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# The Relationship between Nutrition and the Prevalence of Subclinical Mastitis under Practical Conditions on Holstein Dairy Farms in Three Regions of Albania

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**Abstract**

This study investigated the relationship between nutrition and the prevalence of subclinical mastitis (SCM) on Holstein dairy farms in three regions of Albania. The study was conducted on nine Holstein dairy farms (with 21–140 cows per farm) in the Durrës, Shkodra, and Korça regions. The 8-month study (December 2023–July 2024) covered winter, spring, and summer and included 1st, 2nd, and 3rd lactation periods. Feeding, housing, and hygiene practices were documented via on-site evaluation and farmer interviews, and no changes were made during the study period. On each farm, 6–11 cows were tested for SCM using the California Mastitis Test (CMT) at the 1st, 2nd, and 3rd lactation periods. Udder quarter-level scores were recorded. Samples of 27 used rations were collected and analyzed for organic nutrients using near-infrared spectroscopy and for calcium (Ca), phosphorus (P), zinc (Zn), and selenium (Se) content using chemical methods. The amount of Ca, P, Zn, and selenium (Se), as well as vitamins E, A, and D, in the mineral-vitamin premixes was calculated based on the providers' declarations. On average, the diets met the recommended requirements for cows producing 14–33 kg of milk per day, though some rations fell below the recommended minimums. Several rations had P and Zn concentrations below the threshold. The mean SCM prevalence was 43.2% (range 0% to 83.3%). The highest prevalence (55.6%) was observed in early lactation. Cows with severe SCM averaged 31.5%, reaching 66.7%. No significant correlation was observed between SCM prevalence and total energy or organic nutrient intake, except for net energy for lactation per kilogram of dry matter. In contrast, SCM prevalence was substantially affected by mineral supply via premixes more than by the same nutrients from basal feeds of diet. Zn, Se, vitamins E and A supplied through premixes showed the strongest association with SCM. These findings underscore the importance of supplementing with Zn, Se, and vitamins E and A to mitigate SCM risk under practical dairy farm conditions in Albania.
**Keywords**: dairy cows; Holstein; subclinical mastitis; mineral nutrition; feeding level

## 1. Introduction

Mastitis, particularly subclinical mastitis (SCM), remains among the most prevalent and economically important diseases in dairy herds in Albania, ranking within the top two three health challenges affecting milk production. Although SCM is clinically unapparent, it contributes substantially to economic losses through reduced milk yield, elevated somatic cell counts (SCC), and altered milk composition, including changes in the protein-to-fat ratio, lactose concentration, and electrical conductivity (Costa et al., 2025; Zalewska et al., 2025). The multifactorial etiology of SCM involves both infectious and noninfectious determinants such as genetics, housing, hygiene, and nutritional management (De Vliegher et al., 2012; Yu et al., 2025; Meçaj et al., 2023). Contemporary mastitis control programs therefore require an integrated approach that consider beside contagious risks, husbandry, udder hygiene, milking hygiene and technics, nutrition and other environmental risks (EFSA, 2009; LKV Kur(h)ier, 2023; Bouchoucha et al., 2024; Meçaj et al., 2025).

Nutrition plays a pivotal role in maintaining mammary gland health and modulating immune competence in dairy cows. Deficiencies in trace minerals, and vitamins, as well as negative energy balance during the transition period, can impair immune cell function, cytokine regulation, and the oxidative–antioxidative balance (Smith et al., 1984; O'Rourke, 2009; Weiss et al., 1997; Khan et al., 2022). Selenium (Se) and vitamin

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E are particularly critical for neutrophil function and antioxidant defense. Furthermore, their deficiency has been consistently associated with increased susceptibility to intramammary infection (Libera et al., 2021; Khan et al., 2022). Moreover, suboptimal calcium (Ca) and phosphorus (P) metabolism can exacerbate SCM risk through impaired parathyroid hormone activity, reduced 1,25-dihydroxyvitamin D synthesis, and compromised phagocytic capacity (Wilde, 2006; Eisenberg et al., 2019; Ganda et al., 2016; Libera et al., 2021; Khan et al., 2024). Targeted prepartum supplementation with Se, vitamin E, and related micronutrients enhances bacterial clearance and reduces SCC, reflecting improved immune resilience (Weiss et al., 1997; Mukherjee, 2008; Wang et al., 2018; Klein et al., 2025). Thus, optimized dietary supply of Zn, Se, Ca, P, and vitamins E, A, D, and folic acid is essential to support udder health and reduce the incidence of SCM in high-yielding cows (NRC, 2005; GfE, 2023). Deficiencies in microelements are frequent in different areas of Europe, especially Western Balkans. Ademi et al. (2015) investigated the status of selenium in some Western Balkans countries such as Kosova, Bosnia and Herzegovina and Serbia. Based on their results, 45.6% of cows had inadequate (≤100 ng/mL) levels of selenium concentration. These results confirm the fact that use of micronutrients in the Western Balkan area is often not sufficient due to many social and economic issues.

