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Article

Influence of lactation period and ketosis on blood ammonia in cows

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A B S T R A C T

Ammonia is a central component in ruminant nitrogen metabolism and serves as a sensitive indicator of the dynamic balance between nitrogen intake, rumen microbial activity, and hepatic detoxification efficiency. Once produced in the rumen, ammonia is absorbed into the bloodstream and must be rapidly cleared by the liver via the urea cycle. As such, blood ammonia concentration reflects both the rate of ammonia production and the liver's detoxification capacity, making it a valuable marker for evaluating protein metabolism and liver function. This study aimed to assess how different lactation stages and the presence of ketosis/hepatic lipidosis affect blood ammonia levels in dairy cows. A total of 50 Holstein-Friesian cows were examined, with 10 animals assigned to each experimental group. Ammonia levels (μmol/L) in blood plasma varied significantly depending on lactation stage and metabolic condition. In early lactation, the mean concentration was 67.64±6.52, which significantly decreased at peak lactation (34.11±7.5), followed by mid-lactation (29.93±7.9), and reached its lowest in the dry period (21.61±6.1). Cows diagnosed with ketosis exhibited significantly higher ammonia levels (96.52 ± 8.2). A significant positive correlation was found between ammonia and BHB (r = 0.54) as well as with the index of hepatocyte steatosis index (nonesterified fatty acid: cholesterol, NEFA:CHOL ratio) (r = 0.81), and significant negative correlations with the liver functionality index (r = –0.82), urea (r = –0.47), total protein (r = –0.38), and albumin (r = –0.36). These findings support the use of ammonia concentration as a reliable biomarker for metabolic and liver function assessment in dairy cows.

Keywords: cow, metabolic profile, liver function, ammonia, ketosis, lactation

И З В О Д

Амонијак представља централну компоненту у метаболизму азота код преживара и служи као осетљив показатељ равнотеже између уноса азота, микробне активности у бурагу и ефикасности детоксикације путем хепатоцита. Након што се произведе у бурагу, амонијак се апсорбује у крвоток и мора бити брзо елиминисан из организма путем циклуса урее у јетри. Због тога концентрација амонијака у крви одражава како степен његове продукције, тако и способност јетре да га уклони, што га чини вредним биомаркером за процену метаболизма протеина и функције јетре. Циљ овог истраживања био је да се испита утицај различитих фаза лактације, као и присуства кетозе/хепатичне липидозе, на концентрацију амонијака у крви млечних крава. Укупно је испитано 50 крава Холштајн-фризијске расе, са по 10 јединки у свакој експерименталној групи. Концентрације амонијака (μmol/L) у крвној плазми значајно су се разликовале у зависности од фазе лактације и метаболичког стања. У раној лактацији просечна вредност износила је 67,64±6,52, а затим се значајно смањивала у пику лактације (34,11±7,5), у средњој лактацији (29,93±7,9) и достигла најнижу вредност у засушењу (21,61±6,1). Краве са дијагностикованом кетозом имале су значајно повећане вредности амонијака (96,52±8,2). Установљена је статистички значајна позитивна корелација између амонијака и бета хидроксибутирата (BHB) (r = 0,54), као и индекса замашћења хепатоцита (неестерификоване масне киселине: холестерол, NEFA:CHOL) (r = 0,81), док су негативне корелације забележене са индексом функције јетре (r = –0,82), уреом (r = –0,47), укупним протеинима (r = –0,38) и албумином (r = –0,36). Ови налази подржавају примену концентрације амонијака као поузданог биомаркера за процену метаболичког и хепатичног статуса код млечних крава.

