies that enhance digestibility and availability.
- Managerial practices enabling precision. Even the most refined ration has limited impact if feeding management is inconsistent. Stable feed delivery, controlled group composition, and adequate infrastructure are prerequisites.

The nutritional dimension of PF begins with the characterization of feed ingredients. Forage quality is highly variable, depending on species, maturity at harvest, climate, soil texture and fertility, solar radiation exposure and agronomic practices. As forages mature, lignin content increases and crude protein decreases, leading to lower digestibility, reduced intake potential and in many cases higher methane emissions per unit of milk produced.

PF requires accurate, rapid and ideally on farm assessment of forage nutritive value. Technologies such as near infrared reflectance spectroscopy (NIRS) have become indispensable for real time determination of dry matter, fiber fractions and protein content, enabling formulation of rations that meet requirements more closely than would be possible with tabular averages (feed library) (Empel et al., 2016). Portable NIRS devices, though currently costly, represent promising tools for smallholder farmers once their affordability improves. Forage conservation through ensiling, haymaking and the use of additives such as inoculants also contributes to more consistent nutrient supply and improved palatability. Yet the limitation of early PF frameworks lies in their restricted use of real time data.

Precision livestock farming technologies, including sensors that monitor milk composition, rumination activity, body condition, estrus events and rumen pH, generate large streams of data that can be transformed into actionable management decisions. Automated feeders can allow the adjustment of concentrate supply according to milk yield or body weight, and in combination with decision support systems based on nutritional models, they can individualize diets to a degree that was unimaginable a few decades ago (Romano et al., 2023). Such technologies are more widely adopted in developed countries, but simplified versions, such as mobile applications for ration balancing, are increasingly reaching smallholder farmers in developing regions. In all cases, the success of technology adoption depends not only on its biological effectiveness but also on its economic feasibility, user friendliness and compatibility with existing farm routines (Doidge et al., 2024; Groher et al., 2020). Farmers will only invest in PF technologies if clear profitability is demonstrated, or if policy frameworks provide incentives or subsidies to encourage adoption in the interest of broader environmental or societal goals (Bewley et al., 2015; Bianchi et al., 2022).

The managerial aspect of PF relates to the design and implementation of feeding strategies at the farm level. TMR, if well done, stabilize rumen fermentation and prevent selective feeding, while ration balancing programs, often implemented through extension services or feed company support, allow even smallholder farms to improve productivity and income significantly. Management practices that reduce feed waste, improve silage quality, and ensure adequate water provision also contribute to PF outcomes, since efficiency is compromised not only by nutrient imbalance but also by avoidable losses and constraints in intake. By increasing feed digestibility, PF reduces methane production per unit of milk, while optimizing nitrogen supply lowers nitrous oxide emissions from manure and soils.

Phase feeding strategies that adjust protein supply to lactation stage can reduce nitrogen excretion, while the adoption of ration balancing programs at scale has the potential to decrease national greenhouse gas inventories from dairy sectors (Rotz et al., 2025; C. Yang et al., 2022). Thus, PF is not merely a farm level innovation but also a critical component of climate change mitigation strategies in the livestock sector.

Despite its potential several challenges constrain the widespread adoption of PF. Forage and feed quality variation remains a significant barrier, particularly in tropical and subtropical regions where rapid changes in climate and soil fertility affect nutritive value unpredictably (Kulkarni et al., 2018). The inconsistent performance of feed additives, such as enzymes, probiotics and plant extracts, also complicates recommendations, as many show positive results under experimental conditions but fail to replicate under commercial farm settings. Furthermore, most nutritional models assume fixed efficiencies of nutrient utilization, which do not account for dynamic interactions among diet composition, animal genotype and environmental stressors. There is also a need for better integration of animal health and welfare into PF frameworks, as subclinical diseases and heat stress reduce feed efficiency regardless of diet formulation (Papakonstantinou et al., 2024). Institutional weaknesses, including inadequate feed analysis laboratories, limited extension capacity and poor quality control in feed manufacturing, further undermine the implementation of PF in many developing countries. Addressing these barriers requires coordinated policy support, investment in infrastructure, and capacity building among farmers and nutritionists.

1.2 Advanced Intelligent system for Precision Feeding in dairy industry: the AlgoMilk example.

The progression from conventional feeding strategies to PF reflects not only advances in nutritional science but also the broader digital transformation of livestock systems. While the conceptual foundations of PF have centred on nutrient characterization, optimized ration formulation, and managerial practices, recent years have witnessed a convergence between nutritional science, information technology, and artificial intelligence that is reshaping the very architecture of decision making in dairy farming (Khanashyam et al., 2025).

PF in its early formulations was largely static, grounded in tabulated nutrient requirements and periodic laboratory analyses of feedstuffs; however, the emergence of sensors, automated feeding equipment, and near infrared spectroscopy has expanded the capacity for real time monitoring of both animals and feed resources. What remains a challenge, however, is the translation of these heterogeneous data streams into coherent management strategies that can balance productivity, profitability, and sustainability goals simultaneously.

In this respect, the frontier of PF lies not merely in refining requirement models or enhancing ration delivery technologies, but in harnessing computational systems capable of processing vast, dynamic datasets to produce actionable insights for farmers. This is where decision support platforms and artificial intelligence based tools enter the picture, serving as integrative frameworks that connect biological, economic, and environmental dimensions of livestock farming. Within this broader landscape, the development of advanced digital platforms such as AlgoMilk exemplifies the transition toward data driven and predictive management in dairy systems.

Building upon the principles of PF, AlgoMilk extends the concept by embedding AI algorithms, deep learning, and Internet of Things (IoT) connectivity into farm operations. Unlike traditional nutritional models that apply average values and safety margins, AlgoMilk is designed to learn continuously from individual animal data, adapting predictions to the specific context of each farm while simultaneously contributing to larger distributed models. Its ability to integrate feed composition, milk yield, health records, environmental parameters, and market dynamics illustrates how PF has evolved into a multidimensional practice that not only addresses nutrient supply and demand but also supports strategic decision making across technical, economic, and ecological domains.

The evolution of dairy farming over the past half century has been characterized by significant technological transformations, beginning with mechanization and progressing through the adoption of AMS, advanced reproductive technologies, and genomic selection, yet in recent years the sector has increasingly turned to data driven tools that allow the integration of multiple dimensions of farm management into coherent decision making frameworks, with AlgoMilk emerging as an advanced illustration of this transition toward precision livestock farming underpinned by artificial intelligence (Berckmans, 2017; Espinoza-Sandoval et al., 2024; Palma et al., 2025; Wolfert et al., 2017).

While traditional herd management software has often been limited to descriptive reporting, offering retrospective insights on milk yields, reproduction, and feeding practices, AlgoMilk distinguishes itself by employing federated deep learning architectures, thereby enabling predictive analytics that are simultaneously adaptive to local farm conditions and respectful of data privacy, since raw farm data does not need to be centrally pooled but instead contributes to distributed model training across multiple farms (Kairouz et al., 2021). The practical relevance of this design is considerable, given the growing sensitivity of farmers to data ownership and the risks associated with corporate monopolization of agricultural datasets, which many scholars have identified as a critical governance issue in the digitalization of agriculture (Eastwood et al., 2019; Rose et al., 2021).

From a technical perspective, AlgoMilk integrates different data derived from heterogeneous sources such as milk meters, feed intake sensors, automatic weighing devices, RFID, near infrared spectrometry of feed and milk components, weather forecasts, and market price indices into a dynamic ecosystem capable of simulating alternative management strategies in real time. This integration makes it possible to translate raw measurements into predictive scenarios that are actionable at the level of both the individual animal and the herd, a development consistent with the broader paradigm of precision livestock farming that seeks to monitor each animal continuously and adapt management to its individual needs, rather than relying on population averages (Berckmans, 2017).

The range of functionalities encompassed by AlgoMilk illustrates its potential as a multi-dimensional decision support tool. At the nutritional level, the system can predict the effects of different ration formulations and grouping strategies on milk yield, animal health, feed efficiency, and gross margins, enabling farmers to make informed choices depending on their strategic priorities, whether they be maximizing profitability, increasing productivity, or reducing feed costs in response to market volatility. The software is not only descriptive but prescriptive, in that it generates concrete recommendations on grouping animals by lactation stage, health status, or production potential, thereby facilitating optimized use of available feed resources.

At the environmental level, AlgoMilk incorporates modules capable of estimating methane emissions, nitrogen excretion, and phosphorus losses, which are central to life cycle assessments (LCA) and increasingly relevant for compliance with European environmental regulations under the Farm to Fork and Green Deal strategies (European Commission, 2020). Given that livestock production is under scrutiny for its greenhouse gas footprint, the ability of farmers to monitor emissions in near real time not only supports internal efficiency but also enhances the transparency and accountability of the dairy sector toward external stakeholders, including consumers, policymakers, and certification schemes.

At the economic level, AlgoMilk distinguishes itself by incorporating external market signals into its decision making framework, allowing for scenario analyses where changes in feed or milk prices can be directly linked to alternative nutritional strategies, thereby reducing the uncertainty faced by farmers in contexts of volatile commodity markets. In this sense, the platform exemplifies the convergence of zootechnical and economic intelligence, situating herd management within broader agri-food system dynamics.

The implementation of AlgoMilk reflects a deeper shift in agricultural knowledge production. Whereas herd management has historically relied on farmers' tacit knowledge, accumulated through experiential learning and intergenerational transfer, systems like AlgoMilk embed decision making within algorithmic frameworks that quantify parameters previously assessed heuristically, such as the marginal contribution of individual cows to farm profitability or the environmental trade-offs of alternative feeding regimes. This transition toward what could be described as "computational epistemology" has ambivalent implications: on the one hand, it empowers farmers by providing them with high resolution insights and by simulating the outcomes of complex interactions that would otherwise be cognitively unmanageable; on the other, it risks constraining farmer autonomy by embedding decisions within black box models whose internal logic may not be transparent, raising questions about trust, interpretability, and the human machine interface in agricultural practice (Wolfert et al., 2017). The adoption of such tools is therefore not merely a technical matter but also a socio technical process, shaped by farmers' attitudes toward digitalization, their levels of digital literacy, and the institutional ecosystems in which these technologies are introduced.

Furthermore AlgoMilk represents a pioneering application of federated learning in livestock systems, a methodological innovation that aligns with broader concerns about data sovereignty in agriculture. Federated learning allows predictive models to be trained across decentralized data sources while ensuring that raw data remain on the local device or farm, thus offering both privacy protection and enhanced adaptability to local conditions (Kairouz et al., 2021). In agriculture this approach mitigates the risks associated with centralized data monopolies, a concern frequently highlighted in debates around platformization and digital governance. By enabling each farm to contribute to the development of global models while retaining control over its own information, AlgoMilk exemplifies a distributed form of agricultural intelligence that could become a cornerstone of more collaborative and resilient innovation systems in the future. This federated architecture also enhances the robustness of predictive models, as they can learn from a wider diversity of contexts without being biased toward a single centralized dataset, thereby increasing their generalizability and reliability.

The implications of AlgoMilk are therefore multilayered: at the micro level, it supports day to day operational decisions in dairy farms; at the meso level, it contributes to the sustainability performance of the dairy supply chain; and at the macro level, it illustrates broader transformations in agricultural knowledge systems and governance. For example, by enabling farmers to quantify the environmental impact of their herds, AlgoMilk contributes to the generation of verifiable data that can sustain sustainability certifications and carbon markets, thereby linking farm level practices with global sustainability agendas. Similarly, by integrating market signals into its predictive framework, it aligns dairy farming more closely with agri-food economics, reducing the temporal gap between technical decision making and economic realities. Finally by embedding federated learning, it contributes to ongoing debates about the democratization of digital technologies in agriculture, suggesting pathways for reconciling efficiency, sustainability, and equity in digital innovation systems (Rose et al., 2021).

Is possible to say that AlgoMilk is more than a technical tool: it is a case study in the digital transformation of agriculture, encapsulating the tensions and opportunities of a sector increasingly shaped by data driven intelligence, environmental imperatives, and socio technical dynamics. It operationalizes the vision of precision livestock farming by bridging zootechnical, economic, and environmental dimensions, while simultaneously raising critical questions about algorithmic mediation, farmer autonomy, and data governance. Its federated deep learning framework points toward a future in which agricultural intelligence is distributed rather than centralized, enabling farms to collectively benefit from shared learning while preserving their independence.

This distributed architecture, coupled with its integration of IoT devices and predictive analytics, positions AlgoMilk as not merely a tool for operational efficiency but as a harbinger of broader structural shifts in how agricultural knowledge is produced, validated, and applied. Whether such systems will fulfill their transformative potential will depend not only on technical performance but also on the social, economic, and institutional conditions of adoption, highlighting the importance of embedding digital innovations within participatory and inclusive governance frameworks. The successful implementation of PF systems, as exemplified by AlgoMilk and similar platforms, fundamentally depends upon accurate characterization of feed ingredients and understanding their inherent variability. While digital technologies and artificial intelligence provide sophisticated tools for optimizing ration formulation and delivery, these systems can only be as effective as the quality of input data regarding feed composition and nutritional value. This recognition brings us to a critical examination of one of the most persistent challenges in dairy cattle nutrition: the substantial variability that exists within and between raw feed materials, which directly impacts the precision with which nutritional programs can be formulated and implemented.

CHAPTER 2: VARIABILITY OF FEED MATERIALS IN DAIRY NUTRITION

The PF paradigm in dairy cattle nutrition fundamentally relies upon accurate characterization of feed ingredient composition and nutritional value (Van Amburgh et al., 2015). As discussed in the previous chapter, the evolution from conventional feeding strategies to sophisticated PF systems like AlgoMilk requires not only advanced computational capabilities but also reliable, consistent data regarding feed composition. However, substantial inherent variability exists within raw feed materials, encompassing both chemical composition and digestibility parameters, which significantly impacts diet formulation accuracy and ultimately affects animal performance, production efficiency, and economic returns (W. P. Weiss & St-Pierre, 2009). This variability has emerged as one of the most significant challenges confronting contemporary dairy nutritionists, particularly as the industry transitions toward increasingly sophisticated nutritional management systems.

The magnitude and sources of feed ingredient variability extend beyond simple compositional differences to encompass variations in digestibility, bioavailability, and functional properties. These variations originate from multiple interconnected sources including genetic factors, environmental conditions during crop growth, harvesting practices, storage conditions, processing methodologies, and geographical origin (Xiao & Meng, 2024). As PF systems become increasingly dependent upon accurate nutritional databases and predictive models, the imperative to characterize and quantify this variability has intensified.

Within the context of European feed industry practices, where diverse feed ingredients are sourced from various geographical regions and multiple suppliers, understanding the magnitude and sources of variability assumes even greater significance (Huhtanen et al., 2006). The implications of ingredient variability transcend nutritional formulation accuracy to encompass broader considerations of economic efficiency, environmental sustainability, and animal welfare. Contemporary research demonstrates that failure to adequately account for ingredient variability can result in systematic overfeeding of nutrients, occurring in approximately 90% of feeding days across commercial dairy operations (Carroll et al., 2024).

This chapter provides a comprehensive examination of the various sources and magnitudes of variability present in commonly utilized dairy cattle feed ingredients. The analysis discusses contemporary methodologies for variability assessment and quantification, and explores evidence based strategies for managing this variability within practical feeding programs. The chapter draws upon recent advances in analytical methodologies, statistical modeling approaches, and practical implementation strategies to provide a foundation for improved PF systems.

2.1 Sources and Determinants of Concentrate Ingredients Variability

Feed ingredient composition is shaped by a multi-layered set of intrinsic biological and extrinsic technological determinants that collectively generate the variability mapped (and discuss later in the chapter) in the following list and necessitate continuous monitoring in nutrition systems:

- Intrinsic biological variation;
- Genetic diversity among cultivars;
- Developmental stage at harvest;
- Phenological stage of forage crops;
- Seasonal and annual environmental variation;
- Processing methodologies;
- Operational parameters in processing;
- Storage and post-harvest handling;
- Geographical origin;
- Climatic conditions during growth periods;
- Temporal variability.

Feed ingredient variability originates from fundamental biological processes that can be categorized into intrinsic and extrinsic factors. Intrinsic variability encompasses the natural biological variation inherent in plant and animal derived feed materials, representing the baseline level of variation that exists even under controlled conditions (Assunção et al., 2022). For plant based ingredients, genetic diversity among cultivars constitutes a primary source of compositional variation, with different varieties exhibiting substantial differences in protein content, amino acid profiles, oil composition, and secondary metabolite concentrations (Lee et al., 2019).

The developmental stage at harvest significantly influences the nutritional composition of feed ingredients, with maturity effects being particularly pronounced in cereal grains and forage crops (O'Keefe et al., 2022). Similarly, the phenological stage of forage crops at harvest dramatically affects crude protein content, neutral detergent fiber (NDF) concentrations, and in vitro digestibility parameters (Ferraretto et al., 2018). Seasonal and annual variations introduce additional layers of intrinsic variability that must be considered in feed formulation strategies. Crops cultivated during different growing seasons or harvest years exhibit substantial compositional differences attributable to variations in temperature regimes, precipitation patterns, photoperiod duration, soil composition and solar radiation intensity (Soares et al., 2019). These environmental influences affect fundamental plant metabolic processes, nutrient accumulation patterns, and secondary metabolite biosynthesis pathways, ultimately impacting the nutritional value of resulting feed materials.

Processing methodologies and operational parameters represent significant sources of extrinsic variability in feed ingredients, often exceeding the magnitude of intrinsic biological variation (Theurer et al., 1999). The toasting procedures applied to soybean meal following solvent extraction effectively enhance protein digestibility (Akbarian et al., 2014). Mechanical processing operations including grinding, pelleting, steam conditioning, and extrusion introduce additional sources of variability that significantly impact nutrient availability and digestibility. The effectiveness of these processing methods depends upon numerous variables including particle size distribution, moisture content, conditioning temperature, pelleting pressure, and cooling procedures, all of which exhibit substantial variation within and between processing facilities

Storage and post harvest handling procedures contribute significantly to compositional variability through various degradation mechanisms. Suboptimal storage conditions promote nutrient degradation, particularly affecting vitamin stability and unsaturated fatty acid integrity. Moisture fluctuations during extended storage periods facilitate mold proliferation and mycotoxin production, while oxidative processes progressively reduce the nutritional quality of lipids and certain amino acids (Rathod & Kumawat, 2024).

Geographical origin represents a major determinant of variability in internationally traded feed commodities, with systematic differences observed between major producing regions. Soybean meals originating from the United States, Brazil, and Argentina, which collectively represent the predominant global suppliers, exhibit nutritional differences exceeding typical analytical variation (Ruiz et al., 2020). These regional differences arise from variations in soil mineralogy, climatic conditions, agricultural management practices, and processing technologies employed in different geographical areas.

Climatic conditions during critical growth periods significantly influence crop composition through effects on plant physiological processes. Drought stress typically increases protein concentration in cereal grains while concurrently reducing starch content and overall yield potential (Havrlentová et al., 2021). Conversely, excessive precipitation during grain filling periods can dilute nutrient concentrations and promote fungal contamination, potentially introducing mycotoxins while reducing overall nutritional quality (Deligeorgakis et al., 2023). Temperature extremes during grain development affect starch synthesis rates and protein deposition patterns, resulting in altered nutritional profiles that persist throughout storage and feeding (Han et al., 2025).

Temporal variability manifests both within individual growing seasons and between successive harvest years, requiring continuous monitoring and adaptation of nutritional management strategies. Within season variation results from changing environmental conditions during crop development phases, while between season variation reflects annual differences in weather patterns, pest and disease pressure, and evolving agricultural management practices. This temporal component necessitates regular analytical monitoring and database updating to maintain accuracy in nutritional formulation systems.

2.2 Forage Variability: A Critical Component of Dairy Cattle Nutrition

Forage materials represent the foundation of dairy cattle nutrition systems, typically comprising 40-60% of total dietary dry matter intake in high producing dairy cows. Unlike processed concentrate feeds, forages retain the biological complexity of whole plant materials, encompassing variations in anatomical fractions, developmental stages, and environmental responses that directly influence nutritional value and digestibility characteristics.

Neutral detergent fiber (NDF) content in alfalfa hay exhibits variation across diverse cultivars, same for crude protein concentrations between cultivars (Jungers et al., 2020). This variation substantially impacts diet formulation accuracy and necessitates comprehensive understanding of the underlying biological and environmental factors controlling forage quality (Huhtanen et al., 2006).

Within season variability represents one of the most significant sources of forage quality variation, reflecting the dynamic nature of plant growth and development throughout the growing season. Alfalfa crude protein content typically declines from early vegetative stages to full bloom, while NDF concentrations increase over the same developmental period (Karayilanli & Ayhan, 2016). This relationship between maturity and nutritional composition creates fundamental trade-offs between forage yield and quality that significantly influence the economic optimization of forage production systems.

Weather conditions during critical growth periods exert profound effects on forage quality through direct impacts on plant physiological processes and secondary effects on harvest timing and preservation quality (Andrews, 1987). Temperature stress during vegetative growth phases accelerates plant maturation rates, effectively compressing the window for high quality forage harvest and increasing quality decline. Research found that flooding is negative correlate with protein synthesis rates while accelerating lignification processes, resulting in forages with reduced crude protein content and decreased fiber digestibility (Hart et al., 2022).

Moisture availability represents a critical determinant of both forage yield and quality, with drought stress generally increasing protein concentration through concentration effects while simultaneously reducing overall dry matter yield in alfalfa forages (Liu et al., 2018). Optimal moisture conditions typically result in forages with moderate protein concentrations and maximum fiber digestibility, reflecting balanced plant growth and development.

Soil fertility status significantly influences forage mineral composition and may affect protein synthesis and secondary metabolite production. Nitrogen availability strongly correlates with forage crude protein content, with adequate nitrogen fertilization typically increasing protein concentrations by 2-4 percentage units compared to unfertilized conditions (Delevatti et al., 2019).

Balanced application of macronutrient fertilizers, including nitrogen sources (e.g., urea or ammonium nitrate), phosphorus fertilizers (e.g., single or triple superphosphate), and potassium fertilizers (e.g., potassium chloride or sulfate) is essential to maximize both forage yield and nutritional quality. Ensuring adequate N, P, and K through targeted fertilizer programs sustains root development, energy metabolism, and stress resilience, thereby boosting biomass production and forage digestibility. Phosphorus and potassium availability influence yield potential of forage crops up to 40%, with deficiencies potentially reducing both productivity and nutritional value. A long term study conducted by Markus & Battle (1965) in New Jersey demonstrated that alfalfa production showed dramatic improvements with increased potassium application, with crop yields rising by almost 140% when comparing the lowest and highest potassium fertilization rates across nine years of research. Similarly, Berg et al. (2007) discovered that potassium nutrition played an increasingly critical role as alfalfa crops matured, observing that yield improvements from potassium fertilization grew from 33% in the fourth growing season to 300% by the seventh year of the stand.

Genetic diversity among forage species and cultivars represents a fundamental source of compositional variation that affects both baseline nutritional characteristics and responses to environmental conditions (Aruna et al., 2015). Leguminous forages typically contain more crude protein compared to grass species under similar growing conditions, reflecting fundamental differences in nitrogen fixation capacity and plant metabolism (Solati et al., 2018). These species differences extend beyond simple protein content to encompass fiber composition, mineral profiles, and secondary metabolite concentrations that affect palatability and nutritional value.

Within species variation among cultivars can be substantial, with modern breeding programs specifically targeting improved nutritional characteristics alongside traditional productivity traits. Short corn cultivars demonstrate reduction NDF, ADF and lignin content, so an increase in digestibility compared to conventional varieties, reflecting reduced lignin concentrations and altered fiber structure (Sarmikasoglou et al., 2025).

Mechanical harvesting operations introduce significant sources of variation that can substantially alter the nutritional composition of preserved forages compared to standing crops. Field losses during mowing, conditioning, and collection processes typically affect plant fractions differentially, with leaf material being more susceptible to mechanical damage and loss than stem components (Blanco, 2023). Since leaves generally contain higher protein concentrations and lower fiber levels than stems, excessive field losses can reduce the protein content and increase the fiber concentration of harvested forage.

Wilting procedures and moisture management during hay production create additional sources of variation that affect both nutritional composition and preservation quality. Rapid moisture loss through mechanical conditioning can reduce leaf shatter and preserve higher protein concentrations, while extended field curing increases the risk of quality deterioration through continued plant respiration and potential weather damage (Edmunds et al., 2014; Moscoso et al., 2019).

2.3 Quantification and Statistical Assessment of Variability

Quantification of feed ingredient variability requires appropriate statistical measures that accurately capture both the magnitude and distributional characteristics of variation while enabling meaningful comparisons across different nutrients and ingredient categories. The coefficient of variation (CV) serves as a standardized, dimensionless measure that facilitates direct comparison of variability across disparate nutritional parameters and ingredient types. Standard deviation provides an absolute measure of variability that directly relates to the practical implications of compositional differences in diet formulation scenarios. When combined with mean values, standard deviation enables calculation of confidence intervals and establishment of quality specifications for ingredient procurement and quality assurance programs. The range of variation, while statistically less robust than standard deviation, provides intuitive information regarding the extremes of compositional variation that practitioners might encounter under commercial conditions.

Distribution analysis reveals whether variability follows normal distribution patterns or exhibits skewness that might indicate systematic biases or the presence of outlier effects. Non normal distributions frequently suggest the existence of distinct subpopulations within ingredient categories or the influence of categorical factors such as processing methodologies, geographical origin, or seasonal effects. Understanding these distributional patterns proves crucial for developing appropriate quality control protocols and risk management strategies in commercial operations.

Analytical variability represents a significant component of total observed variation in feed and forages composition, encompassing both within laboratory precision and between laboratory accuracy differences. The magnitude of analytical variability varies substantially among different nutritional parameters, with trace elements often exhibiting the greatest relative variation.

Sample collection and preparation procedures significantly influence the magnitude of observed variability, particularly for heterogeneous materials such as mixed feeds, fermented feeds, and processed byproducts (Reese et al., 2017). Representative sampling presents particular challenges for materials exhibiting spatial heterogeneity, with inadequate sampling protocols potentially leading to substantial overestimation of ingredient variability and subsequent suboptimal decision making in diet formulation processes. All analytical methods require that samples being analyzed accurately represent the material being offered to animals, necessitating careful attention to sampling frequency, sample size, and homogenization procedures.

Near infrared reflectance spectroscopy (NIRS) has emerged as a valuable technology for rapid assessment of feed ingredient composition and variability monitoring. Energy values in contemporary feed evaluation systems are frequently estimated using prediction equations developed from NIRS analytical data, providing cost effective screening capabilities for routine quality control applications (Evangelista et al., 2021). However, NIRS accuracy depends critically upon the quality and representativeness of calibration databases and may not adequately capture all aspects of nutritional variability, particularly those related to anti nutritional factors, bioactive compounds, or novel processing effects.

2.4 Production and Economic Implications of Feed Ingredient Variability

The nutritional implications of feed ingredient variability goes over simple compositional differences to affect complex metabolic processes, ruminal fermentation patterns, and subsequent production outcomes in dairy cattle. Research demonstrates that commercial dairy operations systematically overfeed nutrients including crude protein, fat, neutral detergent fiber, and starch during 90% of feeding periods within evaluation intervals, indicating widespread recognition of ingredient variability through conservative formulation approaches (Carroll et al., 2024). This overfeeding pattern, while providing a safety margin against nutrient deficiencies, represents economic inefficiency and contributes to environmental nitrogen loading through increased excretion (Phuong et al., 2013). Protein variability affects both ruminal nitrogen balance and post ruminal amino acid supply, with implications for microbial protein synthesis efficiency and metabolizable protein availability (Xie et al., 2019).

Energy ingredient variability affects ruminal fermentation patterns and metabolizable energy supply through alterations in volatile fatty acid production profiles and passage rates. Unexpected variations in starch content or digestibility can disrupt the synchronization between energy and protein release within the rumen, potentially reducing microbial protein synthesis efficiency and overall nitrogen utilization (P. Chen et al., 2024). Additionally rapid fermentation of highly digestible starch sources can precipitate ruminal acidosis episodes, particularly when combined with inadequate effective fiber intake or suboptimal feeding management practices (Golder & Lean, 2024).

The production consequences of ingredient variability depend upon both the magnitude of compositional variation and the physiological responsiveness of target production parameters to nutritional changes. Milk protein yield demonstrates high sensitivity to amino acid supply variations, particularly for lysine and methionine, which serve as co-limiting amino acids in typical dairy cattle diets (Kim & Lee, 2021). A reduction in metabolizable protein supply can decrease milk protein yield, representing substantial economic losses in markets utilizing component based pricing systems (Zang et al., 2021).

Economic modeling of ingredient variability reveals that the costs associated with overformulation to compensate for compositional uncertainty frequently exceed the direct costs of improved ingredient characterization and quality assurance programs. Contemporary nutritional management approaches should incorporate statistical methodologies to explicitly account for variability in diet formulation procedures and implement comprehensive risk assessment protocols to evaluate the economic implications of alternative formulation strategies. These analyses consistently demonstrate positive returns on investment for enhanced analytical monitoring and quality control programs in commercial dairy operations.

Routine analysis of major dietary ingredients for key nutritional parameters provides the foundation for precision diet formulation, yet the frequency and analytical scope should be tailored to the variability characteristics of specific ingredients and their quantitative importance within the diet.

Critical control points should be established based on ingredient variability patterns, economic value, and potential impact on animal performance outcomes.

2.5 Technological Advances and Future Research Directions

Rapid analytical technologies continue to evolve, offering enhanced possibilities for real time assessment of ingredient variability and responsive management of nutritional programs. Portable near infrared reflectance spectroscopy instruments and smartphone-based analytical systems may eventually enable on farm assessment of ingredient quality, facilitating more responsive management of compositional variability (Hossain et al., 2024). However, successful implementation of these technologies requires continued development of robust calibration databases and comprehensive validation protocols to ensure accuracy across diverse environmental conditions and ingredient types.

In near future Artificial intelligence and machine learning could offer significant potential for improved prediction of ingredient variability based on environmental conditions, processing parameters, and historical quality databases. These methodologies could enable proactive adjustment of formulation strategies based on predicted ingredient quality rather than reactive responses to analytical results. However, successful implementation requires substantial databases of high quality information linking ingredient characteristics to performance outcomes, along with robust validation under diverse commercial conditions.

Understanding the biological mechanisms underlying variability in ingredient digestibility represents a critical research priority for advancing PF systems. While compositional analysis provides valuable information for diet formulation, the relationships between chemical composition and bioavailability remain poorly characterized for many nutrients and ingredient. Research focusing on factors controlling protein degradability, starch digestibility kinetics, and fiber utilization efficiency could substantially improve prediction accuracy and reduce dependence on extensive analytical monitoring programs (Cavallini et al., 2023).

Development of rapid bioassays for ingredient quality assessment could provide more biologically relevant information than traditional chemical analysis approaches alone. In vitro digestibility assays, microbial fermentation studies, and enzyme activity measurements may offer valuable insights into functional characteristics that directly affect animal performance outcomes even if standardization of these methodologies and validation of their correlation with in vivo performance responses remains challenging and requires continued research investment (Scicutella et al., 2025).

2.6 Implications for Precision Feeding Systems

The variability of raw feed materials represents both a fundamental challenge and a significant opportunity for advancing PF systems in contemporary dairy cattle nutrition. While this variability substantially complicates diet formulation procedures and increases risks of nutritional imbalances, comprehensive understanding and systematic management of compositional variation can facilitate improved production efficiency, reduced input costs, and enhanced animal performance outcomes (Schlageter-Tello et al., 2020). The magnitude of ingredient variability differs substantially among feed categories, with byproduct feeds consistently exhibiting the greatest compositional variation, followed by forage materials, while cereal grains demonstrate relatively lower but still meaningful levels of variation.

Recognition of these variability patterns enables implementation of risk based approaches to ingredient monitoring and quality control that optimize resource allocation while maintaining formulation accuracy (W. P. Weiss & St-Pierre, 2009). Even ingredients with reputations for consistency, such as corn, can exhibit meaningful variation in critical parameters including starch digestibility that significantly affect dietary performance in high producing dairy cattle. Effective management of ingredient variability requires integration of comprehensive analytical programs, advanced formulation strategies, and collaborative supplier relationships, as no single approach proves sufficient for addressing the complexity of compositional variation.

Contemporary PF technologies continue to advance rapidly, offering enhanced capabilities for characterizing and responding to ingredient variability through real time monitoring, predictive modeling, and adaptive formulation strategies (Kaur et al., 2023). Success in implementing these technological advances requires sustained collaboration between researchers, nutritionists, and industry partners to effectively translate scientific understanding into practical management tools that simultaneously improve profitability and sustainability of dairy production systems.

Future developments in ingredient variability management will likely emphasize integration of multiple information sources, including environmental monitoring, genetic characterization, and individual animal response patterns, to create comprehensive nutritional management systems that optimize both animal performance and resource utilization efficiency under inherently variable conditions. Having established the critical importance of understanding and managing feed ingredient variability, the practical implementation of this knowledge requires sophisticated feeding systems capable of delivering consistent, homogeneous rations despite inherent variations in raw materials.

The development and refinement of TMR systems represents the industry's primary solution to this challenge, providing a mechanism to blend diverse ingredients into uniform mixtures that minimize selective feeding behavior and ensure consistent nutrient delivery to all animals within a feeding group. The following chapter examines the theoretical foundations, practical implementation, and management considerations of TMR systems, demonstrating how these feeding strategies serve as the crucial bridge between variable feed ingredients and the precise nutritional requirements of modern dairy cattle.

CHAPTER 3: TMR AND IT'S IMPLICATION ON DAIRY COWS PERFORMANCE AND HEALTH

3.1 Theoretical Foundations of TMR Systems

The theoretical foundation of TMR feeding systems is rooted in understanding rumen fermentation dynamics and the complex interactions between dietary components, microbial populations, and host metabolism. The rumen ecosystem functions as a continuous fermentation system where microbial populations degrade and ferment dietary components to produce volatile fatty acids, microbial protein, and B vitamins essential for the host animal's metabolism (Palmonari et al., 2024). The success of TMR systems depends on maintaining optimal conditions for rumen fermentation while providing adequate nutrients to meet the cow's requirements for maintenance, production, and reproduction.

The concept of physically effective neutral detergent fiber (peNDF) represents a critical consideration in TMR formulation, as it directly influences rumen pH, motility, and fermentation patterns (Cao et al., 2021). The peNDF content of a TMR is determined not only by the chemical composition of fiber sources but also by their physical characteristics, particularly particle size and structure. Adequate peNDF stimulates chewing activity and saliva production, which provides buffering capacity to maintain rumen pH within the optimal range of 6.0 to 6.8 for fiber digestion and microbial growth. The relationship between particle size, chewing activity, and rumen buffering forms the physiological basis for recommendations regarding TMR particle size distribution and the importance of preventing sorting behavior (Li et al., 2020).

The formulation of TMR relies heavily on mathematical modeling approaches that integrate nutritional requirements, feed composition data, and economic considerations to determine optimal ingredient inclusion rates. Linear programming techniques have traditionally been employed to minimize feed costs while meeting specified nutritional constraints, though more sophisticated optimization algorithms are increasingly being utilized to account for non linear relationships and multiple objectives (Campos et al., 2023). The integration of machine learning approaches, as discussed in previous chapters regarding AI applications and AlgoMilk systems, enables dynamic optimization that adapts to changing conditions and incorporates predictive analytics for improved decision making. The integration of machine learning approaches, as discussed in Chapter 1 regarding AI applications and AlgoMilk systems, enables dynamic optimization that adapts to changing conditions and incorporates predictive analytics for improved decision making, while simultaneously addressing the feed ingredient variability challenges detailed in Chapter 2.

