Original Paper

Analysis of the mineral profile in the urine of dry and lactating cows: a case study

Tomáš Kanka[1], Ivan Imrich[1], Eva Mlyneková[1], Martina Pšenková[1], Martin Fik[1], Cyril Hrnčár[1], Michal Rolinec[2]*
[1]*Slovak University of Agriculture in Nitra, Institute of Animal Husbandry, Slovakia*
[2]*Slovak University of Agriculture in Nitra, Institute of Nutrition and Genomics, Slovakia*

Article Details: Received: 2023-09-11 | Accepted: 2023-10-13 | Available online: 2023-12-31

https://doi.org/10.15414/afz.2023.26.04.360-363

In this case study, the urinary mineral profile of dairy cows was investigated. Determined urinary mineral concentrations were evaluated in relation to the production cycle (dry cows versus lactating cows) and were also compared to the physiological optimum. The study was carried out on twelve Holstein-Friesian dairy cows. Calcium, phosphorus, magnesium, sodium and urea concentrations were determined in urine samples. The urine of dry cows showed demonstrably higher concentrations of Ca (1.86 vs. 1.17 mmol.l^{-1}), P (0.88 vs. 0.43 mmol.l^{-1}), Na (4.97 vs. 2.70 mmol.l^{-1}) and Mg (16.46 vs. 14.24 mmol.l^{-1}) compared to the urine of lactating cows. Compared to the physiological optimum, significantly lower urinary Na concentrations were determined in all cows. This indicates a significant deficiency of Na in the diet of both dry cows and lactating cows. Marked differences between the minimum and maximum concentrations of all minerals studied in the urine of dairy cows were determined. The mineral profile obtained from urine samples can be an important diagnostic method for detecting preclinical stages of metabolic disorders. At the same time, urine sample collection is stress-free for dairy cows.

Keywords: Holstein cattle, bovine urine, trace elements, metabolic profile

1 Introduction

Dairy farms are constantly under increasing pressure to produce milk economically and also to maintain their competitive ability, particularly in the domestic market. To survive in this challenging environment, dairy farmers need healthy animals. Maintaining good health is mainly due to good nutrition, on which both the production and reproductive performance of dairy cows depend. However, its management to ensure the proper functioning of all metabolic processes in the organism is often not easy (Dvořák, 2005; Rolinec et al., 2021; Zábranský et al., 2019 and 2022). The metabolism and nutrition are closely related and functionally inseparable (Juráček et al., 2021). Thus, the mechanism of metabolic disorders can be illustrated by the principles of intake of necessary nutrients and specifically active substances as well as the achievement of the expected production. The above set of metabolic disorders also includes production diseases in which high animal production is a risk factor (Mihok et al., 2021; LeBlanc et al., 2006). Metabolic disorders, particularly in high-producing animals, thus contribute significantly to overall losses in animal production. Their incidence is generally less with developed clinical signs. However, they mostly occur in latent forms which are extremely serious from an economic point of view. They cause serious economic losses in performance, impair feed utilization, and negatively affect the reproductive process and the viability of the young. The later clinical stage is already evident and is accompanied by culling or death (Kraft and Dürr, 2013). For a thorough examination of the herd or individual animals, collection of biological material is necessary. By analysing it using laboratory diagnostic methods, which are an integral part of the metabolic profile test (MPT), we can determine the exact cause of production disorders (Bertoni et al., 2009; Kabir et al., 2022). The authors (Kantíková and Balážik, 2003) recommend periods when animals are physiologically most stressed. In the case of dairy cows, this is the drying-

***Corresponding Author:** Michal Rolinec. Institute of Nutrition and Genomics, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 94976 Nitra, Slovak Republic
michal.rolinec@uniag.sk https://orcid.org/0000-0003-4966-7909

off period and the period up to 15 weeks after calving. In recent years, studies have been published on the use of urine for the prediction or confirmation of metabolic disorders (Zhang et al., 2021; Eom et al., 2021; Dervishi et al., 2018). Urine sampling is a non-invasive method that does not cause stress to the animals.