Кључне речи: краве, метаболички профил, функција јетре, амонијак, кетоза, лактација

1. Introduction

Ammonia plays a pivotal role in ruminant nitrogen metabolism, serving as a key indicator of the balance between nitrogen intake, ruminal microbial activity, and liver function (Đoković et al., 2014). In dairy cows, ruminal microbes produce ammonia from dietary protein and non-protein nitrogen. This ammonia is absorbed into the bloodstream and must be efficiently detoxified by the liver through the urea cycle (Cincović and Belić, 2021). Consequently, blood ammonia levels reflect both ammonia production and the liver's clearance capacity, making it a valuable marker of protein metabolism and hepatic health. While the liver typically maintains ammonia homeostasis, elevated blood ammonia (hyperammonemia) can arise from disruptions in hepatic function, often linked to lactation

stage and metabolic disorders like ketosis (Belić and Cincović, 2021). In addition, the use of non-protein nitrogen in the diet of cows may be significantly associated with ammonia concentration, where an increase indicates inadequate utilization of these nitrogen sources (Zurak et al., 2023).

Under normal physiological conditions, the liver effectively converts ammonia to urea, which is then excreted via urine or recycled to the rumen (Røjen et al., 2011; Li et al., 2019). However, several physiological and pathological conditions can disrupt this balance, leading to elevated blood ammonia levels (hyperammonemia). Major factors influencing this process are, among others, the lactation stage and metabolic disorders, such as ketosis and fatty liver, both of which can impair hepatic function and nitrogen clearance (Pinedo and Melendez, 2022). The transition period, defined as three weeks before to three weeks after parturition, is one of the most metabolically challenging phases in the cow's productive cycle. During this time, cows often experience a negative energy balance (NEB) due to a sudden increase in energy demand for milk production, accompanied by insufficient dry matter intake. This energy deficit prompts the mobilization of body fat reserves, leading to elevated blood non-esterified fatty acids (NEFAs), which are taken up by the liver. Excessive lipid uptake can exceed the liver's metabolic capacity, resulting in hepatic lipidosis. Such hepatic dysfunction impairs several metabolic pathways, including the urea cycle, thus reducing the liver's ability to detoxify ammonia effectively (Mudron et al., 2004). Fatty liver leads to urea cycle enzyme dysregulation and increased blood ammonia levels (Gallego-Durán et al., 2022). These metabolic changes, accompanied by inflammation and oxidative stress in the liver and adipose tissue, lead to increased blood ammonia concentrations (Thomsen et al., 2023). Changes in feed quality and postabsorptive metabolism due to climatic and other environmental changes (Cincović et al., 2023; Glijan Pantović, 2025) can significantly affect metabolic pathways involving ammonia.

Despite the recognized importance of ammonia in nitrogen metabolism, few studies have systematically evaluated its dynamics in relation to both lactation stage and ketosis. The current literature provides fragmented insights, often focusing either on ammonia metabolism in general or on ketosis diagnosis without directly linking the two. This study aims to fill this knowledge gap by investigating the influence of lactation period and ketosis on blood ammonia levels in dairy cows. Specifically, it will compare blood ammonia concentrations across different lactation stages (early, mid, and late) and between cows with and without subclinical ketosis. The working hypothesis is that cows in early lactation and those affected by ketosis will exhibit significantly higher blood ammonia levels due to increased protein catabolism and compromised hepatic function. The outcomes of this research could support the inclusion of ammonia as a routine biomarker in the metabolic health assessment of dairy cows and contribute to the development of more effective monitoring protocols for the transition period.