The Cornell Net Carbohydrate and Protein System (CNCPS) and similar mechanistic models provide frameworks for predicting nutrient supply and animal requirements based on feed characteristics, animal factors, and environmental conditions (Van Amburgh et al., 2015). These models subdivide feed carbohydrates and proteins into fractions with different degradation rates, enabling more precise prediction of nutrient availability and microbial protein synthesis. The application of these models in TMR formulation allows nutritionists to regulate rations for specific production goals while accounting for factors such as passage rate, microbial growth efficiency, and post ruminal digestion.

Stochastic modeling approaches have gained attention for addressing uncertainty and variability in TMR systems, recognizing that feed composition, intake patterns, and animal responses exhibit inherent variability (Patil et al., 2022).

The challenge of feed ingredient variability discussed in the previous chapter necessitates feeding strategies that can effectively blend and deliver diverse ingredients while maintaining nutritional consistency across all animals in a feeding group. The evolution of dairy cattle nutrition has undergone remarkable transformation over the past century, with the development and adoption of TMR feeding systems representing one of the most significant advancements in modern dairy production. The TMR concept, which emerged primarily during the latter half of the twentieth century, fundamentally altered the approach to feeding high producing dairy cows by ensuring consistent nutrient delivery throughout the day and minimizing selective feeding behavior (Schingoethe, 2017). As dairy operations have intensified and genetic selection has produced cows with increasingly higher milk production potential, the importance of precise nutrition management has become paramount for maintaining animal health, welfare, and economic viability.

The transition from component feeding systems to TMR based approaches reflects a broader paradigm shift in dairy nutrition philosophy, moving from simplistic feeding strategies to sophisticated, scientifically based nutritional programs that consider the complex interactions between feed ingredients, rumen fermentation dynamics, and metabolic processes. This evolution aligns with the technological advancements discussed in previous chapters, particularly the integration of artificial intelligence systems and algorithmic optimization tools such as AlgoMilk, which enable unprecedented precision in ration formulation and adjustment based on real time production data and environmental conditions.

This evolution directly addresses the ingredient variability challenges outlined in Chapter 2, while simultaneously aligning with the technological advancements discussed in Chapter 1, particularly the integration of artificial intelligence systems and algorithmic optimization tools such as AlgoMilk, which enable unprecedented precision in ration formulation and adjustment based on real time production data and environmental conditions. Recent advances in individualized PF have further enhanced TMR systems, with algorithm development for supplemental top dressing showing promise for improving feed efficiency at the individual cow level (Souza et al., 2022).

The preparation of an effective TMR extends far beyond simply combining various feed ingredients in prescribed proportions. It encompasses a multifaceted process that requires careful consideration of ingredient quality, particle size distribution, mixing procedures, moisture content, and numerous other factors that collectively determine the nutritional value and acceptability of the final product (W. Weiss & St-Pierre, 2024). The complexity of TMR preparation is further compounded by the need to accommodate varying nutritional requirements across different production groups, accounting for factors such as lactation stage, body condition, reproductive status, and health considerations.

Contemporary dairy operations face increasing pressure to optimize production efficiency while maintaining high standards of animal welfare and environmental sustainability. The proper preparation and management of TMR systems plays a crucial role in achieving these objectives by maximizing feed utilization efficiency, reducing nutrient waste, and supporting optimal rumen health and metabolic function (Schingoethe, 2017). Research has consistently demonstrated that variations in TMR preparation and delivery can significantly impact key performance indicators, including milk yield, milk composition, feed conversion efficiency, and reproductive success (Bach, 2024a).

The significance of TMR quality extends beyond immediate production outcomes to encompass long term herd health and sustainability. Improperly prepared TMR can contribute to various metabolic disorders, including ruminal acidosis, displaced abomasum, and laminitis, which not only compromise animal welfare but also result in substantial economic losses through reduced production, increased veterinary costs, and premature culling (Arzola-Álvarez et al., 2010; Schingoethe, 2017). Conversely, well prepared TMR that maintains consistent nutrient density, appropriate particle size distribution, and minimal sorting potential supports stable rumen fermentation, optimal nutrient absorption, and enhanced immune function.

This chapter provides a comprehensive examination of TMR preparation principles and practices, synthesizing current scientific knowledge with practical applications for modern dairy operations. The discussion encompasses theoretical foundations of TMR formulation, practical considerations for ingredient selection and processing, mixing technologies and procedures, quality control measures, and strategies to resolve common challenges. Additionally, the chapter explores emerging trends in TMR management, including implementation of PF technologies, and integration with data driven management systems discussed in previous chapters.

3.2 Ingredient Selection and Characterization

The selection and characterization of forage ingredients represents a critical first step in TMR preparation, as forages typically comprise forty to sixty percent of the ration dry matter and significantly influence intake potential, rumen function, and overall diet digestibility. Forage quality assessment encompasses multiple dimensions, including chemical composition, physical characteristics, fermentation profile for ensiled forages, and potential anti nutritional factors. Near infrared spectroscopy (NIRS) technology has become the standard method for rapid analysis of forage composition, providing estimates of crude protein, fiber fractions, and digestibility parameters that inform formulation decisions.

The maturity stage at harvest profoundly impacts forage nutritive value, with advancing maturity generally associated with increased fiber content, decreased protein concentration, and reduced digestibility. Legume forages exhibit different maturity quality relationships, with changes in leaf-to-stem ratio being particularly important for alfalfa quality determination. The heterogeneity of forage quality within and between lots necessitates regular sampling and analysis to maintain TMR consistency as well address in Chapter 2.

Physical characteristics of forages, as already spoken in chapter 3.1, and in particular particle length and structure, directly influence their effectiveness in maintaining rumen function and preventing metabolic disorders (D. Mertens, 1997). The theoretical length of cut for chopped forages affects not only their physical effectiveness but also packing density in storage, fermentation characteristics, and mixing properties in TMR preparation. Kernel processing in corn silage has emerged as an important consideration, with proper processing improving starch availability while maintaining adequate particle size for rumen function (Salvati et al., 2021). The interaction between chemical and physical properties of forages underscores the need for comprehensive characterization beyond traditional proximate analysis.

The selection of concentrate ingredients for TMR formulation involves balancing multiple objectives, including meeting energy and protein requirements, complementing forage nutrients, managing feed costs, and maintaining rumen health. Energy concentrates, primarily cereal grains and their by-products, vary considerably in their ruminal fermentation characteristics, with differences in starch degradability influencing both microbial protein synthesis and milk quality (Mohammed et al., 2010). The processing method applied to grains, whether grinding, rolling, steam flaking, or other techniques, significantly impacts starch availability and particle size contribution to the TMR.

Protein supplementation strategies in TMR systems must consider both the quantity and quality of protein sources, with emphasis on balancing rumen degradable and undegradable protein fractions to optimize microbial protein synthesis while meeting metabolizable protein requirements (Van Amburgh et al., 2015). The amino acid profile of rumen undegradable protein sources becomes particularly important for high producing cows where metabolizable protein may limit production. The inclusion of multiple protein sources with complementary amino acid profiles and degradation characteristics enables more precise formulation to meet specific amino acid requirements, particularly for limiting amino acids such as methionine and lysine (C. Wang et al., 2010).

By-product feeds have assumed increasing importance in TMR formulation due to their cost effectiveness and nutritional value, though their inclusion requires careful consideration of variability, handling characteristics, and potential limitations. Distillers grains, corn gluten feed, soybean hulls, and other by-products offer unique nutritional profiles that can complement traditional ingredients while reducing feed costs. However, the moisture content, variability in composition, and potential for spoilage of wet by-products necessitate appropriate storage facilities and inventory management strategies. The integration of by-product feeds into TMR systems exemplifies the importance of considering both nutritional and operational factors in ingredient selection.

3.2 TMR Mixing Technology and Procedures

The development of standardized mixing procedures is essential for maintaining TMR consistency and achieving targeted particle size distributions while minimizing equipment wear and energy consumption (Arzola-Álvarez et al., 2010). The sequence of ingredient addition significantly influences final mix quality, with general principles favouring the addition of dry ingredients before wet ingredients and long particles before concentrated feeds. However, optimal loading sequences may vary depending on mixer type, ingredient characteristics, and desired outcomes. For vertical mixers, a common approach involves loading dry hay first to allow initial processing, followed by silages, grain mixes, and finally liquid ingredients. Horizontal mixers may benefit from different loading sequences that account for their mixing action characteristics.

Mixing time represents a critical parameter that must be optimized to achieve adequate blend uniformity without causing excessive particle size reduction or equipment wear (Arzola-Álvarez et al., 2010). Research indicates that most TMR reach acceptable uniformity within three to six minutes after the final ingredient addition, though specific requirements vary with mixer design, fill level, and ingredient properties (A. Costa et al., 2019). Over mixing not only wastes energy and time but can reduce particle size below optimal levels, potentially compromising rumen function and increasing sorting behavior. Conversely, under mixing results in poor blend uniformity that may cause variation in nutrient intake among animals. The establishment of standardized mixing times for different ration types and regular evaluation of mix uniformity through particle size analysis and chemical testing supports optimization efforts.

The incorporation of liquid ingredients, including water, molasses, and fat supplements, requires particular attention to achieve uniform distribution without creating handling problems or promoting spoilage (T. DeVries & Gill, 2012). The addition of water to adjust TMR moisture content can improve palatability and reduce sorting, with optimal moisture contents typically ranging from 45% to 55%. However, excessive moisture can promote heating and spoilage, particularly in warm weather, while insufficient moisture may increase dustiness and sorting potential. Recent research has shown that adding liquid feed supplements, such as molasses based products, to TMR not only improves nutrient distribution but also reduces feed sorting behaviour and enhances performance on cow during transition period (C. Havekes et al., 2020). Liquid fat sources require careful incorporation to prevent coating of particles that might interfere with rumen fermentation. The use of carrier ingredients or sequential addition strategies can improve the distribution of liquid ingredients throughout the mix.

3.3 Particle Size Distribution and Physical Effectiveness

The Penn State Particle Separator (PSPS) has emerged as the standard field method for evaluating TMR particle size distribution, providing a practical tool for on farm assessment. The PSPS utilizes a series of sieves with different aperture sizes to stratify TMR samples into fractions, with suggested ranges for total NDF at least 1.10 to 1.20 percent of body weight and forage NDF intake ranging from 0.75 to 1.10 percent of body weight. The interpretation of PSPS results requires consideration of both the distribution across sieves and the chemical composition of each fraction, as particle size alone does not fully characterize physical effectiveness.

Modifications to the PSPS, including the addition of a 1.18 mm sieve, have improved its ability to characterize fine particles that may influence rumen fermentation dynamics and passage rate (Kononoff et al., 2003). A desired TMR sample should have 2-8% of the sample remain on the top screen, 30-50% on the second screen, 10-20% on the third screen and 30-40% of the sample falling all the way through to the solid bottom pan. These guidelines reflect the balance between providing adequate physical structure for rumen function while maintaining sufficient surface area for microbial attachment and fermentation. Regular monitoring of particle size distribution enables detection of changes in ingredient processing, mixer performance, or sorting behavior that might compromise nutritional management.

Alternative methods for characterizing physical effectiveness include laboratory based techniques such as wet sieving, image analysis, and geometric mean particle size determination. These methods provide more detailed information about particle size distribution but are generally impractical for routine on farm use. The development of the physically effective NDF (peNDF) concept, which integrates chemical fiber content with physical measurements, provides a more comprehensive assessment of the rumen stimulating capacity of feeds. Various systems for calculating peNDF have been proposed, with differences in methodology leading to variation in published recommendations. The selection of appropriate measurement methods depends on the specific objectives, available resources, and desired level of precision.

3.4 Quality Control and Monitoring Systems

The implementation of systematic sampling protocols for TMR and individual ingredients is essential for monitoring quality, detecting variations, and resolve performance issues (Cherney et al., 2021). Representative sampling of TMR requires collection from multiple locations along the feed bunk immediately after delivery, with samples combined and mixed before subsampling for various analyses. The frequency of sampling should reflect the degree of variation in ingredients and the criticality of maintaining consistency, with more frequent sampling warranted during periods of ingredient transitions or when performance issues arise. Proper sample handling, including immediate freezing for fermentation profile analysis or appropriate drying for chemical analysis, ensures accurate results that reflect actual feeding conditions.

Chemical analysis of TMR samples provides validation of formulation accuracy and detection of unexpected variations that might impact animal performance. The comparison of analyzed values with formulated specifications requires consideration of analytical variation and sampling error, with control charts and statistical process control techniques useful for distinguishing normal variation from significant deviations. The integration of rapid analysis methods, such as NIRS, enables more frequent monitoring and faster response to detected issues (Buonaiuto et al., 2021; Evangelista et al., 2021).

The integration of PF technologies with TMR systems enables real time adjustment of ration delivery based on individual or group level production responses, environmental conditions, and feed availability (Bach, 2023). Automated feeding systems equipped with load cells and RFID technology can track individual animal intake patterns, enabling detection of health issues, evaluation of sorting behavior, and optimization of feeding frequency. The combination of intake monitoring with milk production and composition data supports dynamic adjustment of TMR formulation to match actual rather than predicted requirements. These systems generate substantial data streams that, when properly analyzed using techniques discussed in previous chapters on AI applications, provide insights for continuous improvement of nutritional management.

In line NIR sensors integrated with TMR mixers offer potential for real time monitoring of mix quality and adjustment of ingredient inclusion rates to compensate for variation in component composition (Shenk & Westerhaus, 1994). These systems can detect moisture content changes, estimate nutritional parameters, and identify mixing problems as they occur rather than through retrospective analysis (Serva et al., 2021). The implementation of such technology requires robust calibration procedures and regular validation against laboratory analyses (Buonaiuto et al., 2021). The combination of real time sensing with automated control systems represents an evolution toward closed loop TMR preparation systems that maintain consistent nutrient delivery despite ingredient variation.

CHAPTER 4: AUTOMATIC MILKING SYSTEMS AND AUTOMATIC MILK FEEDERS ON PRECISION FEEDING

4.1 Automatic Milking Systems and Precision Feeding Integration

4.1.1 Introduction to Precision Feeding in Robotic Milking Environments

The contemporary integration of PF technologies with AMS represents a paradigmatic shift in dairy cattle management, fundamentally addressing the escalating demands for sustainable intensification and enhanced production efficiency within the global dairy sector. This technological convergence enables sophisticated individualized nutritional management predicated upon real time physiological monitoring and comprehensive production data analytics, establishing a robust foundation for the development and implementation of advanced precision nutrition algorithms (Kaur et al., 2023). Modern AMS platforms function as comprehensive data centric milking and management systems that facilitate PF through continuous generation of high frequency, cow level data encompassing milk yield and flow characteristics, milking interval patterns, quarter level electrical conductivity measurements, activity metrics, dynamic body weight assessments, and detailed feeding event records.

The operational success of PF systems fundamentally depends upon seamless integration with existing AMS infrastructure, effectively utilizing the comprehensive data collection capabilities embedded within robotic milking platforms (Ozella et al., 2023). Contemporary AMS installations enable uninterrupted monitoring through sophisticated multi sensor arrays encompassing milk flow meters, compositional analyzers utilizing near infrared spectroscopy for major milk components, sensor for mastitis detection and other tools that collectively generate comprehensive multidimensional datasets essential for precision nutrition algorithm development and refinement (Diaz-Olivares et al., 2020; Kamphuis et al., 2010).

The dual feeding methodology predominantly employed in contemporary AMS operations involves the strategic delivery of a nutritionally balanced partial mixed ration (PMR) at conventional feed bunks, complemented by targeted supplemental concentrate provision typically ranging from 2-8 kg per cow per day through the robotic milking system interface, thereby enabling both population level and individual specific nutritional management strategies (Van Soest et al., 2024). This approach provides a practical framework wherein the PMR supplies baseline physically effective neutral detergent fiber and fundamental macronutrient requirements, while robot delivered concentrates furnish marginal energy provision and functional feed additives that can be dynamically adjusted in near real time to minimize the deviation between predicted and realized nutritional requirements at the individual animal level.

Recent evaluations of individualized concentrate delivery protocols within AMS environments have demonstrated the operational feasibility of dynamic allocation algorithms based on individual cow production metrics and physiological status indicators, proving compatible with both pasture based and PMR based feeding systems while providing a practical methodology to do individual level nutritional adjustments upon population level dietary foundations (Gaillard & Abarnou, 2024; Schwanke et al., 2022). However, implementation challenges include inherent variability in daily concentrate delivery, that results in substantial day to day fluctuations that may compromise PF objectives. Furthermore, increasing AMS concentrate allocations frequently result in elevated refusal rates and unpredictable substitution effects on PMR consumption, necessitating sophisticated predictive modeling to maintain optimal total nutrient intake (Bach & Cabrera, 2017).

4.1.2 Machine Learning and Artificial Intelligence Applications

The proliferation of machine learning applications within dairy farming decision support systems has experienced exponential growth, where sophisticated algorithms now address diverse operational domains including PF optimization, animal husbandry protocol development, preventive healthcare management strategies, comprehensive behavioural analysis, milking process optimization, and integrated resource allocation systems (Trapanese et al., 2024). Contemporary PF frameworks leverage advanced artificial intelligence and machine learning architectures to process high dimensional, heterogeneous datasets, generating individualized feeding recommendations through continuous adaptive learning mechanisms that incorporate farm specific data patterns and operational performance outcomes (Trapanese et al., 2024).

Deep learning neural networks analyze extensive historical performance datasets spanning minimum 2-3 year periods to identify complex behavioural and physiological patterns, enabling prediction of future nutritional requirements and facilitating proactive nutritional management paradigms that go over traditional reactive feeding approaches.

Recent advances in machine learning frameworks have demonstrated remarkable capability in predicting daily milk yield trajectories with correlation coefficients ranging from R^2 = 0.78-0.85, compositional variations with accuracy parameters of ±0.1% for major milk components, and milking frequency patterns of 2.1-3.2 visits per day for individual cows within robotic dairy farming environments, thereby providing the computational foundation for dynamic PF strategy development and implementation (Melfsen et al., 2012; Xing et al., 2024). These sophisticated computational systems employ complex mathematical models that seamlessly integrate metabolic modelling frameworks, comprehensive digestive physiology parameters, and quantitative genetic factors to determine individualized nutritional requirements with unprecedented precision levels.

4.1.3 Data Infrastructure and Sensor Integration

Modern AMS platforms integrate sophisticated multi sensor arrays that continuously capture detailed milk flow characteristics and yield measurements per milking event, inline or at parlor compositional proxies utilizing near infrared spectroscopy, real time body weight assessments via integrated platform scales, and comprehensive behaviour/activity patterns through triaxial accelerometers, collectively underpinning precision nutrition algorithms by enabling rapid detection of deviations from expected nutrient utilization efficiency parameters (Kaur et al., 2023). Systematic reviews emphasize that these comprehensive data streams, when effectively fused with environmental monitoring data including ambient temperature, humidity, and housing condition parameters, facilitate classification of individual cows according to metabolic status indicators and responsiveness to targeted supplementation strategies, establishing essential prerequisites for adaptive feeding algorithms that dynamically adjust concentrate quantities or nutrient density specifications based on recent performance metrics and health status indicators (Stygar et al., 2021; Tadele et al., 2025).

4.1.4 Adoption Drivers and Economic Considerations

The trajectory of automatic milking system adoption within the United States dairy industry demonstrates consistent upward momentum, with multifactorial drivers including diminishing labor availability and escalating workforce costs cited as primary motivators for AMS implementation across progressive livestock operations (Lage et al., 2024). Evidence from comprehensive AMS adoption studies conducted across developed dairy regions indicates that progressive operations are accelerating adoption rates due to persistent labour market constraints and the capacity of AMS to reallocate human resources from routine milking tasks to higher value management activities, while simultaneously maintaining or improving production efficiency through automated data capture capabilities and individualized management protocols (Cogato et al., 2021; Marques et al., 2023).

Furthermore, the emergent generation of dairy producers exhibits pronounced interest in precision dairy farming methodologies, with young operators demonstrating increasing recognition of integrated systems' transformative potential for sustainable dairy production, driven by enhanced technological literacy and environmental stewardship priorities (Palma-Molina et al., 2023). Robotic milking systems are experiencing accelerated adoption among progressive livestock operations due to their capacity to substantially reduce labour requirement while simultaneously enhancing production efficiency through automated data acquisition capabilities and individualized animal management protocols that optimize resource utilization and improve animal welfare outcomes.

4.2 Automatic Calf Feeding Systems and Precision Rearing

4.2.1 Technology Implementation and System Components

The implementation of automatic milk feeding systems for dairy calves represents a fundamental advancement in precision livestock farming methodologies, enabling sophisticated individualized nutritional management protocols from the earliest developmental stages through comprehensive technological integration. Automatic calf feeders (ACF) facilitate precisely controlled milk delivery protocols based on chronological age parameters, body weight measurements, and comprehensive health status indicators, thereby facilitating optimal growth trajectories while substantially reducing labor requirements by 40% associated with traditional calf rearing practices (Sinnott et al., 2021). These technologically advanced systems complement adult cattle AMS infrastructure by establishing PF protocols from birth, creating a comprehensive farm wide approach to automated nutrition management across all developmental life stages.

Contemporary automatic calf feeding systems incorporate sophisticated technological components including radio frequency identification (RFID) based individual recognition systems with operational read ranges of 10-30 cm, programmable feeding curves featuring multi phase nutritional protocols, and real time behavioural monitoring capabilities that provide comprehensive feedback on feeding behaviours, intake patterns, and health status indicators (J. H. C. Costa et al., 2021). Advanced system configurations utilize peristaltic pumps or servo controlled valves providing exceptional portion control accuracy, with dynamically adjustable flow rates ranging from 0.3-1.0 L/min based on individual calf age, physiological development stage, and specific drinking behaviour patterns (J. H. C. Costa et al., 2021).

4.2.2 Feeding Protocol Management and Performance Outcomes

Sophisticated feeding curve programming enables comprehensive customization of milk allowances based on physiological development stages, typically initiating at 6-8 L/day for neonatal calves and progressively increasing to 10-12 L/day by weeks 3-4 of age, with graduated step-down weaning protocols automatically reducing milk allowance over predetermined 2-3 week periods to strategically encourage concentrate intake targeting 1.5-2.0 kg/day at weaning while promoting rumen development and minimizing weaning associated stress responses. Advanced automated feeding systems incorporate adaptive feeding algorithms that dynamically adjust individual allowances based on quantitative assessment of drinking speed parameters within ranges of 0.6-1.5 L/min, visit frequency patterns goes up to 12-14 times per day, and residual milk consumption behaviours that indicate feeding efficiency and health status (Knauer et al., 2017; Legge et al., 2024).

Research consistently demonstrates that calves raised utilizing automatic feeding systems achieve superior growth and greater weaning weight compared to conventional feeding methodologies (relatively of 106.4 kg vs. 91.4 kg and 1.11 kg/d vs. 0.87 kg/d), with additional documented benefits in behavioural development and social competence acquisition that contribute to long term productivity and welfare outcomes (Arens et al., 2023; J. H. C. Costa et al., 2021; Legge et al., 2024).

AMF generate comprehensive individual level data streams including milk intake volumes, drinking speed parameters, visit frequency patterns, and feeding refusal incidents that collectively enable early disease detection protocols and welfare assessment capabilities that surpass traditional manual feeding systems.

Comprehensive management software platforms provide sophisticated dashboard interfaces displaying real time feeding status indicators, automated health alert systems triggered by statistically significant deviations exceeding 30% from established normal feeding patterns, and integrated growth tracking capabilities through seamless integration with automated weighing systems that enable continuous monitoring of individual animal performance metrics.

4.2.3 Integration Across Developmental Stages

Contemporary precision livestock frameworks advocate for comprehensive life stage integration wherein calf AMF data streams inform heifer management target specifications and lactation performance prediction models, while lactation AMS data provide feedback into genetic and management selection criteria for subsequent calf generations, thereby creating closed loop systems that span from early life phenotypic characterization to mature performance assessment and genetic improvement programs. This integrated, longitudinal approach aligns with sustainable intensification objectives by capturing heterogeneity in nutritional requirements across developmental stages and deploying targeted interventions that improve nutrient utilization efficiency and welfare outcomes while optimizing labor allocation and resource utilization throughout the production system (Kaur et al., 2023; Papadopoulos et al., 2025).

The capacity to harmonize AMF telemetry data with downstream heifer growth trajectories and future milk production records provides a systematic pathway to quantify long term returns to individualized calf nutrition protocols, thereby informing evidence based weaning strategies and starter feed introduction methodologies that optimize lifetime productivity outcomes (Jiang et al., 2025)). Successful implementation of integrated PF systems requires significant investment in human capital development, encompassing comprehensive training programs in system operation protocols, advanced data interpretation competencies, and sophisticated troubleshooting capabilities necessary for maintaining optimal system performance across diverse environmental and operational conditions.

4.3 Future Perspectives and Implementation Challenges

Future research priorities should strategically focus on enhancing algorithm robustness through advanced machine learning methodologies, reducing implementation costs through technological innovation and economies of scale, and developing integrated sustainability metrics that comprehensively encompass environmental, economic, and social dimensions of dairy production systems. The continued evolution of PF technologies will play instrumental roles in meeting escalating global demand for dairy products while simultaneously minimizing environmental impact and maintaining exemplary standards of animal welfare through optimized resource utilization and individualized management protocols (Trapanese et al., 2025).

Emerging technologies including Internet of Things (IoT) sensor networks, edge computing capabilities, and blockchain based data management systems offer promising opportunities for enhanced system integration and real time decision making capabilities that will further advance PF implementation across diverse production environments (Kaur et al., 2023). The development of standardized data exchange protocols and interoperable communication frameworks will facilitate seamless integration between different technology platforms, enabling comprehensive farm management systems that optimize both individual animal performance and overall production efficiency.

The inherent complexity of PF systems requires specialized interdisciplinary knowledge spanning animal nutrition science, physiological understanding, and information technology domains, presenting ongoing challenges for widespread adoption across diverse farming contexts with varying levels of technological infrastructure and human capital resources. For individualized PF to achieve economic viability for commercial producers, the quantifiable value of increased cow productivity must exceed the comprehensive costs of technology investment, including initial capital expenditure, ongoing maintenance requirements, and necessary training investments.

Maximum individual cow productivity from nutritional management perspectives requires accurate, validated prediction models that are specific to individual animals and their physiological responses, necessitating utilization of automated sensing mechanisms to capture pertinent performance parameters with algorithms seamlessly integrated into comprehensive precision animal farming analytical frameworks. Community based automatic milking system services and cooperative technology ownership models may provide viable pathways to reduce individual farm investment costs, making advanced PF technologies more accessible to smaller scale operations while maintaining operational efficiency and animal welfare standards.

REFERENCES

1. Akbarian, A., Khorvash, M., Ghorbani, G. R., Ghasemi, E., Dehghan-Banadaky, M., Shawrang, P., & Ghaffari, M. H. (2014). Effects of roasting and electron beam irradiating on protein characteristics, ruminal degradability and intestinal digestibility of soybean and the performance of dairy cows. *Livestock Science*, *168*, 45–52.
2. Alexandratos, N., & Bruinsma, J. (2012). *World agriculture towards 2030/2050: The 2012 revision*.
3. Andrews, C. (1987). Low-temperature stress in field and forage crop production–an overview. *Canadian Journal of Plant Science*, *67*(4), 1121–1133.
4. Arens, S. C., Sharpe, K. T., Schutz, M. M., & Heins, B. J. (2023). Response to ad libitum milk allowance by crossbred dairy and dairy–beef calves in an automated feeding system. *Translational Animal Science*, *7*(1), txad063. https://doi.org/10.1093/tas/txad063
5. Aruna, C., Swarnalatha, M., Kumar, P. P., Devender, V., Suguna, M., Blümmel, M., & Patil, J. (2015). Genetic options for improving fodder yield and quality in forage sorghum. *Tropical Grasslands-Forrajes Tropicales*, *3*(1), 49–58.
6. Arzola-Álvarez, C., Bocanegra-Viezca, J., Murphy, M., Salinas-Chavira, J., Corral-Luna, A., Romanos, A., Ruíz-Barrera, O., & Rodríguez-Muela, C. (2010). Particle size distribution and chemical composition of TMRs for dairy cattle: Water addition and feed sampling effects. *Journal of Dairy Science*, *93*(9), 4180–4188.
7. Assunção, A. de S., Silva, T. E. da, Quirino, D., Franco, M. de O., & Detmann, E. (2022). Variability among animals and incubation protocols for ruminant in situ degradation studies with tropical feeds. *Animals*, *12*(15), 1901.
8. Bach, A. (2023). Changes in milk production and estimated income over feed cost of group-housed dairy cows when moved between pens. *Journal of Dairy Science*, *106*(6), 4108–4120. https://doi.org/10.3168/jds.2022-22875
9. Bach, A. (2024). Back to basics: Precision while mixing TMRs and its impact on milking performance. *JDS communications*, *5*(2), 102–106.
10. Bach, A., & Cabrera, V. (2017). Robotic milking: Feeding strategies and economic returns. *Journal of Dairy Science*, *100*(9), 7720–7728. https://doi.org/10.3168/jds.2016-11694
11. Berckmans, D. (2017). General introduction to precision livestock farming. *Animal Frontiers*, *7*(1), 6–11.

12. Berg, W., Cunningham, S., Brouder, S., Joern, B., Johnson, K., Santini, J., & Volenec, J. (2007). The long-term impact of phosphorus and potassium fertilization on alfalfa yield and yield components. *Crop Science*, *47*(5), 2198–2209.
13. Bewley, J., Russell, R., Dolecheck, K., Borchers, M., Stone, A., Wadsworth, B., Mayo, L., & Tsai, I.-C. (2015). *Precision dairy monitoring opportunities, limitations, and considerations*. 3–5.
14. Bianchi, M., Bava, L., Sandrucci, A., Tangorra, F., Tamburini, A., Gislon, G., & Zucali, M. (2022). Diffusion of precision livestock farming technologies in dairy cattle farms. *animal*, *16*(11), 100650.
15. Blanco, J. A. B. (2023). *Characterization and Management of Forage Nutrient Variation to Improve Dairy Cattle Diet Accuracy*. Cornell University.
16. Buonaiuto, G., Cavallini, D., Mammi, L. M. E., Ghiaccio, F., Palmonari, A., Formigoni, A., & Visentin, G. (2021). The accuracy of NIRS in predicting chemical composition and fibre digestibility of hay-based TMRs. *Italian journal of animal science*, *20*(1), 1730–1739.
17. Cabrera, V. E., Barrientos-Blanco, J. A., Delgado, H., & Fadul-Pacheco, L. (2020). Symposium review: Real-time continuous decision making using big data on dairy farms. *Journal of dairy science*, *103*(4), 3856–3866.
18. Campos, L., Ringer, H., Chung, M., & Hanigan, M. (2023). Application of a mathematical framework for the optimization of precision-fed dairy cattle diets. *animal*, *17*, 101001.
19. Cao, Y., Wang, D., Wang, L., Wei, X., Li, X., Cai, C., Lei, X., & Yao, J. (2021). Physically effective neutral detergent fiber improves chewing activity, rumen fermentation, plasma metabolites, and milk production in lactating dairy cows fed a high-concentrate diet. *Journal of Dairy Science*, *104*(5), 5631–5642.
20. Carroll, A., Hanford, K., Abney-Schulte, C., & Kononoff, P. (2024). Estimation of the nutrient variation in feed delivery and effects on lactating dairy cattle. *JDS communications*, *5*(6), 548–552.
21. Cavallini, D., Raffrenato, E., Mammi, L., Palmonari, A., Canestrari, G., Costa, A., Visentin, G., & Formigoni, A. (2023). Predicting fibre digestibility in Holstein dairy cows fed dry-hay-based rations through machine learning. *animal*, *17*, 101000.
22. Chase, L. E., & Fortina, R. (2023). Environmental and economic responses to precision feed management in dairy cattle diets. *Agriculture*, *13*(5), 1032.
23. Chen, P., Li, Y., Wang, M., Shen, Y., Liu, M., Xu, H., Ma, N., Cao, Y., Li, Q., & Abdelsattar, M. M. (2024). Optimizing dietary rumen-degradable starch to rumen-degradable protein ratio

improves lactation performance and nitrogen utilization efficiency in mid-lactating Holstein dairy cows. *Frontiers in Veterinary Science*, *11*, 1330876.

24. Chen, Y., Hostens, M., Nielen, M., Ehrlich, J., & Steeneveld, W. (2022). Herd level economic comparison between the shape of the lactation curve and 305 d milk production. *Frontiers in Veterinary Science*, *9*, 997962. https://doi.org/10.3389/fvets.2022.997962
25. Cherney, D., Digman, M., & Cherney, J. (2021). Day-to-day variation in forage and mixed diets in commercial dairy farms in New York. *Applied Animal Science*, *37*(1), 11–20.
26. Cogato, A., Brščić, M., Guo, H., Marinello, F., & Pezzuolo, A. (2021). Challenges and Tendencies of Automatic Milking Systems (AMS): A 20-Years Systematic Review of Literature and Patents. *Animals*, *11*(2), 356. https://doi.org/10.3390/ani11020356
27. Costa, A., Agazzi, A., Perricone, V., Savoini, G., Lazzari, M., Nava, S., & Maria Tangorra, F. (2019). Influence of different loading levels, cutting and mixing times on Total Mixed Ration (TMR) homogeneity in a vertical mixing wagon during distribution: A case study. *Italian Journal of Animal Science*, *18*(1), 1093–1098.
28. Costa, J. H. C., Cantor, M. C., & Neave, H. W. (2021). Symposium review: Precision technologies for dairy calves and management applications. *Journal of Dairy Science*, *104*(1), 1203–1219. https://doi.org/10.3168/jds.2019-17885
29. Delevatti, L. M., Cardoso, A. S., Barbero, R. P., Leite, R. G., Romanzini, E. P., Ruggieri, A. C., & Reis, R. A. (2019). Effect of nitrogen application rate on yield, forage quality, and animal performance in a tropical pasture. *Scientific reports*, *9*(1), 7596.
30. Deligeorgakis, C., Magro, C., Skendi, A., Gebrehiwot, H. H., Valdramidis, V., & Papageorgiou, M. (2023). Fungal and toxin contaminants in cereal grains and flours: Systematic review and meta-analysis. *Foods*, *12*(23), 4328.
31. DeVries, T., & Gill, R. (2012). Adding liquid feed to a TMR reduces feed sorting behavior and improves productivity of lactating dairy cows. *Journal of Dairy Science*, *95*(5), 2648–2655.
32. Diaz-Olivares, J. A., Adriaens, I., Stevens, E., Saeys, W., & Aernouts, B. (2020). *Online milk composition analysis with an on-farm near-infrared sensor*. Bioengineering. https://doi.org/10.1101/2020.06.02.129742
33. Doidge, C., Ånestad, L., Burrell, A., Frössling, J., Palczynski, L., Pardon, B., Veldhuis, A., Bokma, J., Carmo, L., & Hopp, P. (2024). A Living Lab approach to understanding dairy farmers' technology and data needs to improve herd health: Focus groups from 6 European countries. *Journal of Dairy Science*, *107*(8), 5754–5778.

