Título:

Magnesium status in Canarian and Holstein cows in early lactation: correlation between serum and urine testing

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Summary

High-producing dairy cows often experience various metabolic disorders, particularly during stressful periods or times of increased production, such as pre- and post- partum stages. These disturbances can lead to health problems for the animals and economic losses for farmers. One of the most important disorders is hypomagnesemia, also known as grass tetany, which is important in cattle worldwide. The main way for the control of this pathology is prevention, as well as continuous monitoring and early detection of those cases that do not show clinical signs (subclinical). The methods for its evaluation are diverse, being mainly used the measurement of magnesium in serum and urine samples through multiple clinical and laboratory procedures. The aim of this study is to assess the usefulness of paired serum and urine magnesium determination, and its comparison between cattle breed with potentially different feeding strategies (Holstein versus Canarian breed), in addition to evaluate the potential use of an alternative (non-validated) urine magnesium methodology using the IDEXX 'Catalyst One' system. This study included a total of 15 cows (n=15), of which 10 belonged to Holstein Friesian cows and 5 to Canaria breed cows, selecting animals that were between 0-60 days of lactation (DIM). Blood and urine samples were collected from each animal.

Urine samples were analysed using two different methods: one by a reference laboratory procedure, and the other by the 'Catalyst One' biochemical analyser, which has not yet been validated for this purpose. The results demonstrated a strong correlation between both methods, stating that 'Catalyst One' can be used for the analysis of magnesium in bovine urine samples with a certain degree of reliability. However, definitive validation requires a study with a larger sample size. Also, it was proved an agreement between the results from blood and urine samples, so both samples are very useful for assessing magnesium levels in cattle.

Future studies should focus on collecting more data and information through the selection of a larger number of animals.

Keywords: magnesium, urine, Friesian, Canaria, Catalyst One

1. Introduction

Animal and plant tissues hold diverse quantities and ratios of mineral elements, varying significantly across species and environments. Some of them are considered essential for life, such as the macronutrient minerals, which include calcium, phosphorus, potassium, sodium, chloride, magnesium, and sulphur. Additionally, micronutrients (also known as trace elements) are also present, among which are iron, iodine, copper, zinc, manganese, chloride… (Suttle, 2022).

Minerals perform four types of functions in animals. The first is their structural function, as they form part of tissues and organs, such as silicon in bones and teeth, or phosphorus and sulfur in muscle proteins. Secondly, minerals have a physiological function, participating in the maintenance of osmotic pressure, acid-base balance, and the transmission of nerve impulses (Byrne & Murphy, 2022; Suttle, 2022). Additionally, another function is catalytic, as minerals are involved in endocrine and enzymatic systems as catalytic agents. Finally, their regulatory function is notable, as minerals modulate cell replication and differentiation.

These mineral requirements may increase during some stressful periods, like reproduction, due to the additional demands associated with the foetus and the products of conception. (i.e. placenta, uterus and foetal fluids), reaching a peak in late gestation (Suttle, 2022).

Periparturient period, and particularly the post-partum phase, represents the most vulnerable time for the development of metabolic disorders in dairy cows. It is estimated that approximately 75% of diseases in dairy cows occur within the first month following calving (LeBlanc et al., 2006). This period is characterized by numerous physiological changes, including significant hormonal fluctuations, the transition from a non-lactating to a lactating state, and a marked decrease in feed intake. Consequently, serum concentrations of macrominerals and glucose often change during this time (DeGaris & Lean, 2008). Furthermore, subclinical forms of mineral deficiencies have been implicated in reduced production levels and diminished feed efficiency, both of which can result in significant economic losses for dairy farmers (Kabir et al., 2022).

Mineral deficiencies are most commonly observed in animals that graze or are fed exclusively on forage or home-produced feeds such as those used in organic farming systems, without the addition of mineral supplements. In contrast, the likelihood of deficiencies is greatly reduced when animals receive substantial amounts of commercially prepared, mineral-fortified concentrate feeds (Scott et al., 2011). Over time, the decreased dependence on home-grown feeds, coupled with the increased utilization of commercially purchased feeds (which are often sourced from regions without the same mineral deficiencies and are frequently fortified with essential minerals and vitamins) has led to a reduction in the prevalence of disorders previously recognized at the local level (Phillips, 2018).

The manifestation of clinical signs related to minerals deficiencies depends on both the extent and duration of insufficient mineral intake. The progression from inadequate mineral consumption to the appearance of clinical disease typically involves four distinct stages. Initially, during the depletion phase, mineral reserves in storage sites (e.g., liver, bone) diminish, though levels in the transport system remain normal. Subsequently, occur a level decline in the transport system (deficiency phase). As the condition progresses into the dysfunction stage, animals are clinically normal, but metabolism may be compromised because of the decrease in enzyme concentrations and functions. Finally, the disease stage emerges when metabolic disruptions lead to observable clinical abnormalities, marking the full manifestation of pathology (Scott et al., 2011). Also, mineral deficiencies can be classified as either primary (or simple), which occur when the dietary intake of minerals are insufficient, or as secondary (or conditioned), which arise when the mineral content in the diet is adequate but other factors interfere with its absorption or utilization by the animal.

