

## Understanding ammonia emissions: the impact of feed chemical composition on dairy Holstein farms

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### ABSTRACT

The aim of this study was to explore the relationship between the chemical composition of cattle feed and the emission of ammonia. To achieve this, we conducted a thorough chemical analysis of the feed, examining factors such as digestible crude protein, rumen undegradable protein, bacterial production of metabolic protein, and levels of methionine and lysine. Our research focused on how these feed components impact both milk yield and ammonia emissions. The study found that there was a positive correlation between digestible crude protein and milk yield, while rumen undegradable protein had a strong positive correlation with milk yield. Additionally, the study revealed a negative correlation between bacterial production of metabolic protein and daily milk yield. The concentration of methionine was weakly positively associated with milk yield, while lysine had a weak negative association with milk yield. The concentration of lysine in the rations was positively correlated with ammonia emissions, while methionine was negatively correlated with it. The study also found that rumen undegradable protein had a significant negative correlation with ammonia emission. Overall, the study highlights the importance of effective feeding management practices in optimizing animal productivity and minimizing ammonia emissions, to promote sustainable agriculture.

**Key words:** ammonia emission; feed chemical composition; milk recording; dairy cows

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### Introduction

Livestock farming is a significant contributor to the release of ammonia (NH<sub>3</sub>) emissions into the environment, which poses a major environmental threat on a global scale. These emissions lead to soil acidification, eutrophication of aquatic ecosystems, and have a significant role in causing global warming ([SANCHIS et al., 2019](#); [YANG](#)

et al., 2022). Livestock emissions are primarily driven by beef and dairy cattle, which account for 77% of all livestock emissions ([ECA, 2021](#)). During the breeding of dairy cows, urea and uric acid are broken down by urease-producing microorganisms, resulting in the release of NH<sub>3</sub> through microbiological hydrolysis. This process

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leads to the formation of  $\text{NH}_4^+$  as a byproduct ([SANCHIS et al., 2019](#); [YANG et al., 2022](#)). The indoor climatic factors that significantly affect  $\text{NH}_3$  emissions from cattle buildings are temperature, air velocity, and relative humidity ([SCHRADER et al., 2012](#); [SAHA et al., 2014](#); [SANCHIS et al., 2019](#); [QU et al., 2021](#); [TABASE et al., 2023](#)). In addition to these climatic factors, there are other factors, such as dairy cow species, feed and manure management, that also significantly contribute to  $\text{NH}_3$  emissions ([SANCHIS et al., 2019](#); [YANG et al., 2022](#)). It is crucial to focus on these parameters to manage and reduce ammonia emissions in livestock operations.

In the rumen, ammonia is produced when the degradable nitrogen (N) fraction of the feed is hydrolysed. This ammonia is then readily utilized by microbes to synthesize microbial protein ([PUTRI et al., 2021](#); [RODRIGUES et al., 2022](#)). In ruminants, an excess of ammonia in the rumen is transformed into urea in the liver. This urea is either recycled back to the rumen through saliva and the epithelium, or excreted in the urine via the kidneys ([CHOJNACKA et al., 2021](#); [SOUZA et al., 2021](#); [RODRIGUES et al., 2022](#)). One of the factors mentioned above is feed. The faeces and urine characteristics of animals are influenced by various factors, such as the chemical composition and physical properties of their feed, the N content and energy-N balance of the diet, as well as the physiological stage of the animal. Specifically, the amount of N excreted is affected by these factors ([DIJKSTRA et al., 2018](#); [SAJEEV et al., 2018](#); [CHEN et al., 2020](#); [RODRIGUES et al., 2022](#)). One potential solution for reducing  $\text{NH}_3$  emissions from manure involves manipulating animal diets to decrease the excretion of nitrogen (N) in manure. This approach presents an opportunity for cost-effective and efficient reduction of  $\text{NH}_3$  emissions from manure ([HRISTOV et al., 2015](#); [KATONGOLE and YAN, 2022](#)). Numerous studies have provided conclusive evidence that there is a positive correlation between dietary crude protein (CP) levels and  $\text{NH}_3$  emissions from cattle manure slurries. In simpler terms, as the CP levels in the diet of cattle increase, so do the emissions of  $\text{NH}_3$  from their manure. [BURGOS](#)