The aim of this case study was to determine the changes in the urinary mineral profile of dairy cows in the pre- and postpartum periods.

2 Material and methods

A case study was conducted to compare the mineral profile of the essential elements Ca^{2+}, P, Na^{+}, Mg^{2+}, and urea in the urine of dairy cows (4 weeks after drying off) and lactating cows (12th week after parturition). Twelve Holstein dairy cows from the VPP-Oponice University Farm (Slovak University of Agriculture in Nitra, Slovakia) were used in the study. The dairy cows were on average in their fourth lactation. At the time of sampling, the feed ratio of the dairy cows corresponded to the nutritional requirements of the category and production use. Urine collection for the determination of mineral profile parameters was carried out by collecting the urine output during the urination of the dairy cows. Urine samples were collected in 50 ml sterile resealable tubes. The labelled tubes of urine were stored in a refrigerator at 4 to 8 °C. Analysis of the urine mineral profile was performed within two days of sample collection. Determination of Ca^{2+}, P, Na^{+}, Mg^{2+}, and urea in the urine of dairy cows was carried out using commercial BIO-LA-TEST kits (Erba Mannheim, Germany), according to the manufacturer's methodology and recommendations. The colour change of the reaction solution was measured with a spectrophotometer (Reasol, Italy). Urine samples from both groups of dairy cows (dry as well as lactating) were analysed in duplicate. The results were processed in the statistical program IBM SPSS v. 20. The significance of differences in mean values between urine collected from dry and lactating cows were tested by *T*-test. A value of $P < 0.05$ was considered to be significant.

3 Results and discussion

The mean urinary calcium concentration of the dry cows was 1.86 mmol.l^{-1} (Table 1). Thus, the value determined exceeds the physiological standard of 1.5 mmol.l^{-1} published by Kahn (2005). During the fourth week post-drying, compared to the physiological optimum, 10 of the 12 samples showed higher urinary Ca concentrations. Compared to dry cows, urine from fully lactating cows contained a demonstrably lower calcium concentration of 1.17 mmol.l^{-1} ($P = 0.002$). During lactation, only three cow urine samples contained a higher Ca concentration compared to the physiological optimum. Urinary Ca concentrations below the minimum physiological optimum were not recorded regardless of the sampling date. Urinary Ca concentrations above the physiological optimum found in 10 of 12 dry cows may be attributed to calcium overfeeding, which according to Schröder and Breves (2006) is ineffective and leads to increased Ca excretion in urine and faeces. There is an increased need for Ca during parturition, during colostrum production and at the beginning of lactation. Kimura et al. (2006) reported that more than 45% of dairy cows are affected by subclinical hypocalcemia, which may be due to the low efficiency of Ca utilization from the ration.

The mean determined phosphorus concentration in the urine of dried cows was 0.88 mmol.l^{-1} (Table 1). No deviations from the physiological norm were detected. Compared with lactating cows, the urinary P concentration of dry cows was lower ($P < 0.001$). Slightly below the physiological limit was found in 2 lactating dairy cows. The authors (Kraft and Dűrr, 2013) state that phosphorus is present in almost all tissues. It provides nutrient transport at the cellular level similar to calcium. Their concentration in the internal environment is controlled by secreted parathyroid hormone, thyrocalcitonin as well as vitamin D. Parathyroid hormone also acts directly on the release of calcium from bone and the excretion of phosphorus by the kidneys (Moreira al.2009). Phosphorus deficiency (hypophosphatemia) in rations is a common cause of vitamin D deficiency,

Table 1 Urine mineral profile of dry cows and lactating cows (mmol.l^{-1})