2. Materials and methods

The study included 50 Holstein-Friesian cows, with 10 cows from each experimental group. Blood samples were taken during early lactation, peak lactation, mid-lactation, and dry period. The cows from these four experimental groups were clinically healthy animals in their second and third lactation and in optimal body condition. The fifth experimental group consisted of cows with ketosis, which had BHB concentrations above 1.2 mmol/L. Blood was collected from the coccygeal vein into EDTA vacutainers. The blood samples were immediately placed on ice at a temperature of 0–4 °C, plasma was separated from cells within the first 15–20 minutes after sampling. Laboratory analyses were performed within two hours of blood sampling. The following parameters were determined: ammonia, total protein, albumin, urea, BHB, NEFA, cholesterol, triglycerides, and total bilirubin. The parameters were measured using standard spectrophotometric methods with reagents from BioSystem (Spain), on a Chemray spectrophotometer (Rayto, China). Liver functionality indices were calculated as follows: liver functionality index (LFI) = (albumin × cholesterol) / total bilirubin, and the ratio of NEFA to cholesterol (NEFA:CHOL). Statistical analysis involved ANOVA to determine the effects of lactation period and ketosis on the examined parameters. A linear relationship between ammonia and the examined parameters was established through linear regression analysis and correlation using the Pearson correlation coefficient. The statistical package SPSS (USA) was used, and graphs were prepared on an online platform based on the Python programming language.

3. Results and discussion

The mean concentrations of ammonia (μmol/L) varied significantly across different stages of lactation and ketosis (Figure 1). In early lactation, the measured value was 67.64±6.52, while at peak lactation the ammonia concentration significantly decreased to 34.11±7.5. During mid lactation, ammonia further declined to 29.93±7.9, and in the dry period it reached its lowest value of 21.61±6.1. Conversely, in animals with ketosis, ammonia concentration was markedly elevated at 96.52±8.2 μmol/L. These differences were statistically significant ($p < 0.01$) (Table 1).

Total protein (g/L) was lowest in ketosis (51.33±4.85) and highest in the dry period (67.75±5.13). Albumin (g/L) followed a similar trend, with the lowest values observed in ketosis (24.1±2.9) and the highest during the dry period (36.72±2.7). Urea levels (μmol/L) remained relatively stable, ranging from 4.25±0.51 in ketosis to 5.39±0.53 in the dry period. NEFA concentrations (mmol/L) were elevated in ketosis (0.96±0.14) and lowest during the dry period (0.35±0.16). BHB (mmol/L) showed a similar pattern, with the highest value in ketosis (1.89±0.12) and the lowest in the dry period (0.60±0.09). Cholesterol levels (mmol/L) were lowest in ketosis (1.85±0.26) and highest during the dry period (3.99±0.27). Triglycerides (mmol/L) were also lowest in ketosis (0.12±0.04) and highest in the dry period (0.34±0.03). Total bilirubin (μmol/L) was significantly increased in ketosis (16.6±3.9) compared to other stages. The LFI index was highest in mid lactation (42.3±16.1) and lowest in ketosis (2.69±0.79). The NEFA to cholesterol ratio (NEFA:CHOL) was highest in ketosis (0.52±0.06) and lowest during the dry period (0.09±0.03). All these differences were statistically significant, with p-values less than 0.05 or 0.01, indicating notable metabolic changes across lactation stages and ketosis.

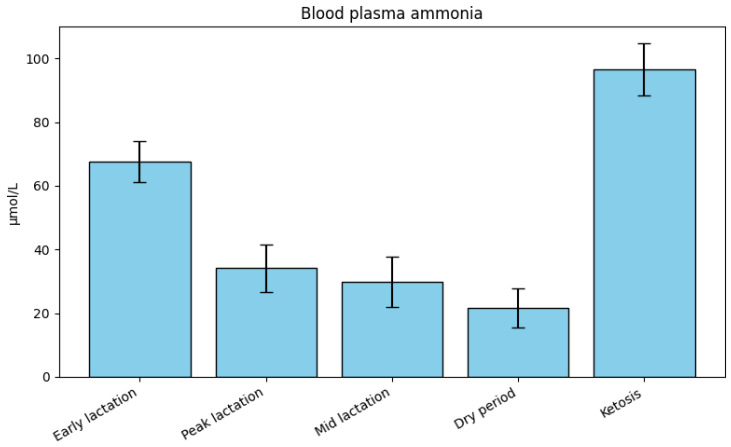

Figure 1. Effect of lactation period and ketosis on ammonia concentration in blood plasma of cows

Ammonia showed statistically significant correlations with several examined parameters. A significant positive correlation was found between ammonia and BHB (r = 0.54; p < 0.01) as well as with the NEFA:CHOL ratio (r = 0.81; p < 0.001). Additionally, ammonia demonstrated significant negative correlations with the LFI index (r = –0.82; p < 0.001), urea (r = –0.47; p < 0.01), total protein (r = –0.38; p < 0.05), and albumin (r = –0.36; p < 0.05). These results are illustrated in Figures 2–6.