1.1. Magnesium functions

Magnesium (Mg^{2+}) is an essential mineral which is crucial for enzymatic reactions after combining with the enzyme or substrate. Magnesium acts as a modulator of synaptic transmission in the central nervous system (Möykkynen et al., 2001) at the motoric endplate, in immunological pathways (Li et al., 2011) and in timekeeping (Feeney et al., 2016). Also, topoisomerases, helicases, and cyclases all require magnesium for their activity. Magnesium also performs non-enzymic functions: folding ribonucleotide chains by binding to phosphate groups (Ahmed and Mohammed, 2019).

Additionally, Mg^{2+} plays a role in the regulation of ion channel gating (Vemana et al., 2008). The modulation of ion channel function in the central nervous system by Mg^{2+} likely explains neurological manifestations such as ataxia, recumbency, convulsions, and tetanic muscle spasms observed in hypomagnesemia. This mechanism has been recognized in cattle for approximately 80 years, clinically termed grass tetany (Martens et al., 2018).

1.2. Magnesium homeostasis

The normal plasma magnesium concentration ranges from 1.8 to 2.3 mg/dL; however, it is important to note that blood values are a poor indicator of total body magnesium status, for his principally intracellular cation (Peek & Divers, 2018). At the same time, extracellular magnesium concentrations are intimately associated with the proper functioning of both nervous and muscular systems.

Approximately 60–70% of the total body magnesium is sequestered within the skeleton. An additional 30% is located in the intracellular compartment, although only 1–5% of intracellular magnesium exists in the ionized form (Houillier, 2014).

Magnesium present in the extracellular space reveals about 1% of the total body magnesium, and its concentration depend on its absorption (principally from the rumen and omasum, which ranges from 10–35%), the requirements for milk production, and renal excretion (Scott et al., 2011).

Between 20% and 40% of plasma Mg^{2+} is bound to albumin and globulin, while approximately 10% is attached to weak acids and forms complexes with small anions such as citrate, phosphate, and bicarbonate. Consequently, 50–70% of plasma magnesium remains in the ionized form (Lopez et al., 2006; Martens et al., 2018).

The majority of magnesium in the body is stored in bone; however, during magnesium deficiency, this reservoir is not readily mobilized, in contrast to the rapid endocrine release of calcium from bone observed in hypocalcaemia. The mobilization of magnesium from the adult bovine skeletal system occurs at an insufficient rate to mitigate the onset of hypomagnesaemia during acute and severe dietary Mg^{2+} depletion (Peek & Divers, 2018; Robson et al., 2004). Instead, serum magnesium concentrations are primarily regulated by daily intestinal absorption and urinary excretion.

Mg^{2+} has no hormonal regulation, so plasma magnesium concentration is primarily determined by various factors: on the absorption of Mg^{2+} (influx) from the gastrointestinal tract into the extracellular space (ECS) (including plasma) and on the efflux from ECS into milk (principally in dairy cows), into the intracellular space (ICS) including soft tissue and bones during growth, as well as the foetus during pregnancy and into the endogenous secretion via intestine. Also, Mg^{2+} not required for plasma, milk and endogenous secretion is eliminated by the kidneys through urine (Martens et al., 2018; Martens & Stumpff, 2019).

1.3. Magnesium absorption

Mg^{2+} is absorbed from the forestomaches (rumen, reticulum and omasum) via an active transport mechanism and principally by the rumen (Martens & Schweigel, 2000). The dissolution of magnesium in the ruminal fluid constitutes a critical prerequisite for its efficient absorption across the rumen epithelium (Goff, 2014).

This absorption from the forestomaches is essential for maintaining normal plasma Mg^{2+} concentrations and constitutes a prerequisite for magnesium homeostasis. A reduction in Mg^{2+} absorption from the forestomaches is not compensated for by increased absorption in the intestine (Martens et al., 2018). It has been demonstrated that, following the dietary transition from milk to solid feed in calves and lambs, Mg^{2+} absorption shifts from the hindgut to the developed rumen. In adult animals, Mg^{2+} absorption from the large intestine persists and may be utilized therapeutically in the management of acute hypomagnesaemia.

