

Article

Influence of Dietary and Ruminal Factors on Microbial and Non-Microbial Nitrogen Flows to the Small Intestine in Lactating Dairy Cows: A Meta-Analysis

Danilo D. Millen ^{1,*} , Gercino F. Virgínio, Jr. ¹ , Fernanda F. Alves ¹, Charles G. Schwab ² and Sergio Calsamiglia ^{3,†}

¹ School of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal 14884-900, Brazil

² Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH 03824, USA

³ Animal Nutrition and Welfare Service, Universidad Autonoma de Barcelona, 08193 Bellaterra, Spain

* Correspondence: danilo.millen@unesp.br

† Deceased author.

Abstract

Improving nitrogen efficiency in dairy cattle requires a better understanding of the dietary and ruminal factors that regulate nitrogen partitioning. This meta-analysis evaluated the effects of ruminal pH and dietary characteristics on microbial nitrogen (MN), non-microbial non-ammonia nitrogen (NANMN), and NAN flows to the small intestine in lactating cows. A dataset was assembled from 44 peer-reviewed *in vivo* studies (163 data points), with dietary intake and ruminal variables standardized across trials. Mixed linear models were developed for each N fraction, and the relative contribution of each predictor to the explained variance was assessed using semipartial coefficients of determination (pR^2). Efficiency of microbial protein synthesis (EMPS), rumen undegraded protein intake (RUP), and organic matter truly digested in the rumen (OMTDR) were the most relevant predictors of NANMN and NAN. Although the ruminal pH itself was not statistically significant in the models, the dietary components that influenced pH, starch concentration, physically effective fiber, and RUP supply were strongly associated with nitrogen flow profiles. Nitrogen utilization was not affected by ruminal pH, but rather by the combination of fermentable substrates and the supply of rumen-degradable and undegraded protein.

Keywords: microbial protein synthesis; nitrogen utilization; rumen metabolism



Academic Editor: Ulrich Meyer

Received: 4 September 2025

Revised: 23 October 2025

Accepted: 5 November 2025

Published: 7 November 2025

Citation: Millen, D.D.; Virgínio, G.F., Jr.; Alves, F.F.; Schwab, C.G.; Calsamiglia, S. Influence of Dietary and Ruminal Factors on Microbial and Non-Microbial Nitrogen Flows to the Small Intestine in Lactating Dairy Cows: A Meta-Analysis. *Dairy* **2025**, *6*, 66. <https://doi.org/10.3390/dairy6060066>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Nitrogen (N) metabolism in ruminants is a central focus in dairy nutrition, both for optimizing milk protein synthesis and reducing N losses to the environment [1]. In the rumen, dietary protein is partially degraded by microbes into ammonia, which can be used for microbial protein synthesis (MN) or absorbed into the bloodstream and excreted as urea [2]. The nitrogenous compounds that escape ruminal protein degradation and reach the small intestine include MN, rumen undegraded protein (RUP), and a fraction referred to as non-ammonia non-microbial N (NANMN). NANMN is defined as the portion of N not associated with MN or ammonia and includes RUP as the main component, together with other nitrogenous compounds such as peptides, free amino acids, and endogenous N derived from sloughed epithelial cells and digestive enzyme [3].

The partitioning of N among these fractions is influenced by multiple dietary and physiological factors. Ruminal pH, modulated by dietary concentrate levels and feeding frequency [4,5], has been proposed as an important factor. However, evidence from in vivo studies remains inconsistent, and its role relative to other dietary drivers of N partitioning is still unclear. This uncertainty highlights the need for a quantitative synthesis, such as the present meta-analysis. Subacute ruminal acidosis (SARA), often defined as ruminal pH below 5.8, is common in high-producing dairy cows receiving high-concentrate diets [5]. However, the exact threshold and measurement method for SARA can vary among studies, contributing to different interpretations of this disorder. SARA may alter microbial activity and N utilization efficiency, particularly by impairing microbial protein synthesis and increasing ruminal escape of dietary protein [6,7].

Continuous culture studies [8] and in situ evaluations [9] have suggested that NANMN outflow may increase under low-pH conditions, likely due to reduced microbial capture of ammonia and greater passage of nitrogenous compounds that escape ruminal degradation, such as undegraded dietary proteins and peptides [10]. Nevertheless, in vivo evidence for this relationship remains scarce and inconsistent, which justifies further investigation through meta-analytical approaches.