Table 1.
Effect of lactation period and ketosis on examined blood parameters and functional indices

	Early lactation	Peak lactation	Mid lactation	Dry period	Ketosis	p
Ammonia (µmol/L)	67.64±6.52^{A}	34.11±7.5^{B}	29.93±7.9^{B}	21.61±6.1^{C}	96.52±8.2^{D}	<0.01
Total protein (g/L)	55.91±5.1^{A}	61.31±4.85^{B}	65.4±5.22^{C}	67.75±5.13^{C}	51.33±4.85^{D}	<0.01
Albumin (g/L)	29.65±2.2^{A}	34.22±2.1^{B}	35.52±2.5^{B}	36.72±2.7^{B}	24.1±2.9^{C}	<0.01
Urea (µmol/L)	4.7±0.49^{A}	5.32±0.58^{B}	5.11±0.6^{B}	5.39±0.53^{B}	4.25±0.51^{A}	<0.05
NEFA (mmol/L)	0.78±0.13^{A}	0.52±0.15^{B}	0.41±0.17^{B}	0.35±0.16^{B}	0.96±0.14^{C}	<0.01
BHB (mmol/L)	1.04±0.09^{A}	0.85±0.08^{B}	0.73±0.11^{C}	0.60±0.09^{D}	1.89±0.12^{E}	<0.01
Cholesterol (mmol/L)	2.23±0.22^{A}	2.56±0.21^{B}	3.2±0.26^{C}	3.99±0.27^{D}	1.85±0.26^{E}	<0.01
Triglycerides (mmol/L)	0.17±0.02^{A}	0.25±0.03^{B}	0.27±0.03^{C}	0.34±0.03^{D}	0.12±0.04^{D}	<0.01
Tot.bilirubin (µmol/L)	7.8±1.6^{A}	2.9±0.89^{B}	2.69±0.99^{B}	3.01±1.02^{B}	16.6±3.9^{C}	<0.05
LFI	8.48 ± 2.99^{A}	30.2±9.5^{B}	42.3±16.1^{B}	48.68±17.12^{B}	2.69±0.79^{C}	<0.01
NEFA:CHOL	0.35±0.05^{A}	0.22±0.04^{B}	0.13±0.04^{C}	0.09±0.03^{D}	0.52±0.06^{E}	<0.01

A, B, C - Values with different superscripts in the rows differ significantly at p < 0.05