Several factors influence the availability of dietary magnesium, including the magnesium content of soil and grasses, which can vary significantly. Elevated potassium levels, often resulting from the application of potash fertilizers, can impede magnesium absorption, whereas sodium is essential for its uptake. Additionally, high concentrations of ammonia (because of the use of nitrogenous fertilizers) may inhibit magnesium absorption by increasing ruminal pH. Furthermore, lush spring pastures that are low in fibre, accelerate the passage of material through the rumen, thereby reducing the time available for magnesium absorption (Cockcroft, 2015; Phillips, 2018; Scott et al., 2011).

1.4. Effects of potassium on magnesium

Potassium has a direct effect on calcium and magnesium's absorption, by modulating the electrochemical transepithelial gradient. For cattle, the optimal potassium concentration in herbage should be less than 10 g/kg; however, maintaining such low levels may adversely affect plant growth (Phillips, 2018). Nevertheless, substituting a portion of the potassium fertilizer with sodium allows sodium to fulfil some of potassium's physiological roles within the plant, thereby reducing the potassium content. One way to increase the sodium concentration in plants is to reduce the application of potassium fertilizers and substitute them with sodium sources, such as common salt or sodium nitrate. Therefore, when Na intake is sufficient, the primary factor influencing magnesium absorption in standard and commercial bovine diets is potassium (Schonewille et al., 2008; Weiss & Wyatt, 2004).

1.5. Magnesium losses

Obligatory losses of magnesium occur through saliva, bile and the gastric and pancreatic secretions. Additionally, magnesium is excreted in milk, and this route of efflux remains constant even in cows experiencing severe hypomagnesemia. Consequently, the loss of magnesium via milk further exacerbates magnesium deficiency and poses a significant challenge to the maintenance of plasma magnesium homeostasis (Martens & Stumpff, 2019).

The concentration of magnesium in milk is significantly higher than in plasma and demonstrates a high degree of heritability in cows (van Hulzen et al., 2009), and this concentration is higher in early colostrum (Kehoe et al., 2007).

Excess dietary magnesium intake is filtered by kidneys and eliminated through urine. Approximately 20–30% of Mg^{2+} is reabsorbed in the proximal tubule of the kidney, while 60–70% is reclaimed in the thick ascending limb of the loop of Henle (Houillier, 2014).

Urinary magnesium concentration is subject to minimal hormonal regulation and is predominantly influenced by dietary intake, the efficiency of intestinal magnesium absorption (which can be inhibited by potassium) and the overall volume of urine excreted (Peek & Divers, 2018). Therefore, a urinary magnesium concentration of less than 1 mmol/L is likely an indicator of inadequate intake or absorption (Martens et al., 2018).

Cattle affected by hypomagnesemia typically present with reduced serum magnesium concentrations (below 0.8 mg/dL) and a fractional excretion of magnesium of less than 4% (Peek & Divers, 2018).

Thus, kidneys demonstrate adaptive regulatory mechanisms in response to fluctuations in absorbed magnesium levels, establishing urinary excretion as a reliable biomarker for evaluating magnesium status. This method proves particularly valuable for assessing the comparative bioavailability of inorganic magnesium sources under conditions of excess intake (Silva-del-Rio et al., 2024). Current research efforts investigating magnesium sources have primarily focused on comparative bioavailability assessments through measurements of urinary magnesium excretion and apparent absorption rates.

Also, apart from urine analysis, the assessment of cerebrospinal fluid or aqueous humour may assist in confirming magnesium deficiency in animals that expected hypomagnesemia (Peek & Divers, 2018).

Test of vitreous humour or cerebrospinal fluid can be utilized to assess magnesium concentrations in cases of sudden death. A magnesium concentration below 0.55 mmol/L in vitreous humour within 48 hours post-mortem is considered hypomagnesaemia. For his part, the average magnesium level in the cerebrospinal fluid of healthy cattle is estimated at 0.86 mmol/L, the same observed in cattle with bacterial encephalitis (Cockcroft, 2015).

1.6. Hypomagnesemia

A deficiency of dietary magnesium may lead to subclinical disorders, resulting in decreased productivity and manifestations of irritability within herds and flocks, even in the absence of overt clinical disease (Dove & McMullen, 2009).

Hypomagnesemia is also known as grass tetany, grass staggers, transport tetany, and winter tetany. This metabolic disorder is most frequently observed in lactating beef cows during the first two months postpartum. It can also affect lactating dairy cows grazing on pasture in the spring, as well as dry cows in the autumn, which are non-supplemented. Furthermore, calves maintained on an all-milk diet and raised in confinement are similarly at risk, particularly between two and four months of age. The clinical manifestation is often triggered by factors such as cold weather, stress, transportation, lush pasture during spring and autumn, reduced feed intake, high potassium and nitrogen in the diet, lactation and pregnancy period and an individual susceptibility has been considered (Cockcroft, 2015; Smith et al., 2020).