Most studies focus primarily on MN or RUP, while the behavior and nutritional significance of NANMN under varying ruminal conditions remain poorly characterized. In our database, continuous or high-resolution measurements of ruminal pH were rarely reported [11,12]. Most studies provided only single or averaged pH values [13,14], limiting the ability to identify studies meeting SARA criteria or to evaluate detailed responses of NANMN to subacute acidosis. This knowledge gap restricts our capacity to fine-tune dietary strategies that maximize N utilization and minimize N excretion.

Besides the potential role of ruminal pH, other factors such as dry matter intake (DMI), organic matter intake (OMI), N intake (NI), organic matter truly digested in the rumen (OMTDR), efficiency of microbial protein synthesis (EMPS), and RUP intake (RUPI) have been consistently shown to influence N partitioning [15,16]. However, the relative contribution of these factors to each N fraction, particularly NANMN, has not been comprehensively quantified in a multivariate framework.

The objective of this study was to evaluate the effect of dietary and ruminal conditions, including ruminal pH, on the ruminal outflow of N fractions in high-producing dairy cows. To this end, a meta-analysis of 44 peer-reviewed in vivo studies, comprising 176 treatment means, was conducted. Using multivariate regression, we aimed to quantify the influence of dietary and ruminal variables on the flow of MN, NPN, and total NAN, and specifically to test whether low ruminal pH conditions, suggested but not consistently confirmed in vivo, are associated with altered N partitioning.

2. Materials and Methods

A systematic literature search was conducted to compile in vivo studies evaluating N metabolism and duodenal N flows in lactating dairy cows. Peer-reviewed articles published over the past 45 years were retrieved from Google Scholar, Web of Science, and PubMed databases. The search strategy combined the following keywords in various combinations: “*ruminal nitrogen flow*”, “*microbial nitrogen*”, “*non-ammonia nitrogen*”, “*duodenal protein*”, “*lactating dairy cows*”, “*ruminal pH*”, and “*rumen undegraded protein*.” Studies published in English and reporting relevant N metabolism data (e.g., microbial N flow, RUP, NANMN, or total N excretion) were screened for inclusion.

Studies were eligible if they met the following criteria: (1) in vivo experiments using lactating dairy cows; (2) reported at least one of the following variables: MN, NANMN, or total NAN flows to the small intestine; (3) included dietary and ruminal parameters, such

as DMI, OMI, NI, CONCI, RUP, OMTDR, EMPS, or ruminal pH. Studies were excluded if ruminal ammonia concentrations were consistently below 5 mg/dL, the average DMI was <15 kg/d, or mean BW was <480 kg to ensure physiological comparability with mid- to late-lactation Holstein cows commonly used in high-producing dairy systems. Data from studies published before 2000 were acknowledged as potentially not fully reflecting contemporary cow genetics or management.

Initially, 95 peer-reviewed studies (372 observations) were identified, primarily from *Journal of Dairy Science*, *Journal of Animal Science*, *British Journal of Nutrition*, *Canadian Journal of Animal Science*, and *Animal Feed Science and Technology*. After applying inclusion criteria and removing studies with incomplete or incompatible data, a final subset of 44 studies (163 observations) was selected for model development and validation (Supplementary File S1).

Studies published between 1983 and 1999 were included because this period encompassed most in vivo trials using omasal or duodenal cannulation, which provide precise and direct estimates of MN, RUP, and NANMN flows. Due to the technical complexity, cost, and animal welfare considerations associated with surgical preparations, few such experiments have been conducted in recent decades. Despite a thorough search of more recent literature, no additional studies meeting the inclusion criteria were found.

When required variables were not explicitly reported, they were estimated from contextual information or calculated using standard nutritional equations. For studies lacking BW, estimates were derived based on parity and lactation stage from comparable studies; studies with estimated BW <480 kg were excluded. Missing CP and NDF values were imputed from NASEM [6]. All variables were converted to consistent units (g/d for N flows, kg/d for intake) to standardize the dataset.

The RUP intake was estimated using the fractional degradation rate (K_d) and fractional passage rate (K_p) approach. This method calculates the proportion of feed protein that escapes ruminal degradation based on the rates at which protein is degraded in the rumen and passes out of the rumen. The RUP fraction of B-type protein is computed using the formula: $RUP = \frac{K_p}{K_d + K_p}$, where K_d is the fractional degradation rate (per hour) and K_p is the fractional passage rate (per hour). Protein intake multiplied by this escape proportion provides the RUP intake estimate [6,17]. For the A fraction, a fixed 6.4% escape is assumed, and the C fraction is considered indigestible in the rumen.

