

**Improving Nitrogen Efficiency and Reducing Environmental Nitrogen in
Dairy Cow Diets**

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Professional Studies in Animal Science — Animal Nutrition Concentration

By Junwei Zhu

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BIOGRAPHICAL SKETCH

Junwei Zhu was born and raised in Sichuan, China. He received his Bachelor of Science in Animal Science from Southwest University. He has learned a lot about animal nutrition, physiology and feed science during his undergraduate life. He participated in a municipal-level project about oligosaccharide fermentation and piglet gut microbiota. He became interested in nutrient metabolism and the environment.

Junwei enrolled in the Master of Professional Studies (MPS) in Animal Science in 2024. He planned to concentrate on animal nutrition. He received basic animal science education during his undergraduate study, including a course in dairy cattle nutrition, companion animal nutrition, reproductive physiology, statistics and analysis methods.

Under the supervision of Mike Van Amburgh, Junwei did his capstone project on improving nitrogen use efficiency in dairy cow diets using CNCPS modeling. He focused on balancing the necessary amino acids and minimizing urinary nitrogen excretion without affecting milk yield.

Junwei also gained hands-on experience through his roles at the Cornell University Ruminant Center and the Goat Breeding Farm of Chongqing Herbivore Research Center, contributing to dairy and small ruminant nutritional management and sampling protocols. He is passionate about advancing precision feeding strategies to improve animal productivity while minimizing environmental impact and aspires to work as a professional feed formulator in the livestock nutrition industry.

This project is dedicated to the mentors and collaborators who have contributed to the advancement of dairy nutrition modeling and nitrogen efficiency research. Their dedication to scientific rigor and sustainable livestock production continues to inspire the next generation of animal nutritionists.

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LIST OF ABBREVIATIONS

- NUE - Nitrogen use efficiency
- UUN - Urinary urea nitrogen
- CP - Crude protein
- RDP - Rumen degradable protein
- NH₃ - Ammonia
- CNCPS - Cornell Net Carbohydrate and Protein System
- PM_{2.5} - Particulate matter in air that is less than 2.5 µm in diameter
- MP - Metabolizable protein
- BCVFA - Branched-chain volatile fatty acid
- ME - Metabolizable energy
- AA - Amino acid
- RUP - Rumen undegradable protein
- NPN - Non-protein nitrogen
- N - Nitrogen
- EAA - Essential amino acids
- MUN - Milk urea nitrogen
- VFA - Volatile fatty acid
- Lys - Lysine
- Met - Methionine
- His - Histidine
- RPL - rumen-protected Lysine

RPM - rumen-protected Methionine

GIT - Gastrointestinal tract

TLRs - Toll-like receptors

EMPS - Efficiency of microbial protein synthesis

OMTD - Organic matter truly digested in rumen

ABSTRACT

Improving dairy cattle nitrogen use efficiency (NUE) is important for both environmental and production economics perspectives. In this project we used CNCPS based modelling via AMTS software to investigate dietary approaches to improve NUE through better AA supply and reducing excess RDP to the hindgut. In Step 1a, three diets differing only in Met supply (0.86, 1.06 and 1.20 g/Mcal ME) were simulated using milk production data from Danese et al. (2023). As expected, milk yield was similar across diets while milk composition improved with increasing Met supply. Productive N increased slightly in response to the higher yields and urinary N increased in response to the higher protein supply, but NUE increased overall. In Step 1b, a diet formulated to contain 1.20 g Met/Mcal ME was balanced and used to simulate increased milk yield and milk true protein output, which led to an increased productive N and reduced urinary N, improving NUE. In Step 2, dietary urea was removed from the high Met diet to reduce RDP. This resulted in a large reduction in rumen NH₃ levels and urinary N losses while maintaining milk output and productive N, improving NUE further. Overall, the results indicate that utilizing AA balancing (Met, Lys and His) and reducing rumen NH₃ and RDP can be used to greatly improve N partitioning and environmental N efficiency in dairy cow diets.

INTRODUCTION

Ammonia emissions from agriculture pose a serious environmental concern as they contribute to air and water pollution through nitrogen (N) deposition and nitrate leaching^[1]. Recent satellite-based analyses confirm that livestock in general, particularly dairy farms, are among the most significant point sources of ammonia^[2]. In dairy production, significant N losses occur primarily through urinary excretion and subsequent ammonia volatilization, contributing to greenhouse gas emissions and soil and water contamination. These inefficiencies not only harm the environment but also represent a loss of economic value in the form of unused dietary N. Improving nitrogen use efficiency (NUE) has become an essential objective in modern dairy nutrition.

Nitrogen is essential for microbial growth and milk protein synthesis in dairy cows; however, a large proportion of dietary N is excreted, in contrast to what is converted to productive outcomes. Theoretical estimates suggest that the NUE in dairy cows could reach 40–45%, yet on-farm values often average around 25%, which is much lower than monogastric animals like pigs, which can exceed 60%^[3-5]. This low N efficiency results in higher feed costs and on farm N loading in the form of urinary urea nitrogen (UUN) and ammonia emissions.

One of the and most significant contributors to low N efficiency in dairy cattle is the overfeeding of crude protein (CP), especially rumen degradable protein (RDP), which leads to a surplus of rumen ammonia (NH₃). The excess NH₃ is rapidly converted to urea in the liver and excreted in urine, with little contribution to milk protein. Additionally, dairy producers' concerns about underfeeding protein, and its potential effects on yield, milk protein percentage, reproductive performance, and body condition typically cause more careful feeding practices where excess N is wasted. However, research has demonstrated that when essential amino acids, such as methionine

and lysine, are sufficient and balanced with sufficient fermentable energy, dietary N can be utilized more efficiently without depressing milk production [6].

Recent advances in nutritional modeling, such as the Cornell Net Carbohydrate and Protein System (CNCPS) (Van Amburgh et al., 2015; Higgs et al., 2015)[6], offer powerful tools to evaluate and optimize dietary formulations. The CNCPS enables precise predictions of microbial protein yield, rumen N dynamics, metabolizable protein (MP) flow, and amino acid (AA) balance[6]. Such parameters allow nutritionists to identify whether microbial growth is limited by fermentable energy, degradable N, or isoacids, and adjust diets according to these parameters.

Furthermore, formulation with the CNCPS can simulate the effects of different protein combinations and AA profiles under varying rumen NH₃ levels while maintaining equal metabolizable energy (ME) supply. Through such evaluations, it becomes possible to test the boundaries of low N feeding and assess its impact on key indicators such as urinary N excretion, productive N: urinary N ratio, microbial protein synthesis, and overall NUE. The present project adopts a formulation approach to investigate how strategic manipulation of dietary protein—specifically the balance between RDP, rumen undegradable protein (RUP), Methionine, Lysine and Histidine—can reduce N losses while sustaining or improving milk yield in mid-lactation Holstein cows.

By applying the CNCPS v6.5.6 model, this project aims to investigate the possibility of developing lower-N, AA-optimized diets that enhance NUE and reduce environmental N excretion for a more sustainable dairy production system at a lower cost.

LITERATURE REVIEW

Agricultural Ammonia Emissions and Dairy Production

On a global basis, NH_3 emissions from agriculture are a growing concern due to their dual environmental and public health impacts. Once released into the atmosphere, ammonia reacts with acidic compounds to form secondary fine particulate matter ($\text{PM}_{2.5}$), contributing to air pollution and increasing the risk of respiratory and cardiovascular diseases^[7].

Globally, agriculture accounts for over 81% of anthropogenic ammonia emissions, and within this sector, livestock manure management—particularly from cattle—is the predominant source^[7]. Of all the livestock species, dairy and beef cattle are responsible for the greatest contribution to NH_3 emissions due to their large body size, high N content in their diets, and high manure output. Cattle manure typically has higher pH and urea content than manure from monogastric animals, which promotes more ammonia volatilization. As dairy production systems intensify globally, particularly in high-income and emerging economies, the scale of these emissions is expected to increase if not well managed.

Satellite-based analyses offer an independent confirmation of agriculture's dominant role in global ammonia pollution (Figure 1). Van Damme et al. (2018) analyzed satellite retrievals to identify ammonia "hotspots" worldwide, many of which correspond to areas of intensive dairy and beef cattle production activities. These hotspots include the Po Valley (Italy), the Central Valley (California, USA), and the North China Plain, all of which have dense ruminant livestock production and high N losses.^[2, 8] These findings suggest a strong spatial correlation between cattle production and ammonia pollution.

Further support comes from emissions inventories such as EDGAR v6.1^[9] (the Emissions Database for Global Atmospheric Research, developed by the European Commission's Joint Research Centre), which compiles harmonized global data on greenhouse gases and air pollutants from multiple statistical and scientific sources. Its standardized methodology allows for consistent comparisons across countries and sectors. EDGAR data (Figure 2) shows that agriculture remains the overwhelming driver of NH₃ emissions in nearly all regions, with manure-related emissions increasing steadily over the past decades^[9]. These trends underscore the need to address N losses in dairy systems.