Furthermore, it must be emphasized that the relationship between the supply of minerals and vitamins and SCM has not yet been studied. Based on this, we assumed that a lack of awareness, limited advisory capacities, and the high cost of mineral and vitamin premixes in the Albanian dairy farming market prevented the implementation of balanced feeding programs, particularly those focusing on mineral and vitamin nutrition. This underscores the need for improved nutritional management as a cornerstone of mastitis prevention during lactation and throughout the year.

This study investigates the relationship between micronutrient nutrition — focusing mainly on calcium (Ca), phosphorus (P), and zinc (Zn) — and the prevalence of subclinical mastitis (SCM) during the lactation period under practical conditions on Holstein dairy farms across three regions of Albania.

## 2. Material and Methods

### *2.1. Experimental design*

An experimental design with repeated measurements was conducted on nine Holstein dairy farms located in Durrës, Shkodra and Korça regions /county of Albania. The study covered an 8-month period (December 2023–July 2024), spanning winter, spring, and summer, and included 1st, 2nd and 3rd lactation periods. To reflect real farm conditions, no changes were made to feeding, housing, or hygiene; these were documented via on-site farm visit and farmer interviews. On each farm, 6–11 cows in their first lactation period were tested individually for SCM using the California Mastitis Test (CMT), repeated during the second and third lactation periods, with udder quarter-level scores recorded. The following average productivity parameters were recorded: farm size ranged from 21 to 140 cows, daily milk production varied between 12 and 30 kg, and the average days in lactation were: 32–83 d for the first testing, 146–213 d for the second, and 241–300 d for the third testing.

### *2.2. Inclusion criteria*

Only cows with a clear clinical history were included in the study. Cows included have been vaccinated from the official veterinarians as per regulation of the Albanian state and individual programs. Moreover, cows had no clinical signs of any diseases or any other medical conditions, confirmed by the official and private veterinarians. All cows with the above mentioned conditions were excluded from this investigation.

### *2.3.Feeding*

#### *Feed Sampling and Analysis*

The feed samples were collected on the day of conducting the CMT testing, in accordance with the official procedure Latimer (2023). High-moisture feeds (silages, TMR, etc.) were placed in polyethylene bags, preserved, and transported at +4 °C immediately to the laboratory. Approximately 0.5 kg of each feed type from each farm was delivered on the sampling day to the Feed Quality Laboratory of Agricultural University of Tirana (AUT). Dry matter content was determined according to the official Weende proximate analysis method. The feed samples were dried and subsequently ground using a Retsch Cutting Mill SM 100 equipped with a 1 mm sieve.

The analysis of mineral content focused on two main macro elements, Ca, P, and Zn as one of microelements of critical importance due to their influence on mastitis. Ca, P, and Zn analyses were conducted at the Environmental Laboratory of AUT which were analyzed using the liquid digestion method according to the procedures cited above for mineral analysis of animal feed. For the digestion of the organic matter in the samples, approximately 0.3 g of ground (Ø 1 mm sieve) dry mass was treated with 8 ml of nitric acid (65%) and 2 ml of $H_2O_2$ (30%) for approximately 40 minutes at a temperature of around 550 °C and a pressure of 30 bar using a Milestone ETHOS EASY Microwave Digestion system. The concentrations of Ca and Zn were quantified using an AAS Nova 400P with an acetylene flame (Atomic Absorption Spectroscopy), while the concentration of P was measured with a UviLine 9100C SECOMAM spectrophotometer at a wavelength of 880 nm. The analysis of the organic matter in the feeds was performed using the NIRS method with a calibrated SpectraStar 2600 XT device (Unity Scientific), using calibrations based on validated reference datasets compliant with ISO 12099:2017, provided by KPM Analytics.