NANMN was calculated as the fraction of N reaching the small intestine that is neither microbial N nor ammonia. This includes RUP as the major component, along with other nitrogenous compounds (peptides, free amino acids, endogenous N).

Each ingredient's contribution to total dietary RUP was estimated using inclusion rate, CP content, and K_d/K_p values. Resulting RUP content (%DM) was multiplied by DMI and converted to RUP-N (g/d). Endogenous N (2 g/kg DMI) was accounted for in the derivation of RUP escape values to avoid bias relative to endogenous-corrected NANMN [1,6]. Descriptive statistics for the final dataset ($n = 163$) are shown in Table 1.

Table 1. Descriptive statistics of dietary, ruminal, and nitrogen-related variables used in the final meta-analysis dataset ($n = 163$).

Item	<i>n</i>	Mean	Minimum	Maximum	Median	SD
BW, kg ¹	163	590.91	480.00	717.00	596.00	49.26
DMI, kg	163	20.14	15.08	26.70	20.00	2.80
DMI, %BW ¹	163	3.42	2.58	4.40	3.50	0.46
OMI ² , kg	151	18.78	14.08	25.90	18.50	2.51
<i>Diet characteristics</i>						
Concentrate, %DM	163	50.36	32.85	75.00	50.0	8.44
CP, %DM	163	17.13	13.08	23.10	17.39	1.47
RUP, %DM ³	163	6.33	2.90	12.00	6.10	1.41
<i>Measured variables</i>						
N intake, g/d	163	553.48	368.19	751.87	550.70	97.41
NAN flow, g/d	163	511.18	308.80	858.00	510.00	101.44
MN ⁴ flow, g/d	163	258.44	108.10	425.00	248.00	63.91
NANMN ⁵ flow, g/d	163	252.73	69.00	433.00	254.00	78.68
NAN flow, %N intake	163	93.34	63.45	140.36	92.81	16.40
MN flow, %N intake	163	47.63	21.92	94.69	46.41	13.45
NANMN flow, %N intake	163	45.71	13.43	85.53	45.16	12.69
MN flow, %NAN	163	51.01	28.69	81.62	48.88	10.75
NANMN flow, %NAN	163	48.99	18.38	71.31	51.12	10.75
Ruminal pH	162	6.11	5.50	6.92	6.05	0.26
Ruminal NH ₃ -N, mg/dl	146	12.46	5.20	40.60	12.00	4.65
OMTDR ⁶ , %	151	50.93	29.30	74.11	50.80	8.60
EMPS ⁷ , g of MN/100 g of OMTDR	151	28.20	12.30	53.70	27.23	8.60

¹ Includes observations for which BW was estimated; ² organic matter intake; ³ calculated by using the K_d - K_p approach; ⁴ microbial nitrogen; ⁵ non-ammonia non-microbial nitrogen; ⁶ organic matter truly digested in the rumen; ⁷ efficiency of microbial protein synthesis.

Several independent variables were initially available, including dietary composition, intake, and ruminal fermentation parameters. Pairwise correlations between potential explanatory variables and dependent variables (MN, NANMN, NAN) were assessed to identify candidate predictors (PROC CORR; SAS v9.4, SAS Institute Inc., Cary, NC, USA). Final model selection and the ranking of predictors were based on corrected Akaike Information Criterion (AICc) to avoid overfitting and account for the number of parameters.

The final models included EMPS, OMTDR, RUPI, DMI, CONCI, OMI, NI, and ruminal pH as continuous explanatory variables, selected based on biological plausibility, statistical relevance, and low collinearity (variance inflation factors).

Multivariate linear mixed models were constructed using the MIXED procedure in SAS. Each N flow fraction (MN, NANMN, NAN) was modeled separately, with study as a random effect. The relative importance of each predictor was quantified using semipartial coefficients of determination (pR^2), isolating the unique contribution of each variable while accounting for shared variance [8]. Model assumptions were assessed via residual analysis (normality, linearity, homoscedasticity).

To assess ruminal pH effects, data were stratified into <5.8 and >5.8 categories, and differences in NANMN flow were tested using independent t -tests. Model fit was evaluated via AIC, residual variance, and overall model R^2 .