	Physiological interval*	Dry cows		Lactating cows		*P*-value
		mean ±s.d.	range	mean ±s.d.	range	
Ca	0.12–1.50	1.86 ±0.41	1.39–2.76	1.17 ±0.54	0.47–2.13	0.002
P	0.32–5.17	0.88 ±0.09	0.74–1.04	0.43 ±0.16	0.26–0.89	<0.001
Mg	6.17–16.50	16.46 ±1.29	14.2–18.2	14.24 ±2.63	10.3–18.6	0.016
Na	20–80	4.97 ±0.61	4.12–6.14	2.70 ±1.01	1.14–4.20	<0.001
Urea	130–300	212 ±42.6	140–285	229 ±45.7	132–288	0.334

* physiological interval according to Kahn (2005); □ sampled cows at fourth week post-drying; ■ sampled cows at the 12th week after parturition; *P*-value indicate the significance of difference of mean values between dry and lactating cows; s.d. – standard deviation

resorption disorders and also increased urinary excretion of phosphorus. In phosphorus deficiency and calcium excess, fertility disorders arise due to alkaline reactions of cervical mucus (Kraft and Dürr, 2013; Elizondo Salazar et al., 2013). In terms of calcium and phosphorus utilization, it is important to maintain a 2 : 1 ratio between them (Goff, 2006). Based on the determined urinary phosphorus concentrations of the studied dairy cows, it can be concluded that the phosphorus content of the dairy cows' diet was correct.

The magnesium concentration in the urine of dry cows did not fall below the lower limit of the physiological optimum (Table 1). On the other hand, the highest value found was 18.24 mmol.l^{-1} and the urine of 6 out of 12 dry cows showed values higher than the physiological optimum. The urine of lactating cows contained a lower concentration of magnesium (P = 0.016) compared to the urine of dry cows. However, the mean magnesium concentration remained within the physiological range. Although 3 of 12 urine samples from lactating cows had magnesium concentrations higher than the physiological optimum. Utilization of magnesium from a normal ration is about 20%. The importance of magnesium is mainly in the effects of neuromuscular excitability. Its deficiency increases the reactivity of muscle fibers causing a risk of tetany. Magnesium utilization is greatly reduced by higher dietary nitrogen and potassium (Holtenius et al., 2008).

Sodium was significantly below the physiological value in all cows (Table 1). According to Slanina and Sokol (1991), hyponatremia is detected in dehydration, chronic metabolic acidosis, renal insufficiency, or long-term feed sodium withdrawal. We consider sodium deficiency in the ration to be the most likely cause of the low urinary sodium concentration in the cows of this study. Sodium deficiency may be due to inadequate supplementation of mineral licks or poor availability of mineral licks on the feed table. This is supported by the results of a study published by Kemp (1964) who found a close correlation between sodium intake and urinary sodium concentration in dairy cows. A demonstrably higher concentration of urinary sodium was found in dry cows compared to lactating cows (P <0.001).

All urinary urea concentrations in cows were within the physiologically normal range, irrespective of the group of cows (Table 1). Differences between dry cows and lactating cows were not demonstrable (P = 0.334). According to the authors (Bannink et al., 1999), urea concentrations should be checked primarily in milk and not in urine. A long-term decrease below the norm implies a deficiency of nitrogen in the diet with all the consequences, especially on the level of performance and animal health. High levels above the norm indicate an excess of rumen soluble nitrogen or unbalanced nutrition, causing health disorders, hoof disease, liver damage, and reproductive disorders (Hanušovský et al., 2017). Therefore, we can conclude that the nitrogen intake in the diet of the cows studied was correct

4 Conclusions

The aim of the study was to determine the mineral profile in the urine of dry-aged and lactating cows. We found demonstrably lower concentrations of Ca, P, Mg and Na in the urine of lactating cows compared to dry cows. Ten out of twelve (for calcium) and six out of twelve (for magnesium) urine samples from dry-feeding cows contained higher concentrations compared to the physiological range. For lactating cows, these counts were significantly lower three out of twelve (for calcium) and two out of twelve (for magnesium). Two urine samples from lactating cows showed phosphorus concentrations below the lower limit of the physiological interface. The urine of all evaluated fasting as well as lactating dairy cows showed Na concentrations below the physiological optimum, indicating a Na deficiency in the ration. Monitoring of mineral concentrations in the urine of dairy cows is non-invasive and stress-free for the animals. Urinary mineral concentrations in dairy cows will allow early detection of imbalance or disease before clinical signs develop.