*2.4. Feeding level and diet characteristics*

The feeding system was barn-based; 6 farms used the TMR, while the other 3 farms fed the components of the ration separately. In the farms that applied TMR, maize silage was used year-round. Corn's silage was part of 23 rations out of 27 in total. Another roughage feed ingredient was lucerne hay, which was used in 25 from 27 diets, while grass hay was used only in 11 diets. In the group of the energy-rich concentrates yellow corn was part of all diets in the studied farms, followed by wheat and barley mainly produced on the farm. The main protein source was soybean meal in 24 diets out of 27 used. In 23 out of the total 27 diets, a premix was included in varying daily amounts of up to 200 g per cow. The following table (Table 1) presents data on dry matter intake and the feeds that composed the rations in the studied farms throughout the entire study period. The concentrate proportion in the dry matter of the ration was in average of 40,8 %. Farmers reported generally high quantities of roughage feeds but were unable to provide information on leftovers in the feed trough.

**Table 1.** Data on feed consumption (dry matter) and the feeds used in the rations of the farms in the study throughout the study period (a total of 27 feed rations).

| Parameters | No. Diets used | Mean, kg | Min, kg | Max, kg |
|---|---|---|---|---|
| **DM Intake** | 27 | 20.1 | 13.5 | 24.3 |
| **Roughage DM** | 27 | 12.0 | 9.9 | 16.8 |
| **Concentrate DM** | 27 | 8.2 | 3.1 | 12.5 |
| ***Roughages:*** | | | | |
| Corn silage | 23 | 20.2 | 10.0 | 25.0 |
| Lucerne hay | 25 | 5.7 | 2.0 | 12.0 |
| Hay | 11 | 5.7 | 3.0 | 11.0 |
| Grass silage | 2 | 4.0 | 4.0 | 4.0 |
| Wheat straw | 5 | 2.0 | 0.5 | 5.0 |
| Green lucerne | 1 | 5.5 | 5.5 | 5.5 |
| Green cereals | 1 | 11.0 | 11.0 | 11.0 |
| ***Concentrates:*** | | | | |
| Yellow corn | 27 | 3.6 | 1.5 | 4.5 |
| Wheat | 17 | 1.6 | 0.8 | 2.0 |
| Barley | 6 | 1.3 | 1.0 | 1.7 |
| Soya bean meal | 24 | 2.2 | 1.0 | 3.6 |
| Dairy performance feed | 9 | 4.8 | 3.0 | 6.0 |
| Wheat bran | 9 | 1.5 | 1.0 | 2.0 |
| Sunflower meal | 1 | 1.0 | 1.0 | 1.0 |
| Dried brewer's spent grain | 2 | 2.0 | 2.0 | 2.0 |
| Sugar beet pulp | 2 | 0.3 | 0.3 | 0.3 |
| Premix | 23 | 0.14 | 0.00 | 0.20 |

**The number of rations where different feeds were used.*

The data on dry matter intake and nutrient supply (Table 2), as well as on nutrient ratios and balancing (Table 3) of the rations used in the studied dairy farms across the three lactation stages, indicate that, on average, they do not deviate significantly from the nutrient requirements recommended by GfE (2023) for lactating cows with a daily milk yield of 12 to 30 kg. Even though the average values of the nutrient ratios in Table 3 align well with the recommended values for these levels of milk production (GfE, 2023), the minimum values found in some of the rations used on the studied farms are still very low compared to the corresponding recommendations. This means that some of farms included in this study, at certain times, were fed with rations with an insufficient balance of energy and various nutrients relative to the DM intake. This was not an unusual situation for farms in Albania, as farmers are generally poorly trained and have great difficulty understanding the criteria for a complete, performance-oriented, and balanced diet for lactating cows. On the other hand, it should be considered that all information about the feed rations was based on farmers' information documented during direct on-farm interviews, i.e. no weighing of the applied diets was conducted.