3. Results

Multivariate linear mixed models were developed to identify the most relevant dietary and ruminal variables associated with the flow of N fractions to the small intestine (Table 2).

Table 2. Estimates and semipartial coefficients of determination (pR^2) for predictors of ruminal N fractions in lactating dairy cows.

Dependent Variable	Predictor	Estimate	pR^2
Non-ammonia non-microbial nitrogen (NANMN) $R^2 = 0.60$	Intercept	396.73	–
	RUPI ¹	640.35	0.47
	OMTDR ²	–4.81	0.10
	EMPS ³	–2.12	0.02
	CONCI ⁴	2.82	0.01
Microbial nitrogen (MN) $R^2 = 0.87$	Intercept	–438.74	–
	EMPS	7.86	0.37
	OMTDR	4.53	0.31
	DMI	12.13	0.19
Non-ammonia nitrogen (NAN) $R^2 = 0.58$	Intercept	–82.32	–
	RUPI	544.89	0.27
	EMPS	5.95	0.21
	OMI ⁵	11.46	0.09
	NI ⁶	0.18	0.01

¹ Ruminal undegraded protein intake; ² organic matter truly digested in the rumen; ³ efficiency of microbial protein synthesis; ⁴ concentrate intake; ⁵ organic matter intake; ⁶ nitrogen intake.

For NANMN flow, the model explained 60% of the variation. RUP intake (RUPI) was the strongest predictor ($p < 0.0001$; $pR^2 = 0.47$), followed by OMTDR ($p < 0.0001$; $pR^2 = 0.10$), EMPS ($p = 0.003$; $pR^2 = 0.02$), and concentrate intake (CONCI; $p = 0.05$; $pR^2 = 0.01$). Although RUPI accounted for nearly half of the explained variance, a substantial portion remains unexplained, indicating additional factors not captured in the dataset. Ruminal pH did not emerge as a significant predictor ($p = 0.13$); however, this result should be interpreted cautiously due to the limited number of low-pH observations ($n = 14$), which reduces statistical power for detecting threshold effects. Mean NANMN flow was 325 g N/d for pH < 5.8 and 241 g N/d for pH > 5.8, but the small sample size in the low-pH category limited statistical inference.

The model for MN flow had high explanatory power, accounting for 87% of the variation in the data. The most influential predictors were: EMPS ($p < 0.0001$; $pR^2 = 0.37$); OMTDR ($p < 0.0001$; $pR^2 = 0.31$); DMI ($p < 0.0001$; $pR^2 = 0.19$).

The model for NAN flow explained 58% of the variation. Significant contributors included: RUPI ($p < 0.0001$; $pR^2 = 0.27$); EMPS ($p < 0.0001$; $pR^2 = 0.21$); OMI ($p < 0.0001$; $pR^2 = 0.09$); NI ($p = 0.01$; $pR^2 = 0.01$).

4. Discussion

The N metabolism in the rumen is a complex process influenced by numerous dietary and physiological factors, and its optimization is essential for enhancing dairy cow performance while minimizing environmental N losses [18,19]. This meta-analysis provides a comprehensive evaluation of the primary drivers of N fractions flowing from the rumen, specifically MN, NANMN, and total NAN, by analyzing data from 44 peer-reviewed in vivo studies encompassing 163 treatment means.

Our results highlight the significant roles of RUPI, OMTDR, EMPS, and dietary CONCI in explaining variations in NANMN flow. Although ruminal pH was not identified as a significant predictor of N partitioning in the dataset, this likely reflects the scarcity of low-pH observations rather than the absence of a biological effect. For observations with ruminal pH < 5.8 ($n = 14$), mean NANMN flow was 325 g N/d, compared to 241 g N/d at pH > 5.8 ($n = 162$); however, the difference was not statistically significant. Due to the limited number of low-pH observations, these results should be interpreted cautiously.

Previous research using continuous culture and in situ techniques has shown that lower ruminal pH, often associated with high-concentrate diets, can impair microbial efficiency and alter N partitioning [8,9]. While a pH of 5.8 does not indicate clinical acidosis, it is commonly used as a threshold for increased risk of subacute ruminal acidosis (SARA). Under such conditions, reductions in fibrolytic and proteolytic bacteria may decrease microbial protein synthesis and increase the passage of undegraded dietary protein to the small intestine [5,20,21]. The limited number of low-pH observations in our dataset ($n = 14$) reduces statistical power and may obscure potential threshold effects. Moreover, inherent variability in diet composition, animal physiology, and measurement techniques can attenuate detectable associations. Consequently, our results do not contradict prior mechanistic studies indicating that low ruminal pH can alter microbial efficiency and N partitioning.