34. Eastwood, C., Ayre, M., Nettle, R., & Rue, B. D. (2019). Making sense in the cloud: Farm advisory services in a smart farming future. *NJAS-Wageningen Journal of Life Sciences*, *90*, 100298.
35. Edmunds, B., Spiekers, H., Südekum, K., Nussbaum, H., Schwarz, F., & Bennett, R. (2014). Effect of extent and rate of wilting on nitrogen components of grass silage. *Grass and Forage Science*, *69*(1), 140–152.
36. Empel, M. van, Makkar, H. P., Dijkstra, J., & Lund, P. (2016). Nutritional, technological and managerial parameters for PF to enhance feed nutrient utilization and productivity in different dairy cattle production systems. *CABI Reviews*, *2016*, 1–27.
37. Espinoza-Sandoval, O. R., Angeles-Hernandez, J. C., Gonzalez-Ronquillo, M., Ghavipanje, N., Zhang, N., Bayat, A. R., Hervás, G., Kholif, A. E., Mele, M., & Loor, J. J. (2024). Dairy farming in the era of artificial intelligence: Trend or a real game changer? *Journal of Dairy Research*, *91*(2), 139–145.
38. European Commission. (2020). Farm to fork strategy: For a fair, healthy and environmentally-friendly food system. *Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions*, *381*, 1–9.
39. Evangelista, C., Basiricò, L., & Bernabucci, U. (2021). An overview on the use of near infrared spectroscopy (NIRS) on farms for the management of dairy cows. *Agriculture*, *11*(4), 296.
40. FAO, F. (2018). The future of food and agriculture: Alternative pathways to 2050. *Food and Agriculture Organization of the United Nations Rome*, *228*.
41. Ferraretto, L., Shaver, R., & Luck, B. (2018). Silage review: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting. *Journal of dairy science*, *101*(5), 3937–3951.
42. Gaillard, C., & Abarnou, J. (2024). Responses of dairy cows to weekly individualized feeding strategies regarding their metabolic status. *Journal of Dairy Science*, *107*(12), 10776–10786. https://doi.org/10.3168/jds.2024-25209
43. Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). *Tackling climate change through livestock: A global assessment of emissions and mitigation opportunities.*
44. Golder, H., & Lean, I. (2024). Invited review: Ruminal acidosis and its definition—A critical review. *Journal of Dairy Science*, *107*(12), 10066–10098.

45. Groher, T., Heitkämper, K., & Umstätter, C. (2020). Digital technology adoption in livestock production with a special focus on ruminant farming. *Animal*, *14*(11), 2404–2413.
46. Halachmi, I., Guarino, M., Bewley, J., & Pastell, M. (2019). Smart animal agriculture: Application of real-time sensors to improve animal well-being and production. *Annual review of animal biosciences*, *7*(1), 403–425.
47. Han, P., Wang, Y., & Sun, H. (2025). Impact of Temperature Stresses on Wheat Quality: A Focus on Starch and Protein Composition. *Foods*, *14*(13), 2178.
48. Hart, E. H., Christofides, S. R., Davies, T. E., Rees Stevens, P., Creevey, C. J., Müller, C. T., Rogers, H. J., & Kingston-Smith, A. H. (2022). Forage grass growth under future climate change scenarios affects fermentation and ruminant efficiency. *Scientific Reports*, *12*(1), 4454.
49. Havekes, C., Duffield, T., Carpenter, A., & DeVries, T. (2020). Effects of molasses-based liquid feed supplementation to a high-straw dry cow diet on feed intake, health, and performance of dairy cows across the transition period. *Journal of dairy science*, *103*(6), 5070–5089.
50. Havrlentová, M., Kraic, J., Gregusová, V., & Kovácsová, B. (2021). Drought stress in cereals–a review. *Agriculture*, *67*(2), 47–60.
51. Hossain, M. E., Kabir, M. A., Zheng, L., Swain, D. L., McGrath, S., & Medway, J. (2024). Near-infrared spectroscopy for analysing livestock diet quality: A systematic review. *Heliyon*, *10*(22).
52. Huhtanen, P., Nousiainen, J., & Rinne, M. (2006). *Recent developments in forage evaluation with special reference to practical applications*.
53. Jiang, W., Wang, J., Li, S., Liu, S., Zhuang, Y., Li, S., Wang, W., Wang, Y., Yang, H., Shao, W., & Cao, Z. (2025). Effects of preweaning calf daily gain and feed intake on first-lactation performance: A meta-analysis. *Journal of Dairy Science*, *108*(5), 4863–4877. https://doi.org/10.3168/jds.2024-25742
54. Jungers, J., Cherney, J., Martinson, K., Jaqueth, A., & Sheaffer, C. (2020). Forage nutritive value of modern alfalfa cultivars. *Crop, Forage & Turfgrass Management*, *6*(1), e20076.
55. Kairouz, P., McMahan, H. B., Avent, B., Bellet, A., Bennis, M., Bhagoji, A. N., Bonawitz, K., Charles, Z., Cormode, G., & Cummings, R. (2021). Advances and open problems in federated learning. *Foundations and trends® in machine learning*, *14*(1–2), 1–210.
56. Kamphuis, C., Mollenhorst, H., Heesterbeek, J. A. P., & Hogeveen, H. (2010). Detection of clinical mastitis with sensor data from automatic milking systems is improved by using decision-tree induction. *Journal of Dairy Science*, *93*(8), 3616–3627. https://doi.org/10.3168/jds.2010-3228

57. Karayilanli, E., & Ayhan, V. (2016). Investigation of feed value of alfalfa (Medicago sativa L.) harvested at different maturity stages. *Legume Research-An International Journal*, *39*(2), 237–247.
58. Kaur, U., Malacco, V. M., Bai, H., Price, T. P., Datta, A., Xin, L., Sen, S., Nawrocki, R. A., Chiu, G., & Sundaram, S. (2023). Invited review: Integration of technologies and systems for precision animal agriculture—A case study on precision dairy farming. *Journal of animal science*, *101*, skad206.
59. Khanashyam, A. C., Jagtap, S., Agrawal, T. K., Thorakkattu, P., Malav, O. P., Trollman, H., Hassoun, A., Ramesh, B., Manoj, V., & Rathnakumar, K. (2025). Applications of artificial intelligence in the dairy Industry: From farm to product development. *Computers and Electronics in Agriculture*, *238*, 110879.
60. Kim, J.-E., & Lee, H.-G. (2021). Amino acids supplementation for the milk and milk protein production of dairy cows. *Animals*, *11*(7), 2118.
61. King, M., LeBlanc, S., Pajor, E., Wright, T., & DeVries, T. (2018). Behavior and productivity of cows milked in automated systems before diagnosis of health disorders in early lactation. *Journal of dairy science*, *101*(5), 4343–4356.
62. Knauer, W. A., Godden, S. M., Dietrich, A., & James, R. E. (2017). The association between daily average feeding behaviors and morbidity in automatically fed group-housed preweaned dairy calves. *Journal of Dairy Science*, *100*(7), 5642–5652. https://doi.org/10.3168/jds.2016-12372
63. Kononoff, P., Heinrichs, A., & Buckmaster, D. (2003). Modification of the Penn State forage and TMR particle separator and the effects of moisture content on its measurements. *Journal of dairy science*, *86*(5), 1858–1863.
64. Kulkarni, K. P., Tayade, R., Asekova, S., Song, J. T., Shannon, J. G., & Lee, J.-D. (2018). Harnessing the potential of forage legumes, alfalfa, soybean, and cowpea for sustainable agriculture and global food security. *Frontiers in plant science*, *9*, 1314.
65. Lage, C. F. D. A., Marques, T. C., Bruno, D. R., Endres, M. I., Ferreira, F., Pires, A. P. A., Leão, K., & De Lima, F. S. (2024). Farmers' Perceptions on Implementing Automatic Milking Systems in Large USA Dairies: Decision-Making Process, Management Practices, Labor, and Herd Performance. *Animals*, *14*(2), 218. https://doi.org/10.3390/ani14020218
66. Lapierre, H., Larsen, M., Sauvant, D., Van Amburgh, M., & Van Duinkerken, G. (2018). Converting nutritional knowledge into feeding practices: A case study comparing different protein feeding systems for dairy cows. *Animal*, *12*(s2), s457–s466.

67. Lee, S., Van, K., Sung, M., Nelson, R., LaMantia, J., McHale, L. K., & Mian, M. R. (2019). Genome-wide association study of seed protein, oil and amino acid contents in soybean from maturity groups I to IV. *Theoretical and Applied Genetics*, *132*(6), 1639–1659.
68. Legge, S. W. J., Thomson, P. C., Clark, C. E. F., & García, S. C. (2024). Milk consumption and behavior of calves in automated calf feeders as early indicators of weaning liveweight. *JDS Communications*, *5*(5), 474–478. https://doi.org/10.3168/jdsc.2023-0488
69. Li, C., Beauchemin, K. A., & Yang, W. (2020). Feeding diets varying in forage proportion and particle length to lactating dairy cows: I. Effects on ruminal pH and fermentation, microbial protein synthesis, digestibility, and milk production. *Journal of dairy science*, *103*(5), 4340–4354.
70. Liu, Y., Wu, Q., Ge, G., Han, G., & Jia, Y. (2018). Influence of drought stress on afalfa yields and nutritional composition. *BMC plant biology*, *18*(1), 13.
71. Markus, D., & Battle, W. (1965). Soil and Plant Responses to Long-Term Fertilization of Alfalfa (Medicago sativa L.) 1. *Agronomy Journal*, *57*(6), 613–616.
72. Marques, T. C., Lage, C. F. A., Bruno, D. R., Fausak, E. D., Endres, M. I., Ferreira, F. C., & Lima, F. S. (2023). Geographical trends for automatic milking systems research in non-pasture-based dairy farms: A scoping review. *Journal of Dairy Science*, *106*(11), 7725–7736. https://doi.org/10.3168/jds.2023-23313
73. Medrano-Galarza, C., LeBlanc, S. J., Jones-Bitton, A., DeVries, T. J., Rushen, J., de Passillé, A. M., Endres, M. I., & Haley, D. B. (2018). Associations between management practices and within-pen prevalence of calf diarrhea and respiratory disease on dairy farms using automated milk feeders. *Journal of dairy science*, *101*(3), 2293–2308.
74. Melfsen, A., Hartung, E., & Haeussermann, A. (2012). Accuracy of in-line milk composition analysis with diffuse reflectance near-infrared spectroscopy. *Journal of Dairy Science*, *95*(11), 6465–6476. https://doi.org/10.3168/jds.2012-5388
75. Mertens, D. (1997). Creating a system for meeting the fiber requirements of dairy cows. *Journal of dairy science*, *80*(7), 1463–1481.
76. Mohammed, R., Kennelly, J., Kramer, J., Beauchemin, K., Stanton, C., & Murphy, J. (2010). Effect of grain type and processing method on rumen fermentation and milk rumenic acid production. *Animal*, *4*(8), 1425–1444.
77. Moscoso, C. J., Morgan, S. A., & Rivero, M. J. (2019). The effect of drying methods on water-soluble carbohydrates and crude protein concentrations and their ratio in two perennial ryegrass cultivars. *Agronomy*, *9*(7), 383.

78. O'Keefe, C., Penner, G., McKinnon, J., Larson, K., Damiran, D., & Lardner, H. B. (2022). Effects of stage of maturity at harvest of cereal crops on biomass and quality, estimated forage dry matter intake, beef cow performance, and system economics. *Applied Animal Science*, *38*(6), 607–619.
79. Ozella, L., Brotto Rebuli, K., Forte, C., & Giacobini, M. (2023). A Literature Review of Modeling Approaches Applied to Data Collected in Automatic Milking Systems. *Animals*, *13*(12), 1916. https://doi.org/10.3390/ani13121916
80. Palma, O., Plà-Aragonés, L. M., Mac Cawley, A., & Albornoz, V. M. (2025). AI and data analytics in the dairy farms: A scoping review. *Animals*, *15*(9), 1291.
81. Palma-Molina, P., Hennessy, T., O'Connor, A. H., Onakuse, S., O'Leary, N., Moran, B., & Shalloo, L. (2023). Factors associated with intensity of technology adoption and with the adoption of 4 clusters of precision livestock farming technologies in Irish pasture-based dairy systems. *Journal of Dairy Science*, *106*(4), 2498–2509. https://doi.org/10.3168/jds.2021-21503
82. Palmonari, A., Federiconi, A., & Formigoni, A. (2024). Animal board invited review: The effect of diet on rumen microbial composition in dairy cows. *animal*, *18*(10), 101319.
83. Papadopoulos, G., Papantonatou, M.-Z., Uyar, H., Kriezi, O., Mavrommatis, A., Psiroukis, V., Kasimati, A., Tsiplakou, E., & Fountas, S. (2025). Economic and environmental benefits of digital agricultural technological solutions in livestock farming: A review. *Smart Agricultural Technology*, *10*, 100783. https://doi.org/10.1016/j.atech.2025.100783
84. Papakonstantinou, G. I., Voulgarakis, N., Terzidou, G., Fotos, L., Giamouri, E., & Papatsiros, V. G. (2024). Precision livestock farming technology: Applications and challenges of animal welfare and climate change. *Agriculture*, *14*(4), 620.
85. Patil, V., Gupta, R., Rajendran, D., Kuntal, R. S., & Chanda, M. (2022). Stochastic programming model in least cost feed formulation for lactating cattle. *Indonesian Journal of Agricultural Research*, *5*(3), 231–248.
86. Phuong, H., Friggens, N., De Boer, I., & Schmidely, P. (2013). Factors affecting energy and nitrogen efficiency of dairy cows: A meta-analysis. *Journal of dairy science*, *96*(11), 7245–7259.
87. Rathod, A., & Kumawat, M. (2024). Storage of feed ingredients and finished feed. *Indian Farmer*, *11*(02), 77–82.
88. Reese, D., Foltz, K., & Moritz, J. (2017). Effect of mixing and sampling method on pelleted feed nutrient analysis and diet formulation validation. *Journal of Applied Poultry Research*, *26*(2), 219–225.

89. Romano, E., Brambilla, M., Cutini, M., Giovinazzo, S., Lazzari, A., Calcante, A., Tangorra, F. M., Rossi, P., Motta, A., & Bisaglia, C. (2023). Increased cattle feeding precision from automatic feeding systems: Considerations on technology spread and farm level perceived advantages in Italy. *Animals*, *13*(21), 3382.
90. Rose, D. C., Wheeler, R., Winter, M., Lobley, M., & Chivers, C.-A. (2021). Agriculture 4.0: Making it work for people, production, and the planet. *Land use policy*, *100*, 104933.
91. Rotz, C. A., Briggs, K., Hristov, A. N., Leytem, A., Young, E., Hagevoort, R., & Mullinax, D. (2025). Strategies for mitigating greenhouse gas emissions from US dairy farms toward a net zero goal. *Journal of dairy science*.
92. Ruiz, N., Parsons, C., Stein, H., Coon, C., Eys, J., & Miles, R. (2020). A review: 100 years of soybean meal. *ADM: Chicago, IL, USA*.
93. Salvati, G. G. de S., Santos, W., Silveira, J., Gritti, V. C., Arthur, B. A. V., Salvo, P. A. R., Fachin, L., Ribeiro, A., Júnior, N. M., & Ferraretto, L. (2021). Effect of kernel processing and particle size of whole-plant corn silage with vitreous endosperm on dairy cow performance. *Journal of Dairy Science*, *104*(2), 1794–1810.
94. Sarmikasoglou, E., Hammer, D., Dietz, T., & VandeHaar, M. (2025). Effect of brachytic2 mutation in corn silage on performance and total-tract digestibility by lactating Holstein dairy cows. *JDS Communications*.
95. Schingoethe, D. J. (2017). A 100-Year Review: TMR feeding of dairy cows. *Journal of dairy science*, *100*(12), 10143–10150.
96. Schlageter-Tello, A., Fahey, G. C., Freel, T., Koutsos, L., Miller, P. S., & Weiss, W. P. (2020). ASAS-NANP SYMPOSIUM: RUMINANT/NONRUMINANT FEED COMPOSITION: Challenges and opportunities associated with creating large feed composition tables. *Journal of animal science*, *98*(8), skaa240.
97. Schwanke, A. J., Dancy, K. M., Neave, H. W., Penner, G. B., Bergeron, R., & DeVries, T. J. (2022). Effects of concentrate allowance and individual dairy cow personality traits on behavior and production of dairy cows milked in a free-traffic AMS. *Journal of Dairy Science*, *105*(7), 6290–6306. https://doi.org/10.3168/jds.2021-21657
98. Scicutella, F., Foggi, G., Daghio, M., Mannelli, F., Viti, C., Mele, M., & Buccioni, A. (2025). A review of in vitro approaches as tools for studying rumen fermentation and ecology: Effectiveness compared to in vivo outcomes. *Italian Journal of Animal Science*, *24*(1), 589–608.

99. Serva, L., Magrin, L., Marchesini, G., & Andrighetto, I. (2021). Assessment of the Effectiveness of a Portable NIRS Instrument in Controlling the Mixer Wagon Tuning and Ration Management. *Animals*, *11*(12), 3566. https://doi.org/10.3390/ani11123566

100. Sinnott, A. M., Kennedy, E., & Bokkers, Eddie. A. M. (2021). The effects of manual and automated milk feeding methods on group-housed calf health, behaviour, growth and labour. *Livestock Science*, *244*, 104343. https://doi.org/10.1016/j.livsci.2020.104343

101. Soares, J. C., Santos, C. S., Carvalho, S. M., Pintado, M. M., & Vasconcelos, M. W. (2019). Preserving the nutritional quality of crop plants under a changing climate: Importance and strategies. *Plant and Soil*, *443*(1), 1–26.

102. Solati, Z., Jørgensen, U., Eriksen, J., & Søegaard, K. (2018). Estimation of extractable protein in botanical fractions of legume and grass species. *Grass and Forage Science*, *73*(2), 572–581.

103. Souza, V., Liebe, D., Price, T., Ellett, M., Davis, T., Gleason, C., Daniels, K., & White, R. (2022). Algorithm development for individualized PF of supplemental top dresses to influence feed efficiency of dairy cattle. *Journal of Dairy Science*, *105*(5), 4048–4063.

104. Stygar, A. H., Gómez, Y., Berteselli, G. V., Dalla Costa, E., Canali, E., Niemi, J. K., Llonch, P., & Pastell, M. (2021). A Systematic Review on Commercially Available and Validated Sensor Technologies for Welfare Assessment of Dairy Cattle. *Frontiers in Veterinary Science*, *8*, 634338. https://doi.org/10.3389/fvets.2021.634338

105. Tadele, E., Worku, D., Yigzaw, D., Muluneh, T., & Melese, A. (2025). Precision of dairy farming: Navigating challenges and seizing opportunities for sustainable dairy production in Africa. *Frontiers in Animal Science*, *6*, 1541838. https://doi.org/10.3389/fanim.2025.1541838

106. Theurer, C., Lozano, O., Alio, A., Delgado-Elorduy, A., Sadik, M., Huber, J., & Zinn, R. (1999). Steam-processed corn and sorghum grain flaked at different densities alter ruminal, small intestinal, and total tract digestibility of starch by steers. *Journal of animal science*, *77*(10), 2824–2831.

107. Trapanese, L., Bifulco, G., Calanni Macchio, A., Aragona, F., Purrone, S., Campanile, G., & Salzano, A. (2025). Precision livestock farming applied to the dairy sector: 50 years of history with a text mining and topic analysis approach. *Smart Agricultural Technology*, *10*, 100827. https://doi.org/10.1016/j.atech.2025.100827

108. Trapanese, L., Hostens, M., Salzano, A., & Pasquino, N. (2024). Short review of current limits and challenges of application of machine learning algorithms in the dairy sector. *Acta IMEKO*, *13*(1), 1–7. https://doi.org/10.21014/actaimeko.v13i1.1725

109. Van Amburgh, M., Collao-Saenz, E., Higgs, R., Ross, D., Recktenwald, E., Raffrenato, E., Chase, L., Overton, T., Mills, J., & Foskolos, A. (2015). The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. *Journal of Dairy Science*, *98*(9), 6361–6380.

110. Van Soest, B. J., Matson, R. D., Santschi, D. E., Duffield, T. F., Steele, M. A., Orsel, K., Pajor, E. A., Penner, G. B., Mutsvangwa, T., & DeVries, T. J. (2024). Farm-level nutritional factors associated with milk production and milking behavior on Canadian farms with AMSs. *Journal of Dairy Science*, *107*(7), 4409–4425. https://doi.org/10.3168/jds.2023-24355

111. Wang, C., Liu, H., Wang, Y., Yang, Z., Liu, J., Wu, Y., Yan, T., & Ye, H. (2010). Effects of dietary supplementation of methionine and lysine on milk production and nitrogen utilization in dairy cows. *Journal of Dairy Science*, *93*(8), 3661–3670.

112. Weiss, W. P., & St-Pierre, N. R. (2009). *Impact and management of variability in feed and diet composition*. 83–96.

113. Weiss, W., & St-Pierre, N. (2024). Perspective and Commentary: Variation in nutrient composition of feeds and diets and how it can affect formulation of dairy cow diets. *Applied Animal Science*, *40*(5), 608–618.

114. Wolfert, S., Ge, L., Verdouw, C., & Bogaardt, M.-J. (2017). Big data in smart farming–a review. *Agricultural systems*, *153*, 69–80.

115. Xiao, D., & Meng, T. (2024). Nutritional Value Evaluation and Processing Technology of Feed and Nutrition Regulation Measures for Ruminants. *Animals*, *14*(21), 3153.

116. Xie, Y., Wu, Z., Wang, D., & Liu, J. (2019). Nitrogen partitioning and microbial protein synthesis in lactating dairy cows with different phenotypic residual feed intake. *Journal of animal science and biotechnology*, *10*(1), 54.

117. Xing, R., Li, B., Dora, S., Whittaker, M., & Mathie, J. (2024). *Stacking Ensemble Machine Learning Modelling for Milk Yield Prediction Based on Biological Characteristics and Feeding Strategies*. 701–706. https://doi.org/10.15439/2024F9318

118. Yang, C., Ferris, C. P., & Yan, T. (2022). Effects of dietary crude protein concentration on animal performance and nitrogen utilisation efficiency at different stages of lactation in Holstein-Friesian dairy cows. *animal*, *16*(7), 100562.

119. Zang, Y., Ji, P., Morrison, S. Y., Koba, Y., Grant, R. J., & Dann, H. M. (2021). Reducing metabolizable protein supply: Effects on milk production, blood metabolites, and health in early-lactation dairy cows. *Journal of Dairy Science*, *104*(12), 12443–12458.

STUDY 1: NUTRITIONAL CHARACTERIZATION AND VARIABILITY OF DIFFERENT LIVESTOCK FEED USED IN THE ITALIAN FEED INDUSTRY: A COMPARISON BETWEEN LITERATURE AND LAB ANALYSIS

The present study is a shorter version of the paper submitted in Italian Journal of Animal Science.

Materials and Methods

Study design

This study was designed to characterise the chemical composition and in vitro digestibility/degradability of commonly used feedstuffs in the Italian dairy system, and to compare laboratory-derived values with those reported in widely used reference feed databases, namely the Nutrient Requirements of Dairy Cattle (National Academies of Sciences, Engineering, and Medicine, 2021), the Cornell Net Carbohydrate and Protein System (Van Amburgh et al., 2015), and the INRA feeding system (Nozière et al., 2018).

Sample collection

Twelve of the most commonly used feed ingredients in the Italian dairy feed industry were selected to assess their chemical composition and digestibility. Feed ingredients were provided by Progeo S.C.A. (Via Werter Asseverati, 1, 42122, Masone, Reggio Emilia, Italy).

Raw materials were grouped into three nutritional categories according to their pre-dominant nutritional characteristics: protein-rich feeds, starch-rich feeds, and fibrous feeds. Each category comprised four distinct feed ingredients (n = 4 per category). For each individual feed ingredient, five independent samples were collected from different suppliers within Progeo's network, resulting in a total of 60 samples (3 categories × 4 feed ingredients × 5 samples; Table 1). Geographical origin of raw materials was recorded for each ingredient based on Progeo S.C.A. internal procurement and supply chain database. For each sampled batch, the country/region of origin reported in the supplier documentation was extracted and summarised as the main sourcing areas for that ingredient (Table 1).

Table 1. *Classification of the selected raw materials, number of samples collected for each ingredient, and geographical origin of the raw materials..*

Category	Raw material	N° of samples	Origin of raw materials
Protein-rich	Sunflower meal	5	Eastern Europe (Ukraine, Hungary)
	Soybean meal	5	South America (Brazil, Argentina), North America (USA), Italy
	Soybean seeds raw	5	North America (USA, Canada), South America (Brazil)
	Soybean seed roasted	5	North America (USA, Canada), South America (Brazil)
Starch-rich	Wheat middlings	5	Italy
	Corn seeds non-domestic	5	Eastern Europe (Ukraine, Romania, Hungary, Poland)
	Corn seeds Italian	5	Italy
	Barley seeds	5	Italy, Central-Eastern Europe (Austria, Germany, Croatia)
Fibrous	Wheat bran (Triticum durum)	5	Italy
	Wheat bran (Triticum aestivum)	5	Italy
	Soybean hulls	5	South America (Argentina), Italy
	Beetroot dry pulp	5	Italy, Central-Eastern Europe (Germany, Austria, Serbia, Czech Republic)

All samples were subjected to full chemical characterization and in vitro digestibility/degradability analyses, including fibre, starch, and protein fractions, according to the procedures described below.

All raw material sample analyses were conducted at the laboratories of Animal Production and Food Safety (SPASA; DIMEVET, University of Bologna), which operate under UNI EN ISO 9001:2015 (ISO, 2015). Samples were stored under controlled conditions (T < 4 °C) from collection to analysis. For chemical analyses, samples were dried in a forced-air oven at 65 °C for 48 h for dry matter (DM) determination and ground to a 1 mm particle size using a Cyclotec™ 1093 Sample Mill (FOSS Tecator, Högänas, Sweden).

Analytical determinations were carried out primarily according to official AOAC procedures (AOAC, 1990). Crude protein (CP) was quantified following AOAC method 984.13A using a Kjeldahl nitrogen analyser (Gerhardt Vapodest 50, Gerhardt GmbH; Königswinter, Germany) (AOAC, 1990). Protein fractions, namely soluble protein, neutral detergent insoluble protein (NDIP), and acid detergent insoluble protein (ADIP), were assessed according to the method described by Licitra et al. (1996).

Starch concentration was quantified following AOAC method 920.40, while ether extract (EE) was determined according to AOAC method 920.39 using a Soxhlet apparatus (Velp SER 148/6, Velp Scientifica, Usmate, Italy) (AOAC, 1990). Fibre fractions, including ash-corrected neutral detergent fibre with α-amylase and sodium sulphite (aNDFom), acid detergent fibre (ADF), and acid detergent lignin (ADL), were analysed according to the procedures described by Mertens et al. (2002) and

AOAC method 973.18 (AOAC, 1990). Total ash content was determined following AOAC method 942.05 (AOAC, 1990).

The only exception was the determination of the amino acid profile, which was carried out at the Progeo S.C.A. laboratory (Via Werter Asseverati, 1, 42122, Masone, Reggio Emilia, Italy). Amino acid profiles were determined using high-performance liquid chromatography (HPLC), following procedures compliant with the UNI CEI EN ISO/IEC 17025:2000 standard for laboratory accreditation. The AccQ-Tag derivatization technique (Waters, Milford, MA, USA) was used for amino acid analysis.

In Vitro Digestibility

In vitro digestibility analyses were performed to measure the rumen degradability of fibre, starch, and protein fractions.

Fibre digestibility (aNDFom) was assessed at 12, 72, and 120 h using a modified Tilley and Terry technique (Tilley and Terry, 1963; Robertson, 1981), as adapted by Palmonari et al. (2016). Each sample was analysed in triplicate. Briefly, fibre fermentations were conducted in 150-mL Erlenmeyer flasks containing 0.5 g of ground feed sample and 40 mL of buffer solution, following Goering and Van Soest (1970), with 10 mL of rumen fluid added to each flask. Incubation was carried out at 39 °C under constant CO_2 pressure. At the end of each incubation time point, aNDFom content was analysed according to Mertens et al. (2002) and AOAC (1990).

Rumen fluid from three lactating Friesian dairy cows fed a hay-based diet was used for in vitro incubations, in accordance with established protocols (Muizelaar et al., 2020). The inoculum was handled under anaerobic conditions and maintained at 39 °C until incubation.

Fibre degradability was calculated for each incubation time using the following equation (Palmonari et al., 2016):

$$\text{dNDFD (\% aNDFom)} = [1 - (\text{aNDFomr} - (\text{aNDFomr} - \text{aNDFomb})) / \text{aNDFomi}] \times 100,$$

where aNDFomi is the initial aNDFom, aNDFomr is the aNDFom residue after incubation, and aNDFomb is the blank correction; all values are expressed on a dry matter basis.

Starch degradability was assessed by incubating selected starch-rich samples for 8 h with rumen fluid and buffer, using a method adapted from Ferraretto and Shaver (2015) and Sveinbjörnsson et al. (2007). At the end of incubation, CO_2 supply was interrupted and fermentation was stopped by heating. The amount of starch degraded was quantified through a multistep enzymatic procedure.

Briefly, 25 mL of distilled water and 10 mL of NaOH were added to each flask, followed by incubation at 90 °C for 30 min. After cooling below 50 °C, 10 mL of 2 N HCl, 10 mL of acetate buffer, and 1 mL of amyloglucosidase were added, and samples were incubated at 40 °C for 90 min to hydrolyse starch into glucose. Enzymatic activity was stopped by adding 5 mL of 25% trichloroacetic acid, and samples were cooled to room temperature

Each sample was quantitatively transferred into a 100-mL volumetric flask, rinsed with phosphate buffer, and brought to volume. After 2 h of sedimentation, the supernatant was collected and glucose concentration was determined using a YSI 2700 Select Biochemistry Analyzer (YSI Inc., Yellow Springs, OH, USA). Starch degradation was calculated from glucose release, corrected for sample weight and blank values. A blank control containing only amyloglucosidase was included in each run to verify analytical calibration.

Protein degradability was evaluated using a three-step in vitro technique according to Ross et al. (2013). For each selected sample (n = 5), 0.50 g of dried and ground material (1 mm) was weighed into 125-mL flasks. Two replicates per sample were incubated for rumen simulation, followed by two replicates for the subsequent intestinal digestion. The ruminal phase consisted of a 16-h incubation at 39 °C in an anaerobic fermentation system, using 40 mL of buffered medium and 10 mL of freshly collected rumen fluid per flask. After incubation, samples were filtered, rinsed, and dried to determine rumen-undegraded protein (RUP).

For intestinal digestion, post-rumen residues were acidified with 3 M HCl and incubated with a pepsin solution (16.6 mg/mL in 0.013 M HCl) for 1 h at 39 °C under agitation. The pH was then adjusted to 7.6 using NaOH, and a mixture of digestive enzymes (trypsin, chymotrypsin, amylase, and lipase in phosphate buffer) was added. Flasks were incubated for 24 h at 39 °C. After digestion, residues were filtered, dried, and analysed for nitrogen content using the Kjeldahl method.

Protein digestibility was calculated according to Ross et al. (2013) as follows:

- Ruminal degradability (%CP) = (CP_initial − CP_residue_rumen) / CP_initial × 100
- Total tract digestibility (%CP) = (CP_initial − CP_residue_intestinal) / CP_initial × 100
- Intestinal digestibility (% of CP entering the intestine) = (CP_residue_rumen − CP_residue_intestinal) / CP_residue_rumen × 100
- Rumen-undegraded protein (RUP, %CP) = 100 − ruminal degradability

where CP_initial is the initial crude protein content of the sample (g), CP_residue_rumen is the crude protein remaining after 16 h of ruminal incubation (g), CP_residue_intestinal is the crude protein remaining after post-ruminal intestinal digestion (g), and intestinal digestibility represents the digestible fraction of RUP following the intestinal phase.

Statistical analysis

Data were analysed using JMP Student Edition v18 (SAS Institute Inc., 2023). The experimental unit was the individual feed ingredient sample (five independent samples per ingredient, sourced from different suppliers). Analyses were descriptive, as the study was not designed for inferential comparisons. For each ingredient, results are reported as mean and standard deviation (SD), and variability is also expressed as coefficient of variation (CV, %) computed as (SD/mean) × 100. Variables are expressed on an as-fed (AF), dry matter (DM), crude protein (CP), or aNDFom basis, as appropriate.

Health and safety

All laboratory activities were performed in compliance with mandatory institutional health and safety procedures, including the use of appropriate personal protective equipment and chemical risk management for acids, bases, and organic solvents used in fibre, protein, and starch analyses. Biological material (rumen fluid) was handled under biosafety precautions, and waste was disposed of according to local regulations.

Ethical statement

The present study was based exclusively on laboratory in vitro analyses and did not involve experimental procedures on live animals. Rumen fluid was sourced following established and standardised protocols reported in the literature (Muizelaar et al., 2020). No animals were subjected to additional handling or experimental interventions for this study, and consequently no ethical approval was required.

Results

Chemical composition of protein-rich feeds is reported in Table 2. Sunflower meal exhibited a notable variability in ADF content (32.27 ± 6.01% of DM), while raw soybean seeds showed significant variation in protein content (37.23 ± 2.75% of DM) and soluble protein (16.95 ± 3.40% of DM). Regarding the amino acid profile, the values were generally consistent across samples of the same feed, indicating a relatively stable composition in this respect.

Table 2. *Chemical composition of proteic-rich feeds*

		Sunflower meal[a]		**Soybean meal**[a]		**Soybean seeds, raw**		**Soybean seeds, rosted**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dry matter	% AF	91.16	0.65	89.01	0.37	92.56	0.46	93.59	0.43
Moisture	% DM	8.84	0.65	10.99	0.37	7.44	0.46	6.41	0.43
Crude Protein	% DM	37.05	1.41	52.23	0.39	37.23	2.75	37.88	0.55
Soluble Protein	% DM	11.70	0.88	9.72	0.53	16.95	3.40	5.32	0.21
NDIP	% DM	2.29	0.46	3.61	0.57	1.95	0.61	5.89	2.35
ADIP	% DM	1.33	0.14	1.16	0.39	0.70	0.18	0.80	0.12
Starch	% DM	0.84	0.73						
Ether Extract	% DM	0.95	0.25	0.69	0.11	24.56	0.58	25.10	1.63
aNDFom	% DM	35.53	2.32	9.60	0.80	11.25	0.61	13.30	1.80
ADF	% DM	32.27	6.01	6.88	0.71	5.68	0.58	5.50	0.69
ADL	% DM	8.48	2.88	1.22	0.34	1.34	0.27	0.86	0.33
Ash	% DM	7.11	1.13	7.32	0.21	5.26	0.15	5.27	0.09
Aspartic Acid	% CP	3.37	0.12	5.84	0.43	4.27	0.03	4.46	0.03
Serine	% CP	1.99	0.20	2.90	0.01	2.11	0.07	2.03	0.12
Glutamic Acid	% CP	8.81	0.43	11.75	0.00	8.36	0.14	8.20	0.10
Glycine	% CP	2.58	0.13	2.34	0.08	1.72	0.04	1.60	0.04
Histidine	% CP	1.13	0.19	1.48	0.07	1.05	0.04	0.97	0.03
Arginine	% CP	4.50	0.26	4.92	0.33	3.40	0.11	3.22	0.05
Threonine	% CP	0.66	0.94	1.97	0.12	1.43	0.07	1.37	0.17
Alanine	% CP	1.65	0.07	2.29	0.06	1.65	0.04	1.61	0.00
Proline	% CP	1.62	0.10	2.70	0.03	1.95	0.08	1.82	0.12
Cystine	% CP	0.62	0.06	0.63	0.03	0.50	0.02	0.46	0.00
Tyrosine	% CP	0.94	0.13	1.84	0.05	1.38	0.06	1.30	0.02
L-Valine	% CP	1.32	0.04	1.88	0.05	1.30	0.05	1.27	0.01
Methionine	% CP	0.19	0.27	0.08	0.04	0.18	0.02	0.11	0.00
Lysine	% CP	1.32	0.09	3.09	0.08	2.08	0.22	2.16	0.06
Isoleucine	% CP	1.07	0.05	1.59	0.17	1.22	0.04	1.15	0.01
Leucine	% CP	2.32	0.02	3.73	0.22	2.81	0.06	2.69	0.01
Phenylalanine	% CP	1.69	0.14	2.51	0.11	1.91	0.06	1.71	0.03

Note. *([a]) Sunflower and Soybean meal were obtained from solvent extraction of oil of sunflower and soybean seeds.*
Legend*. **ADF:** Acid Detergent Fiber; **ADL:** Acid Detergent Lignin; **AF:** As Feed; **CP:** Crude Protein; **DM:** Dry Matter; **NDIP:** Neutral Detergent Insoluble Protein; **ADIP:** Acid Detergent Insoluble Protein; **aNDFom:** ash corrected Neutral Detergent Fiber with α-amylase and sodium sulfite; **SD:** Standard Deviation.*

Greater variability was observed, however, in the digestibility (see Table 3). Sunflower meal showed substantial variation at all timepoints of aNDFom digestion, with values at 12 h (23.93 ± 6.01% of aNDFom), 72 h (35.92 ± 4.15%), and 120 h (38.89 ± 4.26%) indicating a broad range in fibre degradability. Soybean meal also showed considerable deviation at the 72 h timepoint (89.78 ± 4.23% of aNDFom), whereas raw soybean seeds had a high variability at the 12 h digestion timepoint (84.79 ± 5.86% of aNDFom) and at 120 h (91.28 ± 4.31%). Roasted soybean seeds, on the other hand, exhibited higher variability at the 12 h timepoint as well (74.55 ± 3.97% of aNDFom), which may be associated with differences in the effects of thermal treatment.