**Table 2**. Data on DM intake and nutrients supply by the rations used in the studied cow farms during the three lactation stages (n=27 rations).

| Nutrients | Supply | | |
|---|---|---|---|
| | Mean | Min | Max |
| DM intake, kg | 20.1 | 13.5 | 24.3 |
| NEL in diet, MJ | 135.4 | 81.3 | 171.4 |
| ME ruminant in diet, MJ | 198.8 | 132.9 | 243.0 |
| Crude protein (XP), g | 3127.8 | 1231.0 | 4151.0 |
| Duodenal available Crude Protein (nXP), g | 3110.8 | 1682.0 | 4001.0 |
| Crude fiber (XF), g | 3513.3 | 2900.0 | 4866.0 |
| Structural fiber (Str-XF), g | 2967.0 | 2395.0 | 4490.0 |
| Starch (XS), g | 5170.1 | 2242.0 | 7326.0 |
| Sugar (XZ), g | 831.8 | 539.0 | 1525.0 |
| Starch + Sugar (XS_XZ), g | 6002.0 | 2889.0 | 8151.0 |
| Degradable Carbohydrates (rdHCO), g | 4610.3 | 2240.0 | 6387.0 |
| Crude fat (XL) | 546.4 | 313.0 | 673.0 |
| NDF, g | 7603.0 | 6339.0 | 10233.0 |
| ADF, g | 4433.6 | 3693.0 | 5931.0 |
| Roughage NDF (rNDF), g | 5842.5 | 4751.0 | 8745.0 |
| Roughage DM (rDM), kg | 12.0 | 9.9 | 16.8 |
| Concentrate DM (cDM, kg | 8.2 | 3.1 | 12.5 |

**according GfE (2023)*

**Table 3.** Descriptive data on nutrient ratios of the diets used in the studied cow farms during the three lactation stages (n=27 rations).

| Parameters | Supply | | |
|---|---|---|---|
| | Mean | Min | Max |
| NEL, MJ/kg DM | 6.7 | 5.7 | 7.1 |
| XP/DM, g/kg | 153.9 | 91.4 | 170.9 |
| nXP/DM, g/kg | 153.6 | 125.0 | 164.7 |
| % XF in DM | 17.7 | 14.9 | 25.4 |
| % Str-XF in DM | 15.1 | 11.0 | 25.0 |
| % XS in DM | 25.4 | 13.4 | 30.5 |
| % XS_XZ in DM | 29.6 | 18.1 | 34.0 |

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| | | | |
|---|---|---|---|
| % NDF in DM | 38.1 | 33.6 | 48.1 |
| % Roughage NDF in DM | 29.7 | 21.9 | 45.2 |
| % Concentrate in DM | 39.6 | 18.8 | 52.5 |

Given that this study focuses on the influence of mineral and vitamin supply on the prevalence of SCM, the supply of mineral and vitamin nutrients was analyzed separately. Data on the amount of minerals and vitamins relative to dry matter intake are presented in Table 4 in three formats: as the total content, and disaggregated by source—namely, from the ration feeds (Ca, P and Zn supply) and from mineral-vitamin premixes for Ca, P, Zn and Se content, as well as for vitamin E, A and D content. The premix employed originated from a commercial supplier (Austria Premix), which provided a substantial portion of the mineral supplements used by dairy farms in Albania during the study period of this research project. This disaggregated analysis was undertaken to demonstrate under usual conditions of Albanian dairy farms the role of mineral vitamin supplements in meeting the nutritional requirements of dairy cows and, consequently, the influence on the occurrence of SCM. As previously noted, in only 23 of the 27 rations examined was the premix incorporated as a component of the feed, while in 4 cases, it was omitted entirely.

As described in the chapter of methodology, the mineral composition of the feedstuffs utilized in the studied farms was analyzed for Ca, P and Zn at the Environmental Laboratory of AUT. In contrast, the contents of other micronutrients known to influence intramammary infection and subclinical mastitis—specifically selenium (Se) and vitamins E, A, and D—were not analyzed in the feedstuffs included in the rations. This omission was due to limited laboratory capacity and the high analytical cost, which could not be accommodated within the financial framework of the present research project. Consequently, in this subsection, the quantities of Se and vitamins E, A, and D originating from the feed ingredients were not calculated; instead, only the amounts supplied via the mineral–vitamin premix used in the rations of each farm and at each measurement period were considered. This approach was consistent with contemporary dairy nutrition practice (DLG, 2023), which assumes that the primary and most reliable source of trace elements—particularly Zn and Se—and fat-soluble vitamins (E, A, and D) was the standardized supply provided through mineral–vitamin premixes included in the diet. This assumption is justified by the fact that the natural concentrations of these micronutrients and vitamins in conventional feed materials for dairy cows are generally low, highly variable, and dependent on agronomic and environmental factors. Their accurate quantification would require sophisticated and costly analytical procedures that are beyond the scope of routine feed evaluation. For these reasons, current feeding practice for dairy cows (DLG, 2023) commonly disregards the micronutrient and vitamin contribution of plant-based feed ingredients and ensures that the full physiological requirements for these nutrients are met through supplementation. Accordingly, the quantity of mineral–vitamin premix incorporated into the rations of both lactating and pregnant cows was calculated to fully covering their physiological requirements for each trace element and fat-soluble vitamin (DLG, 2023).