NANMN represents the portion of N escaping microbial assimilation and ammonia, including RUP as the main component, but also peptides, free amino acids, and endogenous N [22]. While RUP was the strongest predictor of NANMN in our models, more than half of the variance remained unexplained ($pR^2 = 0.60$), likely reflecting additional factors such as differences in feed ingredient digestibility, RUP amino acid profile, microbial efficiency under specific dietary conditions, and endogenous N contributions. These findings highlight the multifactorial nature of NANMN flow and the importance of considering both dietary RUP and ruminal fermentation dynamics to optimize N delivery to the small intestine [23].

RUP's influence on NANMN is not merely quantitative; it reflects a physiological balance between the supply of rumen-degradable N and the availability of fermentable energy [24]. Insufficient degradable protein relative to carbohydrate fermentation limits microbial growth via ammonia, favoring the greater passage of undegraded dietary protein to the small intestine [25]. Conversely, excess degradable protein can lead to ammonia accumulation, inefficient microbial capture, and increased N recycling and excretion [26,27]. Thus, the association between RUP and NANMN flow supports the concept that the synchronization of N and energy release is critical for maximizing N utilization efficiency in the rumen.

Despite these mechanistic insights, in vivo evidence on the effect of ruminal pH on N partitioning remains limited and inconsistent. In our dataset, ruminal pH was not a statistically significant predictor of NANMN flow. Controlled in vivo studies with continuous pH monitoring and detailed N fraction analyses are required to clarify the role of ruminal pH in nitrogen partitioning [20,21,28].

Predictive modeling based on dietary factors, including physically effective NDF (peNDF; >8 mm), starch content, and dry matter intake (DMI), can estimate the duration of ruminal pH < 5.8 and the associated risk of SARA [29]. These dietary factors also modulate microbial activity, ammonia assimilation, and the passage of undegraded dietary N to the small intestine [1,30].

Strong positive associations between MN flow, EMPS, and OMTDR partly reflect that EMPS is calculated from MN. Dietary starch generally increases OMTDR and MN flow but may decrease EMPS, reflecting reduced microbial protein synthesis efficiency at higher substrate supply [31]. These results emphasize the tight linkage between microbial protein synthesis, substrate availability, and the conversion efficiency of digested organic matter into microbial biomass, in agreement with the concept that maximizing ruminal organic matter digestion enhances microbial growth and the flow of high-quality protein to the intestine [16,26].

Carbohydrate fermentation provides the primary source of ATP for rumen microbes, fueling amino acid incorporation into microbial biomass [32,33]. Under optimal conditions,

increased organic matter digestion enhances MN yield; however, excessive fermentation rates can reduce ruminal pH, suppress fibrolytic activity, and shift the microbial community toward amylolytic species such as *Streptococcus bovis* and *Selenomonas ruminantium*, which exhibit lower N assimilation efficiency [34,35]. This trade-off illustrates how excessive starch fermentation can decouple energy supply from N incorporation, reducing EMPS despite increased substrate availability.

The EMPS is a critical factor influenced by diet composition, feeding strategy, and ruminal environment [36]. Diets rich in fermentable carbohydrates promote microbial proliferation but can reduce ruminal pH, potentially offsetting gains in microbial protein synthesis if acidosis develops [37]. Thus, balancing fermentable energy sources and protein supply is key to optimizing MN yield in dairy cows [38]. Although RUP contributes directly to NANMN flow, its interaction with ruminal digestibility and microbial efficiency shapes post-ruminal N supply. Total nutrient intake, reflected by OMI and nitrogen intake (NI), also affects NAN, consistent with Giallongo et al. [15], who reported that increasing metabolizable protein enhances milk protein yield, emphasizing the importance of intestinal amino acid supply and RUP amino acid profile.

The CONCI had a minor but significant effect on NANMN flow. High-concentrate diets increase ruminal fermentation rates and volatile fatty acid production, lowering ruminal pH and affecting microbial population [20,39]. This effect suggests that diet composition influences N partitioning via mechanisms not solely mediated by pH.