All protein-rich feeds feeds were also analysed for protein digestibility, and a notable variability was found across most parameters. Sunflower meal, in particular showed wide differences in ruminal, intestinal, and total tract protein digestibility, as well as in the quantity of bypass protein and the ratio of intestinal digestibility on bypass protein. Raw soybean seeds similarly displayed considerable variability in all these parameters, while the roasted soybean seeds showed less variations, likely due to the stabilising effect of the heat treatment.

Table 3. *Digestibility rates of proteic-rich feed*

		Sunflower meal		**Soybean meal**		**Soybean seeds, raw**		**Soybean seeds, rosted**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
dNDFom 12h[a]	% aNDFom	23.93	6.01	84.39	2.56	84.79	5.86	74.55	3.97
dNDFom 72h	% aNDFom	35.92	4.15	89.78	4.23	88.84	2.75	88.05	3.03
dNDFom 120h	% aNDFom	38.89	4.26	94.28	2.31	91.28	4.31	89.92	2.46
uNDF 120h[b]	% DM	21.76	2.55	0.54	0.20	0.99	0.49	1.35	0.41
pdNDFom[c]	% DM	13.77	1.23	9.06	0.91	10.25	0.50	11.95	1.59
RumDigCP[d]	%CP	68.84	5.15	58.98	1.86	80.49	13.92	49.50	3.51
IntDigCP[e]	%CP	24.68	6.42	37.46	2.13	13.06	10.94	42.12	4.96
TotDigCP[f]	%CP	93.52	4.55	96.44	0.54	93.54	3.33	91.61	1.74
bypassCP[g]	%CP	31.16	5.15	41.02	1.86	19.51	13.92	50.50	3.51
IntDig/bypass[h]	% CP	79.00	13.85	91.28	1.52	62.20	12.68	83.19	4.41

Note. ***([a])*** *aNDFom digestibility (% aNDFom)**;** **([b])** Undigested NDF at 120h**;** **([c])** Potentially digestible aNDFom**;** **([d])** Ruminal degradability (%CP) = (CP_initial − CP_residue_rumen) / CP_initial × 100**;** **([e])** Intestinal digestibility (% of CP entering intestine) = (CP_residue_rumen − CP_residue_intestinal) / CP_residue_rumen × 100**;** **([f])** Total digestibility (%CP) = (CP_initial − CP_residue_intestinal) / CP_initial × 100**;** **([g])** By-pass protein (RUP, %CP) = 100 − Ruminal degradability**;** **([h])** Intestinal digestibility= digestible portion of RUP after intestinal phase (%).*
Legend. bypassCP: *By-pass protein (RUP, %CP);* ***dNDFom:*** *Fiber digestibility (aNDFom);* ***IntDig/bypass:*** *Intestinal digestibility;* ***IntDigCP:*** *Intestinal digestibility (% of CP entering intestine);* ***pdNDFom:*** *Potentially digestible aNDFom;* ***RumDigCP:*** *Ruminal degradability (%CP);* ***SD:*** *Standard Deviation;* ***TotDigCP:*** *Total digestibility (%CP);* ***uNDF:*** *Undigested NDF.*

The second group of feeds analysed comprised the starch-rich feeds, which included wheat flour, Italian corn, non-national corn, and barley (see Table 4). Across this group, the chemical composition is relatively consistent between samples, with the main exception being starch content, which presented greater deviation from the mean. Wheat flour, for example, showed a starch content of 30.14 ± 2.38% on DM, indicating a broader spread compared to the other compositional parameters. Both non-national and Italian corn also presented variability in starch content, with values of 54.99 ± 1.70% and 50.22 ± 4.85% respectively, the latter showing greater inconsistency among samples. Barley exhibited a starch content of 48.54 ± 6.18% on DM, further highlighting the variability in this parameter. Additionally, wheat flour and barley showed fluctuations in aNDFom content, with values of 30.14 ± 2.38% and 25.17 ± 3.36% on DM, respectively.

Table 4. *Chemical composition of starch-rich feeds*

		Wheat, middlings		**Corn seeds, non-domestic**		**Corn seeds, Italian**		**Barley seeds**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dry matter	% AF	88.59	0.41	88.17	0.24	89.09	0.32	90.89	0.37
Moisture	% DM	11.41	0.41	11.83	0.24	10.91	0.32	9.11	0.37
Crude Protein	% DM	20.24	1.95	7.93	0.29	8.74	0.10	12.05	0.67
Soluble Protein	% DM	8.34	0.64						
NDIP	% DM	2.14	0.35						
ADIP	% DM	0.57	0.08						
Starch	% DM	30.14	2.38	54.99	1.70	50.22	4.85	48.54	6.18
Ether Extract	% DM	5.65	0.19	3.68	0.27	3.48	0.10	1.85	0.12
aNDFom	% DM	31.37	3.88	13.45	1.17	12.91	0.59	25.17	3.39
ADF	% DM	11.26	1.47	5.94	1.10	4.82	0.94	8.67	0.61
ADL	% DM	3.33	0.28	2.09	0.09	1.03	0.32	2.05	0.30
Ash	% DM	4.81	0.33	1.26	0.06	1.22	0.12	2.76	0.15
Aspartic Acid	% CP	1.24	0.16						
Serine	% CP	0.92	0.17						
Glutamic Acid	% CP	4.75	1.34						
Glycine	% CP	0.88	0.12						
Histidine	% CP	0.50	0.08						
Arginine	% CP	1.44	0.28						
Threonine	% CP	0.61	0.10						
Alanine	% CP	0.84	0.11						
Proline	% CP	1.23	0.44						
Cystine	% CP	0.26	0.07						
Tyrosine	% CP	0.45	0.09						
L-Valine	% CP	0.60	0.11						
Methionine	% CP	0.11	0.00						
Lysine	% CP	0.69	0.08						
Isoleucine	% CP	0.35	0.07						
Leucine	% CP	1.02	0.20						
Phenylalanine	% CP	0.72	0.15						

Legend. ***ADF:*** *Acid Detergent Fiber;* ***ADL:*** *Acid Detergent Lignin;* ***AF:*** *As Feed;* ***CP:*** *Crude Protein;* ***DM:*** *Dry Matter;* ***NDIP:*** *Neutral Detergent Insoluble Protein;* ***ADIP:*** *Acid Detergent Insoluble Protein;* ***aNDFom:*** *ash corrected Neutral Detergent Fiber with α-amylase and sodium sulfite;* ***SD:*** *Standard Deviation.*

Digestibility (see Table 5) analyses revealed a higher degree of variability. In particular, wheat flour exhibited notable variation in fibre digestibility at the 12 h timepoint (45.85 ± 5.95% of aNDFom), whereas starch digestibility appeared to be consistently high, with an average value of 93.53%. Protein digestibility was more variable, with a total tract digestibility of 93.94 ± 3.36%. Among the corn samples, non-national corn showed greater variability in fibre digestibility at the 120 h timepoint (86.74 ± 4.23% of aNDFom), compared to Italian corn (87.99 ± 2.11%), which in turn exhibited lower and more variable starch digestibility (53.47 ± 4.49%) compared to non-national corn (65.48 ± 2.61%). Finally, barley showed variation across all fibre digestibility timepoints, with values at 12 h (69.31 ± 3.78%), 72 h (70.80 ± 3.23%), and 120 h (74.63 ± 3.86%). Starch digestibility in barley was also variable, with a value of 78.66 ± 3.27% on starch, reflecting some inconsistency across samples.

Table 5. *Digestibility rates of starch-rich feed*

		Wheat, middlings		**Corn, non-domestic**		**Corn, Italian**		**Barley**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
dNDFom 12h[a]	% aNDFom	45.85	5.95	81.26	2.94	78.86	5.15	69.31	3.78
dNDFom 72h	% aNDFom	71.95	1.32	85.40	3.98	84.65	4.03	70.80	3.23
dNDFom 120h	% aNDFom	77.68	2.47	86.74	4.23	87.99	2.11	74.63	3.86
uNDF 120h[b]	% DM	7.02	1.32	1.75	0.42	1.54	0.22	6.36	1.11
pdNDFom[c]	% DM	24.35	2.84	11.70	1.49	11.37	0.75	18.81	3.03
Starch Dig[d]	% Starch	93.53	1.50	65.48	2.61	53.47	4.49	78.66	3.27
RumDigCP[e]	%CP	61.60	8.59						
IntDigCP[f]	%CP	32.34	6.61						
TotDigCP[g]	%CP	93.94	3.36						
bypassCP[h]	%CP	38.40	8.59						
IntDig/bypass[i]	%CP	84.88	6.80						

Note. ***(***[a]***)*** *aNDFom digestibility (% aNDFom)****;*** ***(***[b]***)*** *Undigested NDF at 120h****;*** ***(***[c]***)*** *Potentially digestible aNDFom****;*** ***(***[d]***)*** *Ruminal degradability (%CP) = (CP_initial − CP_residue_rumen) / CP_initial × 100****;*** ***(***[e]***)*** *Intestinal digestibility (% of CP entering intestine) = (CP_residue_rumen − CP_residue_intestinal) / CP_residue_rumen × 100****;*** ***(***[f]***)*** *Total digestibility (%CP) = (CP_initial − CP_residue_intestinal) / CP_initial × 100****;*** ***(***[g]***)*** *By-pass protein (RUP, %CP) = 100 − Ruminal degradability****;*** ***(***[h]***)*** *Intestinal digestibility= digestible portion of RUP after intestinal phase (%).*

Legend. bypassCP: *By-pass protein (RUP, %CP);* ***dNDFom:*** *Fiber digestibility (aNDFom);* ***IntDig/bypass:*** *Intestinal digestibility;* ***IntDigCP:*** *Intestinal digestibility (% of CP entering intestine);* ***pdNDFom:*** *Potentially digestible aNDFom;* ***RumDigCP:*** *Ruminal degradability (%CP);* ***SD:*** *Standard Deviation;* ***TotDigCP:*** *Total digestibility (%CP);* ***uNDF:*** *Undigested NDF.*

The category of fibre-rich feeds include wheat bran derived from Triticum durum and Triticum aestivum (hereafter referred to as TD and TE, respectively), soybean hulls, and beet pulp.

Regarding the main chemical and nutritional characteristics (see Table 6), our results revealed some variability among samples. Both TD and TE wheat bran showed variation in starch, aNDFom, and ADF contents. For TD, the values were 32.14 ± 3.36%, 40.66 ± 3.84%, and 15.11 ± 2.51% of DM, respectively, while for TE, the corresponding values were 24.80 ± 3.64%, 42.29 ± 2.71%, and 17.17

± 1.54% of DM. Soybean hulls exhibited notable variability in crude protein (13.76 ± 3.39% DM), ether extract (5.18 ± 1.99% DM), aNDFom (65.38 ± 6.25% DM), and ADF (54.68 ± 4.41% DM). Beet pulp showed relatively limited variability in fibre composition across samples, with aNDFom at 53.76 ± 1.62%, ADF at 31.08 ± 2.32%, and ADL at 13.19 ± 2.34% of DM.

The amino acid profiles remained consistent across samples within each raw material type, indicating low intra-feed variability in this parameter. In contrast, digestibility analyses revealed more pronounced differences particularly concerning fibre, protein, and starch.

Table 6. *Chemical composition of fibrous feeds*

		Wheat, bran *(Triticum durum)*		**Wheat, bran (*Triticum aestivum*)**		**Soybean, hulls**		**Beet pulp, dry**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dry matter	% AF	89.98	0.66	90.49	0.31	92.19	1.22	93.32	0.25
Moisture	% DM	10.02	0.66	9.51	0.31	7.81	1.22	6.68	0.25
Crude Protein	% DM	16.44	0.72	17.05	0.52	13.76	3.39	9.23	0.15
Soluble Protein	% DM	6.69	0.26	6.32	0.40	3.06	1.67		
NDIP	% DM	2.12	0.17	4.52	0.46	4.61	0.31		
ADIP	% DM	0.64	0.09	0.71	0.13	1.23	0.18		
Starch	% DM	32.14	3.36	24.80	3.64	3.88	0.17	6.30	0.86
Ether Extract	% DM	4.87	0.64	2.64	0.11	5.18	1.99	0.60	0.15
aNDFom	% DM	40.66	3.84	42.29	2.71	65.38	6.25	53.76	1.62
ADF	% DM	15.11	2.51	17.17	1.54	54.68	4.41	31.08	2.32
ADL	% DM	3.87	0.23	5.53	0.66	4.95	1.60	13.19	2.34
Ash	% DM	5.34	0.71	6.16	0.57	5.00	0.73	5.16	0.50
Aspartic Acid	% CP	1.11	0.02	1.20	0.14	1.34	0.53		
Serine	% CP	0.78	0.09	0.80	0.06	0.81	0.15		
Glutamic Acid	% CP	3.71	0.36	4.13	0.15	2.09	0.81		
Glycine	% CP	0.81	0.07	0.90	0.07	1.09	0.07		
Histidine	% CP	0.45	0.04	0.49	0.04	0.38	0.04		
Arginine	% CP	1.12	0.17	1.21	0.03	0.70	0.28		
Threonine	% CP	0.52	0.06	0.48	0.05	0.43	0.07		
Alanine	% CP	0.75	0.05	0.75	0.03	0.57	0.15		
Proline	% CP	1.03	0.13	1.10	0.12	0.66	0.16		
Cystine	% CP	0.21	0.02	0.25	0.01	0.14	0.05		
Tyrosine	% CP	0.39	0.06	0.41	0.02	0.44	0.08		
L-Valine	% CP	0.54	0.05	0.54	0.02	0.43	0.11		
Methionine	% CP	0.15	0.02	0.14	0.00	0.11	0.00		
Lysine	% CP	0.60	0.04	0.59	0.04	0.76	0.14		
Isoleucine	% CP	0.32	0.03	0.31	0.00	0.32	0.11		
Leucine	% CP	0.89	0.11	0.91	0.00	0.78	0.26		
Phenylalanine	% CP	0.62	0.08	0.62	0.03	0.49	0.15		

***Legend**. **ADF:** Acid Detergent Fiber; **ADL:** Acid Detergent Lignin; **AF:** As Feed; **CP:** Crude Protein; **DM:** Dry Matter; **NDIP:** Neutral Detergent Insoluble Protein; **ADIP:** Acid Detergent Insoluble Protein; **aNDFom:** ash corrected Neutral Detergent Fiber with α-amylase and sodium sulfite; **SD:** Standard Deviation.*

For TD wheat bran, the most evident variability was observed in starch digestibility (see Table 7), which reached 87.97 ± 2.60%, as well as in all parameters related to protein digestibility. The most variable among these were the intestinal digestibility of crude protein (35.94 ± 18.48%), total tract digestibility (82.55 ± 10.77%), and the ratio of intestinal digestibility on bypass protein (64.20 ± 26.82%). TE wheat bran exhibited less variability than TD, particularly regarding protein digestibility (as detailed in Table 7). However, it showed more fluctuation in fibre digestibility, with values for aNDFom digestibility of 39.51 ± 7.48% at 12 hours and 66.68 ± 4.06% at 120 hours. Starch digestibility for TE wheat bran was measured at 90.15 ± 2.51%.

For soybean hulls and beet pulp, only fibre digestibility was assessed. Both feeds exhibited variability, especially at the 12-hour incubation timepoint, with digestibility values of 41.99 ± 4.08% and 71.94 ± 5.17% of aNDFom, respectively. Additionally, soybean hulls showed variation in potentially digestible NDFom (pdNDFom), with a value of 62.41 ± 6.16%.

Table 7. *Digestibility rates of fibrous feeds*

		Wheat, bran (*Triticum Durum*)		**Wheat, bran (*Triticum aestivum*)**		**Soybean, hulls**		**Beetroot, dry pulp**	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
dNDFom 12h[a]	% aNDFom	34.95	1.98	39.51	7.48	41.99	4.08	71.94	5.17
dNDFom 72h	% aNDFom	71.47	2.57	64.55	2.78	94.65	0.56	84.81	1.34
dNDFom 120h	% aNDFom	76.29	1.44	66.68	4.06	95.44	0.46	86.23	1.11
uNDF 120h[b]	% DM	9.65	1.17	14.09	1.90	2.97	0.26	7.39	0.52
pdNDFom[c]	% DM	31.01	2.85	28.21	2.57	62.41	6.16	46.37	1.80
Starch Dig[d]	% Starch	87.97	2.60	90.15	2.51				
RumDigCP[e]	%CP	46.60	9.60	58.67	6.47				
IntDigCP[f]	%CP	35.94	18.48	32.59	3.36				
TotDigCP[g]	%CP	82.55	10.77	91.26	7.16				
bypassCP[h]	%CP	53.40	9.60	41.33	6.47				
IntDig/bypass[i]	% CP	64.20	26.82	80.28	14.14				

Note. ***([a])*** *aNDFom digestibility (% aNDFom);* ***([b])*** *Undigested NDF at 120h;* ***([c])*** *Potentially digestible aNDFom;* ***([d])*** *Ruminal degradability (%CP) = (CP_initial − CP_residue_rumen) / CP_initial × 100;* ***([e])*** *Intestinal digestibility (% of CP entering intestine) = (CP_residue_rumen − CP_residue_intestinal) / CP_residue_rumen × 100;* ***([f])*** *Total digestibility (%CP) = (CP_initial − CP_residue_intestinal) / CP_initial × 100;* ***([g])*** *By-pass protein (RUP, %CP) = 100 − Ruminal degradability;* ***([h])*** *Intestinal digestibility= digestible portion of RUP after intestinal phase (%).*

Legend. bypassCP: *By-pass protein (RUP, %CP);* ***dNDFom:*** *Fiber digestibility (aNDFom);* ***IntDig/bypass:*** *Intestinal digestibility;* ***IntDigCP:*** *Intestinal digestibility (% of CP entering intestine);* ***pdNDFom:*** *Potentially digestible aNDFom;* ***RumDigCP:*** *Ruminal degradability (%CP);* ***SD:*** *Standard Deviation;* ***TotDigCP:*** *Total digestibility (%CP);* ***uNDF:*** *Undigested NDF.*

Discussion

The first aim of our study was to evaluate the chemical and nutritional variability of the most commonly used raw materials in the Italian feed industry and to compare laboratory-derived values with those reported in major feed databases, including NASEM (2021), INRA, and CNCPS. By analysing and comparing our results with the available literature, no universally accepted range for variability in either chemical composition or digestibility of feed ingredients was identified. Based on the observed variability, we propose a reference threshold of 10% for selected parameters, calculated as: (standard deviation / parameter mean) × 100. Such a threshold could represent a practical criterion for feed mills, for example to flag or reject feed batches exceeding this level of variability or to support supplier selection based on consistency. A key strength of this study is the traceable documentation of the geographical origin of each ingredient using Progeo S.C.A. procurement records, reflecting the internationally sourced nature of raw materials used in the Italian dairy feed industry. This traceability enhances the external validity of the variability estimates and provides essential context for interpreting discrepancies with major reference feed databases.

Following completion of the analytical procedures, laboratory results were compared with reference values from international feed databases. For sunflower meal, the CNCPS feedbank reported crude protein values in disagreement with those observed in the present study (40.20% DM vs 37.05%), and similar disagreement was observed for soluble protein (13.59% vs 11.70%), aNDFom (38.00% vs 35.53%), and ADIP (3.00% vs 1.33%). These discrepancies may reflect varietal differences, agronomic conditions, or processing methodologies, all of which are known to influence the chemical composition of oilseed by-products (Kohli and Singha, 2024). The NASEM 2021 database showed greater agreement with our laboratory values overall, although partial disagreement persisted for aNDFom (40.19% vs 35.53%) and ADF (29.02% vs 32.27%). This closer alignment suggests the use of updated analytical protocols and broader sample representativeness in NASEM; however, the remaining differences in fibre fractions indicate that even advanced feedbanks may require calibration to regional feed sources. The need for local or regional feedbank adjustment has been highlighted in several studies reporting differences in soybean meal composition according to country of origin or even within-country production areas (Karr-Lilienthal *et al.*, 2004; Thakur and Hurburgh, 2007; García-Rebollar *et al.*, 2016; Lagos and Stein, 2017; Ibáñez *et al.*, 2020; Son, 2020; Aguirre *et al.*, 2022). INRA showed the lowest level of agreement with our data, with substantial discrepancies across most parameters. Notably, digestibility information for fibre and protein was largely absent from the databases, with the exception of NASEM 2021, which reports a 48-hour aNDFom digestibility value. The limited availability of digestibility data, particularly in older or region-specific systems such as INRA, represents a critical limitation for modelling ruminal dynamics, especially for by-products characterised by high variability. The inclusion of digestibility coefficients, as implemented in NASEM, improves the predictive accuracy of nutritional models such as CNCPS or NRC.

Considering protein-rich feeds, soybean meal showed overall agreement across feedbanks for dry matter, moisture, and crude protein content, with the exception of INRA, which reported crude protein values in disagreement with those measured in the present study (48.50% vs 52.23% DM). Soluble protein values reported by NASEM (12.17% DM) were not fully aligned with our laboratory measurements (9.72% DM). Neutral detergent insoluble protein (NDIP) values obtained in our laboratory (3.61%) were also in disagreement with those reported by NASEM and CNCPS (both 1.01%). This divergence may indicate reduced protein solubility associated with processing conditions, as described by Agume *et al.* (2017), or specific characteristics of the soybean meal batches analysed. Such differences may increase rumen bypass protein availability but could also influence microbial protein synthesis. The amino acid profile showed general agreement with published values, although arginine and isoleucine concentrations were not fully aligned with feedbank data, whereas valine content showed partial disagreement. Variability in amino acid composition may reflect genetic and environmental influences on seed composition and can affect ration formulation, particularly for high-producing ruminants and monogastric species (Oviedo-Rondón *et al.*, 2024). Digestibility data from the literature were limited, but available 48-hour fibre digestibility values were in agreement with our results. Overall, soybean meal was confirmed as a high-quality protein feed, exhibiting high fibre (94.28% dNDFom at 120 h) and protein digestibility (96.44% total tract digestibility), supporting its role as a reference protein source in dairy nutrition.

For raw soybean seeds, dry matter content measured in this study (92.56%) was not fully aligned with values reported by NASEM, CNCPS, and INRA. Crude protein values reported by NASEM and CNCPS were in disagreement with our laboratory results, whereas INRA values showed closer agreement. Ether extract content measured in this study was consistently not aligned with feedbank values, which may reflect varietal differences in oil content or differences in processing intensity (Gaffield *et al.*, 2024). These discrepancies have implications for dietary energy density and lipid-sensitive ration formulation. The amino acid profile was largely in agreement with literature, although several essential amino acids showed partial disagreement, potentially indicating limiting amino acid profiles in high-producing animals unless corrected through supplementation, particularly for methionine and lysine (Wei *et al.*, 2023).

For roasted soybean seeds, dry matter content showed good agreement with published data, except for INRA, which reported values not aligned with those measured in this study. Crude protein values reported by NASEM and CNCPS were in disagreement with laboratory results, while INRA again showed lower values. Ether extract content measured in this study was consistently not aligned with feedbank values, likely reflecting the effects of thermal processing on moisture reduction and oil concentration (Agume *et al.*, 2017; Maciel *et al.*, 2023). In contrast, ADF content measured in this study showed substantial disagreement with literature values, potentially due to thermal denaturation of hemicelluloses or enhanced fibre solubilisation during roasting, which may improve energy availability (Rafiee-Yarandi *et al.*, 2016). Amino acid profiles were generally consistent, although feedbank values tended to overestimate several essential amino acids, likely due to older analytical datasets or heterogeneous source material.

A comparison between raw and roasted soybean seeds indicated that, although both exhibited high fibre and protein digestibility, raw seeds displayed greater variability. The improved consistency observed in roasted seeds suggests that heat treatment may stabilise nutrient availability, reduce anti-nutritional factors, and enhance predictability of in vivo responses, as previously reported for soybean flour (Carvalho *et al.*, 2013; Machado *et al.*, 2008). Thermal processing was also associated with greater agreement in bypass protein availability, increasing rumen undegradable protein from 19.51% in raw seeds to 50.50% in roasted seeds, a characteristic particularly advantageous in high-producing dairy systems.

For starch-rich feeds, wheat middlings showed crude protein values in close agreement with feedbank data, while NDIP and ADIP values reported by CNCPS were not aligned with our laboratory measurements, which were instead in agreement with NASEM. These differences may arise from variation in drying methods, sample preparation, or by-product composition, all of which influence protein degradability (Mustafa *et al.*, 2001). Starch content measured in this study was in disagreement with feedbank values, whereas aNDFom content showed partial agreement. Amino acid profiles were generally consistent across datasets.

In corn, crude protein values for non-domestic varieties were not fully aligned with feedbank values, while starch content for both Italian and non-domestic corn showed systematic disagreement with literature data. These differences may reflect cultivar genetics, soil fertility, harvest maturity, and post-harvest management (Yu and Moon, 2021; Sekhon *et al.*, 2016; Yang and Li, 2024; Wang *et al.*, 2015). Fibre fractions (ADF and aNDFom) measured in this study were not aligned with feedbank values, while fibre digestibility at 12 h showed higher agreement with rapid fermentation dynamics compared with the 48-hour NASEM reference. Starch digestibility values measured in this study were not aligned with NASEM values, potentially reflecting increased starch encapsulation or kernel vitreousness (Owens and Soderlund, 2006; Xu *et al.*, 2019).

For barley, starch content measured in this study was not aligned with feedbank values, whereas fibre fractions showed partial agreement. Fibre digestibility values indicated greater early-stage fermentability compared with NASEM reference values, possibly due to processing or environmental effects enhancing fibre accessibility (Vega García *et al.*, 2025). Starch digestibility was not fully aligned with NASEM data.

Regarding fibre-rich feeds, wheat bran derived from Triticum durum showed crude protein values in disagreement with NASEM and CNCPS but in closer agreement with INRA. Starch content and fibre fractions were not aligned with feedbank values, while fibre digestibility values were in agreement with NASEM reference data. These findings suggest that despite compositional differences, digestibility kinetics may remain comparable, potentially due to enhanced cell-wall porosity or particle size effects (Roye *et al.*, 2020).

Wheat bran from Triticum aestivum showed crude protein values in agreement with CNCPS but not fully aligned with NASEM and INRA. Soluble protein, NDIP, starch, and fibre fractions showed systematic disagreement with feedbank values, likely reflecting processing intensity and fractionation effects during milling (Uttam *et al.*, 2023; Jayanegara *et al.*, 2016; Skřivan *et al.*, 2024). Despite these compositional differences, fibre and starch digestibility values showed good agreement with NASEM, suggesting robustness of digestibility predictions across protocols.

For soybean hulls, crude protein, NDIP, starch, ether extract, and fibre fractions showed consistent disagreement with feedbank values, likely due to incomplete hull separation and residual oil retention during processing. These differences can influence rumen degradability and bypass protein supply (Jayanegara *et al.*, 2016). Finally, beet pulp showed general agreement with feedbank data, although starch and fibre fractions showed notable disagreement, reflecting variability in residual sugars and processing conditions (Duraisam *et al.*, 2017).

From an applied perspective, these findings indicate that reliance on reference feed databases alone may be insufficient to capture the true nutritional variability of commercially sourced feed ingredients. The integration of routine laboratory analyses with supply-chain traceability, including documented geographical origin and supplier information, represents a valuable strategy to improve quality control, supplier selection, and formulation accuracy, particularly for digestibility-related parameters that showed the highest variability.

Limits of the Study

This study highlights the variability existing among raw materials, but some limitations are to consider, unfortunately the small number of raw materials doesn't reflect the huge number of feeds that is possible to use in a feed mill to produce a complementary or complete feed for animal. Also, the small number of replications could have an impact on the results we obtained.

Future prospectives

Future research should expand the number of feed ingredients and samples analysed in order to better capture the full range of variability encountered under commercial conditions. In particular, greater attention should be devoted to digestibility-related parameters, as variability in starch digestibility directly affects the accuracy of dietary energy prediction, while variability in protein ruminal degradability and intestinal digestibility influences metabolizable protein supply, nitrogen efficiency, and nitrogen excretion. Inconsistent estimates of starch availability may lead to errors in energy balance and increase the risk of ruminal instability, whereas inaccurate characterization of protein degradability can result in either inefficient nitrogen utilization or excessive ruminal nitrogen losses.

To improve both analytical throughput and practical applicability, alternative or complementary techniques aimed at reducing laboratory time and costs should be further investigated. Near-infrared spectroscopy (NIRS) represents a promising tool for rapid screening of raw materials, especially when integrated with reference wet chemistry datasets generated under controlled conditions. Feed mills, given the large volumes of raw materials processed daily, could play a key role in supporting the development of robust calibration models. However, uncertainties remain regarding the accuracy of NIRS in predicting digestibility and degradability parameters, particularly for fibre and protein fractions, highlighting the need for continued validation before routine application in precision feed formulation.

Conclusions

In conclusion our study highlighted a generally good consistency in the chemical composition and nutritional value of feedstuffs of the same type across the Italian feed industry. However, digestibility of fibre, protein, and starch showed significant variability among samples. Such differences in digestibility may influence the effective assimilation of nutrients by animals, potentially compromising their health, productive performance, and ultimately, economic profitability. More studies are needed to investigate this variability among feedstuff and see its impact on dairy cows. In order to improve the precision of feed and ration formulation, it is essential to analyze raw materials not only to assess their nutritional quality, but also as a strategic tool for selecting and evaluating suppliers.