1.6.1. Clinical signs

Clinical signs range from sudden death (peracute form, it is not very common) to subclinical disease. By the one hand, the acute form of hypomagnesaemia in cattle is characterized by an initial period of increased nervousness and hyperaesthesia, with affected cows often displaying a startled expression, exophthalmos, and markedly erect, twitching ears. Early clinical signs include anorexia, separation from the herd, muscle fasciculations (particularly around the head and neck) and an uncoordinated gait. As the condition progresses, cows may exhibit ataxia, nystagmus and tremors. In severe cases, animals become recumbent, with paddling movements of the limbs, teeth grinding, opisthotonus, and clonic convulsions. Additional signs may include uncontrolled diarrhoea and urination, foaming at the mouth, tachycardia, hyperthermia, and loud heart sounds. Seizure activity is often precipitated by external stimuli, such as restraint or clinical examination, and relapses are common even after apparent recovery. The subacute form presents with similar clinical signs, although these are prolonged over several days (Cockcroft, 2015; Phillips, 2018; Scott et al., 2011).

On the other hand, subclinical or chronic disease is common, but frequently goes undetected. This condition is characterized by low blood magnesium levels, dullness, reduced appetite, subtle changes in behaviour, odd facial expressions, and decreased production. Affected cows may appear slightly nervous, reluctant to be milked or herded, and often exhibit reduced dry matter intake and poor milk yield. Investigations have revealed an annual incidence of 3–4% in lactating dairy cows. Alterations in channel function within excitable tissues mediated by magnesium are likely responsible for the neurological manifestations observed in hypomagnesemia, including unsteady gait, bruxism, hypersalivation, ataxia, recumbency, convulsions, and ultimately, tetanic muscle spasms (Blowey, 2016; Martens & Stumpff, 2019).

1.6.2. Differential diagnosis

When acute form of hypomagnesemia is present, clinical signs can be confused with other pathological processes that affect ruminants. For example, diseases that produce forebrain signs, like infectious bovine rhinotracheitis, vitamin A deficiency, rabies or nervous ketosis. Also, other diseases that occur with spasticity or tremors, like cerebellar hypoplasia, bovine viral diarrhea or bluetongue. Finally, we cannot forget other differential diagnoses such as tetanus, hypocalcemia, lead, organophosphate poisoning or downer cows (animals that remain recumbise to their feet) (Buergelt et al., 2017; Smith et al., 2020).

1.6.3. Treatment

In case of seizures, the veterinary may act to avoid a fatal consequence. Firstly, sedatives help to maintain the animal in a calm state and assist in preventing muscle injury, through the administration of pentobarbitone, xylazine or acetylpromazine. Thus, when seizure activity has been controlled, should be administered calcium borogluconate plus magnesium sulphate slowly intravenously. Then, another magnesium sulphate administration is proportionate by subcutaneous injection. Subsequently, the animal should receive 70 g of calcined magnesite orally, or a comparable preparation, in order to restore intestinal magnesium levels. Nevertheless, remaining cows are highly likely to exhibit subclinical hypomagnesaemia, placing them at risk of developing acute grass tetany (Blowey, 2016). A rapid response to magnesium treatment serves to confirm the diagnosis; however, in untreated clinical cases, mortality rates can reach up to 30% (Donovan et al., 2004).

1.6.4. Management

To prevent this pathology, an adequate supply of magnesium is necessary. Various salt forms of magnesium, which are commercially available, are frequently added to the diets of dairy cows to ensure their nutritional requirements for magnesium are adequately met. Some of them are Mg sulfate ($MgSO_4$), Mg chloride ($MgCl_2$), Mg oxide (MgO), Ca-Mg carbonate [$CaMg(CO_3)_2$], Ca-Mg hydroxide [$CaMg(OH)_4$]. The most commonly added is Mg oxide (MgO), that contains 51 to 59% Mg^{2+} and also alkalizes the rumen.

Also, supplementation of drinking water is possible, using magnesium acetate. Furthermore, calcined magnesite can be supplied to the rations or dusted into the pastures in periods of risk. While providing unrestricted access to high-magnesium mineral supplements can be beneficial, it often results in some cows consuming more than their needs and others consuming insufficient amounts, so in general magnesium salts and minerals are not palatable. Finally, magnesium bullets are large cylindrical metallic objects administered orally and designed to remain in the reticulum, where they gradually dissolve to release small amounts of magnesium. They are regarded as the most reliable option in high-risk areas; however, there remains a slight possibility that they may be expelled from the rumen during rumination, which could leave certain animals inadequately protected (Bach et al., 2018; Urdaz et al., 2003).

1.7. Canarian breed cow

Canarian breed cow is a rustic breed present in the Canary Islands, mainly on the islands of Gran Canaria and Tenerife.