Improving N utilization efficiency is essential for reducing N excretion and its environmental consequences, such as nitrate leaching and greenhouse gas emissions [19,40]. Although our dataset lacked sufficient low-pH observations (<5.8) to evaluate the effects of SARA on NANMN, maintaining rumen health and stability through appropriate feeding management remains crucial [5]. Controlled experiments with repeated pH measurements and detailed N fraction analyses, covering a range of diet compositions and RUP levels, are needed to disentangle direct pH effects from diet-mediated changes in microbial activity.

Collectively, these findings highlight that N partitioning in the rumen arises from the dynamic interplay among degradable and undegradable protein fractions, carbohydrate fermentation rates, and the ruminal physicochemical environment. While ruminal pH did not emerge as an independent predictor in our models, its biological effects are likely mediated through shifts in microbial composition, ATP generation efficiency, and synchronization between N and energy metabolism. This integrative perspective underscores that accurate prediction of N flow requires understanding both diet composition and the microbial and energetic context of N degradation and assimilation.

Finally, previous continuous culture and in situ studies demonstrate clear effects of low ruminal pH on N partitioning under controlled conditions. In contrast, our meta-analysis synthesizes in vivo studies reflecting realistic production environments with variable diets, animal physiology, and measurement methods, highlighting the importance of integrating mechanistic and empirical approaches to understand ruminal N metabolism. Altogether, the results of this meta-analysis contribute quantitative evidence to refine predictive models of ruminal nitrogen flows and guide dietary strategies aimed at improving nitrogen efficiency in dairy systems.

5. Conclusions

This meta-analysis integrated data from 44 in vivo studies to identify the key dietary and ruminal factors affecting N flow to the small intestine in lactating dairy cows. The results demonstrate that rumen undegraded protein intake, organic matter truly digested in the rumen, microbial protein synthesis efficiency, and concentrate intake were the most influential predictors of non-ammonia, NANMN flow. Although ruminal pH did not reach

statistical significance in the final models, this result should be interpreted cautiously due to the limited representation of low-pH conditions, which may have reduced the power to detect threshold effects. Overall, the findings highlight that dietary composition, particularly the balance between fermentable energy and rumen-degradable versus undegradable protein, plays a predominant role in regulating N partitioning within the ruminant system. Future research should emphasize controlled evaluations of ruminal pH dynamics, microbial protein synthesis, and N fractionation to elucidate the complex interactions among acid-base status, microbial efficiency, and post-ruminal N supply.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/dairy6060066/s1>, File S1: References of studies included in the meta-analysis.

Author Contributions: Conceptualization, D.D.M., S.C. and C.G.S.; methodology, D.D.M., S.C. and C.G.S.; validation, D.D.M., S.C. and C.G.S.; formal analysis, D.D.M.; investigation, D.D.M. resources, S.C. and C.G.S.; data curation, D.D.M., G.F.V.J. and F.F.A.; writing—original draft preparation, D.D.M., G.F.V.J. and F.F.A.; writing—review and editing, D.D.M. and G.F.V.J.; supervision, S.C. and C.G.S. Author S.C. passed away prior to the publication of this manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES) for the scholarship granted to the second author (process n°: 88887.102494/2025-00).