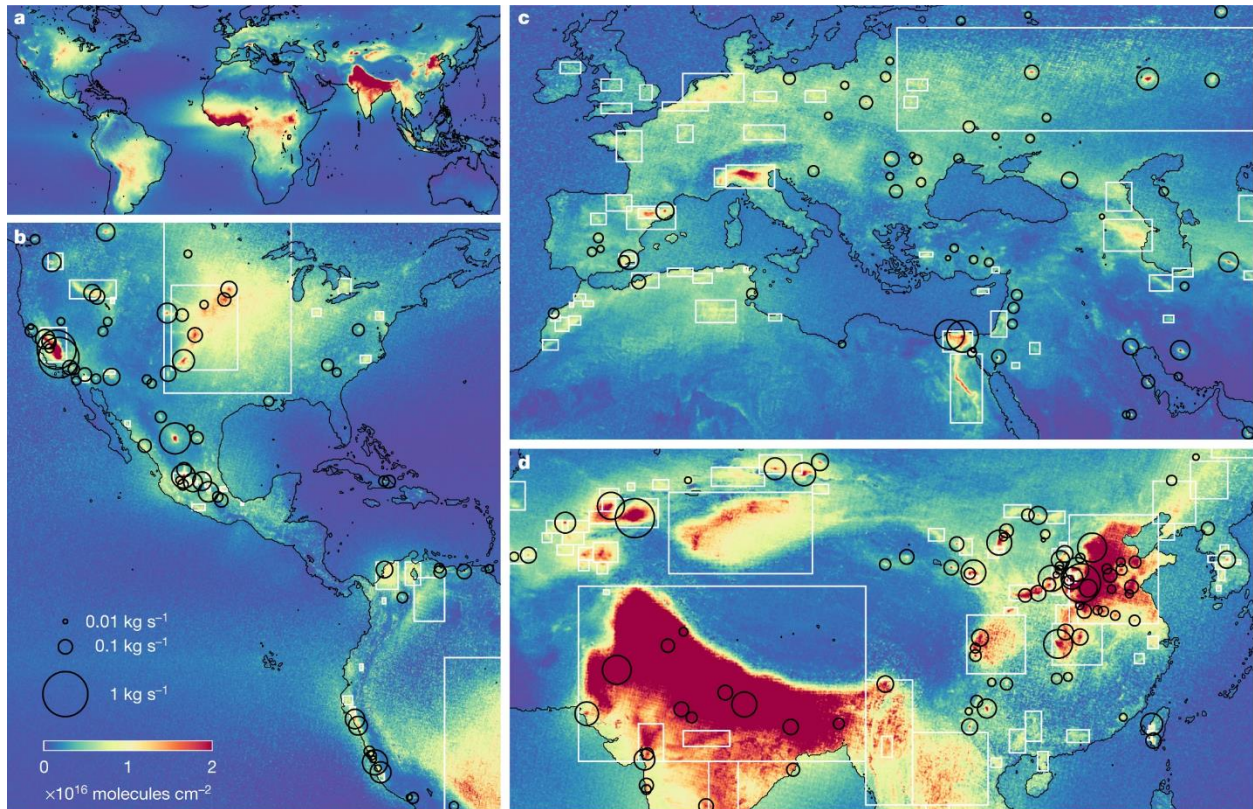


Figure 1. Global distribution of ammonia (NH_3) emission hotspots as observed by satellite.

Panels show total column NH_3 concentrations ($\times 10^{16}$ molecules cm^{-2}) derived from IASI satellite data, with color intensity indicating NH_3 abundance. Circle markers represent detected point sources scaled by emission rate (kg s^{-1}).

Panel a: Global overview; b: North and Central America; c: Europe and North Africa; d: South and East Asia.

Adapted from Van Damme, M., et al. (2018). Nature, 564, 99–103.

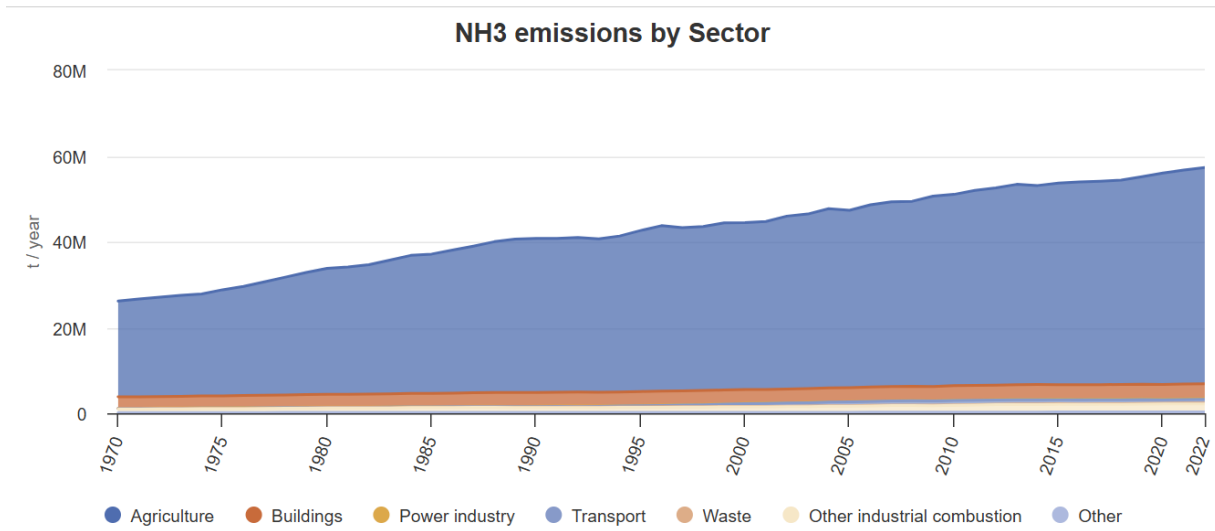


Figure 2. Global ammonia (NH₃) emissions by the economic sector from 1970 to 2022.

Agriculture remains the dominant source of NH₃ emissions, with which emissions have steadily risen in the last 50 years.

Data were taken from EDGAR v6.1, European Commission Joint Research Centre.

Nitrogen Flow in the Rumen

Nitrogen is one of the costliest and closely managed components in dairy cow nutrition, due to its essential role in supporting milk production. Past inefficient utilization by agricultural and manure management practices has resulted in excessive N deposition into ecosystems, with significant environmental impacts. In ruminants, N metabolism begins in the rumen, where N is introduced from both exogenous and endogenous sources. Exogenous N is primarily derived from dietary CP and non-protein nitrogen (NPN) sources such as urea, typically found in feed ingredients including legume forages (e.g., alfalfa silage), oilseed meals (e.g., soybean and canola meal), and protein-rich byproducts like distillers grains^[10, 11]. Endogenous N originates from urea recycled to the rumen via saliva or diffusion across the rumen wall, as well as from sloughed rumen epithelial and microbial cells^[12].

Once in the rumen, N exists in several chemical forms: intact protein, peptides, free AA, and NH_3 . The portion of feed protein that is subject to microbial degradation is known as rumen-degradable protein (RDP). Through microbial enzymatic activity, RDP is hydrolyzed into peptides and AA, which are then deaminated to generate NH_3 ^[11]. Ammonia becomes a key N source for rumen microbial protein synthesis, provided that sufficient fermentable carbohydrates and BCVFAs are concurrently available to fuel microbial growth.^[13]

Microbial protein synthesized in the rumen is later digested in the small intestine and represents the dominant source of MP for the host animal. Thus, maximizing microbial protein yield is a core objective in ruminant nutrition, contributing directly to milk protein synthesis while reducing N excretion^[14].

However, when dietary RDP supply exceeds the microbial capacity to assimilate ammonia—often due to excess CP or limited fermentable energy—ruminal NH_3 accumulates. This excess

ammonia diffuses across the rumen epithelium into the bloodstream, where it is converted into urea by the liver. Urea is either excreted via urine or partially recycled to the gastrointestinal tract [15, 16]. Urea in urine accounts for 50-90% of urinary N. 4-41% of the N in urine is volatilized, while only 1–13% of the N in feces is volatilized and lost^[17]. Therefore, urine is the main route of N loss in animals and the main source of environmental N emissions such as ammonia volatilization^[12, 18].

Nitrogen Use Efficiency

In dairy farming, NUE is a useful parameter to assess how much dietary N intake is converted into useful animal products, such as milk protein or N retained as body weight gain. It is an important metric to estimate protein nutritional efficiency and environmental impact. At the individual animal level, NUE is commonly defined as:

$$NUE(\%) = (milk\ N + growth\ N) \times 100 / N\ intake^{[4]}$$

A high NUE means that a greater proportion of N is used for milk protein synthesis in the mammary gland, and less is lost through urine and feces, resulting in better feed conversion efficiency and reduced environmental burden. However, according to Kohn, the average NUE in dairy cows is only 23%, which is substantially lower than that of other livestock species, such as pigs (35%), and exhibits considerable variation across studies^[4]. This means that approximately three-quarters of the dietary N is wasted rather than utilized. More concerningly, despite decades of research advancements between the 1960s and 2008, NUE in U.S. dairy cows has shown no significant improvement^[19, 20].

Given the environmental implications of N excretion, dietary strategies to reduce N losses are of increasing interest in dairy nutrition. One widely adopted approach is to reduce the CP content of the diet while balancing MP and essential amino acids (EAA).

A field case study applying the updated Cornell Net Carbohydrate and Protein System (CNCPS v6.1) to two commercial dairy farms in New York demonstrated the practical effectiveness of this strategy. Diets were reformulated to reduce CP by approximately 1% of dietary dry matter. The results were dramatic: milk and protein yields remained unchanged on both farms, milk urea nitrogen (MUN) decreased by 1.5 and 3.4 mg/dL, respectively, and urinary N excretion decreased by 26 and 46 g/d, respectively, while fecal and milk N output remained essentially unchanged^[21].

These results are consistent with broader trends observed across studies evaluating N partitioning. As shown in Figure 3, reductions in N intake—primarily via decreased dietary CP—are strongly associated with reduced urinary N excretion. At the same time, N output in milk and feces remains relatively stable. This pattern supports the strategic focus on dietary CP management to enhance NUE and mitigate environmentally labile N losses.

According to a nationwide survey, 69% of U.S. dairy nutritionists reported formulating diets with lower crude protein than 3–5 years ago. The primary motivation was to reduce feed costs (25.4%) and improve the balance of MP, RDP, or EAA (19.4%). Notably, 89% of respondents considered methionine requirements when formulating rations, and over half acknowledged that AA balancing facilitated lower CP levels. These results highlight an industry-wide shift toward precision protein nutrition.^[22]

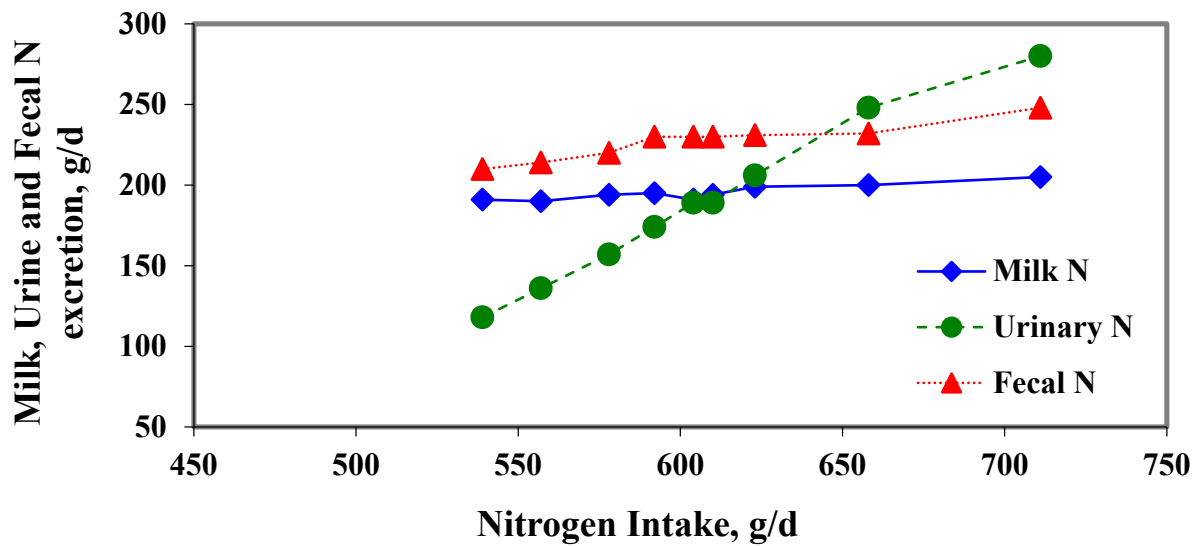


Figure 3. Effects of nitrogen intake on nitrogen partitioning in lactating dairy cows.