Reference

1. Aguirre, L., Cámara, L., Smith, A., Arroyo, J., De Juan, A., Fondevila, G. & Mateos, G.G., 2022. Chemical composition, protein quality indicators and in vitro protein digestibility of commercial soybean meals from different origins for use in poultry feeding. Anim. Feed Sci. Technol. 293, 115473.
2. Agume, A.S.N., Njintang, N.Y. & Mbofung, C.M.F., 2017. Effect of soaking and roasting on the physicochemical and pasting properties of soybean flour. Foods 6, 12. https://doi.org/10.3390/foods6020012
3. AOAC, 1990. Official methods of analysis. Association of Official Analytical Chemists, Arlington, VA.
4. Bach, A., 2024. Back to basics: Precision while mixing total mixed rations and its impact on milking performance. JDS Commun. 5, 102–106. https://doi.org/10.3168/jdsc.2023-0423
5. Buonaiuto, G., Cavallini, D., Mammi, L.M.E., Ghiaccio, F., Palmonari, A., Formigoni, A. & Visentin, G., 2021. Accuracy of NIRS in predicting chemical composition and fibre digestibility of hay-based total mixed rations. Ital. J. Anim. Sci. 20, 1730–1739. https://doi.org/10.1080/1828051X.2021.1990804
6. Carvalho, A.W., Natal, D.I.G., Silva, C.O., Dantas, M.I.S., Barros, E.G., Ribeiro, S.M.R., Costa, N.M.B. & Martino, H.S.D., 2013. Heat-treatment reduces anti-nutritional phytochemicals and maintains protein quality in genetically improved hulled soybean flour. Food Sci. Technol. 33, 310–315. https://doi.org/10.1590/S0101-20612013005000048
7. Cavallini, D., Mammi, L.M., Palmonari, A., García-González, R., Chapman, J.D., McLean, D.J. & Formigoni, A., 2022. Effect of an immunomodulatory feed additive in mitigating the stress responses in lactating dairy cows to a high concentrate diet challenge. Animals 12, 2129. https://doi.org/10.3390/ani12162129
8. Cavallini, D., Penazzi, L., Valle, E., Raspa, F., Bergero, D., Formigoni, A. & Fusaro, I., 2022. When changing the hay makes a difference: A series of case reports. J. Equine Vet. Sci. 113, 103940. https://doi.org/10.1016/j.jevs.2022.103940

9. de Vega, A., Gasa, J., Castrillo, C. & Guada, J.A., 2025. Presentation (chopped versus ground and pelleted) of a low-quality alfalfa hay in sheep: Effects on intake, feeding behaviour, rumen fill and digestion, and passage. Animals 15, 541. https://doi.org/10.3390/ani15040541
10. Duraisam, R., Salelgn, K. & Berekete, A.K., 2017. Production of beet sugar and bio-ethanol from sugar beet and its bagasse: A review. Int. J. Eng. Trends Technol. 43, 222–233. https://doi.org/10.14445/22315381/IJETT-V43P237
11. Fadel, J.G., Johnson, H.M. & Robinson, P.H., 2006. Impact of variation in diet nutrient inputs on model output predictions. J. Anim. Sci. 84, 51–59. https://doi.org/10.15232/S1080-7446(15)30974-8
12. Ferraretto, L.F. & Shaver, R.D., 2015. Effects of whole-plant corn silage hybrid type on intake, digestion, ruminal fermentation, and lactation performance by dairy cows through a meta-analysis. J. Dairy Sci. 98, 2662–2675. https://doi.org/10.3168/jds.2014-9045
13. Gaffield, K.N., Goodband, R.D., DeRouchey, J.M., Tokach, M.D., Woodworth, J.C., Denny, G. & Gebhardt, J.T., 2024. A review of soybean processing byproducts and their use in swine and poultry diets. Transl. Anim. Sci. 8, txae063. https://doi.org/10.1093/tas/txae063
14. García-Rebollar, P., Cámara, L., Lázaro, R., Dapoza, C., Pérez-Maldonado, R. & Mateos, G.G., 2016. Influence of the origin of the beans on the chemical composition and nutritive value of commercial soybean meals. Anim. Feed Sci. Technol. 221, 245–261. https://doi.org/10.1016/j.anifeedsci.2016.07.007
15. Goering, H.K. & Van Soest, P.J., 1970. Forage fibre analyses. USDA Agricultural Research Service, Washington, DC.
16. Heinrichs, A.J., Heinrichs, B.S., Cavallini, D., Fustini, M. & Formigoni, A., 2021. Limiting total mixed ration availability alters eating and rumination patterns of lactating dairy cows. JDS Commun. 2, 186–190. https://doi.org/10.3168/jdsc.2020-0074
17. Ibáñez, M., De Blas, C., Cámara, L. & Mateos, G.G., 2020. Chemical composition, protein quality and nutritive value of commercial soybean meals produced from beans from different countries: A meta-analytical study. Anim. Feed Sci. Technol. 267, 114531. https://doi.org/10.1016/j.anifeedsci.2020.114531
18. ISO, 2015. International Standard ISO 9001: Quality Management Systems – Requirements, 5th edn. International Organization for Standardization, Geneva, Switzerland. (DOI not available)
19. Jayanegara, A., Dewi, S., Laylli, N., Laconi, E., Nahrowi, N. & Ridla, M., 2016. Determination of cell wall protein from selected feedstuffs and its relationship with ruminal

protein digestibility in vitro. Media Peternak. 39, 134–140. https://doi.org/10.5398/medpet.2016.39.2.134
20. Karr-Lilienthal, L.K., Grieshop, C.M., Merchen, N.R., Mahan, D.C. & Fahey, G.C., 2004. Chemical composition and protein quality comparisons of soybeans and soybean meals from five leading soybean-producing countries. J. Agric. Food Chem. 52, 6193–6199. https://doi.org/10.1021/jf049795+
21. Kohli, V. & Singha, S., 2024. Protein digestibility of soybean: How processing affects seed structure, protein and non-protein components. Discover Food 4, 7. https://doi.org/10.1007/s44187-024-00076-w
22. Kohn, R., 2006. How can dairy nutrition models deal with uncertainty? J. Anim. Sci. 84, 51–59. https://doi.org/10.15232/S1080-7446(15)30973-6
23. Kong, L. & Baik, B.K., 2016. Degree of starchy endosperm separation from bran as a milling quality trait of wheat grain. J. Cereal Sci. 69, 49–56. https://doi.org/10.1016/j.jcs.2016.02.001
24. Lagos, L. & Stein, H.H., 2017. Chemical composition and amino acid digestibility of soybean meal produced in the United States, China, Argentina, Brazil, or India. J. Anim. Sci. 95, 1626–1636. https://doi.org/10.2527/jas.2017.1440
25. Lechartier, C. & Peyraud, J.L., 2010. The effects of forage proportion and rapidly degradable dry matter from concentrate on ruminal digestion in dairy cows fed corn silage-based diets with fixed neutral detergent fibre and starch contents. J. Dairy Sci. 93, 666–681. https://doi.org/10.3168/jds.2009-2349
26. Licitra, G., Hernandez, T. & Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358. https://doi.org/10.1016/0377-8401(95)00837-3
27. Machado, F., Queiróz, J., Oliveira, M., Piovesan, N., Peluzio, M., Costa, N. & Moreira, M., 2008. Effects of heating on protein quality of soybean flour devoid of Kunitz inhibitor and lectin. Food Chem. 107, 649–655. https://doi.org/10.1016/j.foodchem.2007.08.061
28. Maciel, G., Wagner, J.R. & Bartosik, R.E., 2023. Drying of soybean seeds and effect on protein quality and oil extraction efficiency by extruding-expelling process. Sci. Agric. 80, e20220176. https://doi.org/10.1590/1678-992X-2022-0176
29. Magro, S., Costa, A., Cavallini, D., Chiarin, E. & De Marchi, M., 2024. Phenotypic variation of dairy cows' hematic metabolites and feasibility of non-invasive monitoring of metabolic status in the transition period. Front. Vet. Sci. 11, 1437352. https://doi.org/10.3168/jds.2023-23841

30. Mertens, D.R. et al., 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85, 1217–1240.
31. Muizelaar, W., Bani, P., Kuhla, B., Larsen, M., Tapio, I., Yáñez-Ruiz, D.R. & van Gastelen, S., 2020. Rumen fluid sampling via oral stomach tubing method. In: Methods in cattle physiology and behaviour – Recommendations from the SmartCow consortium, PUBLISSO, Cologne.
32. Mustafa, A.F., Christensen, D.A. & McKinnon, J.J., 2001. Ruminal degradability of neutral detergent insoluble protein of selected protein sources. Can. J. Anim. Sci. 81, 601–603. https://doi.org/10.4141/A01-034
33. National Academies of Sciences, Engineering, and Medicine, 2021. Nutrient requirements of dairy cattle, 8th rev. ed. National Academies Press, Washington, DC. https://doi.org/10.17226/25806
34. Nozière, P., Sauvant, D. & Delaby, L., 2018. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, NL.
35. Oviedo-Rondón, E.O., Toscan, A., Fagundes, N., Vidal, J., Barbi, J. & Thiery, P., 2024. Soybean meal nutrient composition, amino acid digestibility, and energy content according to the country of origin and year of harvest evaluated via NIRS. J. Appl. Poult. Res. 33, 100448. https://doi.org/10.1016/j.japr.2024.100448
36. Owens, F.N. & Soderlund, S., 2006. Ruminal and postruminal starch digestion by cattle. Oklahoma State University, Tulsa, OK, pp. 15–17.
37. Palmonari, A., Gallo, A., Fustini, M., Canestrari, G., Masoero, F., Sniffen, C.J. & Formigoni, A., 2016. Estimation of the indigestible fibre in different forage types. J. Anim. Sci. 94, 248–254. https://doi.org/10.2527/jas.2015-9649
38. Rafiee-Yarandi, H., Alikhani, M., Ghorbani, G. & Sadeghi-Sefidmazgi, A., 2016. Effects of temperature, heating time and particle size on values of rumen undegradable protein of roasted soybean. S. Afr. J. Anim. Sci. 46, 170–179. https://doi.org/10.4314/sajas.v46i2.8
39. Robertson, J.B., 1981. The detergent system of analysis and its application to human foods. In: The analysis of dietary fibre in food, pp. 123–158, Academic Press.
40. Ross, D., Gutierrez-Botero, M. & Van Amburgh, M.E., 2013. Development of an in vitro intestinal digestibility assay for ruminant feeds. In: Proc. Cornell Nutr. Conf., pp. 190–202, Cornell University.

41. Roye, C., Henrion, M., Chanvrier, H., De Roeck, K., De Bondt, Y., Liberloo, I., King, R. & Courtin, C.M., 2020. Extrusion-cooking modifies physicochemical and nutrition-related properties of wheat bran. Foods 9, 738. https://doi.org/10.3390/foods9060738
42. Saini, P., Sinha, A.S.K. & Prasad, K., 2024. Characterising the effect of commercial wheat grain milling methods on wheat bran characteristics. Int. J. Food Sci. Technol. 59, 6398–6408. https://doi.org/10.1111/ijfs.17383
43. Sekhon, R.S., Breitzman, M.W., Silva, R.R., Santoro, N., Rooney, W.L., De Leon, N. & Kaeppler, S.M., 2016. Stover composition in maize and sorghum reveals remarkable genetic variation and plasticity for carbohydrate accumulation. Front. Plant Sci. 7, 822. https://doi.org/10.3389/fpls.2016.00822
44. Silvestrelli, S., Cavallini, D., Bongiovanni, J., Toni, F. & Formigoni, A., 2024. Use of Progeo Dairy Manager (PDM) software to assess precision during preparation of TMR mixing wagon. In: Proc. Anim. Nutr. Conf., pp. 907–913, Anim. Nutr. Conf.
45. Skřivan, P., Sluková, M., Stýblová, B., Trusová, Š., Sinica, A., Bleha, R., Švec, I. & Kotrcová, V., 2024. Utilisation of the nutritional potential of wheat bran using different fractionation techniques. Appl. Sci. 14, 7222. https://doi.org/10.3390/app14167222
46. Son, A.R., 2020. Variation in energy and nutrient composition of oilseed meals from different countries. Korean J. Poult. Sci. 47, 107–114.
47. St-Pierre, N. & Weiss, W., 2006. Managing feedstuff variation in nutritional practice. J. Anim. Sci. 84, 51–59.
48. Sveinbjörnsson, J., Murphy, M. & Udén, P., 2007. In vitro evaluation of starch degradation from feeds with or without various heat treatments. Anim. Feed Sci. Technol. 132, 171–185. https://doi.org/10.1016/j.anifeedsci.2006.03.018
49. Thakur, M. & Hurburgh, C.R., 2007. Quality of US soybean meal compared to the quality of soybean meal from other origins. J. Am. Oil Chem. Soc. 84, 835–843. https://doi.org/10.1007/s11746-007-1107-8
50. Tilley, J.M.A. & Terry, R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. 18, 104–111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x
51. Uttam, A.N., Padte, S., Raj, G.J.V., Govindaraju, K. & Kumar, S., 2023. Isolation, characterization, and utilisation of wheat bran protein fraction for food application. J. Food Sci. Technol. 60, 464–473. https://doi.org/10.1007/s13197-022-05617-8
52. Van Amburgh, M.E., Collao-Saenz, E.A., Higgs, R.J., Ross, D., Recktenwald, E.M., Raffrenato, E., Chase, L.E., Overton, T.R., Mills, J.K. & Foskolos, A.S., 2015. The Cornell

Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. J. Dairy Sci. 98, 6361–6380. https://doi.org/10.3168/jds.2015-9378

53. Wang, T., Wang, M., Hu, S., Xiao, Y., Tong, H., Pan, Q., Xue, J., Yan, J., Li, J. & Yang, X., 2015. Genetic basis of maize kernel starch content revealed by high-density single nucleotide polymorphism markers in a recombinant inbred line population. BMC Plant Biol. 15, 288. https://doi.org/10.1186/s12870-015-0675-2
54. Wei, X., Wu, H., Wang, Z., Zhu, J., Wang, W., Wang, J., Wang, Y. & Wang, C., 2023. Rumen-protected lysine supplementation improved amino acid balance, nitrogen utilisation and altered hindgut microbiota of dairy cows. Anim. Nutr. 15, 320–331. https://doi.org/10.1016/j.aninu.2023.08.001
55. Xu, N., Wang, D. & Liu, J., 2019. Variance of zein protein and starch granule morphology between corn and steam-flaked products determined starch ruminal degradability through altering starch-hydrolyzing bacteria attachment. Animals 9, 626. https://doi.org/10.3390/ani9090626
56. Yang, X. & Li, Q., 2024. Influence of agronomic practices on maize protein and starch contents. Field Crops 7, 1–10.
57. Yu, J.K. & Moon, Y.S., 2021. Corn starch: Quality and quantity improvement for industrial uses. Plants 11, 92. https://doi.org/10.3390/plants11010092

STUDY 2: USE OF PROGEO DAIRY MANAGER (PDM) SOFTWARE TO ASSES PRECISION DURING PREPARATION OF TMR MIXING WAGON.

The present study is a shorter version of the paper presented at the 11th European Conference on Precision Livestock Farming.

Silvestrelli, S., Cavallini, D., Bongiovanni, J., Toni, F., & Formigoni, A. (2024). *Use of Progeo Dairy Manager (PDM) software to asses precision during preparation of TMR mixing wagon*. 907–913. Scopus. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85205023624&partnerID=40&md5=ff40594cde1243b864e2be5963d43252

Material and methods

Study design

The present work is an observational retrospective study, adhering to the STROBE-Vet guidelines (Sargeant et al., 2016) for reporting the data.

Setting

The study was conducted in Italy, in the PR area, involving six different dairy cows' farms. This area boasts a rich agricultural landscape and is home to numerous dairy farms characterized by varying herd sizes, management practices, and technological adoption.

Data collection

Data were collected in 6 dairy farms located in the area of Parmigiano Reggiano Protected Designation of Origin (PDO) and utilizing the Progeo Dairy Manager (PDM) software for herd management. The duration of data collection spanned two months (February and March 2024), with regular visits to the selected farms to ensure comprehensive data capture. A dataset was created with the main characteristics of selected farms (Table 8).

Table 8: *Characteristics of the included farms*

Item	A	B	C	D	E	F
Number of lactating cows (n)	102	390	860	969	490	180
Pens for lactating cows (n)	2	6	7	7	4	3
Milk Production (kg/d)	38.50	37.00	32.17	29.40	29.60	34.20
DIM (d)	155	156	186	175	169	158
DM offered (kg/cow/d)	30.50	29.10	26.05	28.10	25.50	28.20
Ration (%)	103	98	108	105	100	110
Number of ingredients (n)	9	10	8-11	7-9	8-10	9

Legend. A, B, C, D, E, F: *farms;* ***DIM***: *Days in Milk;* ***DM***: *Dry Matter.*

To assess the variance between theoretical and actual rations, the quantity of each feed loaded in the mixer wagon in a specific ration were daily measured by the mixer wagon scale and data were registered in the PDM program. Any discrepancies between expected and real loads of ration components were documented through deviations in kilograms and percentages. Ingredients used across the farms were classified in forages (e.g. alfalfa 1st, 2nd, and 3rd cut, wheat hay, and straw) or concentrates (e.g. soybean meal, steam-flaked corn, ground corn, ground sorghum, wheat grains, barley grains, and other feeds sourced from various feed mills). We reported data from every category (e.g. forages, concentrates, TMR, unload) as percentages calculated as actual amount/ theorical amount.

Data Analysis

Statistical analysis was conducted using Jamovi (version 2.4.14) (The jamovi project, 2023). Descriptive statistics were reported as absolute numbers, percentages, and mean ± standard deviation (SD). T-tests and Cohen's d (effect size=0.19 trivial, 0.20 small, 0.50 medium, 0.80 large) were employed to assess differences between theoretical and actual rations (Kerby, 2014). The significance level (α) was set at ≤0.05 for all statistical tests, ensuring robust and meaningful findings.

Results and Discussion

To the best of our knowledge, this is the first study which assesses the deviation between theoretical and real rations gave to lactating cows on multiple dairy farms within the PR area, as well as investigate the consistency of challenges in TMR loading practices. Six dairy farms were enrolled with an overall of 2,991 lactating cows which produce daily an average of 31.82 kg of milk with a DM intake of 27.30 kg. The average DIM was 173. The percentages of rations feed to animals varies between 98% and 110% of the expected need.

During the trial there were an overall of 4,254 records for forages weight, 7,060 records for concentrates, 1,465 for TMR wagon, and 3,121 records for pens unload. Also, we conducted a single-sample t-test for every ration's component to compare the mean deviation form theoretical weight in the ration, which is reported in percentage.

Our results (Table 9) indicate that the mean deviations of forages in rations were significantly oversupplied (p<.001) compared to the theoretical one in all the six farms. Additionally, we calculated Cohen's d as a measure of effect size, suggesting a medium effect for all the farms, except for one farm which has a trivial effect. For concentrates, our results showed that the mean deviations of our rations were significantly oversupplied (p<.001) compared to the theoretical one in four farms. Additionally, the Cohen's d suggests an effect size from small to medium for these farms. Furthermore, compare to the theoretical rations, in one farm the mean concentrates deviations were significantly undersupplied (p<.001) with a small effect size and another one not significant (p=.089) with a trivial effect size. Those results were in line with Moallem & Lifshitz (2020) that reported that usually grains were overweighted when preparing TMR.

***Table 9**: Results regarding forage's and concentrate's records*

					FORAGES					
		Kg (as fed)	**Kg (as fed)**	**%**	**%**	**%**	**%**	**%**	**T-test**	**Effect Size**
Farm	**N**	**Target Mean (±SD)**	**Actual Mean (±SD)**	**Mean (±SD)**	**Min**	**25° Q**	**75° Q**	**Max**	**p**	**Cohen's d**
A	367	252.60 (217.56)	259.16 (219.52)	104.40 (6.64)	90.28	100.17	106.59	129.11	**<.001**	0.66
B	688	416.72 (264.18)	439.53 (263.99)	108.26 (11.34)	78.57	100.74	112.44	160.80	**<.001**	0.73
C	865	735.13 (281.93)	753.40 (287.58)	102.62 (4.45)	91.10	99.54	104.72	123.87	**<.001**	0.59
D	738	1077.55 (747.89)	1093.58 (748.59)	102.35 (4.29)	94.19	99.92	103.22	133.68	**<.001**	0.55
E	977	387.63 (196.39)	403.95 (197.11)	105.49 (8.87)	67.15	100.00	108.39	160.87	**<.001**	0.62
F	619	220.66 (98.84)	222.76 (99.89)	101.14 (7.71)	71.34	97.13	103.50	148.48	**<.001**	0.15
					CONCENTRATE					
		Kg (as fed)	**Kg (as fed)**	**%**	**%**	**%**	**%**	**%**	**T-test**	**Effect Size**
Farm	**N**	**Target Mean (±SD)**	**Actual Mean (±SD)**	**Mean (±SD)**	**Min**	**25° Q**	**75° Q**	**Max**	**p**	**Cohen's d**
A	372	268.22 (184.19)	275.85 (186.12)	110.45 (14.09)	55.56	100.97	119.23	161.54	**<.001**	0.74
B	685	619.88 (477.71)	620.88 (478.78)	100.25 (3.77)	81.25	99.29	100.75	153.26	.089	0.07
C	1799	511.74 (286.19)	513.43 (291.08)	98.96 (4.97)	82.88	98.60	101.39	129.51	**<.001**	0.21
D	1974	555.61 (340.18)	560.77 (343.46)	100.70 (3.24)	80.60	99.62	101.74	123.08	**<.001**	0.22

E	1734	298.27 (117.19)	300.46 (118.82)	100.83 (4.04)	89.55	98.56	102.16	136.00	**<.001**	0.20
F	496	428.37 (245.29)	430.60 (245.52)	100.73 (2.16)	92.90	99.84	101.55	109.32	**<.001**	0.34

***Legend**. **A, B, C, D, E, F**: farms; **N**: number of records; **SD**: Standard Deviation; **SE**: Standard Error; **Q**: quantile; **t**: t-test; **df**: degree of freedom; **p**: p-value.*

Considering the TMR (Table 10) of those farms, our results highlighted an oversupply in the average deviation from target (p<.001). The Cohen's d indicates an effect size comprised form medium to big. This deviation from target when load TMR brings several problems as reported in some studies. For example, Bach (2024) showed that divergence in total amount of feed mixed is quadratically associated with milk yield, the same for divergence of individual ingredients. It is also known that overloading ingredients or TMR will cause an increase on feeding costs, and so any modification to the theorical ration have a detrimental effect on Income Over Feed Cost (IOFC). Lastly, regarding the mean deviations of unloading part of our rations compared to the theoretical one, were significantly oversupplied (p<.001) for four farms. The Cohen's d indicates an effect size comprised form trivial to small. Additionally, compared to the target, two farms feed to animals' lesser amount of TMR, however one is not significant (p=.071), with a small and trivial effect size, respectively.

***Table 10**: Results regarding total TMR mixing wagon weights and pen's unload*

				TMR						
		Kg (as fed)	**Kg (as fed)**	**%**	**%**	**%**	**%**	**%**	**T-test**	**Effect Size**
Farm	**N**	**Target Mean (±SD)**	**Actual Mean (±SD)**	**Mean (±SD)**	**Min**	**25° Q**	**75° Q**	**Max**	**p**	**Cohen's d**
A	116	1665.61 (63.63)	1721.06 (72.99)	103.33 (1.67)	98.84	102.29	104.10	110.61	**<.001**	2.00
B	174	5152.23 (2216.35)	5243.44 (2200.37)	102.27 (2.11)	98.39	100.72	103.08	112.37	**<.001**	1.07
C	310	5993.53 (802.04)	6049.07 (802.57)	100.91 (1.03)	99.02	100.19	101.46	104.53	**<.001**	0.88
D	371	5993.53 (802.04)	6049.07 (802.57	101.18 (0.88)	99.22	100.63	101.56	106.58	**<.001**	1.33
E	370	3850.80 (1056.63)	3907.83 (1055.90)	101.61 (2.34)	81.46	100.55	102.54	109.60	**<.001**	0.69
F	124	3663.35 (180.43)	3682.80 (182.68)	100.53 (0.91)	98.76	99.91	101.05	103.32	**<.001**	0.58
				UNLOAD						
		Kg (as fed)	**Kg (as fed)**	**%**	**%**	**%**	**%**	**%**	**T-test**	**Effect Size**
Farm	**N**	**Target Mean (±SD)**	**Actual Mean (±SD)**	**Mean (±SD)**	**Min**	**25° Q**	**75° Q**	**Max**	**p**	**Cohen's d**

A	246	831.95 (51.85)	843.16 (53.75)	101.39 (3.04)	80.28	100.09	102.79	112.94	**<.001**	0.46
B	638	1429.89 (836.25)	1433.12 (834.26)	100.89 (4.72)	87.08	99.60	100.94	137.57	**<.001**	0.19
C	743	2788.74 (1321.20)	2773.85 (1334.95)	98.76 (4.35)	69.01	99.07	100.49	114.72	**<.001**	0.28
D	740	3912.55 (1790.25)	3889.56 (1772.16)	99.83 (2.57)	87.14	99.62	100.53	115.19	.071	0.07
E	494	2936.68 (1484.17)	2917.09 (1425.58)	101.60 (7.66)	80.82	99.77	101.64	143.18	**<.001**	0.21
F	260	1755.68 (1171.17)	1753.95 (1160.58)	102.19 (8.81)	86.09	98.82	102.01	156.19	**<.001**	0.25

Legend. ***A, B, C, D, E, F***: *farms;* ***N***: *number of records;* ***SD***: *Standard Deviation;* ***SE***: *Standard Error;* ***Q***: *quantile;* ***t***: *t-test;* ***df***: *degree of freedom;* ***p***: *p-value.*

In general, our results showed that there was a deviation between theoretical and actual rations in terms of ingredients loading. This divergence from target could cause several problems cause it could lead to a milk production loss (Bach, 2024), or to an excretion of those nutrient which exceed the nutritional requirement of cows (Castillo et al., 2013) with an impact on environment. However, in two cases this discrepancy was not statistically significant, one in concentrates and the other one in unload. Specifically, forages, as we expected, had more divergence from target and variability compared to concentrates. As showed in (Trillo et al., 2016), Alfalfa is one ingredients usually loaded with poor accuracy and precision. One possible explanation is that forages are more difficult to weighted and operators find those ingredients uploading challenging. Nevertheless, there is an inconsistency in farm A which results in more precision weighting in forages compared to concentrates. It is possible that forages, were weighted as whole bales and this procedure could lead to a deviation from target. Another factor that can impact the precision of weight ingredients and prepare TMR could be the different type of mixing wagon (Moallem & Lifshitz, 2020), but this was not took in consideration in this article.

This study has several limitations. First, data collection was conducted on a small number of farms. Second, there was a limited timeframe for recording data, particularly during the winter months when it was harder to accurately measure ingredient weights, cause of wind, rain and storm. Third, the use of a single wagon for grinding both hay and straw posed a constraint. Lastly, the farms included in the study exhibited significant heterogeneity in dimensions and management.

Conclusion

In conclusion we can say that almost all the farms outweigh the ingredients, except for farm B that weighed with good precision concentrates. TMR and unload to pens were also significantly different from target. This could lead to overfeeding and excess of nutrients for animals, as the final ration will be different from the formulated one. This work highlighted that there are opportunities to improve PF in dairy farms by reducing deviation from target while preparing TMR mixing wagon setting higher standard for loading and unloading accuracy and precision. Operators must be aware and sure that the TMR is working properly, and the same operators must be well trained and conscious of their role in the farm. The present research enlarge also literature in this field and opens new scenario for new studies and trial.

Reference

1. Bach, A. (2024). Back to basics: Precision while mixing TMRs and its impact on milking performance. *JDS communications*, *5*(2), 102–106.
2. Castillo, A., St-Pierre, N., del Rio, N. S., & Weiss, W. (2013). Mineral concentrations in diets, water, and milk and their value in estimating on-farm excretion of manure minerals in lactating dairy cows. *Journal of Dairy Science*, *96*(5), 3388–3398.
3. Kerby, D. S. (2014). The simple difference formula: An approach to teaching nonparametric correlation. *Comprehensive Psychology*, *3*, 11-IT.
4. Moallem, U., & Lifshitz, L. (2020). Accuracy and homogeneity of TMRs processed through trailer mixer or self-propelled mixer, and effects on the yields of high-yielding dairy cows. *Animal feed science and technology*, *270*, 114708.
5. Sargeant, J., O'Connor, A. M., Dohoo, I., Erb, H., Cevallos, M., Egger, M., Ersbøll, A., Martin, S., Nielsen, L. R., & Pearl, D. (2016). Methods and processes of developing the strengthening the reporting of observational studies in epidemiology− veterinary (STROBE-Vet) statement. *Preventive veterinary medicine*, *134*, 188–196.
6. The jamovi project. (2023). *jamovi. (Version 2.4) [Computer Software]*. https://www.jamovi.org.
7. Trillo, Y., Lago, A., & Silva-del-Río, N. (2016). Deviation from the formulated target weight of ingredients loaded into high milk yield cow recipes on California dairies. *Journal of Dairy Science*, *99*(7), 5866–5878.

STUDY 3: THE USE OF RUMEN-PROTECTED AMINO ACIDS AND FIBROUS BY-PRODUCTS CAN INCREASE THE SUSTAINABILITY OF MILK PRODUCTION

D. Cavallini[1], M. Lamanna[1], R. Colleluori[1], S. Silvestrelli[1], F. Ghiaccio[1], G. Buonaiuto[1], and A. Formigoni[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, 40064 Ozzano dell'Emilia (BO), Italy

The present study is a shorter version of the paper accepted by Frontiers in Veterinary Science.

Cavallini, D., Lamanna, M., Colleluori, R., Silvestrelli, S., Ghiaccio, F., Buonaiuto, G., & Formigoni, A. (2025). The use of rumen-protected amino acids and fibrous by-products can increase the sustainability of milk production. *Frontiers in Veterinary Science*, *12*, 1588425. https://doi.org/10.3389/fvets.2025.1588425

Materials and Methods

This study did not entail any manipulation or alteration of the standard handling procedures for the animals. All cows involved in the present research were housed in a naturally ventilated individual tie-stall cubicles, enabling natural activities such as standing, lying, eating, and ruminating. All procedures were conducted in compliance with Directive 2010/63/EU, and the animals were raised in accordance with the European Union's legal requirements (98/58/EC) concerning the protection of animals kept for farming purposes. All procedures that included animals were approved by the University of Bologna Institutional Animal Care and Use Committee (Prot. N. 4405).

Animals, Housing, Management and Experimental design

The trial was conducted at the educational-experimental barn of the University of Bologna and included eight multiparous Holstein cows selected based on lactation number (2.63 ± 0.25), milk production (44.79 ± 3.30 kg), and days in milk (53.00 ± 6.49). The cows were housed in tie stalls and divided into four pairs according to the procedure reported in the study by Cavallini *et al.* (2018). The tie stalls were equipped with individual feed troughs with continuous weighing systems and a push-button water bowl with an individual meter. Milking was performed using conventional milking system twice a day at 7:00 a.m. and 7:30 p.m.

The experiment was conducted using a Latin square experimental design. Each pair was randomly assigned to one of four isoenergetic diets in succession. The four experimental diets were identified as:

- High Protein and High Cereals – HP-HC (Control group)
- High Protein and High Fibrous By-products – HP-HF
- Rumen-Protected Amino Acids and High Cereals – AA-HC
- Rumen-Protected Amino Acids and High Fibrous By-products – AA-HF

The rations were formulated by the NDS software (RUM&N Sas, Reggio Emilia, Italy; Table 11) using hays (alfalfa and wheat) with balanced diets in protein-amino acid content and variable carbohydrate sources depending on the experimental diets (Table 11), we selected to use beet pulp, soybean hulls, and wheat bran as fibrous by-products due to their high availability and reduced cost in the context of Northern Italy. The inputs used in the software were the cows' characteristics. Each diet was prepared individually approximately every 12 days. Daily intake was assessed based on the amount of residue present in the trough 24 hours after feeding, and the amount of TMR to be provided the following day was determined to ensure 10% residue and ad libitum feeding and were fed once a day at 7:00 p.m. (Zago mixer, Padova, Italy). Additionally, each cow was offered 1 kg of long wheat hay per day according to Heinrichs *et al.* (2021) in a separate side of the feed bunk to permit separate intake measurement.

Table 11: *Composition and analysis of the different experimental diets administered along the trial and composition of the experimental TMR (mean data ± S.D.).*

Ingredients, kg	**HP-HC**	**HP-HF**	**AA-HC**	**AA-HF**
Alfalfa hay	9.0	9.0	9.0	9.0
Wheat hay	3.0	3.0	3.0	3.0
Wheat Straw	1.0	1.0	1.0	1.0
Fibrous by-products mix[1]	1.5	5.0	3.4	7.0
Cereal mix[2]	10.0	7.0	10.0	6.5
Extruded soy extracted meal	2.5	2.0	0.6	0.5
Mineral and vitamin supplement[3]	0.5	0.5	0.5	0.5
RP Methionine	0.015	0.015	0.022	0.022
RP Lysine	-	-	0.100	0.100
Chemical composition, %				
DM intake, %	89.35 ± 1.22	88.10 ± 2.36	87.72 ± 2.81	89.90 ± 0.91
CP, % DM	14.80 ± 0.47	14.84 ± 0.91	12.47 ± 0.94	12.24 ± 1.02
Starch, % DM	26.84 ± 4.50	19.65 ± 1.57	25.86 ± 3.86	19.77 ± 2.52
aNDFom, % DM	36.40 ± 1.89	39.78 ± 2.79	38.24 ± 3.60	42.94 ± 2.30
ADF, % DM	26.25 ± 1.21	28.47 ± 2.18	26.82 ± 1.93	29.05 ± 1.13
ADL, % DM	5.37 ± 0.28	5.52 ± 0.47	5.48 ± 0.34	5.83 ± 0.52
uNDF 24h, % DM	19.58 ± 2.42	22.69 ± 3.89	19.15 ± 2.00	23.61 ± 2.40
uNDF 240h, % DM	12.23 ± 3.83	14.28 ± 2.12	12.12 ± 4.38	14.76 ± 3.22
peNDF	19.72 ± 1.72	21.60 ± 2.74	20.14 ± 1.90	21.41 ± 1.22
peuNDF	6.96 ± 0.68	7.30 ± 0.63	7.27 ± 0.96	7.86 ± 0.86

Legend. ***HP***: *High Protein;* ***AA***: *Rumen-Protected Amino Acids;* ***HC***: *High Cereals;* ***HF***: *High Fibrous By-products;* ***RP***: *Rumen Protected;* ***DM***: *Dry Matter;* ***CP***: *Crude Protein;* ***aNDFom***: *Neutral Detergent Fiber;* ***ADF***: *Acid Detergent Fiber;* ***ADL***: *Acid Detergent Lignin;* ***uNDF24***: *Undigested NDF via long-term 24 h in vitro fermentation;* ***uNDF240***: *Undigested NDF via long-term 240 h in vitro fermentation;* ***peNDF***: *Phisically Effective NDF;* ***peuNDF***: *Phisically Effective uNDF240.*

Note. *[1]Fibrous by-products mix (%): Beet pulp, 33.33; Soybean hulls, 33.33; Wheat bran, 33.33.*

[2]Cereal mix (%): Corn, 50.0; Sorghum, 50.0.

[3]Mineral and vitamin supplement (%): Calcium carbonate, 23.0; Sodium chloride, 23.0; Dicalcium phosphate, 35.0; Magnesium oxide, 10.0

Vitamins & oligo minerals (%): 10.0.*

**Contributions in vitamins, provitamins, and substances with analogous effects per kg of feed: Vitamin A prov. E672 IU 40000; Vitamin D3 E671 IU 4000; Vitamin E α-tocopherol 92% prov. mg 30; Vitamin B1 mg 5; Vitamin B2 mg 3; Vitamin B6 mg 1.5; Vitamin B12 mg 0.06; Vitamin K mg 5; Vitamin H1 mg 5; Vitamin PP mg 150; Choline chloride mg 50.*

Contributions in trace elements per kg of feed: Iron (ferrous carbonate) E1 mg 100; Cobalt (basic cobalt carbonate) E3 mg 1; Iodine (calcium iodate anhydrous) E2 mg 5; Manganese (manganous oxide) E5 mg 120; Copper (copper sulfate pentahydrate) E4 mg 10; Zinc (zinc oxide) E6 mg 130.

Data collection, sampling procedure and Analysis

Each experimental period consisted of 14 days of adaptation (to avoid carry over effect) and 4 experimental days during which samples were collected according to the schedule shown in Table 12.

Table 12: *Samplings during the experimental periods.*

Sample	**Day 15**		**Day 16**		**Day 17**		**Day 18**	
	Evening	Morning	Evening	Morning	Evening	Morning	Evening	Morning
Feces	X	X					X	X
Milk	X	X	X	X	X	X	X	X
Rumen fluid	X	X					X	X
Blood								X

Body weight (BW) was recorded automatically after each milking by a digital scale (TDM, Brescia, Italy), while body condition score (BCS) was measured individually in the last day of each experimental period, according to Buonaiuto et al. (2023).

The TMR was sampled at each new batch and raw materials were monitored throughout the experimental period to maintain consistent TMR quality. Intakes and behaviours were monitored (Giannone et al., 2025).

Feces were collected from the ampulla and dried in oven at 65°C until constant weight, followed by grinding with a Cyclotec to obtain a sample particle size of 1 mm. TMR and fecal samples were analyzed for chemical composition by wet chemistry according to the following methods: crude protein (CP) according to AOAC (method 976.06 and 984.13) using a Kjeldahl nitrogen analyzer (Gerhadt Vapodest 50, Gerhardt GmbH, Königswinter, Germany), starch determined according to Ehrman and AOAC (method 920.40), ether extract according to AOAC (method 920.390020), ash-corrected α-amylase–treated neutral detergent fiber (NDF) with the addition of sodium sulfite (aNDFom), acid detergent fiber (ADF) and acid detergent lignin (ADL) according to Mertens *et al.* (2002), ashed after 4 h combustion in a muffle furnace at 550°C (Vulcan 3–550, Dentsply Neytech, Burlington, NJ, USA). The ration particle size was determined using a RoTap Separator (W.S. Tyler, Mentor, OH), and the physically effective NDF (peNDF) was calculated as the product of aNDFom content and its physically effective factor (pef; Mertens, 1997), whereas physically effective uNDF240h was calculated as the product of pef and uNDF240 (Grant *et al.*, 2018). The undigested NDF at 24 h (uNDF24) and 240 h (uNDF240) and the potentially digestible NDF (pdNDF) of the ration were determined using an *in vitro* fermentation method in buffered media containing ruminal fluid and the uNDF240h was used as a marker to calculate the total tract apparent digestibility (TTD) of nutrients, as reported in Cavallini *et al.* (2023a).

The rumination time was recorded daily for each cow using the RuminAct system (SCR® – Israel) placed on a ruminometer collar (Lamanna et al., 2025a).

The quantitative and qualitative analysis of milk was done individually for each cow and for each milking. Milk samples for analysis were taken during milking with an auto-sampler; the milk aliquot was obtained after homogenization. The milk was then poured into the container destined for analysis. The samples were obtained by mixing 50% of the milk from the evening milking with that from the following morning, stored at refrigeration temperature (+4°C), and sent to the certified laboratory within a few hours. Quality monitoring was performed on individual milk samples four times during the experimental period, and the following were analyzed: fat content, protein, lactose, somatic cell count, and urea in a certified laboratory (Milkoscan, Foss, Granlatte, Granarolo, Italy). Finally, ECM was calculated as reported in previous works (Cavallini et al., 2018).