Its origin lies in the crossbreeding of various cattle breeds from the north of the Iberian Peninsula. It is a hardy and rustic breed, with an excellent environment adapted, and known for its docile temperament and longevity. While it is primarily used for milk production, it is considered a dual-purpose breed (meat and milk). It is also employed for field work, and participation in local festivals. Milk production is generally modest and mainly intended for self-consumption, but it is also used in the production of mixed-milk cheeses together with milk from other species, such as sheep and goats. The management systems are highly traditional, with minimal mechanization and, in most cases, exclusively family labour. The animals' diet tends to be quite basic, relying on local flora and other low-energy local products collected by the farmer (such as products from the brewing industry), although supplementation with forage and formulated rations is sometimes provided.

The average milk yield per lactation is approximately 3,500 kg, with a lactation period of around 240 days and a milk fat content of 3.35%. Regarding reproductive data, females reach sexual maturity at 23,65 months, and the average age at first parturition is 33 months. Additionally, the interval between parturitions is 13.5 months, with an average of 0.9 parturitions per year and a prolificacy rate of 1.125.

Currently, the population in the archipelago is estimated at around 1,030 animals, playing a fundamental role on the islands, supporting the livelihood of small businesses in the northern regions, as well as having significant cultural importance through its participation in various local festivities and traditions (Ministerio de Agricultura, 2025). Particularly, in the island of Gran Canaria, there are a total of 379 animals of which 269 are reproductive females (Consejería de Agricultura, Ganadería y Pesca, 2024).

1.8. Objectives

Based on the relevance of magnesium metabolism in cattle and in order to understand the usefulness of serum and urine detection of this mineral for the evaluation of potential variation between cattle breed with different feeding management strategies, the following objectives were set:

- To determine urine and blood serum magnesium concentration in a cohort of Friesian and Canarian cattle.

- To evaluate the usefulness of the Catalyst system (IDEXX) for the detection of magnesium in cattle urine.

2. Materials and methods

2.1. Animals

To carry out the study, a total of fifteen cows were selected, distributed in two groups: ten Holstein Friesian cows and five Canaria breed cows. The animals belonging to the Holstein Friesian breed came from an intensive farm specialised in dairy production, located in the municipality of Arucas, in the north of the island of Gran Canaria. The Canarian breed was selected from several small-scale farms located in the municipalities of Agüimes and Valleseco.

All animals were chosen based on their stage of lactation, ensuring that it was equal to or less than 60 days.

Both urine and blood samples were obtained from the selected animals. Urine samples were collected by spontaneous urination, using sterile containers designed for this purpose. On the other hand, blood samples were obtained by puncture of the mammary and coccygeal veins using a Vacutainer®, a vacuum extraction system (Becton, Dickinson and company). The samples were stored at 4°C until subsequent analysis.

After the samples were obtained, they were prepared for analysis. To obtain the serum from the blood sample, the blood was allowed to clot for a minimum of 20 minutes. Then, 45 minutes after collection, the sample was centrifugated at 3500 rpm for 10 minutes and the appropriate volume of sample was immediately transferred to a Catalyst sample cup using a transfer pipette.

2.2. Sample analysis

In the case of urine samples, these were transferred to a disposable sample tube, centrifuged and then deposited in a Catalyst sample cup following the same procedure as described for serum.

The serum and urine samples were analysed using the 'Catalyst One', a next-generation biochemical analyser for veterinary use only, belonging to the commercial brand 'IDEXX'. Urine samples were diluted 1/10 in sterile saline solution prior use in order to produce values withing the dynamic detection range of the Catalyst system. The determination of magnesium concentration was determined by employing a specific reagent, which triggers the following chemical reactions (IDEXX, 2024):

$$Mg^{2+} + Ca^{2+} \xrightarrow{\text{chelator}} Mg^{2+} + Ca^{2+} \text{-chelator complex}$$

$$Mg^{2+} + \text{formazan dye derivative} \xrightarrow{\text{pH 9.75}} Mg^{2+} \text{-dye complex}$$

In addition, the urine samples were evaluated by two procedures. On the one hand, all samples were evaluated with the biochemical analyser 'Catalyst One'. On the other hand, five samples from Holstein Friesian cows and four samples from Canarian breed cows were sent to the laboratory 'Eurofins Lgs Megalab Analisis Clinicos, S.L.U.' (Animal-Lab), located in Las Palmas de Gran Canaria. The urine samples were analysed in a DxC 700 AU Chemistry Analyzer (Beckman Coulter), following the reaction described below:

$$Mg^{2+} + \text{Xylidyl blue} \xrightarrow{\text{pH 11.4}} \text{Purple complex}$$

2.3. Statistical analysis

Descriptive data from each animal and magnesium concentration results in urine and serum were collected in a Microsoft® Excel database. Mean, range, standard deviations (±), 95% confidence intervals (CI) and Pearson's correlation statistical analyses was conducted using IBM® SPSS® Statistics version 26 (IBM Corp., Armonk, NY, USA). A p-value of <0.05 was considered statistically significant.