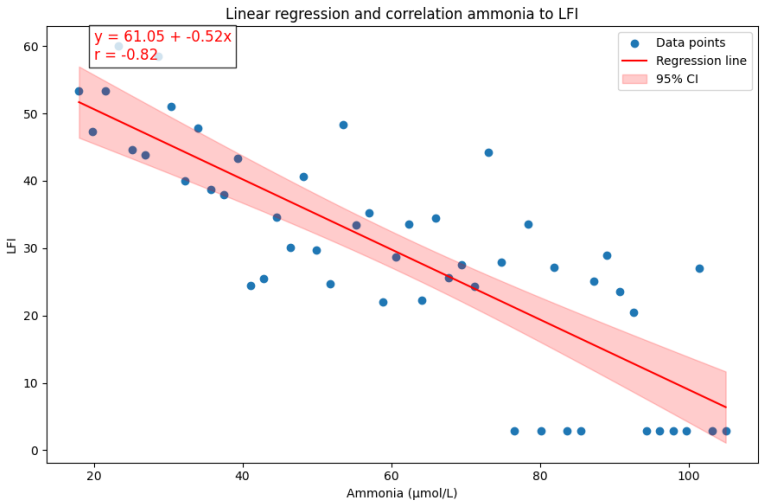

Figure 2. Linear relation between ammonia and LFI in blood plasma of cows

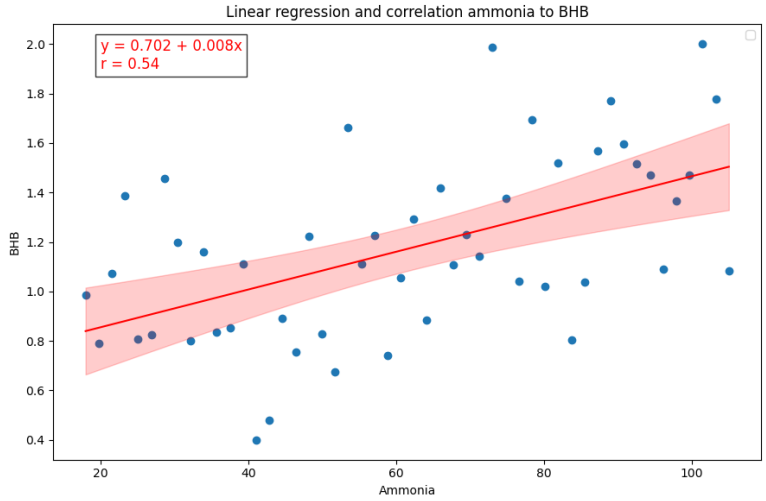

Figure 3. Linear relation between ammonia and BHB in blood plasma of cows

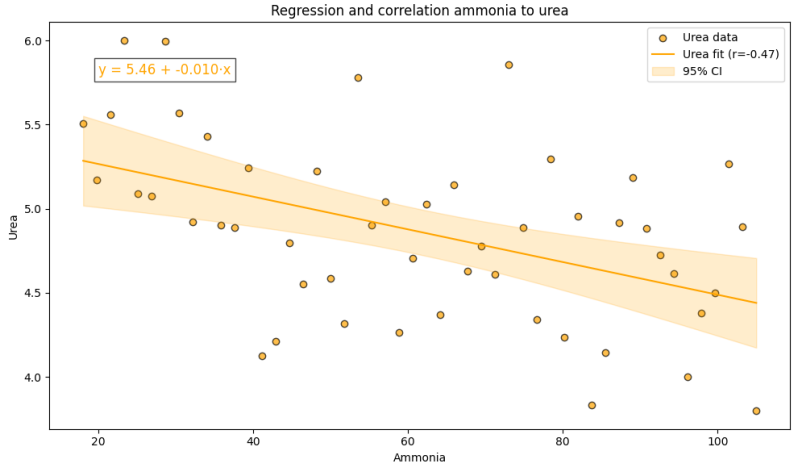

Figure 4. Linear relation between ammonia and urea in blood plasma of cows

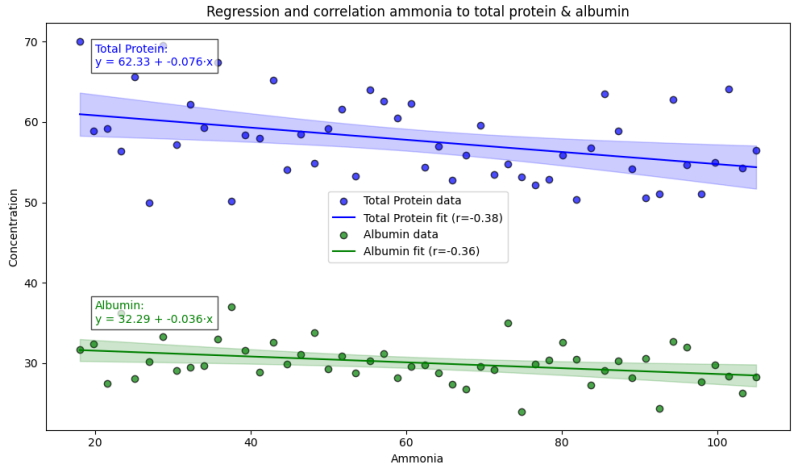

Figure 5. Linear relation between ammonia and total protein and albumin in blood plasma of cows