Acknowledgments

This work was supported by grant KEGA No. 039SPU-4/2019.

References

Bannink, A., Valk, H., & Van Vuuren, A. M. (1999). Intake and excretion of sodium, potassium, and nitrogen and the effects on urine production by lactating dairy cows. *Journal of Dairy Science*, 82(5), 1008–1018. https://doi.org/10.3168/jds.S0022-0302(99)75321-X

Bertoni, G., Trevisi, E., & Lombardelli, R. (2009). Some new aspects of nutrition, health conditions and fertility of intensively reared dairy cows. *Italian Journal of Animal Science*, 8(4), 491–518. https://doi.org/10.4081/ijas.2009.491

Dervishi, E., Zhang, G., Hailemariam, D., Mandal, R., Wishart, D. S., & Ametaj, B. N. (2018). Urine metabolic fingerprinting can be used to predict the risk of metritis and highlight the pathobiology of the disease in dairy cows. *Metabolomics*, 14, 1–15. https://doi.org/10.1007/s11306-018-1379-z

Dvořák, R. (2005). *Výživa skotu z hledisek produkční a preventivní medicíny* [*Cattle nutrition from the point of view of production and preventive medicine*]. Česká buiatrická společnost.

Elizondo Salazar, J. A., Ferguson, J. D., Beegle, D. B., Remsburg, D. W., & Wu, Z. (2013). Body phosphorus mobilization and deposition during lactation in dairy cows. *Journal of Animal Physiology and Animal Nutrition*, 97(3), 502–514. https://doi.org/10.1111/j.1439-0396.2012.01291.x

Eom, J. S., Kim, E. T., Kim, H. S., Choi, Y. Y., Lee, S. J., Lee, S. S., Kim, S. H., & Lee, S. S. (2021). *Metabolomics* comparison of serum and urine in dairy cattle using proton nuclear magnetic resonance spectroscopy. *Animal Bioscience*, 34(12), 1930. https://doi.org/10.5713/ab.20.0870

Goff, J. P. (2006). Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders. *Animal feed science and technology*, 126(3–4), 237–257. https://doi.org/10.1016/j.anifeedsci.2005.08.005

Hanušovský, O., Šimko, M., & Bíro, D. (2017). *Kontinuálne sledovanie parametrov bachorového prostredia využitím prenosu dát nízkofrekvenčným signálom* [*Continuous monitoring of the parameters of the rumen environment using data transmission with a low-frequency signal*]. Slovak University of Agriculture in Nitra.

Holtenius, K., Kronqvist, C., Briland, E., & Spörndly, R. (2008). Magnesium absorption by lactating dairy cows on a grass silage-based diet supplied with different potassium and magnesium levels. *Journal of Dairy Science*, 91(2), 743–748. https://doi.org/10.3168/jds.2007-0309

Juráček, M., Vašeková, P., Massányi, P., Kováčik, A., Bíro, D., Šimko, M., Gálik, B., Rolinec, M., Hanušovský, O., Kolláthová, R., Mixtajová, E., & Kalúzová, M. (2021). The effect of dried grape pomace feeding on nutrients digestibility and serum biochemical profile of wethers. *Agriculture*, 11(12), 1194. https://doi.org/10.3390/agriculture11121194

Kabir, M., Hasan, M. M., Tanni, N. S., Parvin, M. S., Asaduzzaman, M., Ehsan, M. A., & Islam, M. T. (2022). Metabolic profiling in periparturient dairy cows and its relation with metabolic diseases. *BMC Research Notes*, 15(1), 231. https://doi.org/10.1186/s13104-022-06130-z

Kahn, C. M. (2005). The Merck Veterinary Manual (9th ed.). White house station, NJ, USA, Merck & CO.