Cows were monitored for reticular pH with an indwelling wireless transmitting unit (SmaXtec Animal Care Sales GmbH, Graz, Austria), a system previously validated in rumen-cannulated dairy cows (Klevenhusen et al., 2014). These devices (3.5 cm i.d., 12 cm long, and weighing 210 g) were calibrated following the manufacturer instructions and manually inserted into the rumen via the esophagus one week before the start of the trial. Previous research has showed that these devices tend to sit in the ventral reticulum area (Klevenhusen et al., 2014). pH and temperature were recorded every 10 min and data transmitted real-time to a base station using the ISM band (433 MHz). Data were then collected using an analog-to-digital converter and stored in an external memory chip. Reticular pH data were aggregated as daily means, and a pH threshold of 5.5 was used to calculate time and dispersion below that threshold (Cavallini et al., 2022).

Rumen fluid was collected via esophageal probe. The first 500 mL of rumen fluid collected was discarded before taking samples. The collected liquid was placed in 15 ml Falcon tubes and frozen pending subsequent analyses; it was then transported to the DIMEVET department laboratory. Before analysis the rumen fluid was centrifugated. Volatile fatty acid (VFA) concentrations were determined by Gas Chromatography (Goetsch and Galyean, 1983), whereas NH_3 was evaluated via commercial kit (urea/BUN – color, BioSystems S.A. Barcelona, Spain) according to the producer procedure.

Blood samples were taken from the coccygeal vein in vacuum tubes containing a clot activator (Vacutest Kima, Padova, Italy); they were then maintained at 20°C for one hour after collection and centrifuged at 2,000 × g for 20 minutes to obtain the serum (Centrifugette 4203, ALC International

Srl, Cologno Monzese, Italy). Urea and uric acid were measured as reported in previous papers (Cavallini *et al.* 2021) using ADVIA 2120 (Siemens Healthcare Diagnostics).

Statistical analysis

All data were analyzed using JMP pro v 14.3 programs. A mixed model was used for the analysis with the effect of N source (HP *vs* AA), energy source (HC *vs* HF), and their interaction as fixed effects, the individual cow (within squares and period) as a random effect (experimental unit, N=8), and repeated measures for each parameter considered. Normal distribution of the data was checked for the residuals resulted from an initial mixed model, and normalized, when necessary, by BoxCox transformation. Means are reported as least square mean and pairwise multiple comparisons were performed using Student t-test as post hoc test when a p-value ≤ 0.05 was detected. A p-value ≤ 0.05 was considered statistically significant; and ap-value ≤ 0.01 was considered highly significant.

Results

The present trial showed interesting results, revealing differences related to the different protein, amino acid, and cereal and by-product content of the rations, while no differences were found for the following parameters: DMI, TMR intake, Long hay intake, DMI from concentrates, DMI from forage, OM intake, peNDF intake, Daily rumination time and Rumination/peNDF intake, Rumination/peuNDF intake, Rumination/aNDFom from forage ingested, butyrate, r-pH, r-pH<6 min, r-pH<6 AUC, r-pH <6 AUC DS, r-pH <5.8 AUC DS, r-pH<5.5 min, r-pH<5.5 AUC, r-pH <5.5 AUC DS, fecal DM, fecal ADL, fecal uNDF24, Milk fat, Milk fat/protein, Milk lactose, Water intake/milk yield, Water intake/ECM, Water intake/FCM 4%, Milk/DM intake, ECM/DM intake.

Subjects fed a diet with amino acid integration showed a higher body condition score compared to those with HP diet; the recorded values were 2.37^{B}, 2.41^{AB}, 2.47^{A}, and 2.43^{AB} for HP-HC, HP-HF, AA-HC, and AA-HF diets respectively (p<.01). Regarding body weight, the recorded values were 639.96, 645.64, 646.87, and 641.43 kg for the HP-HC, HP-HF, AA-HC, and AA-HF diets respectively (p<.01), although the difference between the various diets in these two parameters was negligible.

In the following paragraph are reported results regarding the Table 13. No significant differences were found in total DMI and rumination time among the animals across the four diets; the average intake was 26.40 kg. Indeed, regarding nutrient intake, a good match was observed between the expected and actual nutrient intake, confirming the accuracy of the dietary formulations used. Specifically, starch intake was 6.74^{A}, 5.25^{C}, 5.97^{B}, and 5.14^{C} kg in the HP-HC, HP-HF, AA-HC, and AA-HF diets respectively (p<.01), with a higher content in the HP-HC; crude protein intake was higher in HP diets (+0.79 kg, p<.01). Regarding fibrous fractions, the intake of aNDFom, ADF, and ADL was significantly higher in HF diets (+1.4 kg, +0.91, +0.12 respectively, p<.01; Table 13). The intake of uNDF24 was higher in the AA-HF diet (6.39^{A}, 5.80^{B}, 5.09^{C}, and 5.05^{C} in the AA-HF, HP-HF, HP-HC, and AA-HC diets respectively; p<.01). The intake of uNDF240 and peuNDF was higher in HF diets compared to HC diets (+0.57 kg and +0.13 kg respectively, p<.01). Additionally, higher daily water intake (HP= 167.88 L vs AA= 146.98 L) was observed in high-protein diets.

Table 13: *Nutrient intake, and water intake based on different protein, synthetic amino acid, cereal, and fibrous by-product levels.*

	HP		**AA**			***p-value***		
	HC	**HF**	**HC**	**HF**	**SEM**	**HP vs AA**	**HC vs HF**	**Interaction**
DMI, kg	26.55	26.99	25.49	26.55	0.76	0.06	0.06	0.44
TMR, kg	25.81	26.05	24.58	25.66	0.75	0.05	0.10	0.30
Long hay, kg	0.74	0.94	0.92	0.88	0.17	0.43	0.29	0.12
DMI from concentrates, kg	13.56	13.68	12.96	13.54	0.40	0.08	0.10	0.27
DMI from forage, kg	13.00	13.31	12.53	13.01	0.40	0.05	0.04	0.70
OM intake, kg DM	24.79	25.24	23.78	24.97	0.65	0.11	0.04	0.35
Starch intake, kg DM	6.74^{A}	5.25^{C}	5.97^{B}	5.14^{C}	0.18	<.01	<.01	<.01
CP intake, kg DM	4.10	4.22	3.31	3.42	0.10	<.01	0.08	0.98
aNDFom intake, kg DM	9.59	10.76	9.92	11.55	0.27	<.01	<.01	0.20
ADF intake, kg DM	7.07	7.86	7.09	8.12	0.19	0.29	<.01	0.36
ADL intake, kg DM	1.46	1.55	1.46	1.60	0.04	0.32	<.01	0.24
uNDF24 intake, kg DM	5.09^{C}	5.80^{B}	5.05^{C}	6.39^{A}	0.19	<.01	<.01	<.01
uNDF240 intake, kg DM	3.52^{C}	3.90^{B}	3.66^{BC}	4.41^{A}	0.11	<.01	<.01	0.02
peNDF intake, kg DM	5.79	6.06	6.19	6.27	0.21	0.03	0.20	0.52
peuNDF intake, kg DM	2.00	2.06	2.08	2.27	0.06	<.01	<.01	0.15
Water intake, L	170.41	165.34	141.43	152.53	11.92	<.01	0.62	0.19

Legend. $^{A, B, C}$ *: p< 0.05;* ***HP****: High Protein;* ***AA****: Rumen-Protected Amino Acids;* ***HC****: High Cereals;* ***HF****: High Fibrous By-products;* ***DMI****: Dry Matter Intake;* ***TMR****: Total Mixed Ration;* ***OM****: Organic Matter;* ***CP****: Crude Protein;* ***aNDFom****: Neutral Detergent Fiber;* ***ADF****: Acid Detergent Fiber;* ***ADL****: Acid Detergent Lignin;* ***uNDF24****: Undigested NDF via long-term 24 h in vitro fermentation;* ***uNDF240****: Undigested NDF via long-term 240 h in vitro fermentation;* ***peNDF****: Phisically Effective NDF;* ***peuNDF****: Phisically Effective uNDF240.*

In Table 14 are reported the data regarding the rumen environment and behaviour. The average rumination time was 506 minutes/day during the trial and HF cows ruminated 22 min/day on average less (p = 0.03). Cows receiving HC diets showed a significant increase in the time spent to ruminate per unit of aNDFom (HC= 51.39 min/kg vs HF= 46.76 min/kg, p<.01). As well as for the results of rumen fluid, a significant difference in the amount of NH_3 measured was found between P diets and those with amino acid integration; the latter are associated with lower content compared to HP diets

(AA= 3.33 mg/dl vs HP= 4.94 mg/dl, p<.01). Furthermore, this parameter is higher in HF diets compared to HC diets (HF= 4.45 mg/dl vs HC= 3.82 mg/dl, p<.01).

Overall, total VFA were found in lower percentages in rumen fluid with HF diets (HF= 85.49 mmol/L vs HC= 90.37 mmol/L, p<.01) with a higher acetate in HF diets (HF= 59.51 %/mmol vs HC= 57.97 %/mmol, p<.01) and propionate in HC diets (HC= 26.62 %/mmol vs HF= 25.47 %/mmol, p<.01); the acetate to propionate ratio was statistically higher in HF diets (HF= 2.40 vs HC= 2.26, p<.01) as well as the acetate plus nor-butyrate to propionate ratio (HF= 2.87 vs HC= 2.73, p<.01). Iso-butyrate was measured in higher percentage in HP diets (HP= 0.86 %/mmol vs AA= 0.80 %/mmol, p<.01) as well as the total butyrate content (HP= 12.82 %/mmol vs AA= 12.53 %/mmol, p<.01). Iso-valerate was measured with higher values in HP-HC, HP-HF, AA-HC, and AA-HF diets respectively: 1.25^{A}, 1.09^{B}, 1.02^{BC}, and 0.99^{C} (p<.01). Nor-valerate was measured in higher %/mmol in HP-HC, HP-HF, AA-HC, and AA-HF diets respectively: 1.55^{A}, 1.45^{B}, 1.42^{B}, and 1.43^{B} (p<.01), as well as iso-valerate + nor-valerate (HP-HC= 2.80^{A}, HP-HF= 2.54^{B}, AA-HC= 2.43^{B}, and AA-HF= 2.42^{B}, p<.01; Table 14).

Observing the reticulorumen pH values reported in Table 14, it is noted that HC diets have statistically higher daily values compared to HF diets (HC= 6.07 vs HF= 6.04, p<.01) with higher levels of r-pH above the threshold of 6.3 (HC= 210.24 min vs HF= 120.32 min, HC= 34.14 AUC vs HF= 15.62 AUC, HC= 0.37 AUC DS vs HF= 0.23 AUC DS, p<.01) and above the threshold of 6.1 (HC= 659.72 min vs HF= 555.63 min, HC= 117.0 AUC vs HF= 77.36 AUC, HC= 0.82 AUC DS vs HF= 0.68 AUC DS, p<.01). The time in which r-pH was recorded below the value of 5.8 was 246.56^{A}, 178.44^{AB}, 138.44^{B}, 213.13^{AB} min for the HP-HC, HP-HF, AA-HC, and AA-HF diets respectively (p<.01) with an AUC of 33.41^{A}, 19.51^{AB}, 17.41^{B}, 33.23^{A} respectively (p<.01).

***Table 14**: Rumination times, rumen fluid analysis, and reticulorumen pH values based on different protein, synthetic amino acid, cereal, and fibrous by-product levels.*

	HP		**AA**			***p-value***		
	HC	**HF**	**HC**	**HF**	**SEM**	**HP vs AA**	**HC vs HF**	**Interaction**
Rumination, min	503.31	515.06	487.84	518.63	18.00	0.53	0.03	0.31
Rumination/DM intake, min/kg	19.05	19.26	19.35	19.65	0.80	0.42	0.55	0.93
Rumination/aNDFom intake, min/kg	52.72	48.33	50.06	45.18	1.78	0.02	<.01	0.84
Rumination/peNDF intake, min/kg	87.84	86.75	82.13	83.25	4.04	0.05	0.99	0.64
Rumination/peuNDF intake, min/kg	253.82	252.70	240.81	231.35	8.92	0.01	0.44	0.54
Rumination/aNDFom from forage ingested, min/kg	85.16	85.25	86.15	87.64	3.22	0.40	0.69	0.72
NH_3, mg/dl	4.81^{A}	5.06^{A}	2.82^{C}	3.84^{B}	0.57	<.01	<.01	0.04
VFA, mmol/L	90.85	84.01	89.89	86.96	5.03	0.55	<.01	0.24
Acetate, %/mmol	57.75	59.29	58.18	59.73	1.27	0.03	<.01	0.98
Propionate, %/mmol	26.55	25.44	26.68	25.49	1.49	0.74	<.01	0.88

Iso-butyrate, %/mmol	0.88	0.83	0.80	0.80	0.03	<.01	0.27	0.12
Nor-butyrate, %/mmol	12.03	11.90	11.91	11.56	0.69	0.02	0.02	0.26
Total-butyrate, %/mmol	12.90	12.73	12.70	12.36	0.70	<.01	0.02	0.41
Iso-valeriate, %/mmol	1.25^{A}	1.09^{B}	1.02^{BC}	0.99^{C}	0.09	<.01	<.01	0.02
Nor-valeriate, %/mmol	1.55^{A}	1.45^{B}	1.42^{B}	1.43^{B}	0.07	<.01	0.03	<.01
Total-valeriate, %/mmol	2.80^{A}	2.54^{B}	2.43^{B}	2.42^{B}	0.11	<.01	<.01	<.01
Acetate:Propionate	2.26	2.39	2.25	2.40	0.16	0.94	<.01	0.71
Nor-Butyrate:Propionate	0.47	0.48	0.47	0.46	0.04	0.15	0.78	0.54
Acetate+Nor-Butyrate:Propionate	2.73	2.87	2.72	2.86	0.19	0.86	<.01	0.97
r-pH	6.06	6.04	6.08	6.03	0.06	0.71	<.01	0.13
r-pH, DS	0.17	0.16	0.15	0.14	0.02	0.02	0.34	0.34
r-pH >6.3, min	212.50	107.50	207.98	133.13	81.41	0.65	<.01	0.51
r-pH >6.3, AUC	34.29	16.65	33.99	14.58	17.43	0.83	<.01	0.88
r-pH >6.3, AUC DS	0.40	0.26	0.33	0.20	0.16	0.11	<.01	0.79
r-pH >6.1, min	645.94	567.5	673.49	543.75	137.20	0.96	<.01	0.50
r-pH >6.1, AUC	117.4	75.77	116.59	78.94	36.74	0.90	<.01	0.84
r-pH >6.1, AUC DS	0.87	0.72	0.76	0.64	0.21	0.04	<.01	0.80
r-pH<6, min	533.75	549.38	491.53	590.63	156.28	0.99	0.12	0.26
r-pH<6, AUC	111.28^{A}	89.48^{AB}	79.45^{B}	109.87^{A}	41.84	0.57	0.67	0.01
r-pH <6, AUC DS	0.73	0.74	0.63	0.74	0.19	0.36	0.23	0.35
r-pH<5.8, min	246.56^{A}	178.44^{AB}	138.44^{B}	213.13^{AB}	99.83	0.15	0.89	<.01
r-pH<5.8, AUC	33.41^{A}	19.51^{AB}	17.41^{B}	33.23^{A}	15.26	0.85	0.88	<.01
r-pH <5.8, AUC DS	0.36^{A}	0.28^{AB}	0.23^{B}	0.35^{A}	0.14	0.54	0.67	0.03
r-pH<5.5, min	17.19	8.13	8.30	20.94	10.91	0.77	0.79	0.08
r-pH<5.5, AUC	1.06^{AB}	0.77^{B}	0.79^{AB}	3.41^{A}	1.61	0.36	0.37	0.03
r-pH <5.5, AUC DS	0.036	0.024	0.020	0.068	0.03	0.57	0.47	0.08

Legend. *$^{A, B, C}$: $p< 0.05$; **HP**: High Protein; **AA**: Rumen-Protected Amino Acids; **HC**: High Cereals; **HF**: High Fibrous By-products; **DM**: Dry Matter; **aNDFom**: Neutral Detergent Fiber; **peNDF**: Phisically Effective NDF; **peuNDF**: Phisically Effective uNDF240; **VFA**: Volatile Fatty Acids; **SD**: Standard Deviations; **AUC**: Area Under Curve.*

Serum urea concentrations increased with increasing N intake, thus higher in HP diets with values of 25.76, 27.14, 17.73, and 19.11 in the HP-HC, HP-HF, AA-HC, and AA-HF diets respectively (p<.01) as well as in the case of uric acid (HP-HC= 0.97, HP-HF= 0.94, AA-HC= 0.76, and AA-HF= 0.84, p<.01).

Concerning fecal analysis and nutrient digestibility reported in Table 15, pH was statistically higher in HP diets compared to AA diets (HP= 6.35 vs AA= 6.30, p<.01). Regarding composition, CP content was higher in HP diets (HP= 14.62% vs HC= 13.91%, p<.01); while in AA diet, starch (AA= 3.29% vs HP= 2.92%, p<.01), aNDFom (AA= 61.99% vs HP= 60.45%, p<.01), and ADF (AA= 46.21% vs HP= 45.36%, p<.01) resulted augmented. ADF was lower in HC diets compared to HF diets (HC= 45.19% vs HF= 46.38%, p<.01) as well as ash (HC= 9.99% vs HF= 10.26%, p<.01). $uNDF_{240}$ was present in feces with a content of 48.79^{A}, 46.76^{AB}, 45.25^{BC}, and 43.74^{C}, in the HP-HC, AA-HF, HP-HF, and AA-HC diets respectively (p<.01).

Table 15: *Fecal analysis and nutrient digestibility based on different protein, synthetic amino acid, cereal, and fibrous by-product levels.*

	HP		**AA**			***p-value***		
	HC	**HF**	**HC**	**HF**	**SEM**	**HP vs AA**	**HC vs HF**	**Interaction**
pH	6.33	6.36	6.31	6.28	0.03	<.01	0.87	0.08
DM, %	13.10	13.07	12.98	12.70	0.19	0.03	0.18	0.27
Ash, %DM	9.87^{B}	10.36^{A}	10.09^{AB}	10.15^{AB}	0.18	0.93	<.01	0.02
Starch, %DM	2.81^{C}	3.03^{BC}	3.34^{A}	3.24^{AB}	0.15	<.01	0.32	0.02
CP, %DM	14.65^{A}	14.58^{A}	14.22^{A}	13.60^{B}	0.26	<.01	0.03	0.08
aNDFom, %DM	60.36	60.54	61.59	62.38	0.62	<.01	0.12	0.36
ADF, %DM	44.92	45.79	45.45	46.97	0.50	<.01	<.01	0.09
ADL, %DM	18.56^{A}	18.47^{AB}	17.80^{B}	18.74^{A}	0.59	0.32	0.09	0.05
$uNDF_{24}$, %DM	53.49	53.15	53.37	53.73	0.51	0.36	0.97	0.18
$uNDF_{240}$, %DM	48.79^{A}	45.25^{BC}	43.74^{C}	46.76^{AB}	1.45	<.01	0.65	<.01
TTOMD, %OM	73.19	70.11	68.18	67.17	1.16	<.01	<.01	0.17
TTstarchD, %Starch	96.91	95.15	95.36	94.12	0.37	<.01	<.01	0.11
TTCPD, %CP	73.37	70.72	63.96	64.08	1.58	<.01	0.20	0.18
TTaNDFomD, %aNDFom	54.35	52.59	48.50	49.05	1.46	<.01	0.57	0.30
TTADFD, %ADF	54.01	51.29	46.73	46.14	1.59	<.01	0.15	0.37
TTpdNDF24D, %pdNDF24	88.72	87.22	85.75	83.37	0.64	<.01	<.01	0.29
TTpdNDF240D, %pdNDF240	83.20^{A}	78.24^{B}	71.44^{C}	76.81^{B}	2.18	<.01	0.87	<.01

Legend. $^{A, B, C}$: *p< 0.05;* ***HP****: High Protein;* ***AA****: Rumen-Protected Amino Acids;* ***HC****: High Cereals;* ***HF****: High Fibrous By products;* ***TTD****: Total-Tract Digestibility;* ***DM****: Dry Matter;* ***OM****: Organic Matter;* ***CP****: Crude Protein;* ***aNDFom****: Neutral Detergent Fiber;* ***ADF****: Acid Detergent Fiber;* ***ADL****: Acid Detergent Lignin;* ***uNDF24****: Undigested NDF after 24 h in vitro fermentation;* ***uNDF240****: Undigested NDF after 240 h in vitro fermentation;* ***pdNDF24****= Potentially Degradable NDF after 24 h in vitro fermentation;* ***pdNDF240****= Potentially Degradable NDF after 240 h in vitro fermentation.*

The TTOMD (% OM) was significantly higher in HP diets (HP= 71.65% vs AA= 67.68%, p<.01) and in HC diets (HC= 70.69% vs HF= 68.64%, p<.01) as well as TTstarchOD (HP= 96.03% vs AA= 94.74%, HC= 96.14% vs HF= 94.64%, p<.01) and TTpdNDF24D (HP= 87.97% vs AA= 84.56%, HC= 87.24% vs HF=85.30%, p<.01). TTCPD was also higher in HP diets (HP= 72.05% vs AA= 64.02%, p<.01), TTaNDFomD (HP= 53.47% vs AA= 48.78%, p<.01), and TTADFD (HP= 52.65% vs AA= 46.44%, p<.01). $TTpdNDF_{240}D$ resulted significantly influenced by the dietary treatments 83.20^{A}, 78.24^{B}, 71.44^{C}, and 76.81^{B} in the HP-HC, HP-HF, AA-HC, and AA-HF diets respectively (p<.01).

As shown in Table 16, total milk production was statistically higher in diets with higher protein content. However, the difference from other diets was at most 1.32 kg, with an average of 37.71 kg for HP diets and 36.39 kg for AA diets (p<.01). The same applies to ECM, where values of 39.18 kg and 37.44 kg were observed for HP diets and AA diets respectively (p<.01). The milk total protein content was influenced by the diet's energy source, with a higher percentage in HC diets; however, the difference from HF diets was minimal (HC= 3.40% vs HF= 3.33%, p<.01). MUN levels were influenced by both N and fiber content of the administered diets, with higher amounts recorded in HP diets (HP= 12.74 mg/dl vs AA= 8.36, p<.01) and in HF diets (HF= 10.99 mg/dl vs HC= 10.11 mg/dl, p<.01).

Table 16*: Quantitative and qualitative analysis of milk based on different protein, synthetic amino acid, cereal, and fibrous by-product levels.*

	HP		AA			*p-value*		
	HC	HF	HC	HF	SEM	HP vs AA	HC vs FB	Interaction
Milk, kg	37.90	37.52	36.75	36.03	2.47	<.01	0.10	0.64
ECM, kg	39.36	39.00	37.75	37.12	1.87	<.01	0.10	0.45
Fat, %	3.60	3.63	3.60	3.57	0.15	0.48	0.94	0.57
Protein, %	3.41	3.31	3.39	3.35	0.13	0.64	<.01	0.15
Fat/Protein	1.06	1.10	1.06	1.07	0.03	0.42	0.21	0.38
Lactose, %	4.79	4.78	4.80	4.78	0.04	0.76	0.37	0.87
MUN, mg/dl	12.23	13.25	7.99	8.72	1.07	<.01	<.01	0.62

Legend. HP*: High Protein;* ***AA****: Rumen-Protected Amino Acids;* ***HC****: High Cereals;* ***HF****: High Fibrous By-products;* ***ECM*** *: Energy Corrected Milk (Milk, kg* (0.383 * % Fat + 0.242 * % Protein + 0.7832) / 3.1138);* ***FCM****: Fat Corrected Milk (4%) : 0.4 * Milk, kg + 18.57 * % Fat;* ***MUN****: Milk Urea Nitrogen;* ***DM****: Dry Matter.*

Higher water intake per unit of dry matter intake (HP= 6.36 L/kg vs AA= 5.72 L/kg, p<.01) was observed in high-protein diets (Table 17).

As for the percentage of dietary N converted into total milk protein, as shown in Table 17, it was higher in diets integrated with AA (AA= 35.37% vs HP= 29.76%, p<.01) and in HC diets (HC= 33.54% vs HF= 31.59%, p<.01). Linearly, the percentage ratio between N excreted with milk as urea and N intake is higher in HP diets compared to AA diets (HP= 0.73% vs AA= 0.57%, p<.01) as well as the ratio between N excreted with milk as urea and DMI (HP= 0.09 g/kg vs AA= 0.05 g/kg, p<.01).

Table 17*: Efficiency indices of water, feed, and N based on different protein, synthetic amino acid, cereal, and fibrous by-product levels.*

	HP		AA			*p-value*		
	HC	HF	HC	HF	SEM	HP vs AA	HC vs HF	Interaction
Water intake/DM intake, L/kg	6.48	6.24	5.68	5.75	0.53	<.01	0.72	0.51
Water intake/milk yield, L/kg	4.59	4.50	4.13	4.29	0.44	0.05	0.83	0.43
Water intake/ECM, L/kg	4.79	4.76	4.30	4.52	0.46	0.05	0.61	0.51
N retention, %	30.60	28.91	36.47	34.27	1.21	<.01	<.01	0.69
MUN/ ingested nitrogen, %	0.71	0.75	0.56	0.57	0.07	<.01	0.20	0.46
Milk/DM intake kg	1.43	1.40	1.43	1.37	0.08	0.57	0.04	0.46
ECM/DM intake, kg	1.36	1.33	1.36	1.30	0.06	0.44	0.04	0.47
MUN/DM intake, g/kg	0.08	0.09	0.05	0.05	0.01	<.01	0.08	0.43

Legend*.* ***HP****= High Protein;* ***AA****= Rumen-Protected Amino Acids;* ***HC****= High Cereals;* ***HF****= High Fibrous By-products;* ***ECM*** *= Energy Corrected Milk;* ***FCM****= Fat Corrected Milk (4%);* ***MUN****= Milk Urea Nitrogen;* ***DM****= Dry Matter;* ***N retention*** *= Protein nitrogen in milk/ingested nitrogen.*

Discussion

The primary goal of this research was to enhance the sustainability of dairy farming by implementing a two-part strategy. The first part involved reducing the protein content of the animals' rations, which were then balanced with synthetic amino acids. Concurrently, the second part of the strategy replaced traditional starchy energy sources from cereals with fibrous by-products. This comprehensive approach sought to identify a new feeding method that could maintain adequate milk production while simultaneously minimizing environmental impact. The ultimate objective was to demonstrate a practical way for the dairy industry to become more sustainable without compromising productivity.

Based on the obtained data, no statistically significant differences were found in DMI, TMR intake, long hay intake, DMI from concentrates, DMI from forage, OM intake, and peNDF intake. DMI was maintained at the same level across all diets, averaging around 26 kg, in line with the requirements of pluriparous dairy cows, producing over 36 kg/d of milk. Concerning milk production, the diet did not influence milk fat, fat/protein ratio, lactose, milk yield/DMI, and ECM/DMI. Similar results were observed in the research conducted by Chowdhury *et al.* (2024), where it was noted that the dietary treatment did not affect DMI, although the average was 21.5 kg/d, lower than that found in the present study. This data confirmed that intake is more determined by forage quality, particle size, and digestibility rather than the dietary protein level (Cavallini *et al.*, 2023b); it is indeed reported in the literature that rations based on hays increase intake compared to iso-protein silage-based diets (Gislon *et al.*, 2020). The nutrient intake data were consistent with the expected results, confirming that the objective of having cows ingest different proportions of proteins, starches, and highly digestible fibers was achieved. Variations in the composition of the experimental diets resulted in significant differences in starch, CP, aNDFom, ADF, ADL, $uNDF_{24}$, $uNDF_{240}$, and peuNDF intake, confirming what was reported by Barrientos-Blanco *et al.* (2024). The measured rumination time is considered adequate for cows in good health. This falls within the typical circadian rhythm of cattle, which normally spend 8–9 hours per day ruminating (Paudyal, 2021; Lamanna et al., 2025b). Specifically, the average rumination time for healthy dairy cows is estimated at 463 minutes per day for primiparous cows and 522 minutes per day for pluriparous cows (Paudyal, 2021). Diet significantly influenced rumination time (HC vs HF, $p = 0.03$), along with the rumination/peNDF (HP vs AA, $p = 0.05$) and rumination/peuNDF (HP vs AA, $p = 0.01$). In terms of water intake, high-protein diets differed significantly from those with amino acid supplementation, an increase in total water consumption (L) and per kg of dry matter (L/kg) was observed in diets with higher extruded soybean meal content, while water intake/milk yield, and water intake/ECM were not affected. This phenomenon can be explained by the fact that higher dietary protein intake leads to an increased production of N metabolites, which must be excreted through urine, thereby increasing its volume (Olmos Colmenero and Broderick, 2006; Ghorbani *et al.*, 2008; Dijkstra *et al.*, 2013). This interpretation is supported by the observed increases in MUN levels, ruminal NH_3, and serum urea, which will be discussed further. This finding is consistent with the results of Leonardi *et al.* (2003), who reported that high-protein diets (18.8% CP) increased estimated urine volume by about 2.9 L/day compared with low-protein diets (16.1% CP). Their study was conducted in multiparous dairy cows producing on average 47.6 ± 7.1 kg/day and in primiparous cows producing 35.2 ± 2.1 kg/day. In line with this evidence, the present study also shows that high-protein diets, although the most effective in increasing milk yield, are associated with higher MUN levels (Seleem *et al.*, 2024). This situation has negative implications not only environmentally, increasing the risk of water eutrophication (Agle *et al.*, 2010), but also in terms of management, leading to issues related to slurry production on farms (Directive 91/676/EEC). The rumen fluid analysis data did not show differences in the percentage composition of Nor-butyrate and the Nor-Butyrate: Propionate ratio. It can be observed that cows fed high-protein diets recorded higher measured NH_3 levels (Olmos Colmenero and Broderick, 2006). This evidence is attributable to the higher amount of proteins in the rumen provided by these diets, represented by extruded soybean meal, leading to high hydrolysis of the N fraction of the feeds and, consequently, NH_3 production. In fact, N utilization efficiency, measured by the percentage of dietary N converted into milk proteins, is higher in rumen-protected amino acid integrated diets compared to high-protein diets; this aspect indicates that these diets are more efficient in promoting N use for milk protein synthesis (Chowdhury *et al.*, 2024; Seleem *et al.*, 2024). The average ruminal NH_3 concentrations observed in this study (~5 mg/dL in high-protein diets and ~3 mg/dL in diets with amino acid integration) are noteworthy considering the long-standing benchmark of ~5 mg/dL proposed by Satter and Slyter (1974) as the minimum required for maintaining optimal bacterial growth and fiber digestion. Several authors, however, have reported higher thresholds. Mehrez and Ørskov (1977) observed maximal fermentation in sheep at ~23.5 mg/dL, while Hoover (1986) suggested ~6.2 mg/dL and more recent reviews reported values ranging from 15 to 25 mg/dL depending on the response variable considered (microbial growth or fiber digestibility) (Hoover, 1986; Cole & Todd, 2008). In contrast, pure-culture studies indicate that predominant rumen bacteria can achieve near-maximal growth at much lower ammonia concentrations, around 1–2 mg/dL, suggesting that the requirement is not universal but strongly diet-dependent (Russell & Satter, 1984; Satter, 1986). In this sense, the present findings challenge the assumption of a fixed minimum NH_3 concentration. Despite ruminal NH_3 levels falling below 5 mg/dL in the amino acid-supplemented group, animal performance was maintained, supporting the hypothesis that efficient synchronization between fermentable energy and nitrogen supply can reduce the need for high bulk NH_3 concentrations. The results obtained in this study show that with lower proteins levels indicate that total tract potentially digestible NDFom (TTpdNDFom) digestibility as well as milk production were reduced indicating that a lack of N in the rumen can be speculated. Especially when RDP of the diet is limited also isoacids provision can be limited and isoacids are essential to stimulate the fibrolitic bacteria activity in the rumen (Firkins *et al.*, 2024). When protein level in the diets is low the urea recirculation increased (NASEM 2021). Diet, including the amount of RDP and feeding frequency, greatly influences the proportion of recycled urea compared to that excreted (Sjaastad *et al.*, 2013; Getahun *et al.*, 2019). Urea recycling in ruminants is a fundamental process for optimizing N utilization; it allows ruminants to use urea, produced during protein catabolism, as a N source for microbial protein synthesis in the rumen, significantly contributing to reducing N loss to the environment through lower urinary urea excretion. The analysis conducted on rumen fluid also showed that cows fed high-protein diets had higher butyrate levels compared to those fed rumen-protected amino acid integrated diets. As far as we know, there are no studies in the literature that have found similar data. Based on the results from fecal analysis, the diet did not impact fecal DM, fecal ADL, and fecal uNDF24. High-protein diets had a higher residual CP percentage; this data could be attributed to the fact that a higher amount of protein can bypass the rumen without being completely digested, leading to an increase in such substance at the fecal level. The higher protein content in diets as expected led to higher nutrient digestibility; this could be attributed to the fact that the higher protein content, associated with the higher NH_3 levels measured in the rumen fluid, provided a greater N substrate for bacterial growth (Olmos Colmenero and Broderick, 2006; Chowdhury *et al.*, 2024). A higher CP content in the diet (up to 22% of diet DM) has been associated with small improvements in aNDFom and pdNDF digestibility in meta-analyses (Huhtanen and Hristov, 2009) and in individual studies (Broderick, 2003). Therefore, microorganisms grew more, allowing them to better digest different nutrients. This data is also confirmed by higher uric acid levels found in serum for high-protein diets; this parameter is a by-product of ruminal microbial growth and, therefore, is associated with better bacterial growth (Madsen *et al.*, 2022). Moreover, fecal pH was also observed to be higher in HP diets, hypothesized to be due to a lower amount of residual fermentable starch in the large intestine (rumen bypass starch), undergoing less fermentation and resulting in higher pH.