et al. (2010) and [EDOUARD et al. \(2019\)](#) have reported that increasing the dietary CP contents from 15 to 21% DM and 120 to 180 g/kg DM, respectively, resulted in 2.3- to 4.6-fold increases in  $\text{NH}_3$  emissions associated with manure slurries from dairy cows. The excretion of N, specifically urinary N, and the consequent emission of  $\text{NH}_3$  from slurry are heavily influenced by the dietary concentration of CP ([YAN et al., 2006](#); [SPIEHS and VAREL, 2009](#); [ANGELIDIS et al., 2019](#)). Elevating the level of rumen undegradable protein (RUP) in animal diets is likely to boost the overall nitrogen (N) efficiency and facilitate the delivery of sufficient metabolizable protein (MP) to the small intestine ([CALSAMIGLIA et al., 2010](#); [DE MELO COELHO et al., 2022](#)).

Methionine and lysine are two crucial amino acids classified as essential due to their inability to be synthesized by the organism. [SAJID et al. \(2024\)](#) stated that most proteins, including those present in livestock feed, do not contain all the essential amino acids required for optimal milk production and tissue development in high or medium-producing dairy cattle. Methionine is one of the essential amino acids that is often lacking in these proteins. Although the protein synthesized by bacteria is a valuable source of amino acids (AA), it is not enough to satisfy the requirements for tissue growth and milk production in dairy cows. This fact has been supported by recent studies conducted by [ROBINSON et al. \(2004\)](#), [MAVROMMATIS et al. \(2021\)](#), and [SAJID et al. \(2024\)](#). Supplementing dairy cow rations with methionine faces challenges due to microbial activity in the rumen. To improve its bioavailability, rumen-undegraded or rumen-encapsulated versions of this amino acids can be provided. Optimizing the lysine to methionine ratio in the diet is crucial for maximizing microbial protein synthesis. Achieving this balance allows for a reduction in the crude protein content in the diet, improving dairy cow reproduction, and reducing nitrogen excretion for environmental sustainability ([PARK et al., 2020](#); [SAJID et al., 2024](#)).

The aim of this study was to investigate the link between the chemical makeup of cattle feed and the release of ammonia. The chemical analysis of cattle feed consisted of an examination of

several factors, such as digestible crude protein, rumen undegradable protein, bacterial production of metabolic protein, and levels of methionine and lysine.

### Materials and methods

This research gathered test-day data from Holstein cows in Croatia, specifically near Osijek in Slavonia, between January 2020 and December 2023, during their regular milk recording. Milk yield measurements were taken every four weeks using the AT4/BT4 milk recording method, and corresponding samples were analysed. The milk samples collected were labelled with the corresponding animal code and sampling date, and then sent to the Central Laboratory for Milk Quality Control, which is under the auspices of the Croatian Agency for Agriculture and Food. Infrared spectrophotometry was used to determine the proportion of urea in the milk samples. The milk sample collection procedures during recording and laboratory testing were carried out following the specifications set forth by the International Committee for Animal Recording ([ICAR, 2017](#)). After conducting a thorough analysis of milk recording data, adhering to ICAR standards, and correcting any illogical variable values, we were able to include a total of 35,245 Holstein test-day records in the updated database. SAS/STAT ([SAS, 2019](#)) was used to prepare the data and conduct dispersion and correlation analysis. The equations below can be used for computation of the milk urea nitrogen (MUN) and ammonia emissions (AM-EMISS) in dairy cows:

$$MUN \text{ (mg/dL)} = UREA * 0.46 \text{ ([SPIEKERS and OBERMAIER, 2012](#))}$$

$$AM-EMISS \text{ (g/cow daily)} = 25.0 + 5.03 * MUN \text{ ([BURGOS et al., 2010](#))}$$

In technical terms, UREA signifies the amount of urea present in the milk on a daily basis, measured in milligrams per decilitre (mg/dL). MUN, on the other hand, represents the concentration of milk urea nitrogen in mg/dL. Lastly, AM-EMISS denotes the quantity of ammonia emission per cow on a daily basis, measured in grams (g).

Near Infrared Spectroscopy (NIR) is an analytical method used for determining the digestible crude protein, rumen undegradable protein, and bacterial production of metabolic protein, methionine, and lysine in a ration. It is a versatile and reliable technology that can be used for analysing a diverse range of sample types. NIR works by detecting changes in the transmission or reflection of NIR energy from a sample, which is dependent on its chemical composition. However, to establish an NIR calibration, a primary reference method is required as NIR is a secondary technique. This calibration is essentially a mathematical correlation linking the raw NIR data obtained from samples to the specific chemical constituents or properties of interest.