3. Results

Samples from a total of 15 animals were analysed, divided into 5 Canarian breed (33.3%) and 10 Holstein breed (66.7%).

The range of lactations varied between 1 (n=3; 20%) and 5 (n=1; 6.7%), with 3 being the maximum number observed (n=6; 40%). Mean age in years was 4.7, ranging from 2.01 to 6.1 years (±0.97; 95% CI=3.8-5.6).

Regarding DIM, mean observed was 25.5, ranging from 6 to 53 DIM (±18.58; 95% CI=8.4-42.7).

Magnesium concentration in urine ranged from 7.7 to 25.2 mg/dL, with a mean value of 19.8 mg/dL (±5.8; 95% CI=14.4-25.2). When classified by breed, Holstein cattle presented a mean urine magnesium concentration of 18.06 mg/dL, ranging from 11.9 to 25.2 mg/dL (±6.1; 95% CI=10.4-25.7), while the Canarian cattle breed presented a mean urine magnesium concentration of 20.2 mg/dL, ranging from 7.7 to 24.8 mg/dL (±8.3; 95% CI=6.90-33.4).

In regard to the magnesium concentration in serum, the values ranged from 1.13 to 2.73 mg/dL, with a mean value of 2.3 mg/dL (±0.54; 95% CI=1.8-2.8). Holstein cattle presented a mean serum magnesium concentration of 2.1 mg/dL, ranging from 1.13 to 2.58 mg/dL (±0.74;

95% CI=1.6-2.6), while the Canarian cattle breed presented a mean urine magnesium concentration of 2.2 mg/dL, ranging from 1.8 to 2.7 mg/dL (±0.46; 95% CI=1.6-2.8).

Based on Martens et al., (2018) classification for serum magnesium concentration in cattle, 80% of the Holstein cattle were classified as normal, while 10% of the animals were grouped in the clinical and 10% in the uncertain group. For Canarian breed cattle, 80% were classified as normal, while 20% were classified as subclinical.

Urine magnesium concentration classification (Kemp. A, 1983) clustered the Holstein breed animals in normal (100%), while for the Canarian cattle breed animals, 75% were classified as normal, while 25% were classified at risk.

All samples classified as normal for magnesium in serum presented a 100% agreement with the normal classification observed in urine in both Holstein and Canarian breed cattle. One sample from Canarian breed cattle classified as subclinical based on magnesium serum concentration was classified as at risk based on urine magnesium concentration. The only lack of agreement was observed in one sample classified as clinical based on serum magnesium concentration from a Holstein cow, that was classified as normal based on urine magnesium concentration classification. Animals did not present clinical signs at the time of sampling.

Table 1. Pearson's correlation of results between IDEXX and Animal Lab urine magnesium concentration determination.

		Animal Lab
IDEXX	Pearson's correlation	0.919
	p-value	0.027

The Pearson correlation coefficient (r) is a statistical measure, a coefficient that ranges from –1 to +1. A positive correlation ($r > 0$) signifies a direct relationship between the variables, whereas a negative correlation ($r < 0$) indicates an inverse relationship. A correlation value between 0 and 0.10 denotes a poor correlation, while values approaching 1 reflect increasingly strong correlations; for instance, an 'r' between 0.70 and 1.00 represents a very strong correlation.

Therefore, this table displays a Pearson's correlation analysis between the variables 'IDEXX' and 'Animal Lab', revealing a very strong, positive association (r= 0.919) that is statistically significant (p = 0.027), indicating that both variables increase simultaneously.

4. Discussion

The present study aims to increase our knowledge on the most appropriate determination strategy for magnesium in common biological samples, such as bovine blood serum and urine, in order to obtain results that may assist in the determination of clinical and subclinical cases of hypomagnesemia in cattle. The proportion of samples from the two breeds evaluated (66.7% Friesian and 33.3% Canaria) reflects the predominance of the Holstein Friesian breed in intensive systems, which has led to a significant decline in the population of native breeds, which are found in a very low proportion, making it difficult to find these native animals for the study, particularly when the study requires specific inclusion criteria, such as the one we have carried out by selecting females that were under 60 days of lactation, a period that is the greatest physiological need for magnesium in cattle. The average age of the animals was 4.7 years, with a range from 2.01 to 6.1 years, representing an age interval that corresponds to peak productive life.

In addition, the range of lactations observed (from 1 to 5, with a predominance of animals in the third lactation), as well as the variability in days in milk (from 6 to 53, with an average of 25.5 days in milk), are relevant parameters. These factors are significant, as both age and number of lactations can influence mineral metabolism, especially during periods of high metabolic demand, when magnesium is usually more compromised (Martens & Schweigel, 2000).