This figure illustrates how N consumed through the diet is partitioned among milk, urine, and feces. As dietary N intake increases, urinary N excretion greatly rises, while N excretion via milk and feces remains relatively stable, which means urinary N is the major route of N loss, indicating a potential nutritional strategy of reducing CP to improve NUE without compromising productive N^[6].

Reproduced from Van Amburgh et al. (2015), Journal of Dairy Science, 98:6361–6380, under the terms of the CC BY-NC-ND 3.0 license.

Amino Acid Balance

High-producing dairy cows have specific and heightened requirements for EAAs, particularly Lysine (Lys), Methionine (Met), and Histidine (His) which are widely recognized as the first-, second- and third limiting AAs in typical dairy diets^[23]. Recent modeling data suggest that dietary Met supply should approach 1.15–1.20 grams per megacalorie of metabolizable energy (g/Mcal ME) for optimal milk protein synthesis and N efficiency^[6]. The Lys value should be 3.2 g/Mcal ME or 2.7 times the grams of Met and His should be equal to Met to meet the requirements for lactating dairy cattle (Higgs et al, 2023). When the supply of these AAs is insufficient or imbalanced, it can lead to inefficient N use, altered nutrient partitioning, and suboptimal milk protein synthesis. However, achieving adequate post-ruminal delivery of Lys and Met is challenging, as unprotected forms are prone to degradation by rumen microbes. To address this, commercial rumen-protected Lys (RPL) and Met (RPM) supplements have been developed, allowing more consistent delivery to the small intestine and potentially supporting improved lactation outcomes^[24, 25].

Despite these technological advances, the response to RPL and RPM varies considerably among studies. Factors such as the basal diet composition, cow physiological status, and the bioavailability of the protected AAs contribute to inconsistent outcomes^[24]. Some studies have found that supplementing Met alone yields little to no improvement in milk yield, likely because Lys remains the first limiting AA in those situations^[24, 26, 27]. As a result, combined supplementation with both RPL and RPM is generally more effective at achieving a balanced EAA profile and enhancing production responses. Meta-analyses suggest that cows are more responsive to RPL than RPM alone, particularly when diets are deficient in Lys or when cows are in early lactation and facing elevated metabolic demands^[28].

The mammary gland is the primary site of EAA utilization during lactation, yet the efficiency with which different AAs are transferred from absorption to milk protein varies. For instance, Lys is rapidly taken up by the mammary tissue, especially under conditions of dietary deficiency, which explains why plasma Lys levels often remain unchanged following RPL supplementation—it is immediately directed toward mammary protein synthesis^[29-31]. Similarly, variation in the bioavailability of different RPL and RPM products, largely driven by coating materials and protection methods, can influence their effectiveness^[28, 32].

Recent evidence has reinforced the value of targeted AA supplementation. In early-lactation cows, increasing intestinally absorbable RPL or RPLM was associated with linear gains in milk production, feed efficiency, and milk protein yield^[33]. Notably, the predictive power of combined RPLM supplementation was greater than RPL alone, suggesting that delivering a more complete AA profile may be key to maximizing performance^[28]. These effects are particularly pronounced when MP supply is marginal or when energy balance is high, highlighting the importance of timing and nutritional context in AA interventions.

Rumen Microbiota

The rumen harbors an extremely complex and diverse microbial ecosystem, composed mainly of bacteria, protozoa, fungi, and archaea^[34], which plays a central role in nutrient acquisition and N transformation in ruminants. Rumen microbes convert RDP and NPN sources into microbial protein, which becomes the primary source of MP for the host animal.

Beyond fermentation, some microbial metabolites may modulate the host's AA pool and N turnover through interactions with gastrointestinal tract (GIT) receptors. For instance, Toll-like receptors (TLRs) in the gut epithelium can detect bacterial lipopolysaccharides and lipopeptides,

activating signaling cascades (e.g., TLR4, TLR5, and TLR9) that trigger the secretion of flagellins and bioactive peptides such as cholecystokinin, which in turn influence dietary N digestion and absorption^[35].

Supporting this, Ribeiro reported that repeated inoculation of cattle rumen with bison rumen contents enhanced protein digestibility and N retention, while altering the profile of ruminal volatile fatty acids.^[36]

Despite variability in host species, dietary composition, and environmental conditions, ruminants such as cattle consistently harbor a relatively stable "core rumen microbiome", primarily composed of *Firmicutes* (e.g., *Ruminococcus*, *Butyrivibrio*) and *Bacteroidetes* (notably *Prevotella*)^[37, 38]. High-throughput sequencing of the 16S rRNA gene has confirmed *Prevotella*, *Butyrivibrio*, and *Ruminococcus* as dominant genera in the rumen, each with specialized contributions to N metabolism. *Prevotella* spp., which often account for 42–60% of total bacterial rRNA gene copies, are particularly associated with proteolysis and ammonia production^[38-40], however, its species-level functional diversity remains poorly characterized in high-producing dairy cows. *Fibrobacter* and *Butyrivibrio* are more involved in fiber degradation and microbial AA biosynthesis. *Fibrobacter succinogenes* and *Ruminococcus albus*, in particular, possess superior cellulolytic capacity and enhance volatile fatty acid (VFA) production, thereby stimulating microbial proliferation and N capture.^[41]

Importantly, this core community exhibits a high degree of functional redundancy, whereby multiple microbial taxa can perform overlapping N-transforming functions^[42], such as ammonia capture, proteolysis, and microbial AA biosynthesis. This redundancy enhances the stability of N metabolism under conditions of dietary or microbial fluctuation^[43]. Notably, besides bacteria, rumen protozoa also play a pivotal role in N and carbohydrate metabolism. Williams and Coleman

observed that apparent N digestibility was positively correlated with protozoal counts and the relative abundance of the bacterial family *Christensenellaceae*, but negatively associated with the BS11 gut group, suggesting a broader microbial involvement in NUE regulation^[44].

Microbial Protein Synthesis

Microbial protein synthesis in the rumen is central to N metabolism in dairy cows. Rumen microbes convert carbohydrate and nitrogenous substrates into microbial biomass, which serves as a significant source of MP for the host animal for its high digestibility and balanced AA profile^[34, 40]. Efficient microbial protein synthesis relies on the synchronized availability of fermentable carbohydrate and N—mostly in the form of ammonia derived from RDP—and fermentable carbohydrates. BCVFAs, either synthesized from AA fermentation or supplied from the diet, are essential for the growth of some microbial populations, especially cellulolytic bacteria.

The Efficiency of microbial protein synthesis is commonly expressed as grams of microbial N per kilogram of organic matter truly digested in the rumen (OMTD), typically ranging from 25 to 32 g N/kg OMTD under optimal conditions^[45]. However, several nutritional and ruminal factors may reduce this efficiency:

(a) An imbalance between N and energy supply—such as excess ammonia with limited fermentable energy—can lead to inefficient microbial growth and increased N loss via ammonia absorption. (b) Low ruminal pH, often resulting from high-starch diets, suppresses cellulolytic bacteria and microbial diversity, impairing fiber digestion and protein synthesis. (c) Rapid rumen passage rates reduce microbial retention time and limit biomass accumulation. (d) Deficiencies in microbial growth precursors such as BCVFAs, sulfur, or ammonia may also restrict microbial replication and protein yield.

Targeted nutritional interventions aimed at correcting these limiting factors can effectively enhance EMPS. These strategies contribute to improving NUE and reducing urinary N excretion.

PROJECT OBJECTIVES

This project focuses on improving NUE and productive efficiency in lactating dairy cows through dietary formulation strategies. It aims to evaluate how targeted adjustments in AA supply and N source impact both milk component synthesis and N excretion dynamics.

The study employed diet formulations using AMTS.Nutrition (based on CNCPS v6.5.6) to simulate nutrient metabolism and N partitioning in dairy cows under controlled dietary scenarios. The primary nutritional strategies evaluated were AA balancing, with a specific focus on Met adequacy, and adjustment of RDP levels in the diet, aimed at improving NUE by reducing urinary N.

The modeling process involved three sequential steps:

Step 1a: Compare three diets differing in metabolizable Met supply (0.86, 1.05, and 1.20 g/Mcal ME), based on formulations from Danese et al. (2023)^[46]. In all treatments, metabolizable Lys and His were formulated to meet the cows' requirements at 3.2 g/Mcal ME and 1.19 g/Mcal ME, respectively, ensuring they were not limiting. Met supply was then titrated to create treatments to evaluate its specific effects. This step assessed how increasing Met levels affects milk production (protein, fat, lactose and milk yield) and if the product would deliver the methionine to the small intestine.

Step 1b: Update milk yield or milk protein values of the control diet (0.86 g/Mcal ME) in line with the performance trend observed in Step 1a. This approach aimed to simulate how AA balancing, if biologically effective, could enable higher N conversion into milk protein and lead to improved NUE through increased productive N.

Step 2: In the balanced diet (1.19 g/Mcal ME Met; Lys and His meeting requirements), the initial rumen N balance was high at 137%, indicating excessive RDP supply relative to microbial requirements. This created an opportunity to reduce the balance toward ~120%, a level more aligned with optimal rumen function. To achieve this adjustment, dietary urea (0.074%) was removed, thereby lowering NPN input. This final step tested whether minimizing NPN supply could further reduce rumen NH₃ concentration and urinary N excretion without compromising milk output, representing an integrated strategy to enhance environmental N efficiency.

By evaluating how dietary Met balancing—conducted with Lys and His formulated at requirement levels—and N source reduction influence both milk production and N excretion, this study offers practical insights into precision feeding strategies that support both environmental sustainability and nutrient use efficiency in modern dairy systems.