### Results

Table 1 presents the basic statistical parameters of the feed ingredients analysed, including digestible crude protein, rumen undegradable

Table 1. Basic statistical parameters of analysed feed chemical composition (proteins and amino acids)

Variable	N	Mean	SD	CV	SE	Min	Max
Digestible crude protein, % DM	432	16.12	1.10	6.84	0.05	11.60	18.70
Rumen undegradable protein, % DM	394	5.51	0.72	13.06	0.04	3.30	6.80
Bacterial production of metabolic protein, % Total MP	372	53.86	4.72	8.76	0.25	46.20	69.40
Methionine, % Rqd	386	100.13	6.80	6.79	0.35	83.00	115.00
Lysine, % Rqd	386	91.23	4.80	5.26	0.24	80.00	108.00

protein, bacterial production of metabolic protein, methionine, and lysine.

The following are the statistical details of the nutritional composition of animal feed. On average, the digestible crude protein in the animals' rations was 16.12%, with a standard deviation of 1.10%. The minimum and maximum values were 11.60% and 18.70%, respectively. Additionally, the average rumen undegradable protein was 5.51%, with a standard deviation of 0.72%. The minimum and maximum values for this metric were 3.30% and 6.80%. Moreover, the average bacterial production of metabolic protein was 53.86%, with a standard deviation of 4.72%. The minimum and maximum values for this metric were 46.20% and 69.40%. Lastly, the average methionine value was 100.13%, with a standard deviation of 6.80%. The minimum and maximum values for this metric were 83.00% and 115.00%. Similarly, the average lysine value was 91.23%, with a standard deviation of 4.80%. The minimum and maximum values for this metric were 80.00% and 108.00%.

Table 2 shows the correlation analysis results between the chemical composition of the feed (specifically, proteins and amino acids) and the derivatives of test-day records (namely, daily milk yield and ammonia emissions).

Table 2 shows the relationship between the examined feed components, including digestible crude protein, rumen undegradable protein, bacterial production of metabolic protein, methionine, and lysine, and the test-day performance traits, namely daily milk yield and ammonia emissions. The statistical analysis conducted of the data revealed interesting insights. Specifically, the results showed that there was a weak positive correlation between the amount of digestible crude protein and the daily milk yield ( $r=0.366$ ,  $P<0.0001$ ). On the other hand, a strong positive correlation was observed between the amount of rumen undegradable protein and the daily milk yield ( $r=0.597$ ,  $P<0.0001$ ). The results of the study indicate that there is a substantial relationship between two important factors in milk

Table 2. Correlation between the analysed feed chemical composition (proteins and amino acids) and test-day records derivatives (daily milk yield and ammonia emissions)

Trait	Daily milk yield	Ammonia emissions
Ammonia emission, g/cow daily	-0.057	1.000
	<.0001	
	94868	94868
Digestible crude protein, %	0.366	0.040
	<.0001	<.0001
	62215	61139
Rumen undegradable protein, %	0.597	-0.009
	<.0001	0.038
	60700	59648
Bacterial production of metabolic protein in relation to total metabolic protein, %	-0.671	0.051
	<.0001	<.0001
	56267	55269
Methionine, %	0.202	-0.016
	<.0001	0.000
	57371	56362
Lysine, %	-0.179	0.014
	<.0001	0.001
	57371	56362