Kantíková, M., & Balážik, T. (2003). *Diagnostika metabolických porúch alebo prevencia je vždy lacnejšia* [*Diagnosis of metabolic disorders or prevention is always cheaper*]. *Slovenský chov*, 8(7), 39–40.

Kemp, A. (1964). Sodium requirement of milking cows: balance trials with cows on rations of freshly mown herbage and on winter rations. *Netherlands Journal of Agricultural Science*, 12(4), 263–280. https://doi.org/10.18174/njas.v12i4.17513

Kimura, K. A. Y. O. K. O., Reinhardt, T. A., & Goff, J. P. (2006). Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. *Journal of Dairy Science*, 89(7), 2588–2595. https://doi.org/10.3168/jds.S0022-0302(06)72335-9

Kraft, W., & Dürr, U. M. (2013). *Klinische Labordiagnostik in der Tiermedizin* [*Clinical laboratory diagnostics in veterinary medicine*]. Schattauer Verlag.

LeBlanc, S. J., Lissemore, K. D., Kelton, D. F., Duffield, T. F., & Leslie, K. E. (2006). Major advances in disease prevention in dairy cattle. *Journal of Dairy Science*, 89(4), 1267–1279. https://doi.org/10.3168/jds.S0022-0302(06)72195-6

Mihok, T., Bujňák, L., Hreško Šamudovská, A., Maskaľová, I., & Zigo, F. (2021). Lipid metabolism in the cattle in different stages of reproductive cycle. *Asian Journal of Agriculture and Food Sciences*, 9(6), 237–241. https://doi.org/10.24203/ajafs.v9i6.6836

Moreira, V. R., Zeringue, L. K., Williams, C. C., Leonardi, C., & McCormick, M. E. (2009). Influence of calcium and phosphorus feeding on markers of bone metabolism in transition cows. *Journal of Dairy Science*, 92(10), 5189–5198. https://doi.org/10.3168/jds.2009-2289

Rolinec, M., Bíro, D., Šimko, M., Juráček, M., Hanušovský, O., Schubertová, Z., Chadimová, L., & Gálik, B. (2021). Grape pomace ingestion by dry cows does not affect the colostrum nutrient and fatty acid composition. *Animals*, 11(6), 1633. https://doi.org/10.3390/ani11061633

Schröder, B., & Breves, G. (2006). Mechanisms and regulation of calcium absorption from the gastrointestinal tract in pigs and ruminants: comparative aspects with special emphasis on hypocalcemia in dairy cows. *Animal Health Research Reviews*, 7(1–2), 31–41. https://doi.org/10.1017/S1466252307001144

Slanina, Ľ., & Sokol, J. (1991). *Vademecum veterinárneho lekára* [Vademecum of the veterinarian]. Príroda.

Zábranský, L., Galik, B., Poborska, A., Hadačova, V., Šoch, M., Lad, F., Petrašková, E., & Frejlach, T. (2019). Influence of probiotic feed supplements on functional status of rumen. *Journal of Central European Agriculture*, 20(4), 1044–1054. https://doi.org/10.5513/JCEA01/20.4.2157

Zábranský, L., Poborská, A., Gálik, B., Šoch, M., Brož, P., Kantor, M., Kernerová, N., Řezáč, I., Rolinec, M., Hanušovský, O., Strnad, L., & Havrdová, N. (2022). Influence of probiotic strains bifidobacterium, lactobacillus, and enterococcus on the health status and weight gain of calves, and the utilization of nitrogenous compounds. *Antibiotics*, 11(9), 1273. https://doi.org/10.3390/antibiotics11091273

Zhang, G., Mandal, R., Wishart, D. S., & Ametaj, B. N. (2021). A multi-platform metabolomics approach identifies urinary metabolite signatures that differentiate ketotic from healthy dairy cows. *Frontiers in Veterinary Science*, 8. https://doi.org/10.3389/fvets.2021.595983