MATERIALS AND METHODS

Formulation Platform

All diet formulations were conducted using AMTS.Nutrition v1.2.4.0 (Agricultural Modeling and Training Systems), which implements the Cornell Net Carbohydrate and Protein System (CNCPS) version 6.5.6 to simulate nutrient flows, rumen metabolism, AA supply, and N utilization. The model was used to assess how variations in Met supply and rumen degradable protein (RDP) influence productive efficiency and nitrogen utilization efficiency (NUE) in lactating dairy cows while meeting the Lys and His requirements.

Cow Profiles

The diets were formulated for Holstein cattle at approximately 70 days in milk, with the following characteristics derived from Danese et al. (2023) diets^[46] and adjusted in AMTS:

Breed: Holstein (Straightbred)

Days in Milk (DIM): 140

Lactation number: 3

Body condition score (BCS): 3.0

Mature body weight (FBW): ~700 kg (711 in control group)

Average daily gain (ADG): between 0.098 to 0.134 kg/d among diets

Milk yield:

Control diet: 45.4 kg/day

Intermediate diet: 45.3 kg/day

High Met diet: 45.2 kg/day

Milk composition:

True protein: 3.12–3.35%

Crude protein: 3.60–3.85%

Fat: 4.28–4.39%

Lactose: 4.70–4.78%

All other environmental and physiological inputs (e.g., temperature, humidity, activity, housing conditions, and feeding system) were standardized among groups and did not vary among treatments.

Experimental Design Overview

The formulation process consisted of three simulation steps to evaluate how Met balancing and RDP optimization affect N partitioning and efficiency:

Step 1a – Observation of Milk Performance under Varying Methionine Supply

Three baseline diets were formulated based on Danese et al. (2023), metabolizable Lys and His were formulated at 3.21 g/Mcal ME and 1.25 g/Mcal ME, respectively, meeting the cows' requirements to ensure they were not limiting, differing in metabolizable Met supply (g/Mcal ME):

Low Met: 0.86 (no supplemental Met)

Medium Met: 1.06 (intermediate to the requirement)

High Met: 1.20 (Full supplementation)

These diets were input into AMTS with no changes to milk yield or milk composition. Instead, milk performance outputs were fixed based on the Danese trial data to observe how different Met levels impacted milk true protein, fat, lactose, and yield. This step served to identify trends in

productive output and nutrient efficiency at varying Met adequacy levels without introducing modeling assumptions.

Step 1b – Simulation of NUE Improvement via Methionine Balancing

To assess whether AA balancing could mechanistically improve N efficiency, the control diet (0.86 g/Mcal ME, no supplementation) was adjusted in AMTS to simulate a biological response to increased Met supply. Two changes were applied:

Met supply was increased to 1.20 g/Mcal ME (full supplementation).

Milk output (milk yield and/or protein yield) was increased based on performance trends observed in Step 1a.

This simulation tested whether NUE improvement could be achieved through increased productive N, reflecting more efficient partitioning of dietary N into milk protein in response to corrected Met limitation.

Step 2 – Simulation of NUE Improvement via RDP Reduction

In this step, the high Met diet (1.20 g/Mcal ME) from Step 1a was modified by reducing Rumen NH₃ balance from 135% to 123% which allowed for the removal of 0.074 grams of urea from the diet.

Since no experimental milk yield or component data were available for this diet, milk output was held constant at the same values used in Step 1a to isolate the effect of reducing RDP—via urea removal—on N partitioning and urinary N excretion. This conservative assumption allowed changes in NUE to be attributed specifically to changes in N supply, not productivity.

Although milk production was fixed in the simulation, previous studies suggest that modest reductions in RDP—particularly from non-protein N sources like urea—may reduce rumen ammonia toxicity, improve the profile of AAs reaching the small intestine, and enhance MP-AA

utilization. These mechanisms could potentially support a small increase (e.g., +1–2%) in milk protein output. However, to avoid confounding and maintain focus on environmental N efficiency, no such adjustment was made in the simulation.

All other nutrient contents (ME, MP) remained unchanged. This allowed assessment of whether NUE could be further improved by reducing urinary N excretion and ruminal NH₃ concentration, without compromising milk production.

Output Metrics and Model Evaluation

For each dietary scenario, simulation outputs from AMTS. Nutrition were recorded to evaluate both productive performance and environmental N dynamics. The following variables were extracted:

Productive Performance Indicators

Milk yield (kg/day)

Milk true protein, fat, and lactose yields (kg/day), as entered based on Danese et al. (2023) or adjusted per treatment.

Nitrogen Utilization Metrics

Total N intake (g/day)

Productive N (g/day) = energy corrected milk + pregnancy + growth + maintenance requirements,

Urinary N excretion (g/day)

Fecal N excretion (g/day)

Total manure N = urinary N + fecal N

Nitrogen utilization efficiency (NUE) = (Productive N / N intake) × 100

Productive N : Urinary N ratio

Productive N : Total N ratio

Summary Table and Interpretation

All diet scenarios were compared in a consolidated output table, with emphasis on:

Step 1a: Effect of increasing Met supply on milk and N partitioning

Step 1b: Impact of enhanced productive N due to improved AA balance

Step 2: Effect of reduced urinary N through reducing Rumen NH₃ balance from 135% to 123% allowing for the removal of dietary urea.

RESULTS

Step 1a – Milk Production and Composition Response to Methionine Supply

Among the three formulated diets (0.86, 1.06, and 1.20 g Met/Mcal ME) and depicted in figures 4a and 4b, milk yield showed no significant difference, despite the increased Met supply (Figure 4a). In contrast, milk composition improved with higher Met levels. Milk true protein concentration increased from 3.12% at 0.86 g/Mcal ME to 3.35% at 1.20 g/Mcal ME, while milk fat percentage increased slightly from 4.28% to 4.39% (Figure 4b). These results, derived from the Danese et al. (2023), indicate that enhancing Met supply—under conditions where Lys and His requirements were met—mainly improves milk components rather than total milk yield during established lactation.

With increasing levels of metabolizable Met, productive N showed a modest increase from 237.4 to 253.4 g/d (Figure 6). However, fecal N excretion remained largely unchanged, fluctuating slightly between 271.5 and 274.1 g/d among diets. Urinary N excretion showed a slight decrease—from 221.0 g/d at the lowest Met level to 211.4 g/d at 1.20 g/Mcal ME—suggesting a marginal improvement in N retention (Table 1).

Step 1b – Simulation of NUE Improvement via Methionine Balancing

Under conditions of fixed dietary N intake and rumen parameters, formulated increases in milk yield and milk true protein were based on the milk component responses observed in the study of Danese et al. (2023). These observed improvements were then applied in the model to evaluate how enhanced performance could influence NUE.

As shown in Figure 5a, when milk yield increased from 44.4 to 46.2 kg/d, productive N output rose from 230 to 244 g/d, while urinary N excretion declined from 230 to 216 g/d. Fecal N remained relatively unchanged. As a result, NUE increased steadily from 31.9% to 33.9%.

Likewise, in Figure 5b, increases in milk true protein from 3.00 to 3.45 kg/d were associated with a sharp rise in productive N (from 230 to 267 g/d) and a marked reduction in urinary N (from 230 to 201 g/d). Fecal N showed no appreciable change. Consequently, NUE improved significantly from 31.9% to 37.6%.

Step 2 – Simulation of NUE Improvement via RDP Reduction

As shown in Figure 6, Diet 4 was derived from Diet 3 by reducing Rumen NH₃ balance from 135% to 123% which allowed for the removal of 74 grams dietary urea while keeping other nutritional contents, including ME, MP, and Rumen protected Met supplementation, unchanged. Milk yield and composition were fixed to isolate the effects of RDP reduction.

Compared to Diet 3, Diet 4 showed a substantial decline in rumen NH₃ balance (from 135.17% to 123.48%) and urinary N excretion (from 211.4 to 184.8 g/d), while productive N remained constant at 253.4 g/d. Fecal N decreased slightly from 274.1 to 267.4 g/d. Consequently, NUE increased from 35% to 37%, and the productive N-to-urinary N ratio rose from 120% to 127%.

Input/Output variables	<u>Diet 1</u>	<u>Diet 2</u>	<u>Diet 3</u> (Balance for AA)	<u>Diet 4</u> (Balance for AA and Urea removal)
DMI, kg/d	28.23	28.41	28.41	28.34
Smartamine M, g/d	0	24	41	41
Lysine, g/Mcal ME	3.21	3.21	3.20	3.20
Methionine, g/Mcal ME	0.86	1.06	1.20	1.20
Milk production, kg/d	45.4	45.3	45.2	45.2
Milk fat, %	4.28	4.23	4.39	4.39
Milk true protein, %	3.12	3.21	3.35	3.35
ME allowable milk, kg	46.92	47.26	45.97	46.19
MP allowable milk, kg	46.75	46.02	44.05	44.04
N intake, g/d	716.88	726.17	725.95	692.67
Rumen NH ₃ balance, %	134.40	135.11	135.17	123.48
RDP, %DM	9.77	9.76	9.74	9.09
Productive N, g/d	237.42	243.83	253.37	253.37
Urine N, g/d	221.04	221.16	211.42	184.83
Fecal N, g/d	271.50	274.16	274.10	267.41
Manure N, g/d	492.54	495.31	485.52	452.24
Productive N/Total N, %	33	34	35	37
Productive N/Urinary N, %	107	110	120	127
Energy allowable bacteria, g	4828.20	4837.03	4833.06	4838.55
N allowable bacteria, g	5314.38	5339.50	5332.60	5077.30
BCVFA allowable bacteria, g	4525.09	4533.04	4526.04	4531.99

Table 1. Input and output parameters of four dietary scenarios differing in AA balancing and urea removal strategies. Diet 1 represents the control diet with no AA balancing; Diets 2 and 3 incorporate increasing levels of Smartamine to balance Met; Diet 4 further removes urea to reduce RDP content. Variables include DMI, ME and MP allowable milk, milk yield and composition, N intake and partitioning, and microbial protein synthesis estimates.