Studies carried out by Solorzano (1992) and Kronqvist (2011) showed that this period represents the greatest challenge for mineral metabolism. During early lactation, calcium demands for milk production can indirectly affect magnesium status, particularly considering that magnesium is essential for parathyroid function, hence the relevance in the selection of these animals, which potentially could have affected to select a higher number of Canary breed animals in this physiological state.

The mean serum magnesium value was 2.3 mg/dL, with the mean values for the Holstein Friesian and Canarian breed being very similar (2.1 and 2.2 mg/dL respectively). These values fall within the range reported by Cockcroft (2015) with values of 1.46 – 2.67 mg/dL and indicate a stability of magnesium under standard management and controlled conditions.

However, one of the Holstein Friesian cows exhibited a serum magnesium value of 1.13 mg/dL, below the normal values previously described, while presenting normal urine magnesium values. Despite being classified as ‘clinical’ according to (Martens et al., 2018), it did not show clinical signs of hypomagnesemia during the study period. This cow had the highest number of lactations at the time of the study (5), a factor that may be involved in these low serum magnesium values, considering that hypomagnesemia is more frequently reported in cows with three or more lactations (Melendez et al., 2023).

Another determining factor is the post-partum stage of the animal. Normally, magnesium levels in the early post-partum period are around 3.0 mg/dL (Cardoso et al., 2008) or approximately 2.5 mg/dL (Schallenberger Gonçalves et al., 2015), values that are higher than those observed in the late postpartum period, around 2.4 mg/dL (Gonzalez & Rocha, 1998). The animal evaluated had the lowest number of days in milk (6 days) among all the cows included in the sampling.

Another serum magnesium value obtained was classified as ‘uncertainly’ according to Martens et al. (2018), with a value of 2.05 mg/dL, corresponding to a Friesian cow. The Friesian cows tested belonged to a single farm, and most of the animals shared the same yard or area within the farm. Considering that the environmental conditions were homogeneous, the cows received the same diet and were milked simultaneously and under the same procedures, the observed differences can be attributed to individual factors. This suggests that, even under uniform management and environmental conditions, individual variations may occur within the same farm. This value, together with the previously described value of 1.13 mg/dL, shows the complexity of magnesium metabolism described by Melendez et al. (2023), where factors such as stress, fatty acid mobilisation and hormonal changes can temporally affect serum concentrations without necessarily indicating chronic deficiency.

On the other hand, one of the Canarian breed samples was classified as ‘subclinical’ for the classification of Martens et al. (2018) in serum and ‘at risk’ for the classification of Kemp (1983) of magnesium concentration in urine, confirming that this animal had decreased magnesium values, which could compromise the health of the animal if the situation progresses. This situation may be attributed to different circumstances, suggesting differences in absorption, nutritional management or genetic factors, among others. Among the genetic factors, one of the studies carried out by (Greene et al., 1989), showed that magnesium absorption (and therefore the capacity of mobilisation from bone reserves) varies according to the different breeds, being lower in European breeds and higher in the Brahman breed (Indian origin) and its crosses. The study also showed that the incidence of hypomagnesemia in these Indian-origin breeds is lower compared to other breeds.

Therefore, the current small population of the Canarian breed in the islands, together with the isolation of its different population nuclei due to insularity, could favour a certain degree of homozygosis in this breed, reducing magnesium availability in these animals. However, this hypothesis should be studied in greater detail.

Moreover, urinary magnesium concentration ranged from 7.7 and 25.2 mg/dL, with an average of 19.8 mg/dL. The average was slightly higher in the Canarian breed (20.2 mg/dL) compared to the Friesian breed (18.06 mg/dL). Although both mean values are within the ranges established by Kemp (1983), it should be noted that 25% of the Canarian breed animals were classified as ‘at risk’, which may reflect various variations such as diet, production level or management practices, as previously discussed.

The study showed a high level of concordance between urine and serum magnesium results. All animals classified as normal in serum were also normal in urine. Only one exception was identified: a Friesian cow classified as ‘clinical’ according to serum results, but ‘normal’ based on urine results.

Urinary magnesium is a reliable indicator of dietary intake, as a decrease in magnesium intake is immediately reflected in reduced urinary excretion, whereas blood tend not to fluctuate during moderate magnesium deficiency (Martens & Schweigel, 2000). Therefore, it is recommended to consider both types of analysis and evaluate both parameters together, in order to make a comprehensive assessment of magnesium status in cattle and to improve the detection of subclinical states, allowing earlier intervention.

Regarding breeds differences, although the sample size is limited, interesting trends were observed between both breeds. The Canarian breed showed a lower variability in serum (± 0.46 compared to ± 0.74 in Friesian), but a higher dispersion in urine (± 8.3 compared to ± 6.1 in Friesian). The lower serum variability in the Canarian breed could be associated to higher homozygosity (as previously suggested) which reduces genetic diversity and phenotypic variability in certain metabolic parameters. Likewise, the greater dispersion in urine (with similar serum values) may be due to individual variations in renal excretion, possibly influenced by physiological, environmental or management factors.