Institutional Review Board Statement: The present study was conducted exclusively using data previously published in peer-reviewed literature, with no involvement of live animals. Consequently, approval by an Institutional Animal Care and Use Committee was not required.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would also like to acknowledge the support from São Paulo State University and the technical assistance provided by their coworkers.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Hristov, A.N.N.; Bannink, A.; Crompton, L.A.A.; Huhtanen, P.; Kreuzer, M.; McGee, M.; Nozière, P.; Reynolds, C.K.K.; Bayat, A.R.R.; Yáñez-Ruiz, D.R.R.; et al. Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. *J. Dairy Sci.* **2019**, *102*, 5811–5852. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Dijkstra, J.; Oenema, O.; van Groenigen, J.W.; Spek, J.W.; van Vuuren, A.M.; Bannink, A. Diet effects on urine composition of cattle and N₂O emissions. *Animal* **2013**, *7* (Suppl. S2), 292–302. [\[CrossRef\]](#)
3. Bach, A.; Calsamiglia, S.; Stern, M.D. Nitrogen metabolism in the rumen. *J. Dairy Sci.* **2005**, *88* (Suppl. S1), E9–E21. [\[CrossRef\]](#)
4. Mao, J.; Wang, L. Rumen acidosis in ruminants: A review of the effects of high-concentrate diets and the potential modulatory role of rumen foam. *Front. Vet. Sci.* **2025**, *12*, 1595615. [\[CrossRef\]](#)
5. Plaizier, J.C.C.; Krause, D.O.O.; Gozho, G.N.N.; McBride, B.W.W. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *Vet. J.* **2008**, *176*, 21–31. [\[CrossRef\]](#) [\[PubMed\]](#)
6. NASEM. *Nutrient Requirements of Dairy Cattle*, 8th revised ed.; National Academies Press: Washington, DC, USA, 2021; ISBN 978-0-309-67777-6.
7. Christodoulouopoulos, G. Subacute ruminal acidosis in cattle: A critical review of clinical management. *Vet. Res. Commun.* **2025**, *49*, 273. [\[CrossRef\]](#)
8. Calsamiglia, S.; Cardozo, P.W.; Ferret, A.; Bach, A. Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. *J. Anim. Sci.* **2008**, *86*, 702–711. [\[CrossRef\]](#)
9. Devant, M.; Ferret, A.; Calsamiglia, S.; Casals, R.; Gasa, J. Effect of nitrogen source in high-concentrate, low-protein beef cattle diets on microbial fermentation studied in vivo and in vitro. *J. Anim. Sci.* **2001**, *79*, 1944–1953. [\[CrossRef\]](#)
10. Ipharraguerre, I.R.; Clark, J.H. A Meta-analysis of ruminal outflow of nitrogen fractions in dairy cows. *Adv. Dairy Res.* **2015**, *2*, 1–13. [\[CrossRef\]](#)