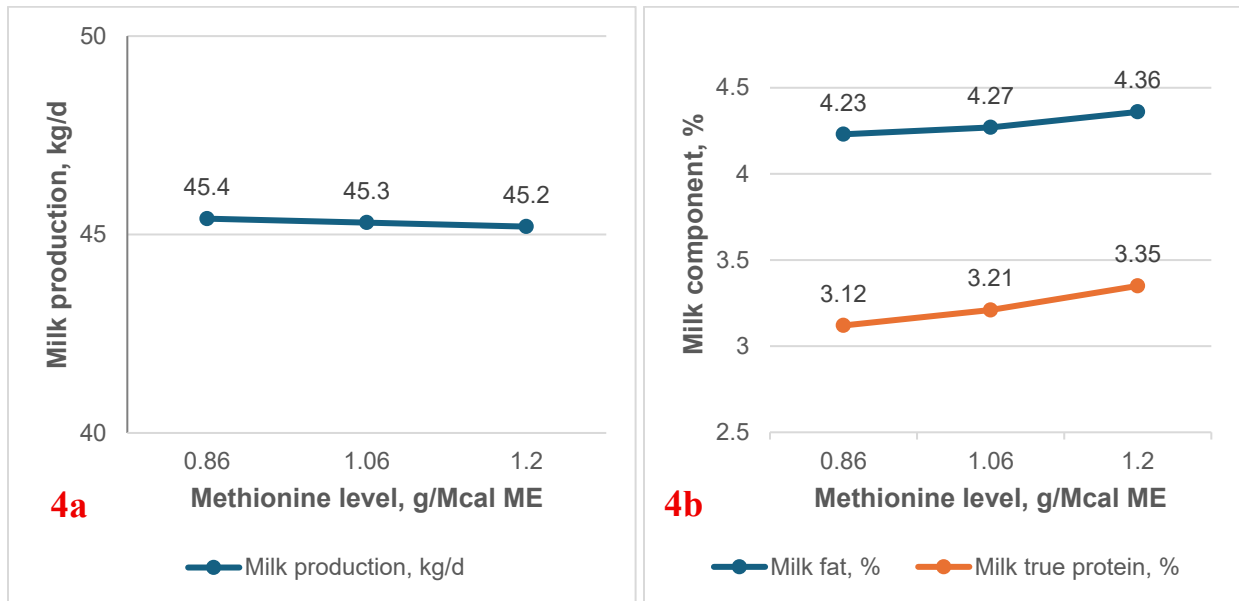


Figure 4a. Effect of increasing metabolizable Met (0.86, 1.06, and 1.20 g/Mcal ME) on milk production (kg/d), based on Danese et al. (2023). Milk yield shows no significant change as Met level approached 1.20 g/Mcal ME.

Figure 4b. Changes in milk fat (%) and milk true protein (%) in response to increasing metabolizable Met supply. Both milk fat and true protein concentrations increased as Met supply approached 1.20 g/Mcal ME.

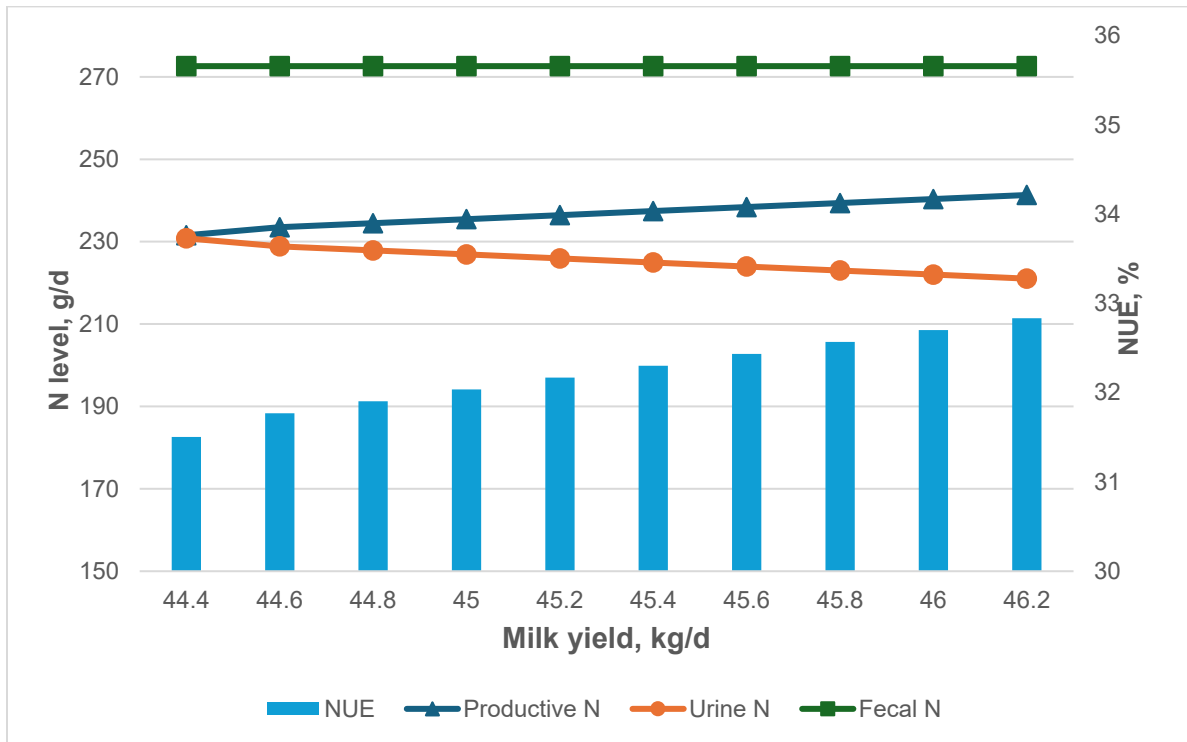


Figure 5a. Simulated effects of increasing milk yield on N partitioning and NUE under a diet with optimized Met supply (1.20 g/Mcal ME).

As milk yield increased from 44.4 to 46.2 kg/d, productive N increased, while urinary N slightly decreased. Fecal N remained unchanged. Consequently, NUE rose from ~31% to 33.5%

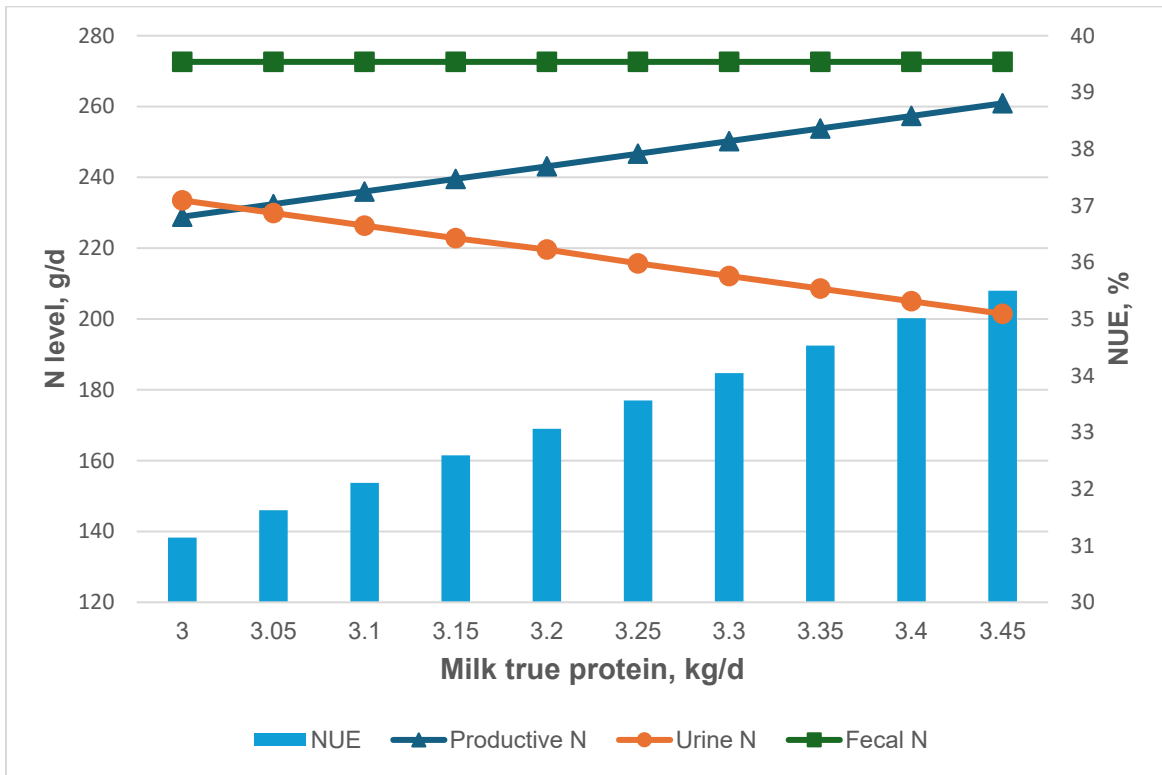


Figure 5b. Simulated effects of increasing milk true protein yield on N partitioning and NUE under a diet with optimized Met supply (1.20 g/Mcal ME) and full supplementation of His (1.25 g/Mcal ME) and Lys (3.21 g/Mcal ME).

As milk true protein increased from 3.00 to 3.45 kg/d, productive N increased sharply while urinary N decreased. Fecal N remained constant. NUE improved markedly from ~31% to ~36%.