The content of Se and vitamins E, A, and D in the premix was calculated based on provider declarations (per kilogram of premix used: Ca 170.0 g; P 20.0 g; Zn 2450 mg; Se 30.0 mg; vitamin E 3000 mg; vitamin A 420,000 IU; vitamin D 75,000 IU). As detailed in Table 4, the premix accounted for the following proportions of the mineral and fat-soluble vitamin requirements of dairy cows on the farms included in this study: 20% Ca, 5% P, 32% Zn, 100 % Se, 53% vitamin E, 52% vitamin A, and 37% vitamin D. These findings highlight the essential role of the premix in meeting the mineral and vitamin requirements of the dairy cow diets evaluated.

When comparing the average concentrations of minerals and vitamins per kilogram of diet DM across the 27 feed rations used on the surveyed farms with the recommendations of GfE (2023)—specifically, the following requirements per kg DM: 5.8 g Ca; 3.7 g P; 50 mg Zn; 0.2 mg Se; 35 mg vitamin E; 5000 IU vitamin A; and 1250 IU vitamin D—it becomes evident that, with the exception of Ca and Se, the concentrations of the remaining elements, namely P and Zn, fall below the recommended thresholds. Moreover, when considering the lowest descriptive values of the analyzed rations from the farms included in this study, the values are significantly lower than the reference values provided by GfE (2023). Even when taking into account the highest measured concentrations per kg DM, P content in particular remains below the minimum of mineral recommendation.

This condition of suboptimal mineral and vitamin supply—identified on several diets (27 diets) fed on the nine dairy farms across the three lactation periods or seasons included in this investigation, suggests that inadequate provision of mineral and vitamin supplements may represent a widespread situation in current dairy nutrition practices in Albania. This phenomenon was likely related to the generally low level of formal agricultural education and limited professional awareness among dairy cow farmers regarding the necessity of use of mineral-vitamin premixes as diet supplement in dairy cow nutrition. These premixes contribute substantially—and for certain nutrients, predominantly—to meeting the mineral and vitamin requirements of dairy cows.

**Table 4**. Data on the mineral and vitamin content per kilogram of dry matter in the feed rations used on the studied farms during all three lactation phases (n=27).

| Nutrients /kg Diet DM | **Total supply per kg DM** | | |
|---|---|---|---|
| | Mean | Min | Max |
| | **Total diet supply on Ca, P, Zn and Se** | | |
| Ca, g | 5.88 | 3.61 | 9.18 |
| P, g | 2.68 | 1.72 | 3.07 |
| Zn, mg | 40.83 | 19.65 | 56.75 |
| Se, mg | 0.19 | 0.00 | 0.36 |
| | **Supply only by feed for Ca, P and Zn** | | |
| Ca, g | 4.75 | 3.12 | 8.12 |
| P, g | 2.51 | 1.72 | 2.91 |
| Zn, mg | 24.98 | 17.42 | 33.81 |
| | **Supply only by supplement for Ca, P, Zn and Se** | | |
| Ca, g | 1.13 | 0.00 | 2.05 |
| P, g | 0.17 | 0.00 | 0.59 |
| Zn, mg | 15.85 | 0.00 | 29.52 |
| Se, mg | 0.19 | 0.00 | 0.36 |
| | **Supply only by supplement for Vitamins E, A and D** | | |
| Vitamin E, mg | 18.57 | 0.00 | 36.14 |
| Vitamin A, IU | 2600 | 0.00 | 5060 |
| Vitamin D, IU | 460 | 0.00 | 900 |