No animal showed clinical signs of hypomagnesemia at the time of sampling, which underlines the importance of preventive monitoring, as subclinical hypomagnesemia can go unnoticed and negatively affect production and animal welfare. Strategic supplementation and dietary adjustment, particularly during periods of increased risk, are recommended measures to prevent deficiencies.

Also, although the present study provides valuable data, the relatively small sample size (n=15) and the concentration of early lactation animals limit the extrapolation of the results. Reference studies, such as the work of Da Silva et al. (2022) in Brazil, which included 450 cows, demonstrate the importance of larger studies to establish firmer reference values.

On the other hand, the method normally used for the measurement of magnesium in urine is the “AU Chemistry Analyzer” from Beckman Coulter. This is a colorimetric method for the quantitative measurement of magnesium in urine (also in serum and plasma), useful for assessing magnesium balance in the organism and assisting in the diagnosis and treatment of conditions such as hypomagnesemia. This method is widely accepted and used in clinical laboratories for its accuracy, automation and reliability in measuring urine magnesium. However, it is a high-cost method and, in most cases, requires send the samples to an external laboratory for evaluation.

Another alternative method is the Catalyst One biochemical analyser, designed for veterinary use and available for testing bovine samples, one of the compatible species. However, there are no specific reference intervals available for this species unlike other species such as dogs, cats and horses, and it is not considered the reference method for magnesium analysis in cattle. For these reasons, we decided to test this analyser to determine whether it is a reliable method for this purpose, with the aim of trying to make the assessment of urinary magnesium in cattle easier and less costly.

Among the advantages of this new method that the Catalyst One device is more widely available in veterinary clinics, particularly in those dedicated to small animals, resulting in greater accessibility for sample evaluation. This eliminates the need to rely on external laboratories, along with the associated costs and logistical aspects, such as sample shipping.

Additionally, this method has a lower cost compared to the determination using the "AU Chemistry Analyzer" from Beckman Coulter, which is validated for the measurement of magnesium bovine urine, unlike the IDEXX analyser.

To confirm the validity of this method, results from the 'Animal Lab' laboratory were compared with those obtained using the 'Catalyst One' analyser, and the Pearson correlation coefficient (r) was calculated. The interpretation of correlation coefficients can vary considerably depending on the scientific field research in which they are applied. In medicine, a correlation coefficient (r) of 0.7 indicates a moderate correlation, while an "r" of 0.8 and 0.9 shows a very strong correlation (Akoglu, 2018).

The strong correlation found between IDEXX and Animal Lab laboratory determinations ($r= 0.919$, $p < 0.05$) validates the methodological robustness of the study, as well as the reliability of the results obtained. Although the sample size is small, the statistical significance observed allows to affirm that both methodologies or instruments provide comparable results, supporting the use of these methods in the routine monitoring of magnesium in cattle.

The results obtained indicate the importance of monitoring magnesium levels in dairy cattle, particularly during the transition period. Although no obvious clinical signs were detected in the animals evaluated, the presence of clinical (serum-based) and subclinical cases shows that the risk of hypomagnesemia is present, which could lead to reduced milk production, convulsive episodes with nystagmus and salivation, or even sudden death without previous signs. This acute hypomagnesemia has an annual prevalence of less than 1%, while subclinical or chronic cases are estimated at 3-4 % in lactating cows (Scott et al., 2011). Moreover, it is advisable to incorporate preventive strategies such as supplementation with magnesium salts in the prepartum and postpartum period, especially in high-risk animals, as well as restricting potassium levels (Suttle, 2022). In the case of native breeds (such as the Canaria), the development of own reference values could be useful, as their physiology may differ from the standard values currently used, which are mostly based on specialised breeds.

In future studies, it is recommended to include a larger number of animals to achieve a larger sample size and greater statistical significance, as well as to consider other variables such as seasonal and environmental factors, or even the inclusion of groups with different levels of mineral supplementation.

5. Conclusions

Based on the results obtained in our study, the following conclusion can be drawn

- Due to the potential incongruencies between serum and urine magnesium concentration values, paired testing should be performed when possible.
- Although based on a small sample size, it can be concluded that IDEXX ‘Catalyst One’ analyser could be considered a viable system for the future determination of magnesium in urine samples, which could facilitate the diagnosis magnesium deficiency in cattle. However, full validation should be carried out through a study including a larger sample size in order to obtain a higher statistical significance.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used artificial intelligence (AI) tools to enhance the readability, clarity, and fluency of the text. After using these tools, the authors reviewed and edited the content as needed and therefore take full responsibility for the content of the documents and for the ethical use of the AI tools.