production – the bacterial production of metabolic protein and the daily milk yield. Specifically, the findings reveal a robust negative correlation between these two variables, with a correlation coefficient of -0.671 and a P-value of less than 0.0001. This suggests that as bacterial production of metabolic protein increases, daily milk yield decreases, and vice versa. The results of this study have important implications for understanding the factors that influence milk production, and may be useful in developing strategies to optimize milk yield. The statistical analysis revealed a weak positive association (correlation coefficient  $r=0.202$ ,  $P<0.0001$ ) between methionine and daily milk yield, indicating that higher levels of methionine were associated with slightly higher milk production. Conversely, a weak negative association (correlation coefficient  $r=-0.179$ ,  $P<0.0001$ ) was found between lysine and daily milk yield, suggesting that higher levels of lysine were associated with slightly lower milk production. The results of our analysis demonstrate a strong correlation between the feed ingredients given to the animals and their daily milk production. This highlights the significance of effective feeding management practices in optimizing animal productivity and reducing feeding costs. The correlation coefficient between the analysed feed ingredients and ammonia emissions was statistically significant ( $P<0.0001$ ;  $P<0.005$ ), although the coefficient itself was low. According to the analysis, there is a negative correlation coefficient between the concentration of methionine and the emission of ammonia. This suggests that increasing the methionine concentration in the animals' rations may help reduce the emission of ammonia. The relationship between the concentration of lysine in the rations and the emission of ammonia was observed to be positively correlated. This implies that a higher concentration of lysine in the rations results in a higher emission of ammonia from the dairy farm. The study highlights the importance of considering lysine concentration in the formulation of dairy farm rations, to minimize ammonia emissions and promote sustainable agriculture. The data indicate a clear and positive relationship between ammonia

emission and the levels of digestible crude protein and the bacterial production of metabolic protein. This suggests that an increase in the amounts of these ingredients in the ration could lead to a corresponding increase in emission. In other words, the more digestible crude protein and metabolic protein bacteria produce, the more ammonia is likely to be emitted. The study conducted on the impact of rumen undegradable protein in the diet found a significant negative correlation between the levels of rumen undegradable protein and ammonia emission. The results indicate that a higher level of rumen undegradable protein in the diet can lead to a reduction in ammonia emissions. This suggests that the inclusion of rumen undegradable protein in animal feed can be an effective strategy to mitigate the release of ammonia into the environment, thereby contributing to environmental sustainability.

## Discussion

Numerous studies have confirmed a significant correlation between milk urea concentration and its derivatives, such as milk urea nitrogen and ammonia emission. The strong association between milk urea content and nitrogen content in animal urine and manure has been established by several researchers, including [BURGOS et al. \(2010\)](#), [ECKERSALL and BELL \(2010\)](#), [KLEIN et al. \(2011\)](#), and [SPEK et al. \(2013\)](#). The dietary CP (crude protein) content has a significant impact on the urinary nitrogen (N) excretion, which in turn affects the level of ammonia (NH<sub>3</sub>) emissions ([SMITS et al., 1995](#); [DE BOER et al., 2002](#); [VAN DUINKERKEN et al., 2005](#); [TODD et al., 2006](#); [POWELL et al., 2008](#); [POWELL et al., 2011](#); [VAN DUINKERKEN et al., 2011](#); [BOUGOUIN et al., 2016](#); [EDOUARD et al., 2019](#); [MÜLLER et al., 2021](#); [DEVANT et al., 2022](#); [SCHRADER et al., 2023](#)). According to [HRISTOV et al. \(2011\)](#), when it comes to reducing dietary crude protein (CP) in livestock feed, it is crucial to assess the impact on animal performance and the associated costs of gain, while also paying attention to minimizing NH<sub>3</sub> emissions. This is because even a slight reduction in CP can potentially lead to negative

effects on the animals' productivity, which, in turn, can affect the overall economic viability of the farm. In their recent study, [DE MELO COELHO et al. \(2022\)](#) recommended the use of various sources of rumen undegradable protein (RUP) in animal feed formulations to lower nitrogen loss through urine. This approach can help reduce the release of harmful gases such as  $N_2O$ ,  $CH_4$ , and  $NH_3$  into the environment.

[KROBER et al. \(2000\)](#) and [HRISTOV et al. \(2011\)](#) found that incorporating methionine in low crude protein diets can significantly reduce nitrogen excretion in urine as a proportion of the total nitrogen excretion. Several studies have examined the efficacy of lysine use in growing cattle, and the findings indicate that roughly 40% of the lysine added is used efficiently by the animals ([BATISTA et al., 2016](#); [HUSSEIN et al., 2016](#)). This finding is based on animal response curves, and suggests that there is significant room for improvement in the use of lysine in cattle feed. Studies conducted by [AWAWDEH et al. \(2005\)](#) and [HUSSEIN et al. \(2016\)](#) have shown that modifying animal metabolism can improve the efficiency of lysine use, leading to a reduction in the cost of dietary lysine supplementation. A feasible strategy to enhance the lysine efficiency in protein synthesis is to administer ammonia ( $NH_3$ ) loads to cattle. When excess  $NH_3$  is fed in the form of feed-grade urea, the liver may spare lysine from catabolism. This preference is given to the removal of toxic  $NH_3$  from the bloodstream ([AWAWDEH et al., 2005](#); [HUSSEIN et al., 2016](#)). It is important to assess the environmental impact of cattle excreting more nitrogen when considering the effectiveness of a particular method. A study by [HUSSEIN et al. \(2016\)](#) found that increasing urea infusions leads to greater daily nitrogen retention, suggesting that increasing ammonia levels in the rumen may improve lysine use efficiency. [SAJID et al. \(2024\)](#) highlighted the fact that ruminant diets that contain high levels of crude protein are linked to elevated nitrogen excretion, which can cause environmental pollution and increase feeding expense. One of the main difficulties in ruminant farming is to enhance the reproductive health and performance of the animals, while simultaneously reducing