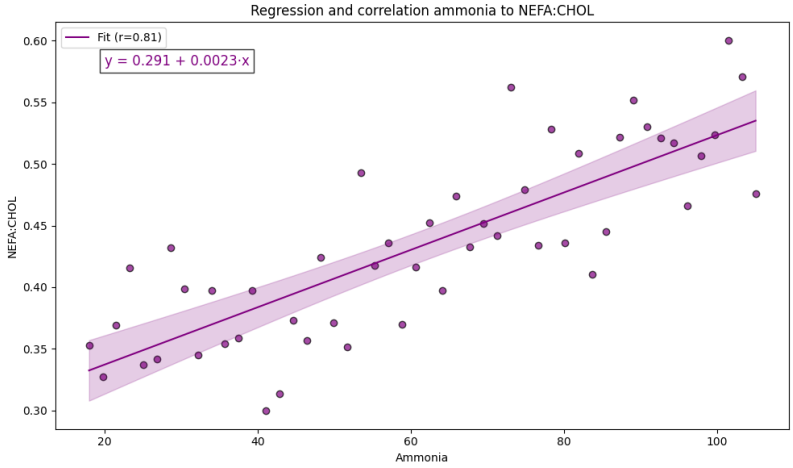

Figure 6. Linear relation between ammonia and NEFA:CHOL ratio in blood plasma of cows

In ruminants, microorganisms in the rumen break down nitrogenous substances from the diet, including urea, into ammonia, which is then utilized for the synthesis of amino acids and proteins. However, when nitrogen intake is excessive or microbial protein synthesis is limited (e.g., due to energy deficiency), ammonia accumulates in the rumen and is absorbed into the bloodstream. The liver plays a crucial role in detoxifying ammonia through urea synthesis, which is subsequently excreted in the urine or returned to the rumen via recirculation (Huntington and Archibeque, 1999). The variability of the examined metabolic parameters, including ammonia, indicates that lactation and ketosis significantly affect metabolic pathways due to changes in energy balance and the functional status of hepatocytes, which aligns with previous findings (Krnjaić et al., 2022; Anđelić et al., 2022; Petrović et al., 2022). It has been shown that hyperammonemia caused by high ammonia concentrations ranging from 193 to over 400 μmol/L negatively impacts feed intake and milk production (Purdie et al., 2009), suggesting that elevated ammonia levels may exacerbate adverse metabolic adaptations in dairy cows.