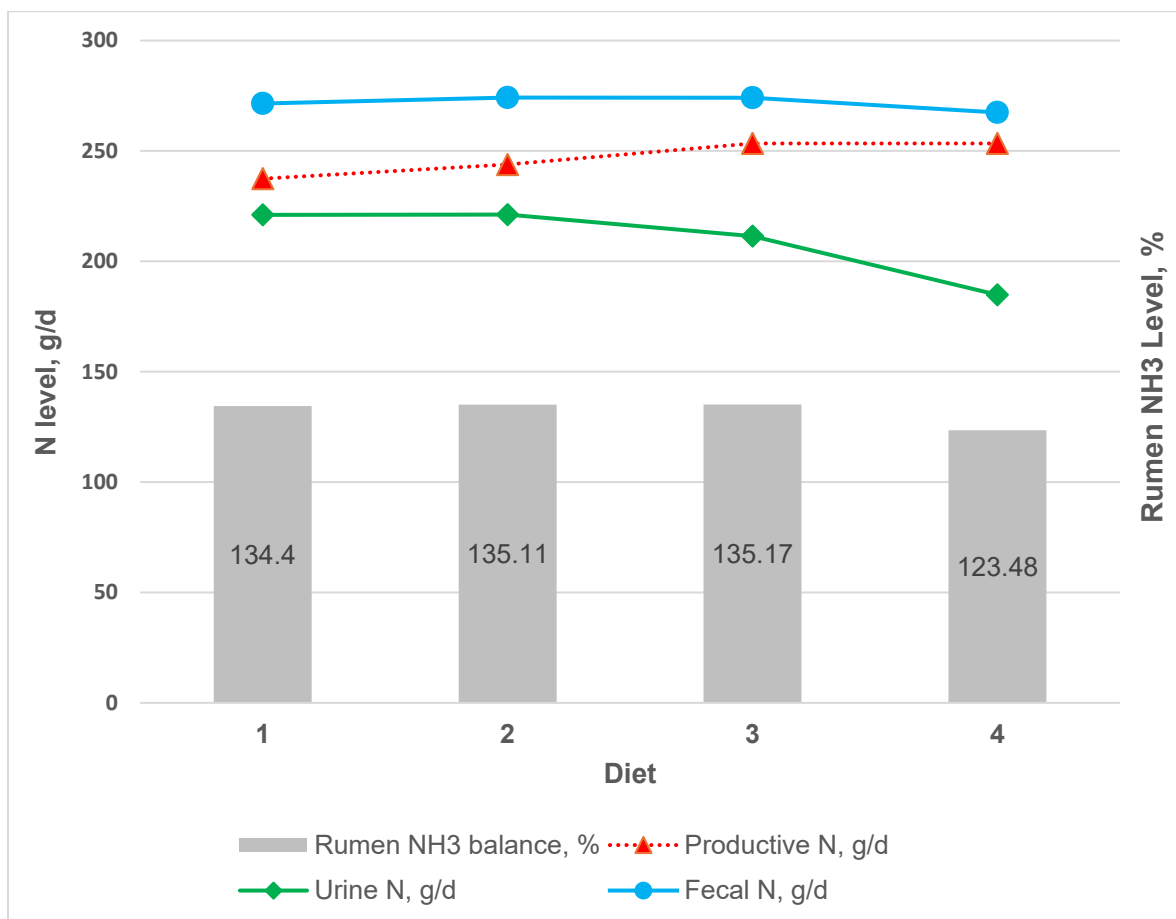


Figure 6. Effects of increased rumen protected Met supplementation and dietary urea removal on N partitioning and rumen NH₃ balance across four diets.

Fecal N and rumen NH₃ remained relatively stable with increasing Met supplementation (Diets 1–3), while productive N showed a slight increase. Diet 3, formulated to meet the recommended Met level, showed a moderate reduction in urinary N. Removal of urea in Diet 4 resulted in a marked decrease in rumen NH₃ concentration and a significant reduction in urinary N compared to Diet 3, with minimal changes in fecal and productive N.

DISCUSSION

This study systematically evaluated how optimizing metabolizable Met supply and reducing rapidly degradable N (RDP) sources, such as urea, affect N partitioning and NUE in lactating dairy cows. The findings demonstrate that strategic AA balancing and protein source refinement can each improve NUE, through distinct mechanisms.

In Step 1a, repeating the diet format of Danese et al. (2023) in the AMTS model revealed that meeting the metabolizable Met requirement, along with Lys and His, improved milk protein and fat concentrations while total milk yield was unaffected. All these effects were accompanied by a small increase in productive N and a small reduction in urinary N, while the fecal N and rumen NH₃ balance were nearly unchanged. Therefore, the changes in N partitioning caused by increasing Met supply can be explained by improved partitioning into milk components, while there were very small changes in total intake and rumen conditions. Similar results have been observed in trials in which Met restriction limited the protein synthesis of the animal despite a sufficient supply with CP^[47, 48].

In Step 1b, simulations confirmed that, under fixed N intake, higher protein output—reflecting a positive biological response to corrected Met deficiency—resulted in higher productive N and reduced urinary N. This shift improved NUE by over 5 percentage points. The results show that AA balancing can improve the metabolic efficiency of the animal and therefore a higher part of the dietary N can be captured in the milk protein and thus retained in the animal instead of being excreted with the urine. This corresponds to the results from numerous trials and reviews, which have shown that a supply with specific AA is more efficient than a simple increase in CP content to enhance N retention and minimize N excretion to the environment^[12, 49].

Step 2 focused on whether environmental N efficiency could be further optimized once AA adequacy was achieved. Removing dietary urea—a rapidly degradable non-protein N source—from the Met-balanced diet significantly reduced rumen NH₃ concentration and urinary N excretion, without compromising productive N. These changes suggest that reducing excess RDP can mitigate NH₃ accumulation and N waste. This corresponds to results from several studies, which have shown that excess RDP can lead to increased rumen NH₃ and urea cycling and thus to a waste of N^[15, 50]. The respective results also show that the removal of excess RDP from the diet leads to an environmental benefit, even when production targets are already achieved.

In total, the results show that both balancing AA and refining protein sources can enhance NUE through different mechanisms. Specifically, that a reduction in excess RDP from the diet, especially from urea, leads to an environmental benefit without any reduction in milk production. Both strategies provide a basis for reducing N losses and improving protein sustainability in dairy farming. Future trials should focus on the long-term production and reproductive performance of animals fed the respective diets under commercial conditions.

To better understand the broader implications of this strategy, we extended our formulation results to estimate its potential environmental and economic impact if applied across a significant portion of the U.S. dairy industry. Assuming a national herd size of 9 million lactating cows, we estimated the impact of implementing this approach in half of the herd (4.5 million cows). Based on our outcomes, the optimized diet resulted in a reduction of 24.21 g/day of feed N intake and 36.21 g/day of urinary nitrogen excretion per cow. The following calculations summarize the total reduction in N use and the corresponding savings in protein ingredients:

Per cow daily feed N reduction:

Initial: 716.88 g/day

Optimized: 692.67 g/day

Difference:

$$716.88 - 692.67 = 24.21 \text{ g/day}$$

Per cow daily urinary N reduction:

Initial: 221.04 g/day

Optimized: 184.83 g/day

Difference:

$$221.04 - 184.83 = 36.21 \text{ g/day}$$

Assuming we can impact 4,500,000 Cows,

Total annual feed N savings:

$$24.21 \times 4,500,000 \times 365 = 39,765,000,000g = 39,765 \text{ metric tons/year}$$

Total urinary N reduction:

$$36.21 \times 4,500,000 \times 365 = 59,475,000,000g = 59,475 \text{ metric tons/year}$$

Conversion to soybean meal equivalent:

Assuming 7% N in soybean meals (from 44% CP / 6.25):

$$39,765 \div 0.07 = 568,071 \text{ metric tons of soybean meal}$$

Conversion to urea equivalent:

Assuming 46% N in urea (from molecular formula $\text{CO}(\text{NH}_2)_2$):

$$39,765 \div 0.46 = 86,445 \text{ metric tons of urea}$$

Estimated cost savings

Soybean meal at \$400/ton:

$$568,071 \times 400 = \$227.2 \text{ million/year}$$

Urea at \$500/ton:

$$86,445 \times 500 = \$43.2 \text{ million/year}$$

These calculations clearly illustrate the potential scalability of this feeding strategy. By improving AA balance and reducing excess RDP—particularly from NPN sources like urea—it would be possible to prevent over 59,000 metric tons of urinary nitrogen emissions annually, while also saving the industry over \$200 million in protein ingredient costs. These outcomes demonstrate the dual benefit of this approach in reducing both N pollution and feed expenses, making it a promising pathway for climate-smart and economically efficient dairy production.

Metric	Estimated Impact
Cow impacted	4,500,000
Feed N reduction per cow, g/day	24.21
Total feed N saved, metric tons/year	39,765
Urinary N reduction per cow, g/day	36.21
Total urinary N avoided, metric tons/year	59,475
Equivalent soybean meal saved, metric tons/year	568,071
Soybean meal cost savings, dollars/year	227,200,000
Equivalent urea saved, metric tons/year	86,445
Urea cost savings, dollars/year	43,200,000

¹Assuming 7% N content in soybean meal (44% CP ÷ 6.25).

²Assuming 46% N content in urea (based on molecular formula CO(NH₂)₂; N = 28/60 = 46.7%).

Table 2. Projected industry-level environmental and economic impact of applying the proposed dietary strategy to 4.5 million U.S. dairy cows.

This table summarizes the estimated annual impact of implementing a strategy combining metabolizable Met and other AA balancing with RDP refinement, based on formulated reductions in feed N intake (−24.21 g/day) and urinary N excretion (−36.21 g/day) per cow. Calculations assume application to 50% of the U.S. dairy herd (4.5 million cows).

CONCLUSION

This study shows that lactating dairy cows can be used to improve NUE with targeted dietary interventions. Increased Met availability enhanced milk protein concentration and resulted in a moderate increase in productive N at the expense of a reduction in urinary N excretion. The simulation of increased milk protein output under Met-sufficient conditions also showed that a greater conversion of dietary N into milk protein markedly increased NUE. Furthermore, a reduction in rapidly degradable N from non-protein sources such as urea markedly decreased rumen NH₃ accumulation and urinary N losses without increasing or decreasing productive N. These findings suggest that a two-component nutritional strategy, first overcoming AA limitations and thereafter optimizing RDP supply, can improve both metabolic and environmental efficiency. Implementation of these strategies into ration formulation is expected to contribute to more sustainable dairy production with a lower environmental N footprint.

APPENDIX

Appendix A–F provide supporting AMTS outputs for the three diets used in the simulation study. Each diet includes both the detailed Diet Summary and the corresponding Excretion & Emission Report.

Blue TMR_Con: Baseline diet with 0.86 g Met/Mcal ME, no Smartamine supplementation.

Green TMR_Int: Intermediate diet with 1.06 g Met/Mcal ME and moderate Smartamine inclusion.

Orange TMR_High: High diet with 1.20 g Met/Mcal ME, before urea removal.

These outputs correspond to diets used in Step 1a simulations.