nitrogen excretion ( $N_2$  and  $NH_3$ ). According to this study, to address this issue, it is recommended to incorporate essential amino acids, especially lysine and methionine, in the diets of ruminants.

### Conclusions

The objective of this research was to establish the correlation between the chemical composition of cattle feed and the emission of ammonia. The chemical assessment of cattle feed involved an in-depth analysis of various factors, including digestible crude protein, rumen undegradable protein, bacterial production of metabolic protein, and levels of methionine and lysine. The study analysed the relationship between feed components and milk yield and ammonia emissions. The results showed a positive correlation between digestible crude protein and milk yield, and a negative correlation between bacterial production of metabolic protein and milk yield. Methionine was found to have a weak positive correlation with milk yield, while lysine had a weak negative correlation. The study also found that increasing methionine concentration in the rations may reduce ammonia emissions, while increasing lysine concentration may increase emissions. The inclusion of rumen undegradable protein in animal feed can help reduce ammonia emissions. Our research findings reveal a significant link between the specific feed ingredients provided to the animals and the amount of milk they produce on a daily basis. This highlights the critical role of efficient and effective feeding management practices in maximizing animal productivity and minimizing the costs associated with feeding. By ensuring that the animals receive the appropriate feed and nutrition, farmers can achieve higher milk yields and reduce unnecessary expense.

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### Declaration of competing interest

No potential conflicting interest was reported by the authors.

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**GAVRAN, M., D. ŠINKA, M. SAMARDŽIJA, R. GANTNER, Z. STEINER, V. GANTNER: Razumijevanje emisija amonijaka: utjecaj kemijskog sastava stočne hrane na farmama mliječnih holštajnskih goveda. Vet. arhiv 95, 187-196, 2025.**

#### **SAŽETAK**

Cilj je rada bio istražiti odnos između kemijskog sastava stočne hrane i emisije amonijaka. Kako bismo to postigli, proveli smo temeljitu kemijsku analizu hrane, ispitujući čimbenike kao što su probavljivi sirovi proteini, nerazgradivi proteini u buragu, bakterijska proizvodnja metaboličkih proteina te razine metionina i lizina. Istraživanje se usredotočilo na to kako su navedeni čimbenici utjecali i na prinos mlijeka i na emisije amonijaka. Otkriveno je da postoji pozitivna korelacija između probavljivih sirovih bjelančevina i prinosa mlijeka, dok je buražno nerazgradivi protein bio u jakoj pozitivnoj korelaciji s prinosom mlijeka. Osim toga, otkrivena je negativna korelacija između bakterijske proizvodnje metaboličkih proteina i dnevne količine mlijeka. Koncentracija metionina bila je u slaboj pozitivnoj korelaciji s dnevnom količinom mlijeka, dok je lizin bio u slaboj negativnoj korelaciji s dnevnom količinom mlijeka. Koncentracija lizina u obrocima bila je u pozitivnoj korelaciji s emisijom amonijaka, dok je metionin bio u negativnoj korelaciji s njim. Istraživanje je pokazalo i da buražno nerazgradivi protein ima znakovitu negativnu korelaciju s emisijom amonijaka. Općenito, u istraživanju je naglašena važnost učinkovitih praksi upravljanja prehranom u optimizaciji proizvodnosti životinja i smanjenju emisija amonijaka za promicanje održive poljoprivrede.

**Ključne riječi:** emisija amonijaka; kemijski sastav obroka; kontrola mliječnosti; mliječne krave

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