11. Lykos, T.; Varga, G.A.; Casper, D. Varying degradation rates of total nonstructural carbohydrates: Effects on ruminal fermentation, blood metabolites, and milk production and composition in high producing Holstein cows. *J. Dairy Sci.* **1997**, *80*, 3341–3355. [[CrossRef](#)] [[PubMed](#)]
12. Yang, W.Z.; Beauchemin, K.A.; Koenig, K.M.; Rode, L.M. Comparison of hull-less barley, barley, or corn for lactating cows: Effects on extent of digestion and milk production. *J. Dairy Sci.* **1997**, *80*, 2475–2486. [[CrossRef](#)] [[PubMed](#)]
13. Putnam, D.E.; Schwab, C.G.; Socha, M.T.; Whitehouse, N.L.; Kierstead, N.A.; Garthwaite, B.D. Effect of Yeast Culture in the Diets of Early Lactation Dairy Cows on Ruminal Fermentation and Passage of Nitrogen Fractions and Amino Acids to the Small Intestine. *J. Dairy Sci.* **1997**, *80*, 374–384. [[CrossRef](#)]
14. Pantoja, J.; Firkins, J.L.; Eastridge, M.L.; Hull, B.L. Effects of Fat Saturation and Source of Fiber on Site of Nutrient Digestion and Milk Production by Lactating Dairy Cows. *J. Dairy Sci.* **1994**, *77*, 2341–2356. [[CrossRef](#)]
15. Giallongo, F.; Harper, M.T.; Oh, J.; Lopes, J.C.; Lapierre, H.; Patton, R.A.; Parys, C.; Shinzato, I.; Hristov, A.N. Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows. *J. Dairy Sci.* **2016**, *99*, 4437–4452. [[CrossRef](#)]
16. Broderick, G.A.; Reynal, S.M. Effect of source of rumen-degraded protein on production and ruminal metabolism in lactating dairy cows. *J. Dairy Sci.* **2009**, *92*, 2822–2834. [[CrossRef](#)]
17. Weiss, W.P.; Conrad, H.R.; St-Pierre, N.R. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. *Anim. Feed Sci. Technol.* **1992**, *39*, 95–110. [[CrossRef](#)]
18. Hristov, A.N.; Hanigan, M.; Cole, A.; Todd, R.; McAllister, T.A.; Ndegwa, P.M.; Rotz, A. Review: Ammonia emissions from dairy farms and beef feedlots. *Can. J. Anim. Sci.* **2011**, *91*, 1–35. [[CrossRef](#)]
19. Kebreab, E.; Dijkstra, J.; Bannink, A.; France, J. Recent advances in modeling nutrient utilization in ruminants. *J. Anim. Sci.* **2009**, *87*, E111–E122. [[CrossRef](#)]
20. Krause, K.M.; Oetzel, G.R. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim. Feed Sci. Technol.* **2006**, *126*, 215–236. [[CrossRef](#)]
21. Zebeli, Q.; Metzler-Zebeli, B.U. Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle. *Res. Vet. Sci.* **2012**, *93*, 1099–1108. [[CrossRef](#)] [[PubMed](#)]
22. Reynal, S.M.; Broderick, G.A. Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. *J. Dairy Sci.* **2005**, *88*, 4045–4064. [[CrossRef](#)]
23. Lapierre, H.; Lobley, G.E. Nitrogen recycling in the ruminant: A review. *J. Dairy Sci.* **2001**, *84*, E223–E236. [[CrossRef](#)]
24. Clark, J.H.; Klusmeyer, T.H.; Cameron, M.R. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *J. Dairy Sci.* **1992**, *75*, 2304–2323. [[CrossRef](#)]
25. Satter, L.D.; Slyter, L.L. Effect of ammonia concentration of rumen microbial protein production in vitro. *Br. J. Nutr.* **1974**, *32*, 199–208. [[CrossRef](#)]
26. Nocek, J.E.; Russell, J.B. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *J. Dairy Sci.* **1988**, *71*, 2070–2107. [[CrossRef](#)]
27. Hristov, A.N.; Etter, R.P.; Ropp, J.K.; Grandeen, K.L. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. *J. Anim. Sci.* **2004**, *82*, 3219–3229. [[CrossRef](#)]
28. Enemark, J.M.D. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *Vet. J.* **2008**, *176*, 32–43. [[CrossRef](#)] [[PubMed](#)]
29. Khorrami, B.; Khiaosa-ard, R.; Zebeli, Q. Models to predict the risk of subacute ruminal acidosis in dairy cows based on dietary and cow factors: A meta-analysis. *J. Dairy Sci.* **2021**, *104*, 7761–7780. [[CrossRef](#)] [[PubMed](#)]
30. Kebreab, E.; France, J.; Mills, J.A.; Allison, R.; Dijkstra, J. A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. *J. Anim. Sci.* **2002**, *80*, 248–259. [[CrossRef](#)] [[PubMed](#)]
31. Reynolds, C.K.; Kristensen, N.B. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. *J. Anim. Sci.* **2008**, *86*, E293–E305. [[CrossRef](#)]
32. Hackmann, T.J.; Firkins, J.L. Maximizing efficiency of rumen microbial protein production. *Front. Microbiol.* **2015**, *6*, 137653. [[CrossRef](#)]
33. Russell, J.B. The energy spilling reactions of bacteria and other organisms. *J. Mol. Microbiol. Biotechnol.* **2007**, *13*, 1–11. [[CrossRef](#)]
34. Russell, J.B. Factors That Alter Rumen Microbial Ecology. *Science* **2001**, *292*, 1119–1122. [[CrossRef](#)]
35. Ramos, S.C.; Jeong, C.D.; Mamuad, L.L.; Kim, S.H.; Kang, S.H.; Kim, E.T.; Cho, Y.I.; Lee, S.S.; Lee, S.S. Diet Transition from High-Forage to High-Concentrate Alters Rumen Bacterial Community Composition, Epithelial Transcriptomes and Ruminal Fermentation Parameters in Dairy Cows. *Animals* **2021**, *11*, 838. [[CrossRef](#)]
36. Dijkstra, J.; France, J.; Davies, D.R. Different mathematical approaches to estimating microbial protein supply in ruminants. *J. Dairy Sci.* **1998**, *81*, 3370–3384. [[CrossRef](#)]
37. Owens, F.N.; Secrist, D.S.; Hill, W.J.; Gill, D.R. Acidosis in cattle: A review. *J. Anim. Sci.* **1998**, *76*, 275–286. [[CrossRef](#)] [[PubMed](#)]

38. Santos, F.A.; Santos, J.E.P.; Theurer, C.B.; Huber, J.T. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. *J. Dairy Sci.* **1998**, *81*, 3182–3213. [[CrossRef](#)] [[PubMed](#)]
39. Beauchemin, K.A.; Colombatto, D.; Morgavi, D.P.; Yang, W.Z. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *J. Anim. Sci.* **2003**, *81*, 37–47.
40. Hristov, A.N.; Heyler, K.; Schurman, E.; Griswold, K.; Topper, P.; Hile, M.; Ishler, V.; Fabian-Wheeler, E.; Dinh, S. CASE STUDY: Reducing dietary protein decreased the ammonia emitting potential of manure from commercial dairy farms. *Prof. Anim. Sci.* **2015**, *31*, 68–79. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.