Appendix A. Diet Summary – Blue TMR_Con (0.86 g Met/Mcal ME, no Smartamine)

Diet Summary

AMTS Nutrition

Farm: x844 Diets FBW: 711 kg DIM: 140 Input DMI: 28.2 kg
 Cattle Group Name: Blue TMR_Con BCS (1-5): 3.00 Milk: 45.40 kg/day Predicted DMI: 27.5 kg
 Barn/Lot: x844 Diets ADG: 0.098 kg/day Milk Fat: 4.28% Milk Prt: 3.12% (True) 3.35% (Crude)

Nutrient	DM	AF
DM (%)	100	40.62
Forage (%)	62.71	82.99
CP (%)	15.87	6.45
RUP (%CP)	38.48	38.48
RDP (%CP)	61.52	61.52
RDP (%)	9.77	3.97
SP (%CP)	42.04	42.04
ME (Mcal/kg)	2.63	1.07
NEI (Mcal/kg)	1.69	0.69
NEm (Mcal/kg)	1.72	0.70
NEg (Mcal/kg)	1.10	0.45
ADF (%)	16.51	6.70
aNDFom (%)	29.28	11.89
Forage (%NDF)	79.32	79.32
Forage NDF (%)	23.23	9.43
Forage NDF (%BW)	92.35	37.51
peNDF (%)	18.60	7.55
Lignin (%NDF)	7.42	79.32
NFC (%)	44.56	18.10
Lactic (%)	5.20	2.11
Silage Acids (%)	6.57	2.67
Other Acids (%)	0	0
Sugar (%)	4.72	1.92
Starch (%)	28.15	11.43
Sol. Fiber (%)	5.12	2.08
EE (%)	3.94	1.60
LCFA (%)	3.27	1.33
Ash (%)	6.91	2.81
Cost (\$/Day)		0.00

Nutrient	DM	AF
DM (%)	100	40.62
Ca (%)	0.74	0.30
P (%)	0.39	0.16
Mg (%)	0.44	0.18
K (%)	1.40	0.57
S (%)	0.18	0.07
Na (%)	0.30	0.12
Cl (%)	0.52	0.21
Fe (ppm)	232.81	94.56
Zn (ppm)	34.97	14.20
Cu (ppm)	8.60	3.49
Mn (ppm)	38.43	15.61
Se (ppm)	0.38	0.15
Co (ppm)	0.05	0.02
I (ppm)	0.02	0.01
Vit-A (KIU/kg)	5.27	2.14
Vit-D (KIU/kg)	1.76	0.71
Vit-E (IU/kg)	21.96	8.92
DCAD1 (meq/kg)	233.27	94.75
DCAD2 (meq/kg)	198.75	80.72

Additive	Total	Diet Conc.
Monensin	399.85 mg	14.17 ppm
Lasalocid	0.00 mg	0.00 ppm
Decoquinat	0.00 mg	0.00 ppm
Yeast	0.00 mg 10 ⁶ cfu	0.00 10 ⁶
Beta Agonist	0.00 mg	0.00 ppm
Virginiamycin	0.00 mg	0.00 ppm
Aureomycin	0.00 mg	0.00 ppm
Chlortetracycline	0.00 mg	0.00 ppm
Oxytetracycline	0.00 mg	0.00 ppm
Salinomycin	0.00 mg	0.00 ppm
Zinc Bacitracin	0.00 mg	0.00 ppm
Enzymes	0.00 act/kg	0.00 act/kg
Toxin Binders	0.00 mg	0.00 ppm
Flavor	0.00 kg	0.00 %DM

Cost/MT: ¥0.00

Ingredient	\$/head	% DM	DM Fed	As-Fed
x844 Soybean Meal	0.00	87.57%	2.24	2.56
x844 Corn Silage	0.00	29.91%	14.20	47.48
x844 Corn Grain	0.00	85.60%	2.97	3.46
x844 Grass Silage	0.00	34.31%	3.50	10.20
Smartamine M	0.00		0.00	0.00
Salt White	0.00	99.50%	0.10	0.10
VTM Mix	0.00	99.50%	0.06	0.06
AjiPro L v3	0.00	97.00%	0.09	0.09
Wheat Midds	0.00	89.00%	2.22	2.49
Selenium 06	0.00	95.00%	0.01	0.01
Palmit 80	0.00	99.50%	0.06	0.06
Sugar	0.00	92.00%	0.85	0.92
Prod_updated				
Soybean Hulls Pellet	0.00	91.00%	0.45	0.49
ProvAAI Lysine	0.00	92.58%	0.45	0.48
MIN AD	0.00	99.50%	0.12	0.12
Magnesium Ox	0.00	99.50%	0.08	0.08
Levucell SC	0.00	95.00%	0.01	0.01
Energy Booster 100	0.00	99.36%	0.08	0.08
Limestone Ground	0.00	99.50%	0.26	0.26
Sodium Bicarbonate	0.00	99.50%	0.11	0.11
Soy Plus	0.00	89.09%	0.30	0.34
Urea 281 CP	0.00	99.00%	0.07	0.07
Rumensin 90.7 gm per lb	0.00	100.00%	0.00	0.00
Totals	0.00	40.62%	28.23	69.49

Appendix B. Diet Summary – Green TMR_Int (1.06 g Met/Mcal ME, moderate Smartamine)

Diet Summary

AMTS Nutrition

Farm: x844 Diets

Cattle Group Name: Green TMR_Int

Barn/Lot: x844 Diets

FBW: 699 kg

BCS (1-5): 3.00

ADG: 0.127 kg/day

DIM: 140

Milk: 45.30 kg/day

Milk Fat: 4.23%

Milk Prt: 3.21% (True) 3.45% (Crude)

Input DMI: 28.4 kg

Predicted DMI: 27.2 kg

Nutrient	DM	AF
DM (%)	100	40.63
Forage (%)	62.65	82.97
CP (%)	15.97	6.49
RUP (%CP)	38.91	38.91
RDP (%CP)	61.09	61.09
RDP (%)	9.76	3.96
SP (%CP)	41.85	41.85
ME (Mcal/kg)	2.63	1.07
NEI (Mcal/kg)	1.69	0.69
NEm (Mcal/kg)	1.72	0.70
NEg (Mcal/kg)	1.10	0.45
ADF (%)	16.48	6.70
aNDFom (%)	29.24	11.88
Forage (%NDF)	79.35	79.35
Forage NDF (%)	23.20	9.43
Forage NDF (%BW)	94.44	38.37
peNDF (%)	18.58	7.55
Lignin (%NDF)	7.40	79.35
NFC (%)	44.50	18.08
Lactic (%)	5.19	2.11
Silage Acids (%)	6.57	2.67
Other Acids (%)	0	0
Sugar (%)	4.72	1.92
Starch (%)	28.09	11.41
Sol. Fiber (%)	5.13	2.09
EE (%)	3.93	1.60
LCFA (%)	3.27	1.33
Ash (%)	6.92	2.81
Cost (\$/Day)		0.00

Nutrient	DM	AF
DM (%)	100	40.63
Ca (%)	0.74	0.30
P (%)	0.39	0.16
Mg (%)	0.43	0.18
K (%)	1.40	0.57
S (%)	0.19	0.08
Na (%)	0.30	0.12
Cl (%)	0.52	0.21
Fe (ppm)	232.36	94.41
Zn (ppm)	34.96	14.21
Cu (ppm)	8.61	3.50
Mn (ppm)	38.39	15.60
Se (ppm)	0.38	0.15
Co (ppm)	0.05	0.02
I (ppm)	0.02	0.01
Vit-A (KIU/kg)	5.24	2.13
Vit-D (KIU/kg)	1.75	0.71
Vit-E (IU/kg)	21.82	8.87
DCAD1 (meq/kg)	224.29	91.13
DCAD2 (meq/kg)	192.92	78.39

Additive	Total	Diet Conc.
Monensin	400.00 mg	14.08 ppm
Lasalocid	0.00 mg	0.00 ppm
Decoquinat	0.00 mg	0.00 ppm
Yeast	0.00 mg 10 ⁶ cfu	0.00 10 ⁶
Beta Agonist	0.00 mg	0.00 ppm
Virginiamycin	0.00 mg	0.00 ppm
Aureomycin	0.00 mg	0.00 ppm
Chlortetracycline	0.00 mg	0.00 ppm
Oxytetracycline	0.00 mg	0.00 ppm
Salinomycin	0.00 mg	0.00 ppm
Zinc Bacitracin	0.00 mg	0.00 ppm
Enzymes	0.00 act/kg	0.00 act/kg
Toxin Binders	0.00 mg	0.00 ppm
Flavor	0.00 kg	0.00 %DM

Cost/MT: ¥0.00

Ingredient	\$/head	% DM	DM Fed	As-Fed
x844 Soybean Meal	0.00	87.57%	2.30	2.62
x844 Corn Silage	0.00	29.91%	14.30	47.81
x844 Corn Grain	0.00	85.60%	2.96	3.46
x844 Grass Silage	0.00	34.31%	3.50	10.20
Smartamine M	0.00	98.00%	0.02	0.02
Salt White	0.00	99.50%	0.11	0.11
VTM Mix	0.00	99.50%	0.06	0.06
AjiPro L v3	0.00	97.00%	0.09	0.09
Wheat Midds	0.00	89.00%	2.22	2.50
Selenium 06	0.00	95.00%	0.01	0.01
Palmit 80	0.00	99.50%	0.06	0.06
Sugar Prod_updated	0.00	92.00%	0.85	0.92
Soybean Hulls Pellet	0.00	91.00%	0.45	0.49
ProvAAI Lysine	0.00	92.58%	0.45	0.48
MIN AD	0.00	99.50%	0.12	0.12
Magnesium Ox	0.00	99.50%	0.08	0.08
Levucell SC	0.00	95.00%	0.01	0.01
Energy Booster 100	0.00	99.36%	0.08	0.08
Limestone Ground	0.00	99.50%	0.26	0.26
Sodium Bicarbonate	0.00	99.50%	0.11	0.11
Soy Plus	0.00	89.09%	0.30	0.34
Urea 281 CP	0.00	99.00%	0.07	0.07
Rumensin 90.7 gm per lb	0.00	100.00%	0.00	0.00
Totals	0.00	40.63%	28.41	69.92

Appendix C. Diet Summary – Orange TMR_High (1.20 g Met/Mcal ME, before urea removal)

Diet Summary

AMTS Nutrition

Farm: x844 Diets

Cattle Group Name: Orange TMR_High

Barn/Lot: x844 Diets

FBW: 696 kg

BCS (1-5): 3.00

ADG: 0.134 kg/day

DIM: 140

Milk: 45.20 kg/day

Milk Fat: 4.39%

Milk Prt: 3.35% (True) 3.60% (Crude)

Input DMI: 28.4 kg

Predicted DMI: 27.4 kg

Nutrient	DM	AF
DM (%)	100	40.63
Forage (%)	62.65	82.97
CP (%)	15.97	6.49
RUP (%CP)	38.99	38.99
RDP (%CP)	61.01	61.01
RDP (%)	9.74	3.96
SP (%CP)	41.85	41.85
ME (Mcal/kg)	2.63	1.07
NEI (Mcal/kg)	1.69	0.69
NEm (Mcal/kg)	1.72	0.70
NEg (Mcal/kg)	1.10	0.45
ADF (%)	16.48	6.70
aNDFom (%)	29.23	11.88
Forage (%NDF)	79.36	79.36
Forage NDF (%)	23.20	9.43
Forage NDF (%BW)	94.85	38.54
peNDF (%)	18.58	7.55
Lignin (%NDF)	7.41	79.36
NFC (%)	44.51	18.09
Lactic (%)	5.19	2.11
Silage Acids (%)	6.57	2.67
Other Acids (%)	0	0
Sugar (%)	4.71	1.91
Starch (%)	28.12	11.43
Sol. Fiber (%)	5.11	2.08
EE (%)	3.93	1.60
LCFA (%)	3.27	1.33
Ash (%)	6.92	2.81
Cost (\$/Day)		0.00