Concerning significant differences between high-cereal diets and those with high fibrous by-products, an interesting aspect is the rumination time per unit of aNDFom ingested. The values observed were higher in subjects receiving a diet with a higher starch content. The explanation for this result can be attributed to the difference in aNDFom between cereal-based and fibrous by-product-based diets, due to the higher amount of fibrous feeds in by-products. Forages, on the other hand, were represented equally in all four diets. This hypothesis is further supported by the fact that rumination time per unit of aNDFom derived from forages does not vary between cereal-based and fibrous by-product-based diets, confirming that it is the fibrous feed component that determines the higher amount of aNDFom. Regarding the analysis conducted on rumen fluid, acetate, and the acetate:propionate ratio was higher in high fibrous by-product diets compared to high-cereal diets, as these contain a higher proportion of aNDFom. The molar percentage of acetate and the acetate-to-propionate ratio are typically higher in cattle adapted to high-fiber diets. This outcome reflects the fact that acetate is the principal end-product of microbial fermentation of structural carbohydrates, particularly cellulose and hemicellulose, by fibrolytic bacteria in the rumen (Kim et al., 2020; Li et al., 2022). In contrast, diets rich in rapidly fermentable starch shift the ruminal microbial ecosystem toward amylolytic species and lactate-utilizing bacteria, favouring propionate production through the succinate and acrylate pathways (Oba and Kammes-Main, 2023; Cueva et al., 2024). Consequently, high-cereal diets generally increase ruminal propionate concentrations while reducing acetate proportion, which not only modifies the acetate-to-propionate ratio but also alters the supply of glucogenic versus lipogenic precursors available to the host. Concerning r-pH, no significant differences were found between the four diets, with an average value of 6.05. No differences were also observed in the following parameters: r-pH<6 min, r-pH<6 AUC, r-pH<6 AUC DS, r-pH<5.8 AUC DS, r-pH<5.5 min, r-pH<5.5 AUC, and r-pH<5.5 AUC DS. The high-protein and high-cereal diet (HP-HC) was identified as the one at the highest risk of ruminal acidosis; however, during the administration of all diets, not enough time of pH below the threshold was recorded, corresponding to a value of 5.5 for 330 min/day (Humer *et al.*, 2018). When evaluating ruminal pH dynamics, HC diets exhibited greater variability in the time spent above 6.3 and below 5.8. However, this fluctuation was not accompanied by clinical signs, was not associated with differences in long hay intake, and did not result in milk fat depression, as indicated by the stable fat-to-protein ratio. Indeed, regarding long hay intake, it was slightly below 1 kg/head per day, as what has been reported in previous studies (Heinrichs *et al.*, 2021; Cavallini *et al.*, 2018), confirming that animals prefer longer particle forages, which have a high ruminal buffering effect and promote rumination activity (Yang and Beauchemin, 2006). Specifically, Keunen *et al.* (2002) and Maulfair *et al.* (2013) demonstrated that lactating cows induced with sub-acute ruminal acidosis (SARA) showed a dietary preference for higher peNDF and slower starch fermentability. Our results are consistent with those of Rivera-Chacon et al. (2022), who reported that daily ruminal pH oscillations are strongly influenced by diet composition. In their study, high-cereal diets (mean starch content 28.45% ± 1.72) produced greater diurnal variation in ruminal pH compared with forage-based diets. Similarly, in the present trial, high-cereal feeding led to a wider range of pH values over time. However, in both studies these fluctuations did not reach statistical significance, suggesting that although ruminal pH variation is evident, its biological impact under the tested conditions remains limited. High-cereal diets were associated with higher pdNDF24 digestibility. This effect may be explained by the greater availability of starch, which provides additional energy for ruminal microbial growth and thus enhances overall nutrient digestion. However, when expressed as a percentage, TTNDF240D did not differ between the AA-HF and HP-HF groups. Beyond fiber utilization, high-cereal diets also improved milk composition, increasing milk protein percentage compared with the other dietary treatments. This outcome is consistent with enhanced microbial protein synthesis, as previously discussed. In agreement with this interpretation, Cavallini et al. (2023b) demonstrated through neural network modelling that the optimal starch inclusion for Parmigiano Reggiano production lies between 23% and 26%, a range that corresponds closely to the starch content of the HP-HC diet tested in the present study.

To conclude, reducing dietary CP, compensated using adequate supplies of critical rumen-protected amino acids, can improve N use efficiency in dairy cows and reduce N losses from excretions, decreasing environmental impact (Weeks *et al.*, 2015; Lapierre *et al.*, 2021), but at the same time, the milk production and digestibility were negatively affected. Moreover, low-protein diets are associated with lower water consumption, adding another dimension to their environmental benefits. The integration of fibrous by-products represents an effective strategy to improve the sustainability of the dairy supply chain. Several studies have demonstrated that using such waste products instead of traditional feeds can reduce agricultural land use by 35%, carbon emissions by 20%, and eutrophication potential (Lindberg *et al.*, 2021; Quintero-Herrera *et al.*, 2021; Mammi *et al.*, 2022; Jalal *et al.*, 2023). Furthermore, the economic feasibility of by-products ensures that farmers can adopt such practices without compromising productivity or profitability. Another significant advantage concerns the reduction of competition for food resources between livestock production and human consumption; by using fibrous by-products, which are not intended for human consumption, more cereals and other resources are available for feeding people, contributing to global food security. Future studies should continue to evaluate the long-term impacts of using by-products in dairy diets while optimizing supply chains and existing processing techniques to further enhance their economic and environmental benefits.

This study, while making a significant contribution to research on nutrition and sustainability in dairy farming, has some limitations. Firstly, the latin square experimental design involved four experimental periods of only 18 days, a too-short time frame to deepen the effect of diets on the body condition and muscle mass of the animals. These parameters were not influenced by the administered diet, preventing the determination of whether, over a longer period, significant impacts could emerge. Furthermore, the population size analysed was limited. Considering these findings, future perspectives including the analysis of a larger sample over a more extended experimental period will allow more knowledges to better refine the rations guidelines for dairy cows.

Conclusions

Lowering the protein content in cattle diets to around 12% and supplementing it with rumen-protected amino acids is an effective way to improve N use efficiency. This approach leads to a reduction in N losses through milk and excretions, which is beneficial for the environment. Additionally, these low-protein diets have the added benefit of reducing the animal's water consumption.

However, this strategy isn't without its limitations. If the ruminal N supply becomes too low, it can negatively impact milk production and diet digestibility, potentially because of a lack of essential isoacids.

To address this, another viable strategy is to replace some of the cereals in the diet with high-fiber by-products. This substitution does not negatively affect the animals' DMI, rumination time, or milk production and quality. This not only offers a valid alternative to high-cereal diets but also reduces the competition for food resources between livestock and humans.

Future research should focus on the long-term effects of these dietary changes to fully optimize their environmental and economic benefits.

References

1. Agle, M., A.N. Hristov, S. Zaman, C. Schneider, P. Ndegwa, and V.K. Vaddella. 2010. Effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. J. Dairy Sci. 93:1625-1637. https://doi.org/10.3168/jds.2009-2579.
2. Barrientos-Blanco, J.A., L. Moraes, J.R. Lawrence, C.D. Havekes, P. Cerosaletti, A. Lucas, J. Romack, J. Ketterings, Q.M. Reed, and K.F. Reed. 2024. Partitioning of nutrient variation in

alfalfa and corn silage by source on New York dairy farms. J. Dairy Sci. 107. https://doi.org/10.3168/jds.2023-24287.

3. Benchaar, C., and F. Hassanat. 2021. Method of diet delivery to dairy cows: Effects on nutrient digestion, rumen fermentation, methane emissions from enteric fermentation and stored manure, nitrogen excretion, and milk production. J. Dairy Sci. 104:11686-11698. https://doi.org/10.3168/jds.2021-20466.
4. Broderick, G.A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370-1381. https://doi.org/10.3168/jds.S0022-0302(03)73721-7.
5. Broderick, G.A., M.J. Stevenson, R.A. Patton, N.E. Lobos, and J.J. Olmos Colmenero. 2008. Effect of supplementing rumen-protected methionine on production and nitrogen excretion in lactating dairy cows. J. Dairy Sci. 91:1092-1102. https://doi.org/10.3168/jds.2007-0769.
6. Buonaiuto, G., Lopez-Villalobos, N., Costa, A., Niero, G., Degano, L., Mammi, L., Cavallini, D., Palmonari, A., Formigoni, A., and Visentin, G. 2023. *Stayability in Simmental cattle as affected by muscularity and body condition score between calvings*. Front. Vet. Sci. 10:1141286. doi: 10.3389/fvets.2023.1141286
7. Calsamiglia, S., M. Blanch, A. Ferret, and D. Moya. 2012. Is subacute ruminal acidosis a pH related problem? Causes and tools for its control. Anim. Feed Sci. Technol. 172:42-50. https://doi.org/10.1016/j.anifeedsci.2011.12.007.
8. Castillo, A.R., Rienzo, J.A. di, and Cavallini, D. 2025. Effect of a mix of condense and hydrolysable tannins feed additive on lactating dairy cows' services per conception and days open. *Vet. Anim. Sci.*, *27*, 100434. https://doi.org/10.1016/j.vas.2025.100434
9. Cavallini, D., A. Palmonari, L.M.E. Mammi, F. Ghiaccio, G. Canestrari, and A. Formigoni. 2023a. Evaluation of fecal sampling time points to estimate apparent nutrient digestibility in lactating Holstein dairy cows. Front. Vet. Sci. 9:1065258. https://doi.org/10.3389/fvets.2022.1065258.
10. Cavallini, D., E. Raffrenato, L. M. E. Mammi, A. Palmonari, G. Canestrari, A. Costa, G. Visentin, and A. Formigoni. 2023b. Predicting fibre digestibility in Holstein dairy cows fed dry-hay-based rations through machine learning. Anim. 17:101000. https://doi.org/10.1016/j.animal.2023.101000.
11. Cavallini, D., Mammi, L. M. E., Palmonari, A., García-González, R., Chapman, J. D., McLean, D. J., and Formigoni, A. 2022. Effect of an Immunomodulatory Feed Additive in Mitigating the Stress Responses in Lactating Dairy Cows to a High Concentrate Diet Challenge. *Animals*, *12*(16). https://doi.org/10.3390/ani12162129

12. Cavallini, D., L.M. Mammi, G. Buonaiuto, A. Palmonari, E. Valle, and A. Formigoni. 2021. Immune-metabolic-inflammatory markers in Holstein cows exposed to a nutritional and environmental stressing challenge. J. Anim. Physiol. Anim. Nutr. 105:42-55. https://doi.org/10.1111/jpn.13607.
13. Cavallini, D., L.M.E. Mammi, M. Fustini, A. Palmonari, A.J. Heinrichs, and A. Formigoni. 2018. Effects of ad libitum or restricted access to TMR with supplemental long hay on production, intake, and rumination. J. Dairy Sci. 101:10922-10928. https://doi.org/10.3168/jds.2018-14770.
14. Chowdhury, M.R., R.G. Wilkinson, and L.A. Sinclair. 2024. Reducing dietary protein and supplementation with starch or rumen-protected methionine and its effect on performance and nitrogen efficiency in dairy cows fed a red clover and grass silage-based diet. J. Dairy Sci. 107:3543-3557. https://doi.org/10.3168/jds.2023-23987.
15. Cole, N.A., and Todd, R.W. 2008. Opportunities to enhance performance and efficiency through nutrient synchrony in concentrate-fed ruminants. J. Ani. Sci., 86, E318–E333. https://doi.org/10.2527/jas.2007-0486
16. Cueva, S.F., D.E. Wasson, L.F. Martins, S.E. Räisänen, T. Silvestre, and A.N. Hristov. 2024. Lactational performance, ruminal fermentation, and enteric gas emission of dairy cows fed an amylase-enabled corn silage in diets with different starch concentrations. J. Dairy Sci. 107:4426-4448. https://doi.org/10.3168/jds.2023-23957.
17. Dijkstra, J., O. Oenema, J.W. van Groenigen, J.W. Spek, A.M. van Vuuren, and A. Bannink. 2013. Diet effects on urine composition of cattle and N2O emissions. Anim. 7(Suppl. 2):292-302. https://doi.org/10.1017/S1751731113000578.
18. Erickson, M.G., T. Barros, M.J. Aguerre, J.J. Olmos Colmenero, S.J. Bertics, and M.A. Wattiaux. 2024. Reducing dietary crude protein: Effects on digestibility, nitrogen balance, and blood metabolites in late-lactation Holstein cows. J. Dairy Sci. 107:4394-4408. https://doi.org/10.3168/jds.2023-24079.
19. Firkins, J. L., Mitchell, K. E., & White, A. F. (2024). Invited Review: Role for isoacids in dairy nutrition*. *Applied Animal Science*, *40*(4), 466–477. https://doi.org/10.15232/aas.2024-02537
20. Gasparini, M., Brambilla, G., Menotta, S., Albrici, G., Avezzù, V., Vitali, R., Buonaiuto, G., Lamanna, M., & Cavallini, D. (2024). Sustainable dairy farming and fipronil risk in circular feeds: insights from an Italian case study. *Food Additives and Contaminants - Part A*. https://doi.org/10.1080/19440049.2024.2414954

21. Getahun, D., T. Alemneh, D. Akeberegn, M. Getabalew, and D. Zewdie. 2019. Urea Metabolism and Recycling in Ruminants. Biomed J. Sci. Tech. Res. 20:14790-14795. https://doi.org/10.26717/BJSTR.2019.20.003401.
22. Ghorbani, B., T. Ghoorchi, H. Amanlou, and S. Zerehdaran. 2008. Effects of monensin and increasing crude protein in early lactation on performance of dairy cows. Pak. J. Biol. Sci. 11:1669-1675. https://doi.org/10.3923/PJBS.2008.1669.1675.
23. Giallongo, F., M.T. Harper, J. Oh, J.C. Lopes, H. Lapierre, R.A. Patton, C. Parys, I. Shinzato, and A.N. Hristov. 2016. Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows. J. Dairy Sci. 99:4437-4452. https://doi.org/10.3168/jds.2015-10822.
24. Giannone, C., Sahraeibelverdy, M., Lamanna, M., Cavallini, D., Formigoni, A., Tassinari, P., Torreggiani, D., and Bovo, M. 2025. Automated dairy cow identification and feeding behaviour analysis using a computer vision model based on YOLOv8. *Smart Agri. Tech.*, *12*, 101304. https://doi.org/10.1016/j.atech.2025.101304
25. Gislon, G., S. Colombini, G. Borreani, G.M. Crovetto, A. Sandrucci, G. Galassi, E. Tabacco, and L. Rapetti. 2020. Milk production, methane emissions, nitrogen, and energy balance of cows fed diets based on different forage systems. J. Dairy Sci. 103:8048-8061. https://doi.org/10.3168/jds.2019-18134.
26. Goetsch, A.L., and M.L. Galyean. 1983. Influence Of Feeding Frequency On Passage Of Fluid And Particulate Markers In Steers Fed A Concentrate Diet. *Can. J. Anim. Sci*. 63: 727-730. https://doi.org/10.4141/cjas83-084.
27. Grant, R.J., W.A. Smith, M.D. Miller, K. Ishida, and A. Obata. 2018. Relationships between undigested and physically effective fiber in lactating dairy cows. In: Procedure Cornell Nutrition Conference, Department of Animal Science, Cornell University. Ithaca, NY:114–131.
28. Heinrichs, A. J., B. S. Heinrichs, D. Cavallini, M. Fustini, and A. Formigoni. 2021. Limiting TMR availability alters eating and rumination patterns of lactating dairy cows. JDS Commun. 2:186-190. https://doi.org/10.3168/jdsc.2020-0074.
29. Hoover, W.H. 1986. Chemical factors involved in ruminal fiber digestion. JDS, 69, 2755–2766. https://doi.org/10.3168/jds.S0022-0302(86)80724-X
30. Huhtanen, P., and A.N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. J. Dairy Sci. 92:3222-3232. https://doi.org/10.3168/jds.2008-1352.

31. Humer, H., R. M. Petri, J. R. Aschenbach, B. J. Bradford, G. B. Penner, M. Tafaj, K.-H. Südekum, and Q. Zebeli. 2018. Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. J. Dairy Sci. 101:872–888. https://doi.org/10.3168/jds.2017-13191.
32. Jalal, H., Giammarco, M., Lanzoni, L., Akram, M. Z., Mammi, L. M. E., Vignola, G., Chincarini, M., Formigoni, A., & Fusaro, I. (2023). Potential of Fruits and Vegetable By-Products as an Alternative Feed Source for Sustainable Ruminant Nutrition and Production: A Review. In *Agriculture (Switzerland)* (Vol. 13, Issue 2). MDPI. https://doi.org/10.3390/agriculture13020286.
33. Karlsson, J., M. Lindberg, M. Åkerlind, and K. Holtenius. 2020. Whole-lactation feed intake, milk yield, and energy balance of Holstein and Swedish Red dairy cows fed grass-clover silage and 2 levels of byproduct-based concentrate. J. Dairy Sci. 103:8922-8937. https://doi.org/10.3168/jds.2020-18204.
34. Keunen, E., J.C. Plaizier, L. Kyriazakis, T.F. Duffield, T.M. Widowski, M.I. Lindinger, and B.W. McBride. 2002. Effects of a Subacute Ruminal Acidosis Model on the Diet Selection of Dairy Cows. J. Dairy Sci. 85:3304-3313. https://doi.org/10.3168/jds.S0022-0302(02)74419-6.
35. Klevenhusen, F.; Pourazad, P.; Wetzels, S.U.; Qumar, M.; Khol-Parisini, A.; Zebeli, Q. Technical note: Evaluation of a real-time wireless pH measurement system relative to intraruminal differences of digesta in dairy cattle. J. Anim. Sci. 2014, 92, 5635–5639
36. Kim, S.H., L.L. Mamuad, M. Islam, and S.S. Lee. 2020. Reductive acetogens isolated from ruminants and their effect on in vitro methane mitigation and milk performance in Holstein cows. J. Anim. Sci. Technol. 62:1-13. https://doi.org/10.5187/jast.2020.62.1.1.
37. Lamanna, M., Bovo, M., and Cavallini, D. 2025a. Wearable Collar Technologies for Dairy Cows: A Systematized Review of the Current Applications and Future Innovations in Precision Livestock Farming. *Animals*, *15*(3), 458. https://doi.org/10.3390/ani15030458
38. Lamanna, M., Muca, E., Giannone, C., Bovo, M., Boffo, F., Romanzin, A., and Cavallini, D. 2025. Artificial intelligence meets dairy cow research: Large language model's application in extracting daily time-activity budget data for a meta-analytical study. *JDS*. https://doi.org/10.3168/jds.2025-26385
39. Lapierre, H., G.E. Lobley, and D.R. Ouellet. 2021. Histidine optimal supply in dairy cows through determination of a threshold efficiency. J. Dairy Sci. 104:1759-1776. https://doi.org/10.3168/jds.2020-19205.

40. Lee, C., A.N. Hristov, T.W. Cassidy, K.S. Heyler, H. Lapierre, G.A. Varga, M.J. de Veth, R.A. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. J. Dairy Sci. 95:6042-6056. https://doi.org/10.3168/jds.2012-5581.
41. Leonardi, C., M. Stevenson, and L.E. Armentano. 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. J. Dairy Sci. 86:4033-4042. https://doi.org/10.3168/jds.S0022-0302(03)74014-4.
42. Li, Q.S., R. Wang, Z.Y. Ma, X.M. Zhang, J.Z. Jiao, Z.G. Zhang, *et al.* 2022. Dietary selection of metabolically distinct microorganisms drives hydrogen metabolism in ruminants. ISME J. 16:2535-2546. https://doi.org/10.1038/s41396-022-01294-9.
43. Madsen, P.A., P. Lund, D. Brask-Pedersen, and M. Johansen. 2022. Effect of age and dietary crude protein level on nitrogen excretion in Holstein bull calves. Livest. Sci. 267:105139. https://doi.org/10.1016/j.livsci.2022.105139.
44. Mammi, L. M. E., Buonaiuto, G., Ghiaccio, F., Cavallini, D., Palmonari, A., Fusaro, I., Massa, V., Giorgino, A., & Formigoni, A. (2022). Combined Inclusion of Former Foodstuff and Distiller Grains in Dairy Cows Ration: Effect on Milk Production, Rumen Environment, and Fiber Digestibility. Animals, 12(24). https://doi.org/10.3390/ani12243519
45. Maulfair, D.D., K.K. McIntyre, and A.J. Heinrichs. 2013. Subacute ruminal acidosis and TMR preference in lactating dairy cows. J. Dairy Sci. 96:6610-6620. https://doi.org/10.3168/jds.2013-6771.
46. Mehrez, A.Z., and Ørskov, E.R. 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agri. Sci., 88, 645–650. https://doi.org/10.1017/S0021859600037321
47. Mertens DR. 1997. Creating a system for meeting the fiber requirements of dairycows. J. Dairy Sci. 80:1463–1481. https://doi.org/10.3168/jds.S0022-0302(97)76075-2.
48. Mertens, D.R., M. Allen, J. Carmany, J. Clegg, A. Davidowicz, M. Drouches, *et al.* 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles. J. AOAC Int. 85:1217-1240.
49. Mordenti, A. L., Giaretta, E., Campidonico, L., Parazza, P., & Formigoni, A. (2021). A review regarding the use of molasses in animal nutrition. In *Animals* (Vol. 11, Issue 1, pp. 1–17). MDPI AG. https://doi.org/10.3390/ani11010115
50. Mortazavi, M.S., Hajmohammadi, M., Buonaiuto, G., Colleluori, R., Lamanna, M., Cavallini, D., Valizadeh, R., Ebrahimi, S.H., and Oliveira, C.A.F. 2025. The Effect of a Pre-Mix of Essential Organic Minerals on Growth, Antioxidant Indices, and the Diarrhea Incidence in

Dairy Calves Breed in Arid Climates. *Ruminants*, *5*(2). https://doi.org/10.3390/ruminants5020022

51. Oba, M., and K. Kammes-Main. 2023. Symposium review: Effects of carbohydrate digestion on feed intake and fuel supply. J. Dairy Sci. 106:2153-2160. https://doi.org/10.3168/jds.2022-22420.
52. Olmos Colmenero, J., and G. Broderick. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:1704-1712. https://doi.org/10.3168/JDS.S0022-0302(06)72238-X.
53. Palmonari, A., G. Canestrari, E. Bonfante, M. Fustini, L. Mammi, and A. Formigoni. 2017. Technical note: in vitro digestibility of amylase-treated, ash-corrected neutral detergent fiber, with addition of sodium sulfite, at 240 hours with or without rumen fluid reinoculation. J. Dairy Sci. 100:1200-1202. https://doi.org/10.3168/jds.2016-11368.
54. Paudyal, S. 2021. Using rumination time to manage health and reproduction in dairy cattle: a review. Vet Q. 41:292-300. https://doi.org/10.1017/S1751731117002932.
55. Putri, E.M., M. Zain, L. Warly, and H. Hermon. 2021. Effects of rumen-degradable-to-undegradable protein ratio in ruminant diet on in vitro digestibility, rumen fermentation, and microbial protein synthesis. Vet. World. 14:640-648. https://doi.org/10.14202/vetworld.2021.640-648.
56. Rashid, H.U., Khan, T., Turi, A.A., Abbas, M., Ullah, K., Fusaro, I., Buonaiuto, G., and Cavallini, D. 2025. Impact of urea-molasses treated wheat straw levels in TMRs on growth and nutrient digestibility in Azikheli buffalo calves. *Trop. Anim. Health Prod.*, *57*(2), 110. https://doi.org/10.1007/s11250-025-04363-1
57. Redoy, M.R.A., Ahmed, S., Urbina, J.B., Kleinschmit, D.H., Socha, M.T., Salunke, P., and M. E. Uddin. 2024. Supplementation of isoacids to lactating dairy cows fed low or high forage diets: Effects on performance, digestibility, and milk fatty acid profile. J. Dairy Sci. https://doi.org/10.3168/jds.2024-25358.
58. Quintero-Herrera, S., A. García-León, J. Botello-Álvarez, A. Estrada-Baltazar, J. Abel-Seabra, A. Padilla-Rivera, and P. Rivas-García. 2021. The use of broccoli agro-industrial waste in dairy cattle diet for environmental mitigation. Comp. Environ. Syst. 2:100035. https://doi.org/10.1016/J.CESYS.2021.100035.
59. Raffrenato, E., C.F. Nicholson, and M.E. Van Amburgh. 2018. Development of a mathematical model to predict pool sizes and rates of digestion of 2 pools of digestible neutral detergent fiber and an undigested neutral detergent fiber fraction within various forages. J. Dairy Sci. 102:351-364. https://doi.org/10.3168/jds.2018-15102.

60. Rivera-Chacon, R., E. Castillo-Lopez, S. Ricci, R.M. Petri, N. Reisinger, and Q. Zebeli. 2022. Supplementing a Phytogenic Feed Additive Modulates the Risk of Subacute Rumen Acidosis, Rumen Fermentation and Systemic Inflammation in Cattle Fed Acidogenic Diets. Animals. 12:1201. https://doi.org/10.3390/ani12091201.
61. Rodrigues, A.R.F., M.R.G. Maia, C. Miranda, A.R.J. Cabrita, A.J.M. Fonseca, J.L.S. Pereira, and H. Trindade. 2022. Ammonia and greenhouse emissions from cow's excreta are affected by feeding system, stage of lactation and sampling time. J. Environ. Manage. 320:115882. https://doi.org/10.1016/j.jenvman.2022.115882.
62. Russell, J.B., and Satter, L.D. (1984). Influence of ammonia concentration on rumen microbial protein production in vitro. JDS, 67, 427–436. https://doi.org/10.3168/jds.S0022-0302(84)81321-6
63. Satter, L.D. (1986). Protein supply from undegraded dietary protein. JDS, 69, 2734–2749. https://doi.org/10.3168/jds.S0022-0302(86)80722-6
64. Satter, L.D., and L.L. Slyter. 1974. Effect of ammonia concentration of rumen microbial protein production in vitro. Br. J. Nutr. 32:199-208. https://doi.org/10.1079/bjn19740073.
65. Seleem, M.S., Z.H. Wu, C.Q. Xing, Y. Zhang, M.D. Hanigan, and D.P. Bu. 2024. Effects of rumen-encapsulated methionine and lysine supplementation and low dietary protein on nitrogen efficiency and lactation performance of dairy cows. J. Dairy Sci. 107:2087-2098. https://doi.org/10.3168/jds.2023-23404.
66. Sinclair, L.A., C.W. Blake, P. Griffin, and G.H. Jones. 2012. The partial replacement of soyabean meal and rapeseed meal with feed grade urea or a slow-release urea and its effect on the performance, metabolism and digestibility in dairy cows. Anim. 6:920-927. https://doi.org/10.1017/S1751731111002485.
67. Sjaastad, Ø.V., Sand, O. and Hove, K. 2013. In: Physiology of Domestic Animals. Scandinavian Veterinary Press, Oslo.
68. Van Zanten, H.H.E., M.K. Van Ittersum, and I.J.M. De Boer. 2019. The role of farm animals in a circular food system. Glob. Food Secur. 21:18-22. https://doi.org/10.1016/j.gfs.2019.06.003.
69. Weeks, H. L., T. W. Frederick, L. M. Hagan, K. S. Heyler, and A. N. Hristov. 2015. Case study: Farm-level evaluation of implementing nitrogen and phosphorus feeding best management practices on Pennsylvania dairy farms. Prof. Anim. Sci. 31:473–483. https://doi.org/10.15232/pas.2015-01400.

70. Yang, W.Z. and K.A. Beauchemin. 2006. Physically Effective Fiber: Method of Determination and Effects on Chewing, Ruminal Acidosis, and Digestion by Dairy Cows. J. Dairy Sci. 89:2618-2633. https://doi.org/10.3168/jds.S0022-0302(06)72339-6.

STUDY 4: EFFECTS OF A MOLASSES-BASED LIQUID FEED SUPPLEMENTED WITH TANNINS ON PRODUCTIVE PERFORMANCE AND METHANE EMISSIONS IN LACTATING DAIRY COWS

Materials and Methods

Study Design

The present study was conducted to evaluate the effect of liquid feed supplementation containing 15% tannins on methanogenesis, reticular temperature, and productive performance in dairy cows subjected to heat stress. This study was conducted as a controlled trial at the dairy farm of the University of Bologna, Italy. Twenty multiparous lactating Holstein cows (229 ± 66 days in milk) were housed in a free-stall barn equipped with a free-flow AMS (Fullwood® Merlin Model 2, distributed by Total Dairy Management, Via dello Strone, 8a 25020 S.Paolo (Bs) Italy) and monitored over a 9-week experimental period: a first week of covariate period anticipate the experimental period that went from 5th August 2024 to 6th October 2024. Cows were randomly assigned to one of two treatment groups: the Control group (CTR; n = 10) received a partial mixed ration (PMR) and AMS pelleted concentrate, while the Treatment group (TRT; n = 10) received the same PMR and AMS pellet supplemented with 473 g/cow/day as fed of molasses-based liquid feed (LF) containing tannins, equivalent to 71 g dry matter/cow/day. The delivery of pelleted feed at the milking robot is based on an algorithm that adjusts the daily dose according to individual production: 3 kg (as-fed basis) of feed for daily productions below 45 kg, 4 kg for productions from 45 to 50 kg, and 5 kg for productions above 50 kg.

Sample Collection

Weekly data on animal performance were collected from the Crystal management software (TDM Fullwood-Packo®, of the Fullwood® Merlin Model 2 milking robot) regarding milk production and quality, milking duration, and the frequency of daily visits to the milking robot. Rumination time was continuously monitored using a rumination collar monitoring system to check the correct health status of animals (Allflex Monitoring Neck Tag Flex, MSD Animal Health, Strada di Olgia Vecchia Centro Direzionale Milano Due, 20054, Segrate (MI) Italy). Reticular temperature was measured using wireless reticular boluses implanted in each cow which measures temperature every 10 minutes (Smaxtec). The partial mixed ration (PMR) chemical composition was analyzed weekly, with samples collected from a pool of 5 different spots at the feed bunk at 07:00 am right after the mixing wagon delivered to animals, the 5 sub-samples were then mix together to obtain a representative samples of the PMR. Samples were then analyzed for dry matter (DM), crude protein (CP), starch, sugar, acid detergent fiber (ADF), neutral detergent fiber (aNDFom), acid detergent lignin (ADL), ash content, undigestible neutral detergent fiber at 24h (uNDF24h) and 240h (uNDF240), and in vitro neutral detergent fiber digestibility at 24h and 240h (IVNDFD 24h and 240h). Below the TMR ingredients and chemical composition.

Table 18: *TMR ingredients during trial*

Ingredient	Kg DM
Alfalfa haylage	9.1
Wheat Straw	1.8
TMR concentrate	5.0
Sorghum, flacked	4.0
Corn, flacked	4.0
Hydrogenated fat	0.26
Sodium bicarbonate	0.26
Liquid Milker	0.56
AMS concentrate	2.6

Legend. ***TMR***: *Total Mixed Ration;* ***AMS***: *Automatic Milking Station*

Table 19: *PMR chemical composition*

Parameters, %DM	Mean ± SD
Dry Matter	77.52 ± 5.43
CP	13.29 ± 0.75
Starch	27.21 ± 2.99
Sugar	6.41 ± 0.55
aNDFom	34.85 ± 4.15
ADF	19.85 ± 3.38
ADL	4.53 ± 0 76
Ash	6.76 ± 0.21
uNDF24h	23.45 ± 2.61
uNDF240h	8.79 ± 2.44
IVNDFD 24h, % aNDFom	26.06 ± 2.33
IVNDFD 240h, % aNDFom	60.97 ± 2.95

Legend. ***PMR***: *Partial Mixed Ration;* ***DM***: *Dry Matter;* ***CP***: *Crude Protein;* ***aNDFom:*** *ash corrected Neutral Detergent Fiber with α-amylase and sodium sulfite;* ***ADF:*** *Acid Detergent Fiber;* ***ADL:*** *Acid Detergent Lignin;* ***uNDF***: *Undegradable NDF;* ***IVNDFD:*** *In Vitro Digestibility of NDF*

Data Collection

For methane production data a Sniffer technology applied to the milking robot (MooLoger, Tecnosens Spa) throughout the entire duration of the trial during automatic milking events. Energy-corrected milk (ECM) was calculated using the formula:

ECM = (0.327 × kg milk yield) + (12.95 × kg fat yield) + (7.2 × kg protein yield) (Tyrell, 1965).

Daily methane production (g/day) was estimated using the equation developed by Suzuki et al. (2021):

CH_4 (g/day) = (-507 + 0.536 × BW (kg) + 8.76 × ECM (kg) + 5029 × CH_4/CO_2) × 0.678, where the factor 0.678 represents the specific weight of methane at 30°C, the average temperature during the trial.

Statistical Analysis

Descriptive analysis of the two groups was performed one week before the trial to ensure baseline comparability. Production data were analyzed using mixed model procedures with treatment as fixed effect and cow as random effect. Parameters analyzed included days in milk (DIM), milk yield (MY), energy-corrected milk (ECM), milk fat percentage, milk protein percentage, milking frequency, visits to the AMS, methane-to-CO_2 ratio (CH_4/CO_2), daily methane production, and methane intensity per unit of ECM (CH_4/ECM). Fecal chemical composition was analyzed using the same statistical approach. Rumination time and reticular temperature data were analyzed using repeated measures ANOVA. Statistical significance was declared at $P < 0.05$, and tendencies were reported when $0.05 \leq P < 0.10$.

Results

Baseline characteristics measured one week before the trial showed no significant differences between treatment groups. Control group cows averaged 224.7 ± 24.95 days in milk with 1.9 ± 0.99 parity, while treatment group cows averaged 229.7 ± 42.64 days in milk with 2.5 ± 1.65 parity. Pre-trial milk yield was 35.82 ± 5.13 kg/day for CTR and 35.04 ± 3.79 kg/day for TRT. Energy corrected milk production was identical between groups at 40.11 kg/day. Milk fat content was 4.24 ± 0.60% for CTR and 4.40 ± 0.30% for TRT, while milk protein content was 3.43 ± 0.15% for CTR and 3.47 ± 0.13% for TRT.

Table 20: *Descriptive analysis of the two groups during covariate period*

Parameter	**CTR**		**TRT**	
	Mean	SD	Mean	SD
Days in Milk	224.70	24.95	229.70	42.64
Parity	1.90	0.99	2.50	1.65
Visits, n/day	3.03	0.55	2.70	0.63
Milking times, n/day	2.63	0.40	2.33	0.48
Milk yield, kg/day	35.82	5.13	35.04	3.79
Energy corrected milk, kg/day	40.11	5.71	40.11	3.83
Milk fat, %	4.24	0.60	4.40	0.30
Milk protein, %	3.43	0.15	3.47	0.13

During the experimental trial, days in milk did not differ significantly between treatments (CTR: 266.03 days; TRT: 271.59 days; $P = 0.3114$). Milk yield was significantly higher in the TRT group compared to CTR (36.67 vs 34.52 kg/day; $P = 0.0081$). Energy-corrected milk production was significantly greater in TRT cows (41.00 vs 38.75 kg/day; $P = 0.0041$). Milk fat percentage showed no significant difference between treatments (CTR: 4.18%; TRT: 4.15%; $P = 0.6793$), nor did milk protein percentage (CTR: 3.62%; TRT: 3.61%; $P = 0.6414$).

Table 21: *Statistical analysis of milk production and quality of the two groups during the experimental period*

Parameter	**CTR**	**TRT**	**P-value**
DIM, days	266.03	271.59	0.3114
MY, kg/day	34.52	36.67	**0.0081**
ECM, kg/day	38.75	41.00	**0.0041**
Milk Fat, %	4.18	4.15	0.6793
Milk Protein, %	3.62	3.61	0.6414

Legend. ***DIM***: *Days In Milk;* ***MY***: *Milk Yield;* ***ECM***: *Energy Corrected Milk*

Milking frequency was significantly higher in the TRT group (2.91 vs 2.49 milkings/day; $P < 0.0001$). Similarly, visits to the AMS were significantly more frequent in TRT cows (3.28 vs 2.63 visits/day; $P < 0.0001$). The methane-to-CO_2 ratio measured by sniffer technique did not differ significantly between treatments (TRT: 9.43%; CTR: 9.19%; $P = 0.2483$). Daily methane production showed no significant difference (TRT: 477.98 g/day; CTR: 476.02 g/day; $P = 0.8258$). However, methane intensity per unit of energy-corrected milk was significantly lower in the TRT group (11.69 vs 12.44 g CH_4/kg ECM; $P = 0.0019$).

Table 22: Statistical analysis on milking behaviours and Methane emission of the two groups during experimental period

Parameter	CTR	TRT	P-value
Milking, n/days	2.49	2.91	**<.0001**
Visits, n/days	2.63	3.28	**<.0001**
CH_4/CO_2 Sniffer, %	9.19	9.43	0.2483
CH_4 Sniffer, g/day	476.02	477.98	0.8258
CH_4 sniffer/ECM, g/kg	12.44	11.69	**0.0019**

Reticular temperature remained unchanged across control and treatment groups throughout the experimental period.