Our results demonstrate significant variation in ammonia concentrations across different lactation stages, with higher levels observed during early lactation compared to other periods, while the highest values were recorded in cows experiencing ketosis. Cows can tolerate high ammonia concentrations that they are able to metabolize, with arterial blood ammonia levels reaching up to 800 μmol/L before signs of intoxication appear (Symonds et al., 1981), although some authors report initial neurological symptoms at approximately 570 μmol/L (Bartley et al., 1981). The concentrations measured in our study were substantially lower, consistent with the absence of neurological dysfunction in the examined cows. Ammonia concentrations have also been assessed in studies involving the intake of highly digestible proteins in the form of urea, with levels ranging from 70 to over 300 μmol/L (Dawuda et al., 2002; Sinclair et al., 2000). In an experiment by Laven et al. (2007), ammonia concentrations varied from below 40 to over 160 μmol/L, depending on whether diets with lower or higher urea content were administered over an extended period. Certain studies have reported elevated ammonia concentrations exceeding 580 μmol/L (Owens and Basalan, 2016), with doubled blood values indicative of intoxication (Owens and Bergen, 1983; Patra, 2015). Measuring ammonia is particularly important for bovine reproduction, as concentrations in ovarian and uterine fluids vary between 93 and 308 μmol/L depending on urea levels (Hammon et al., 2005). The ammonia concentrations determined in our study align with findings reported by Mudron et al. (2004), who observed arterial and venous ammonia levels ranging from 21±8.04 to 30.8±11.3 μmol/L, while concentrations increased to 70.4±34.1 μmol/L (arterial) and 93.0±44.9 μmol/L (venous) in cases of liver damage. Notably, the variability of ammonia in their study was higher compared to our findings. Zhu et al. (2000) reported ammonia concentrations of 64.1±5.4 μmol/L on the first day postpartum, decreasing to 28.1±5.4 μmol/L one month after calving. Additionally, Braun et al. (2000) found that ammonia concentration in non-lactating cows was approximately 15 μmol/L in the jugular vein, while it was about 19 times higher in the portal vein.

Ammonia exhibits the strongest correlation with the indices of the hepatocyte functional status, which is logical given that the liver is the central organ responsible for the metabolism of urea and ammonia through its intensive functional connection with the digestive organs (Patra and Aschenbach, 2018). The Liver Functionality Index (LFI) represents the degree of preservation of the liver's secretory capacity, while the NEFA:CHOL ratio indicates the level of hepatic lipidosis (Cincović, 2016). In ruminants, blood ammonia plays an important diagnostic role in cases of liver disease and ketosis. The liver is the primary organ responsible for ammonia detoxification via conversion into urea within the urea cycle. The severity of hepatic steatosis correlates with ammonia values, and a positive correlation has been found between ammonia concentration and triglyceride levels in hepatocytes (Zhu et al., 2000). In liver diseases (e.g., hepatitis, cirrhosis, toxicoses), this mechanism is disrupted, leading to hyperammonemia, with ammonia concentrations showing a marked increase relative to urea or glucose (West, 1997). Furthermore, reduced urea synthesis can clinically manifest as a decreased blood urea concentration despite the high nitrogen load. A negative correlation between urea and ammonia was observed in cows during studies of diurnal variation (Hwang et al., 2001). Negative correlations between total protein and albumin with ammonia may result from increased protein catabolism

(as occurs in early lactation and during negative energy balance) or decreased liver secretory capacity (as observed in the ketosis-fatty liver complex) (Kroupina et al., 2022; Pinedo and Melendez, 2022).

Ammonia is a useful metabolic parameter that can be incorporated into routine practice. However, it is a sensitive parameter whose value can falsely increase in samples during both preanalytical and analytical phases. It is essential to follow specific recommendations when measuring ammonia concentration in blood: use EDTA vacutainers, separate plasma from cells as soon as possible, keep samples on ice, and complete ammonia analysis within two hours of sampling (Hashim and Cuthbert, 2014). Nonetheless, the use of EDTA vacutainers provides good ammonia stability if samples are kept at +4°C; and if frozen at -70°C, samples remain stable for up to three weeks (Goldstein et al., 2017).

Conclusion

Based on the obtained results, we conclude that the stage of lactation significantly influences plasma ammonia concentration, with the highest levels observed during early lactation. Ammonia levels were highest during early lactation and in cows with ketosis. Correlation analyses revealed that ammonia may serve as a useful parameter in evaluating the functional status of hepatocytes, particularly in relation to lipolysis, ketogenesis, lipid infiltration, and the secretory capacity of liver cells. These findings support the use of blood ammonia as a valuable indicator of hepatic function and metabolic status in dairy cows.

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Declaration of competing interests

The authors declare that they have no personal and/or financial relationships with other people or organizations that could inappropriately influence their work.

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