Nutrient	DM	AF
DM (%)	100	40.63
Ca (%)	0.74	0.30
P (%)	0.39	0.16
Mg (%)	0.43	0.18
K (%)	1.40	0.57
S (%)	0.20	0.08
Na (%)	0.30	0.12
Cl (%)	0.52	0.21
Fe (ppm)	232.18	94.34
Zn (ppm)	34.91	14.18
Cu (ppm)	8.59	3.49
Mn (ppm)	38.34	15.58
Se (ppm)	0.38	0.15
Co (ppm)	0.05	0.02
I (ppm)	0.02	0.01
Vit-A (KIU/kg)	5.24	2.13
Vit-D (KIU/kg)	1.75	0.71
Vit-E (IU/kg)	21.83	8.87
DCAD1 (meq/kg)	217.87	88.53
DCAD2 (meq/kg)	189.12	76.84

Additive	Total	Diet Conc.
Monensin	400.15 mg	14.08 ppm
Lasalocid	0.00 mg	0.00 ppm
Decoquinat	0.00 mg	0.00 ppm
Yeast	0.00 mg	0.00 10 ⁶ cfu
Beta Agonist	0.00 mg	0.00 ppm
Virginiamycin	0.00 mg	0.00 ppm
Aureomycin	0.00 mg	0.00 ppm
Chlortetracycline	0.00 mg	0.00 ppm
Oxytetracycline	0.00 mg	0.00 ppm
Salinomycin	0.00 mg	0.00 ppm
Zinc Bacitracin	0.00 mg	0.00 ppm
Enzymes	0.00 act/kg	0.00 act/kg
Toxin Binders	0.00 mg	0.00 ppm
Flavor	0.00 kg	0.00 %DM

Cost/MT: ¥0.00

Ingredient	\$/head	% DM	DM Fed	As-Fed
x844 Soybean Meal	0.00	87.57%	2.27	2.59
x844 Corn Silage	0.00	29.91%	14.30	47.81
x844 Corn Grain	0.00	85.60%	2.98	3.48
x844 Grass Silage	0.00	34.31%	3.50	10.20
Smartamine M	0.00	98.00%	0.04	0.04
Salt White	0.00	99.50%	0.10	0.10
VTM Mix	0.00	99.50%	0.06	0.06
AjiPro L v3	0.00	97.00%	0.09	0.09
Wheat Midds	0.00	89.00%	2.22	2.50
Selenium 06	0.00	95.00%	0.01	0.01
Palmit 80	0.00	99.50%	0.06	0.06
Sugar	0.00	92.00%	0.85	0.92
Prod_updated				
Soybean Hulls Pellet	0.00	91.00%	0.45	0.49
ProvAAI Lysine	0.00	92.58%	0.45	0.48
MIN AD	0.00	99.50%	0.12	0.12
Magnesium Ox	0.00	99.50%	0.08	0.08
Levucell SC	0.00	95.00%	0.01	0.01
Energy Booster 100	0.00	99.36%	0.08	0.08
Limestone Ground	0.00	99.50%	0.26	0.26
Sodium Bicarbonate	0.00	99.50%	0.11	0.11
Soy Plus	0.00	89.09%	0.30	0.34
Urea 281 CP	0.00	99.00%	0.07	0.07
Rumensin 90.7 gm per lb	0.00	100.00%	0.00	0.00
Totals	0.00	40.63%	28.41	69.92

Appendix D. Excretion & Emission Report – Blue TMR_Con

Excretion & Emissions Report

AMTS Nutrition

Farm: x844 Diets	FBW: 711 kg	DIM: 140	Input DMI: 28.2 kg
Cattle Group Name: Blue TMR_Con	BCS (1-5): 3.00	Milk Fat: 4.28%	Predicted DMI: 27.5 kg
Barn/Lot: x844 Diets	ADG: 0.098 kg/day	Milk Prt: 3.12% (True) 3.35% (Crude)	

Ingredients	DM Fed	As-Fed
x844 Soybean Meal	2.24	2.56
x844 Corn Silage	14.20	47.48
x844 Corn Grain	2.97	3.46
x844 Grass Silage	3.50	10.20
Smartamine M	0.00	0.00
Salt White	0.10	0.10
VTM Mix	0.06	0.06
AjiPro L v3	0.09	0.09
Wheat Midds	2.22	2.49
Selenium 06	0.01	0.01
Palmit 80	0.06	0.06
Sugar Prod_updated	0.85	0.92
Soybean Hulls Pellet	0.45	0.49
ProvAAI Lysine	0.45	0.48
MIN AD	0.12	0.12
Magnesium Ox	0.08	0.08
Levucell SC	0.01	0.01
Energy Booster 100	0.08	0.08
Limestone Ground	0.26	0.26
Sodium Bicarbonate	0.11	0.11
Soy Plus	0.30	0.34
Urea 281 CP	0.07	0.07
Rumensin 90.7 gm per lb	0.00	0.00
Totals	28.23	69.49

Total Fecal (kg)	50.90
Total Urine (kg)	24.11
Total Wet Manure (kg)	75.01

	N	P
Intake (g)	716.88	109.95
Productive (g)	237.42	43.18
Urine (g)	221.04	1.42
Fecal (g)	271.50	65.35
Total Manure (g)	492.54	66.77

Productive N/Total N	33%
Productive N/Urinary N	107%
Manure N/Total N	69%
Productive P/Total P	39%
Manure P/Total P	61%

Methane

Total Liters/day	903.40
Total kg/day	0.60
grams/kg ECM	12.00
kg/day CO2 Equiv	15.10

CO2

Total grams/day	16.00
CO2 Equiv grams/day	31.10
grams/kg ECM	309.30
g CO2 Equiv/kg ECM	618.50

Appendix E. Excretion & Emission Report – Green TMR_Int

Excretion & Emissions Report

AMTS Nutrition

Farm: x844 Diets

FBW: 699 kg

DIM: 140

Input DMI: 28.4 kg

Cattle Group Name: Green TMR_Int

BCS (1-5): 3.00

Milk Fat: 4.23%

Predicted DMI: 27.2 kg

Barn/Lot: x844 Diets

ADG: 0.127 kg/day

Milk Prt: 3.21% (True) 3.45% (Crude)

Ingredients	DM Fed	As-Fed
x844 Soybean Meal	2.30	2.62
x844 Corn Silage	14.30	47.81
x844 Corn Grain	2.96	3.46
x844 Grass Silage	3.50	10.20
Smartamine M	0.02	0.02
Salt White	0.11	0.11
VTM Mix	0.06	0.06
AjiPro L v3	0.09	0.09
Wheat Midds	2.22	2.50
Selenium 06	0.01	0.01
Palmit 80	0.06	0.06
Sugar Prod_updated	0.85	0.92
Soybean Hulls Pellet	0.45	0.49
ProvAAI Lysine	0.45	0.48
MIN AD	0.12	0.12
Magnesium Ox	0.08	0.08
Levucell SC	0.01	0.01
Energy Booster 100	0.08	0.08
Limestone Ground	0.26	0.26
Sodium Bicarbonate	0.11	0.11
Soy Plus	0.30	0.34
Urea 281 CP	0.07	0.07
Rumensin 90.7 gm per lb	0.00	0.00
Totals	28.41	69.92

Total Fecal (kg)	51.28
Total Urine (kg)	24.47
Total Wet Manure (kg)	75.75

	N	P
Intake (g)	726.02	110.66
Productive (g)	243.83	42.96
Urine (g)	221.05	1.40
Fecal (g)	274.11	66.30
Total Manure (g)	495.16	67.70

Productive N/Total N	34%
Productive N/Urinary N	110%
Manure N/Total N	68%
Productive P/Total P	39%
Manure P/Total P	61%

Methane

Total Liters/day	905.70
Total kg/day	0.60
grams/kg ECM	12.00
kg/day CO2 Equiv	15.10

CO2

Total grams/day	16.10
CO2 Equiv grams/day	31.20
grams/kg ECM	316.00
g CO2 Equiv/kg ECM	631.90

Appendix F. Excretion & Emission Report – Orange TMR_High

Excretion & Emissions Report

AMTS Nutrition

Farm: x844 Diets	FBW: 696 kg	DIM: 140	Input DMI: 28.4 kg
Cattle Group Name: Orange TMR_High	BCS (1-5): 3.00	Milk Fat: 4.39%	Predicted DMI: 27.4 kg
Barn/Lot: x844 Diets	ADG: 0.134 kg/day	Milk Prt: 3.35% (True) 3.60% (Crude)	

Ingredients	DM Fed	As-Fed
x844 Soybean Meal	2.27	2.59
x844 Corn Silage	14.30	47.81
x844 Corn Grain	2.98	3.48
x844 Grass Silage	3.50	10.20
Smartamine M	0.04	0.04
Salt White	0.10	0.10
VTM Mix	0.06	0.06
AjiPro L v3	0.09	0.09
Wheat Midds	2.22	2.50
Selenium 06	0.01	0.01
Palmit 80	0.06	0.06
Sugar Prod_updated	0.85	0.92
Soybean Hulls Pellet	0.45	0.49
ProvAAI Lysine	0.45	0.48
MIN AD	0.12	0.12
Magnesium Ox	0.08	0.08
Levucell SC	0.01	0.01
Energy Booster 100	0.08	0.08
Limestone Ground	0.26	0.26
Sodium Bicarbonate	0.11	0.11
Soy Plus	0.30	0.34
Urea 281 CP	0.07	0.07
Rumensin 90.7 gm per lb	0.00	0.00
Totals	28.41	69.92

Total Fecal (kg)	51.30
Total Urine (kg)	24.48
Total Wet Manure (kg)	75.78

	N	P
Intake (g)	725.94	110.49
Productive (g)	253.37	43.89
Urine (g)	211.41	1.39
Fecal (g)	274.10	65.21
Total Manure (g)	485.51	66.60

Productive N/Total N	35%
Productive N/Urinary N	120%
Manure N/Total N	67%
Productive P/Total P	40%
Manure P/Total P	60%

Methane

Total Liters/day	905.50
Total kg/day	0.60
grams/kg ECM	12.00
kg/day CO2 Equiv	15.10

CO2

Total grams/day	16.10
CO2 Equiv grams/day	31.20
grams/kg ECM	321.20
g CO2 Equiv/kg ECM	642.30

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