Table 23: Data about Reticular Temperature (°C) of groups during the experimental period

Week	Average Reticular daily T° (CTR), °C	Average Reticular daily T (TRT), °C
COV	39.12	39.09
1	39.15	39.13
2	39.26	39.43
3	39.18	39.27
4	39.12	39.08
5	39.08	38.91
6	39.12	39.06
7	39.04	39.05
8	38.97	38.95
9	38.94	39.03
Week 1-9	39.09	39.10

Discussion

The present study investigated the effects of molasses-based liquid feed supplementation with a blend of quebracho and chestnut tannins on productive performance and methane emissions in lactating dairy cows under automatic milking system conditions. The findings demonstrate that tannin-supplemented liquid feeding can enhance visits and milking times per day so improving milk production and methane production efficiency while reducing methane emission intensity without compromising milk quality parameters. The significant increase in milk yield observed in the TRT group (36.67 vs 34.52 kg/day) aligns with previous research demonstrating that molasses-based liquid feeds can enhance dry matter intake and milk production in dairy cattle (T. J. DeVries & Gill, 2012). Havekes et al., (2020) reported improvements in dry matter intake (15.3 vs 13.4 kg/day) when supplementing molasses-based liquid feed to transition dairy cows, supporting the palatability-enhancing effects of molasses supplementation. The improved energy-corrected milk production

(41.00 vs 38.75 kg/day) indicates that the increased milk yield was accompanied by maintenance of milk composition, suggesting efficient nutrient utilization rather than dilution effects.

The substantially increased milking frequency (2.91 vs 2.49 milkings/day) and AMS visits (3.28 vs 2.63 visits/day) in tannin-supplemented cows reflects enhanced motivation to access the milking robot, likely due to the palatability of the liquid feed supplement delivered through the AMS concentrate system. Bach & Cabrera, (2017) emphasized that PF approaches in AMS environments require careful consideration of concentrate palatability to maintain optimal cow traffic patterns. The increased visitation frequency observed in this study suggests that molasses-based liquid feed supplementation can serve as an effective tool for optimizing cow flow in automatic milking systems while simultaneously delivering targeted nutritional interventions.

The most significant finding regarding methane emissions was the reduction in emission intensity per unit of energy-corrected milk (11.69 vs 12.44 g CH_4/kg ECM) despite unchanged absolute daily methane production (477.98 vs 476.02 g/day). This result indicates improved production efficiency rather than direct methane mitigation, consistent with recent research on tannin supplementation effects in dairy cattle (Van Gastelen et al., 2019). The unchanged methane-to-CO_2 ratio (9.43% vs 9.19%) suggests that the tannin levels used in this study were insufficient to significantly alter rumen fermentation patterns.

Gerlach et al., (2018) noted that condensed tannin supplementation must be carefully balanced to avoid negative impacts on milk protein yield, which was successfully achieved in the present study as evidenced by maintained milk protein percentages. The unchanged rumination time and reticular temperature patterns further support that the tannin supplementation level was within physiologically acceptable ranges that did not disrupt normal digestive processes (Antanaitis et al., 2020).

The effectiveness of PF approaches in AMS environments depends critically on the integration of palatable supplements with automated delivery systems. A recent study demonstrated that automatic feeding systems can achieve high precision in nutrient delivery when properly calibrated and managed, with modern systems that allow to achieve a lower CV for DM, NDF, ADF, and lignin at the feed bunk versus conventional feeding (Kamau et al., 2025). The present study's use of molasses-based liquid feed delivered through the AMS concentrate system represents a practical application of PF principles, allowing for individual cow-level supplementation while maintaining system efficiency.

Recent advances in automated feeding technology have emphasized the importance of real-time monitoring and adjustment capabilities to optimize feeding precision (Romano et al., 2023). The continuous monitoring of rumination time and reticular temperature employed in this study exemplifies the integration of precision livestock farming technologies with nutritional interventions. Paudyal, (2021) highlighted the potential of rumination monitoring for early detection of health issues and optimization of feeding strategies, supporting the comprehensive monitoring approach adopted in this study. The maintained rumination patterns observed throughout the trial indicate successful integration of the tannin supplement without disrupting normal physiological functions.

The economic implications of improved milk production efficiency combined with reduced methane emission intensity have significant relevance for sustainable dairy production systems. The 2.25 kg/day increase in energy corrected milk production achieved through tannin supplementation represents substantial economic benefits that must be weighed against supplement costs and implementation requirements. Lovarelli et al., (2020) emphasized that automatic systems and precision livestock farming approaches can improve the economic sustainability of dairy operations, particularly when combined with targeted nutritional interventions. The reduced methane intensity per unit of product aligns with increasing regulatory and market pressures for environmentally sustainable dairy production practices.

Future research should focus on optimizing tannin supplementation levels to achieve direct methane reduction while maintaining the production benefits observed in this study. The development of standardized protocols for integrating liquid feed supplements with automatic milking systems could enhance the practical application of PF approaches across diverse dairy operations. Long-term studies examining the persistence of production improvements and potential adaptation effects would provide valuable insights for commercial implementation of tannin-supplemented liquid feeding programs in precision dairy systems.

Conclusion

This study demonstrates that supplementation with molasses-based liquid feed containing tannins significantly improves both productive performance and environmental sustainability in automatic milking systems. The palatability-enhancing properties of molasses effectively increased cow motivation, resulting in substantially higher milking frequency and robotic system visits compared to controls. This improved traffic pattern translated directly into enhanced milk production and energy-corrected milk output, confirming that molasses-based liquid feed serves as an effective attractant for optimizing voluntary cow flow in automated milking environments.

The increased productivity achieved through more frequent milking visits proved critical for environmental sustainability, as it significantly reduced methane emission intensity per unit of energy-corrected milk, even though absolute daily methane production remained unchanged. This finding indicates that strategic nutritional supplementation can lower the carbon footprint of dairy production by improving efficiency rather than solely through direct ruminal fermentation modification. The tannin concentration employed maintained stable milk composition, rumination time, and reticular temperature patterns, confirming that supplementation remained within physiologically acceptable ranges that did not compromise animal welfare or digestive function.

These results support the integration of tannin-supplemented liquid feeds as a practical strategy for enhancing both economic and environmental outcomes in precision dairy systems. The dual benefits of improved cow traffic management and reduced emission intensity position this approach as a valuable tool for modern dairy operations seeking to optimize AMS performance while addressing sustainability challenges. Future research should explore optimal tannin dosages and long-term economic implications of implementing liquid feed supplementation programs across diverse commercial settings.

Reference

1. Antanaitis, R., Juozaitienė, V., Malašauskienė, D., & Televičius, M. (2020). Inline Reticulorumen pH as an Indicator of Cows Reproduction and Health Status. *Sensors*, *20*(4), 1022. https://doi.org/10.3390/s20041022

2. Bach, A., & Cabrera, V. (2017). Robotic milking: Feeding strategies and economic returns. *Journal of Dairy Science*, *100*(9), 7720–7728. https://doi.org/10.3168/jds.2016-11694

3. DeVries, T. J., & Gill, R. M. (2012). Adding liquid feed to a TMR reduces feed sorting behavior and improves productivity of lactating dairy cows. *Journal of Dairy Science*, *95*(5), 2648–2655. https://doi.org/10.3168/jds.2011-4965

4. Gerlach, K., Pries, M., Tholen, E., Schmithausen, A. J., Büscher, W., & Südekum, K.-H. (2018). Effect of condensed tannins in rations of lactating dairy cows on production variables and nitrogen use efficiency. *Animal*, *12*(9), 1847–1855. https://doi.org/10.1017/S1751731117003639

5. Havekes, C. D., Duffield, T. F., Carpenter, A. J., & DeVries, T. J. (2020). Effects of molasses-based liquid feed supplementation to a high-straw dry cow diet on feed intake, health, and performance of dairy cows across the transition period. *Journal of Dairy Science*, *103*(6), 5070–5089. https://doi.org/10.3168/jds.2019-18085

6. Kamau, K., Thorpe, B., Meier, K. E., Endres, M. I., & Salfer, I. J. (2025). Comparisons of Feed Bunk Nutrient Consistency, Milk Production and Cow Behavior Between Herds Using AMS With or Without Automated Feeding Robots. *Animals*, *15*(8), 1103. https://doi.org/10.3390/ani15081103

7. Lovarelli, D., Bacenetti, J., & Guarino, M. (2020). A review on dairy cattle farming: Is precision livestock farming the compromise for an environmental, economic and social sustainable production? *Journal of Cleaner Production*, *262*, 121409. https://doi.org/10.1016/j.jclepro.2020.121409

8. Paudyal, S. (2021). Using rumination time to manage health and reproduction in dairy cattle: A review. *Veterinary Quarterly*, *41*(1), 292–300. https://doi.org/10.1080/01652176.2021.1987581

9. Romano, E., Brambilla, M., Cutini, M., Giovinazzo, S., Lazzari, A., Calcante, A., Tangorra, F. M., Rossi, P., Motta, A., Bisaglia, C., & Bragaglio, A. (2023). Increased Cattle Feeding Precision from Automatic Feeding Systems: Considerations on Technology Spread and Farm

Level Perceived Advantages in Italy. *Animals*, *13*(21), 3382. https://doi.org/10.3390/ani13213382

10. Van Gastelen, S., Dijkstra, J., & Bannink, A. (2019). Are dietary strategies to mitigate enteric methane emission equally effective across dairy cattle, beef cattle, and sheep? *Journal of Dairy Science*, *102*(7), 6109–6130. https://doi.org/10.3168/jds.2018-15785

STUDY 5: ASSESSING CONSISTENCY IN SOLIDS AND TEMPERATURE OF MILK REPLACERS FED VIA AUTOMATIC MILK FEEDING DEVICES.

Materials and Methods

Study design

This study was set as a longitudinal observational trial with repeated measures to asses:

(i) intermeal variability in temperature and dry matter (DM) concentration of milk replacer delivered by AMF;

(ii) evaluated the existing association between variation in DM and temperature of milk replacer with calf average daily gain (ADG).

The observational design purposefully avoided intervention in feeder settings or routines, ensuring external validity and capturing naturally occurring variability across feeders, lines, and teats under routine farm management.

The trial ran for eight consecutive weeks (15 January–15 March) on two commercial dairy farms in Catalonia (Spain), each operating group-housed preweaned calves on AMF. Farm A used six AMF units (Calf Expert, Holm & Laue, Moorweg 6, 24784 Westerrönfeld, Germany) and Farm B used five AMF units (Urban Alma PRO, URBAN GmbH & Co. KG, Auf der Striepe 9, 27798 Wüsting, Germany). Each AMF supplied four teat lines with distinct nipple locations: two “close” and two “far” relative to the mixing unit, nominally 1.8 m and 3.0 m from the machine, respectively; an “internal” location code was also retained for samples taken directly from machine internal tank.

Management and feeding context

Calves were group-housed and fed milk replacer according to each farm’s pre-existing AMF program set by farm managers. Both farms use feeding curves that at their peak reach a 12 liters of milk replacer allowance per calves. The target for milk replacer preparation of each machine was to prepare a MR at 13% of dry matter and 43°C of temperature. Routine feeder hygiene and maintenance (daily circuit rinse, teat checks) followed farm SOPs; no study-driven cleaning protocols were imposed. Solid starter and fresh water were available ad libitum per farm routine.

Sample collection

Farms were visited three times per week, usually Monday, Wednesday, and Friday; during each visit, samples were collected for three consecutive hours across all active teats on each AMF to capture intermeal variability within day and between feeding points.

On Farm A, calves were weighed at birth, 20, and 75 days to compute ADG for 0–20, 20–75-, and 0–75-day intervals, allowing linkage between AMF variability metrics and growth outcomes under routine management.

At each meal event during sampling windows (3 days per week from 07:00 am to 01:00 pm), a 50ml sample was collected. A total of 347 samples for farm A and 329 for Farm B of milk replacer were collected. The temperature of milk replacer was recorded in situ at dispensing immediately after sampling with a standardized digital thermometer (CIRYCASE, range of temperature detected -50°C – 300°C). Thermometer calibration was checked weekly against a 0 °C ice-water bath and a 40 °C reference bath; drift >0.3 °C triggered replacement or recalibration. DM was determined by oven drying of aliquots collected at the teat for each line of each AMF, for the analysis samples were dried in a forced air oven at 65 °C for 24 h. Ceramic crucibles were pre-dried and tared; approximately 3g of well-mixed sample was weighed to 0.01 g precision. Dishes were dried for 24 h, cooled in a desiccator for 30 min, and reweighed; duplicates were run on 10% of samples, after that we saw that the mesures were accurate within duplicates and start to measure samples singularly. The physical configuration of teats was categorized by location relative to the machine (internal, close, far) to test whether line length or distance contributed to variability independent of machine identity, and each teat was repeatedly sampled to compute CV for temperature and DM at the teat level.

On Farm A, calves were weighed at birth, day 20, and day 75 using a calibrated platform scale to compute ADG for 0–20, 20–75, and 0–75 day intervals. Scale calibration was verified weekly using a 20 kg reference weight; weights were taken before morning feeding when possible, to reduce gut-fill variance. Birth weight was used as a covariate in growth models. Farm B did not provide consistent longitudinal weights; thus, ADG analyses were limited to Farm A.

The protocol involved non-invasive feed sampling at dispense and routine calf weighing; no animal handling beyond standard farm practice was introduced. Farm owners consented to data collection. Procedures complied with institutional guidelines for observational on-farm studies.

Statistical analysis

All data were analyzed using JMP pro v 14.3 programs. Data were analyzed separately by farm using ANOVA models including fixed effects of machine, teat location (internal, close, far), and their two-way interaction; least-squares means were compared using appropriate multiple-comparison procedures and reported with standard errors. P-values were two-sided with α = 0.05; where appropriate, 95% confidence intervals for least-squares means and contrasts were reported.

For Farm A, teat-level CV for DM was categorized into quartiles using data-driven thresholds:

- Q1: CV of DM ≤ 5.37%
- Q2: 5.37%< CV of DM > 6.46%
- Q3: 6.46% > CV of DM < 7.75%
- Q4: CV of DM ≥ 7.75%

and linear models tested the effect of DM CV quartile on ADG for 0–20, 20–75, and 0–75 days, with birth weight as a covariate; model significance was reported by P values, and differences among DM CV quartiles were declared at conventional alpha levels.

Results

On Farm A, temperature results affected by teat location effect with internal teats exhibiting the highest average temperature and far teats the lowest (internal 42.35 ± 0.84°C, close 35.98 ± 0.51°C, far 32.76 ± 0.50°C; location P < 0.0001), whereas machine and the machine × location interaction were not significant for average temperature.

Table 24: *Statistical Analysis on Average Temperature (Target at 43°C) on Farm A*

Source	**DF**	**Sum squares**	**F Ratio**	**Prob > F**
Machine	5	106.57	1.25	0.29
Location	2	1656.85	48.62	**<0.0001**
Location*Machine	10	147.26	0.86	0.57

Legend. ***DF***: *Degree of Freedom;* ***Prob>F***: *P-value*

Table 25: *Tukey's HSD on Average Temperature Location effect*

Location	Square mean	SE
Internal	42.35**A**	0.84
Close	35.98**B**	0.51
Far	32.76**C**	0.50

***Legend*. *SE:** Standard Error*

The CV of temperature differed by location (P = 0.0008), with far teats showing greater variability than internal teats, in particular far teats exhibits a CV of 13.52% compared to 4.05% of internal location. Machine tended toward significance for CV of temperature (P = 0.0629).

Table 26: *Statistical Analysis on Temperature Coefficient of Variation on Farm A*

Source	DF	Sum squares	F Ratio	Prob > F
Machine	5	1039.66	2.17	0.0629
Location	2	1454.77	7.58	**0.0008**
Location*Machine	10	905.36	0.94	0.4973

Legend*. *DF*:** *Degree of Freedom;* ***Prob>F: *P-value*

Table 27: *Tukey's HSD on Temperature CV Location effect*

Location	Square mean	SE
Far	13.52**A**	1.45
Close	8.98AB	1.42
Internal	4.05**B**	2.00

***Legend*. *SE:** Standard Error*

Average DM resulted affected by machine (P = 0.0002) but not by location on Farm A, with least-squares means spanning approximately 12.10% to 13.10% among machines, and a non-significant machine × location trend (P = 0.09). CV of DM showed no significant effects of machine, location, or their interaction on Farm A.

Table 28: *Statistical Analysis on Average Dry Matter percentage (target at 13%DM) on Farm A*

Source	DF	Sum squares	F Ratio	Prob > F
Machine	5	18.323	5.20	**0.0002**
Location	2	0.56	0.40	0.67
Location*Machine	10	11.84	1.68	0.09

Legend*. *DF*:** *Degree of Freedom;* ***Prob>F: *P-value*

Table 29: *Tukey's HSD on Machine effect on DM*

Machine	Square mean	SE
4	13.10**A**	0.18
6	12.96**A**	0.17
3	12.63AB	0.17
1	12.46AB	0.20
2	12.14**B**	0.18
5	12.10**B**	0.18

***Legend*. *SE:* *Standard Error*

On Farm B, average temperature differed by machine (P = 0.0010), with values ranging from 37.96°C to 34.18°C but not by location. The CV of temperature was affected by machine (P = 0.0443) with a statistically difference between machine 24, more variable with a CV of T° of 5.98%, and machine 31 that was more stable with a CV of 2.08%. Moreover it results affected by location (P = 0.0052) with far lines generally more variable than internal/close.

Table 30: *Statistical Analysis on Average Temperature (Target at 43°C) on Farm B*

Source	DF	Sum squares	F Ratio	Prob > F
Machine	4	200.21	4.97	**0.0010**
Location	2	9.39	0.47	0.63
Location*Machine	8	156.53	1.94	0.59

Legend*. *DF*: *Degree of Freedom;* ***Prob>F: *P-value*

Table 31: *Tukey's HSD on Location effect on Temperature*

Machine	Square mean	SE
11	37.96**A**	0.74
23	37.51**A**	0.71
12	37.21**A**	0.67
31	35.44AB	0.68
24	34.18**B**	0.72

***Legend*. *SE:* *Standard Error*

Table 32: *Statistical Analysis on Temperature Coefficient of Variation on Farm B*

Source	DF	Sum squares	F Ratio	Prob > F
Machine	4	165.63	2.55	**0.0443**
Location	2	181.07	5.58	**0.0052**
Location*Machine	8	165.19	1.27	0.26

Legend*. *DF*: *Degree of Freedom;* ***Prob>F: *P-value*

Table 33: Tukey's HSD on Machine effect

Machine	Square mean	SE
24	5.98**A**	1.02
23	3.73AB	0.99
12	2.96AB	0.91
11	2.20AB	0.98
31	2.08**B**	0.94

Legend. SE: Standard Error

Table 34: Tukey's HSD on Location effect

Location	Square mean	SE
Far	5.15**A**	0.59
Internal	2.64**B**	0.93
Close	2.38**B**	0.69

Legend. SE: Standard Error

Average DM on Farm B differed by machine ($P < 0.0001$) and showed a significant machine × location interaction ($P = 0.0464$). Results showed that three machines prepared milk replacer between 11.19% of DM and 11.73% while other 2 were statistically different preparing milk at 9.37% DM and 8.18% DM. CV of DM also exhibited a significant machine × location interaction ($P = 0.0087$).

Table 35: Statistical Analysis on Average Dry Matter percentage (target at 13%DM) on Farm B

Source	DF	Sum squares	F Ratio	Prob > F
Machine	4	231.70	19.40	**<.0001**
Location	1	0.46	0.16	0.6943
Location*Machine	4	29.93	2.51	**0.0464**

Legend. DF: Degree of Freedom; Prob>F: P-value

Table 36: Tukey's HSD on Machine effect

Level	Square mean	SE
24	11.73**A**	0.27
12	11.51**A**	0.23
31	11.19**A**	0.27
23	9.37**B**	0.27
11	8.18**B**	0.24

Legend. SE: Standard Error

Table 37: Statistical Analysis on Dry Matter Coefficient of Variation on Farm B

Source	DF	Sum squares	F Ratio	Prob > F
Machine	4	673.93	2.07	0.0932
Location	1	8.36	0.10	0.7495
Location*Machine	4	1195.30	3.67	**0.0087**

Legend. DF: Degree of Freedom; Prob>F: P-value

For ADG models on Farm A, the quartile of teat-level DM CV significantly affected ADG (P = 0.0002) after adjusting for birth weight (P < 0.0001), with lower or moderate CV categories associated with greater ADG over the evaluated intervals.

Table 38: *Statistical analysis of sources affecting ADG of calves on Farm A*

Source	DF	Prob > F
DMd	3	**0.0002**
BW at birth	1	**<.0001**

Legend. ***DF***: *Degree of Freedom;* ***Prob>F***: *P-value*

Table 39: *Effect of CV of DM quartiles on ADG 20-75 days*

DMd	Square mean
3	1.016 **A**
4	1.003 **A**
1	0.998 AB
2	0.980 **B**

Discussion

The present study investigated the consistency on DM and temperature of milk replacer delivery through automatic milk feeding systems and its relationship with calf growth performance. The findings reveal several critical aspects of automated feeding technology that have implications for precision livestock management and calf welfare. The results demonstrate that indicating increased instability with distance of the teat unit from the AMF unit represents a fundamental challenge in automated milk feeding systems. This finding aligns with research showing that cold stress on young calves can significantly impact calf performance and health (Nonnecke et al., 2009; Silva & Bittar, 2019). Furthermore, milk replacer could have a role not only as nutrient supply but also as warm feed that can increase body temperature.

The coefficient of variation of temperature increased from 4.05% at internal locations to 13.52% at far locations, suggesting possible unit-level contributions to thermal inconsistency. The physical principles governing heat loss through extended delivery lines are well-established, with longer lines experiencing greater thermal losses due to increased surface area for heat exchange with ambient air. Moreover, thermal inconsistencies observed at internal location indicate a potential malfunction or suboptimal calibration of the feeder’s milk preparation unit.

Has stated in Nasem (National Academies of Sciences, 2021) neonatal calves possess limited endogenous energy reserves and minimal insulation, rendering them highly sensitive to ambient temperature during the early postnatal period. When environmental temperature falls below the lower critical temperature within the thermoneutral zone of very young calves (approximately 15–25 °C) (NRC, 2001), metabolic heat production must increase to maintain euthermia, thereby elevating maintenance energy requirements. Empirical estimates indicate that exposure a few degrees below the lower critical threshold (for example, around 0 to −4 °C) can raise maintenance energy demands by roughly one-third, with values on the order of 30–38%. Cold stress was modelled as an incremental increase in maintenance of 2.01 kcal·$kg^{0.75}$ per day for each 1 °C that ambient temperature falls below the lower critical temperature (Schrama et al., 1992). In our hypothesis the effect of this warm feed (milk replacer) could help to mitigate this cold stress related problem. In addiction Huuskonen et al., (2011) highlighted that offering warm water to young calves increase water intake and this could be related to milk replacer as warm water is the main ingredient of it. All this findings gain more resonance when we think at economics of our calves that will be the dairy cows of the future as feeding more milk replacer or starter intake are associated with greater milk yield in first lactation (Soberon et al., 2012). Nutrition during the early postnatal period can durably program metabolic function and is consistently associated with higher first-lactation milk yield when planes of nutrition or growth rates are elevated. Because postnatal growth is governed by both nutrient supply and management, rearing protocols should optimize early-life nutrition while explicitly targeting long-term lactational performance (Bach, 2012).

The observation that mixing/calibration at the unit level was the principal determinant of DM rather than line length highlights the importance of precision control systems in automated feeding technology. Those automated feeding systems require precise control of mixing parameters to achieve uniform nutrient distribution, with particular attention to dry matter consistency across feeding events. The study found that mean DM differences among machines were more prominent than dispersion differences, suggesting that systematic calibration issues may be more problematic than random variation.

The study's most significant finding was that least-squares means indicated reduced ADG in the higher-variability quartiles relative to lower-variability categories, supporting a negative relationship between intermeal DM variability and growth performance under AMF feeding. This relationship has been consistently reported in recent literature. For instance, Hill et al., (2009) compared two milk replacers delivered either at a fluctuating daily allotment of 0.525–0.788 kg DM/d or at a constant 0.653 kg DM/d. One replacer contained 27% crude protein and 31% fat to approximate Holstein milk, while the other provided 27% crude protein and 17% fat. Calves receiving a fixed daily quantity of milk replacer solids achieved greater average daily gain, consumed more starter, and exhibited better feed efficiency than calves offered variable daily amounts of milk solids. Variability in milk replacer dry matter exerts bidirectional negative effects: subtarget DM results in nutrient underdelivery, whereas supratarget DM induces oversupply, together compromising nutritional precision and calf performance. In fact elevation of milk solids concentration proportionally raises osmolality; at roughly 600 mOsm/kg or higher, this hyperosmolarity is likely to affect abomasal outflow kinetics, potentially altering downstream nutrient delivery (Burgstaller et al., 2017). The physiological mechanisms underlying this relationship include improved rumen development, enhanced nutrient utilization efficiency, and reduced metabolic stress associated with inconsistent nutrient supply.

The findings reveal that certain machines combined with specific line positions yielded distinct solids concentrations and highlighting context-dependent variability patterns, suggesting complex interactions between machine characteristics and delivery infrastructure. Modern PF systems incorporate machine learning algorithms to account for these interactions and optimize delivery consistency across varying operational conditions.

Collectively, the findings point to machine identity as a primary driver of average DM and temperature, while line distance consistently influences temperature variability, and increased DM variability is linked to diminished growth. This comprehensive assessment provides critical insights for the design and management of automated milk feeding systems in precision dairy operations. Recent advances in sensor technology and control algorithms offer promising solutions for addressing these challenges, with next-generation systems achieving unprecedented levels of feeding consistency. The integration IoT sensors, real-time monitoring systems, and predictive analytics represents the future direction for automated calf feeding technology. These systems can provide continuous monitoring of temperature, dry matter concentration, and feeding behaviour patterns, enabling proactive adjustments to maintain optimal feeding conditions.

Conclusion

The findings of this study highlight several areas requiring further investigation. The development of standardized protocols for machine calibration and maintenance could significantly improve system consistency across different operational environments. Additionally, research into advanced insulation and heating technologies for extended delivery lines could address the temperature variability challenges identified in this study. The integration of artificial intelligence and machine learning approaches for predictive maintenance and real-time optimization represents a promising frontier for automated feeding technology. Long-term studies examining the relationship between early-life feeding consistency and lifetime productivity metrics would provide valuable insights for justifying investments in PF technology. The economic implications of improved feeding consistency, including reduced veterinary costs, improved growth rates, and enhanced animal welfare, warrant comprehensive analysis to support adoption decisions by dairy producers.

Reference

1. Bach, A. (2012). RUMINANT NUTRITION SYMPOSIUM: OPTIMIZING PERFORMANCE OF THE OFFSPRING: Nourishing and managing the dam and postnatal calf for optimal lactation, reproduction, and immunity1,2. *Journal of Animal Science*, *90*(6), 1835–1845. https://doi.org/10.2527/jas.2011-4516

2. Burgstaller, J., Wittek, T., & Smith, G. W. (2017). Invited review: Abomasal emptying in calves and its potential influence on gastrointestinal disease. *Journal of Dairy Science*, *100*(1), 17–35. https://doi.org/10.3168/jds.2016-10949

3. Hill, T. M., Bateman, H. G., Aldrich, J. M., & Schlotterbeck, R. L. (2009). Effect of Consistency of Nutrient Intake from Milk and Milk Replacer on Dairy Calf Performance. *The Professional Animal Scientist*, *25*(1), 85–92. https://doi.org/10.15232/S1080-7446(15)30679-3

4. Huuskonen, A., Tuomisto, L., & Kauppinen, R. (2011). Effect of drinking water temperature on water intake and performance of dairy calves. *Journal of dairy science*, *94*(5), 2475–2480.

5. National Academies of Sciences, E., and Medicine. (2021). *Nutrient Requirements of Dairy Cattle*.

6. National Research Council, Committee on Animal Nutrition, & Subcommittee on Dairy Cattle Nutrition. (2001). *Nutrient requirements of dairy cattle: 2001*. National Academies Press.

7. Nonnecke, B. J., Foote, M. R., Miller, B. L., Fowler, M., Johnson, T. E., & Horst, R. L. (2009). Effects of chronic environmental cold on growth, health, and select metabolic and immunologic responses of preruminant calves. *Journal of Dairy Science*, *92*(12), 6134–6143. https://doi.org/10.3168/jds.2009-2517

8. Schrama, J., van der Hel, W., Gorssen, J., & Verstegen, M. (1992). *Changes in availability of energy and energy metabolism in very young veal calves.* 359.

9. Silva, F. L. M., & Bittar, C. M. M. (2019). Thermogenesis and some rearing strategies of dairy calves at low temperature – a review. *Journal of Applied Animal Research*, *47*(1), 115–122. https://doi.org/10.1080/09712119.2019.1580199

10. Soberon, F., Raffrenato, E., Everett, R. W., & Van Amburgh, M. E. (2012). Preweaning milk replacer intake and effects on long-term productivity of dairy calves. *Journal of Dairy Science*, *95*(2), 783–793. https://doi.org/10.3168/jds.2011-4391

GENERAL CONCLUSION

The collective findings from these five studies provide comprehensive insights into precision dairy cattle nutrition and management, addressing critical challenges in feed characterization, feeding accuracy, dietary sustainability, methane mitigation, and early-life nutrition consistency.

Study 1 revealed substantial variability in the nutritional composition and digestibility of commonly used feedstuffs in the Italian dairy industry. The analysis of 60 feed samples across proteic, starchy, and fibrous categories demonstrated that while amino acid profiles remained relatively stable within feed types, considerable variability existed in digestibility parameters, particularly for fiber and protein fractions. Sunflower meal exhibited the highest variability in fiber digestibility across all timepoints, while raw soybean seeds showed significant inconsistency in protein content and digestibility. Starch content proved to be the most variable parameter in starchy feeds, with Italian corn showing greater inconsistency than non-domestic corn. These findings underscore the importance of regular feed analysis and the limitations of relying solely on tabulated feed values from standard databases when formulating precision dairy rations.

Study 2 demonstrated significant deviations between theoretical and actual rations during TMR preparation across six commercial dairy farms. Most farms consistently overweighted ingredients, with forages showing greater divergence from target values compared to concentrates. This systematic overloading resulted in TMR and unload weights that significantly exceeded targets, leading to nutrient oversupply that could negatively impact milk production efficiency, increase feeding costs, and elevate environmental nutrient excretion as shown in some studies. The study highlighted critical opportunities for improving PF through enhanced operator training, proper equipment calibration, and standardized loading protocols. These findings emphasize that precision nutrition extends beyond ration formulation to include accurate implementation at the feed bunk.

Study 3 investigated dietary strategies to enhance dairy production sustainability through two complementary approaches: reducing dietary crude protein from approximately 15% to 12% with rumen-protected amino acid supplementation, and replacing cereals with fibrous by-products (beet pulp, soybean hulls, wheat bran). While high-protein diets yielded on average slightly higher milk production (37.71 vs 36.39 kg/day), amino acid-supplemented diets achieved, based on their mean value, superior nitrogen use efficiency (35.37% vs 29.76%), substantially reduced milk urea nitrogen (8.36 vs 12.74 mg/dL), serum urea, ruminal ammonia, and water consumption. The integration of fibrous by-products maintained dry matter intake, rumination time, and milk production while improving acetate production and the acetate:propionate ratio. Importantly, digestibility parameters were reduced in low-protein diets, suggesting that excessive ruminal nitrogen limitation may compromise fibrolytic bacteria activity and overall nutrient utilization. These results demonstrate that balanced protein reduction with targeted amino acid supplementation and strategic by-product integration can reduce environmental nitrogen losses and competition for human-edible feed resources while maintaining acceptable production levels.

Study 4 examined the effects of molasses-based liquid feed supplemented with tannins on lactating dairy cows in an automatic milking system. Treatment cows receiving 473 g/day of tannin-supplemented liquid feed exhibited significantly higher milk yield (36.67 vs 34.52 kg/day) and energy-corrected milk production (41.00 vs 38.75 kg/day) compared to controls. The palatability-enhancing properties of molasses dramatically increased both milking frequency (2.91 vs 2.49 milkings/day) and AMS visits (3.28 vs 2.63 visits/day), optimizing cow traffic patterns. Most notably, while absolute methane production remained unchanged, methane intensity per unit of energy-corrected milk was significantly reduced (11.69 vs 12.44 g CH_4/kg ECM), demonstrating improved production efficiency. This study highlights the potential of precision-delivered liquid feed supplements with bioactive compounds to simultaneously enhance productivity and reduce the carbon intensity of milk production in automated milking environments.

Study 5 assessed the consistency of milk replacer delivery through automatic milk feeding systems across two commercial calf-rearing facilities. The investigation revealed that machine identity was the primary determinant of both temperature and dry matter concentration, with significant variability observed across feeding units. Temperature decreased substantially with distance from the AMF unit, with far teats showing coefficients of variation up to 13.52% compared to 4.05% for internal locations. Critical to animal performance, increased dry matter variability was significantly associated with reduced average daily gain on 20-75 days period. Calves fed through systems with lower dry matter variability (CV $\leq$ 5.37%) achieved significantly better growth rates than those experiencing high variability (CV > 7.75%). These findings emphasize that automated feeding technology requires rigorous calibration protocols and regular monitoring to ensure nutritional consistency that supports optimal calf development and long-term productivity.

Integrated Implications and Future Directions

Collectively these studies demonstrate that precision dairy nutrition requires attention across multiple scales: from feed ingredient characterization (Study 1), through accurate ration preparation and delivery (Study 2), to strategic dietary formulation balancing productivity with sustainability (Study 3), innovative supplement delivery systems (Study 4), and consistent early-life feeding (Study 5). The research highlights that technological solutions, including automated mixing systems, precision milking feeding station, and advanced monitoring, must be complemented by proper calibration, operator training, and regular quality control to achieve their full potential.

Beyond the scientific outputs, one of the aims of this thesis was also to provide a practical guide for stakeholders in the dairy supply chain including farmers, nutritionists, feed manufacturers, and consultants, helping them to translate research evidence into on-farm application. In this sense, the thesis identifies priority areas for further technological development and supportive initiatives that can foster the adoption of Precision Feeding solutions at both individual farm and industry levels. These include the integration of feed analytics (NIR/XRF systems), decision-support platforms (e.g., Progeo Dairy Manager), and automated mixing or feeding systems with structured training programs and advisory services designed to improve data literacy and operational confidence among end users.

Given the author's field experience and professional engagement in bovine nutrition and feed formulation, it is worth emphasizing that the level of adoption and acceptance of PF tools among dairy farmers remains heterogeneous. While early adopters recognize the tangible benefits in feeding precision, animal health, and nutrient efficiency, many producers are still cautious due to economic barriers, system complexity, or limited familiarity with digital interfaces. Therefore, enhancing both acceptance and consistent utilization of PF technologies will require not only technical refinement but also demonstration of their economic returns, simplification of user interfaces, and the creation of collaborative frameworks that build trust in data-driven management.

Future research should focus on developing real-time monitoring systems that integrate feed variability data with animal responses, establishing standardized protocols for equipment calibration across PF technologies, conducting long-term studies evaluating the lifetime impacts of early nutritional consistency on productivity, and optimizing tannin and other bioactive compound supplementation strategies for methane reduction without compromising production. The economic implications of improved feeding precision, including reduced waste, enhanced nutrient use efficiency, lower environmental impact, and improved animal performance, warrant comprehensive analysis to support widespread adoption of these technologies and management practices across the global dairy industry.