*Content in kg premix: Ca, 170,0 g; P 20,0; Zn 2450 mg; Se 30,0; Vitamin E 3000 mg; Vitamin A 420000 UI; Vitamin D 75000 UI. Requirements according GfE (2023) (per kg DM): 5,8 g Ca; 3,7 g P; 50 mg Zn; 0,2 mg Se; 35 mg vitamin E; 5000 UI vitamin A; 1250 UI vitamin D.*

### *2.5. Statistical analysis*

The data were analyzed using SPSS for Windows (20.0.1, SPSS Inc., Chicago, IL, USA) and R (4.3.3) of the "MASS" library. A univariate analysis of variance (ANOVA) was carried out to evaluate the effects of two fixed factors: "nutrient" and "season". Post hoc comparisons were subsequently performed using Dunnett's test to assess differences among nutrient levels and Tukey's test to evaluate seasonal effects. To investigate the associations between dietary mineral concentrations in kg diet DM and the prevalence of SCM, regression analysis was applied using curve fitting procedures. Linear, quadratic, and compound models were explored to determine the best-fitting relationship between the variables. For all statistical tests, a significance level of $p < 0.05$ was considered indicative of statistical significance.

## 3. Results & Discussion

### *3.1. Relationship between DM intake, Energy and Organic Nutrient Supply and the Prevalence of Subclinical Mastitis*

The dry matter intake, the amount of energy and nutrients supplied, as well as the degree of ration optimisation used in the nine dairy farms during the three study periods were presented in the previous section, with the corresponding data provided in Tables 1, 2, 3, and 4. The following table (Table 5) presents descriptive data on the prevalence of subclinical mastitis (SCM) grouped by measurement period. These periods correspond to three lactation stages and three seasons: winter, spring, and summer. Table 5 exclusively presents data on the occurrence of SCM to enable analysis of its correlation with the nutritional supply parameters of the 27 rations evaluated across the farms included in this study.

**Table 5**. Data on subclinical mastitis occurrence: aggregated data and seasonal breakdown.

| Parameters | Total no. of cows tested | Days in lactation | Daily milk production, kg/cow | Number of cows in different udder health status (≥1+) [a] | | | | Total no. infected cows (≥1+) [b] | Prevalence of infected bovines (≥1+), % [c] | Prevalence of severe SCM (≥2+), % [d] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | (1+) | (2+) | (3+) | 0 | | | |
| **Winter** | | | | | | | | | | |
| No. farms | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| No. cows | 54 | | | 8 | 12 | 10 | 24 | 30 | | |
| Average | 6.0 | 54.1 | 22.9 | 0.9 | 1.3 | 1.1 | 2.7 | 3.3 | 55.6 | 40.7 |
| Min | 6.0 | 32.8 | 19.0 | 0.0 | 0.0 | 0.0 | 1.0 | 1.0 | 16.7 | 0.0 |
| Max | 6.0 | 83.0 | 30.0 | 3.0 | 3.0 | 2.0 | 5.0 | 5.0 | 83.3 | 83.3 |
| SD | 0.0 | 21.1 | 4.4 | 1.2 | 1.2 | 0.8 | 1.4 | 1.4 | 23.6 | 32.4 |
| **Spring** | | | | | | | | | | |
| No. farms | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| No. cows | 63 | | | 24 | 3 | 1 | 35 | 28 | | |
| Average | 7.0 | 169.7 | 23.7 | 2.7 | 0.3 | 0.1 | 3.9 | 3.1 | 42.0 | 5.3 |
| Min | 6.0 | 109.6 | 15.3 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| Max | 11.0 | 205.0 | 32.8 | 7.0 | 1.0 | 1.0 | 6.0 | 9.0 | 83.3 | 18.2 |
| SD | 1.7 | 37.2 | 6.1 | 2.3 | 0.5 | 0.3 | 2.0 | 2.8 | 31.4 | 8.0 |
| **Summer** | | | | | | | | | | |
| No. farms | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| No. cows | 61 | | | 15 | 5 | 1 | 40 | 21 | | |
| Average | 6.8 | 247.1 | 22.5 | 1.7 | 0.6 | 0.1 | 4.4 | 2.3 | 32.1 | 10.3 |
| Min | 6.0 | 201.1 | 14.5 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| Max | 11.0 | 300.0 | 29.7 | 6.0 | 2.0 | 1.0 | 6.0 | 7.0 | 83.3 | 50.0 |
| SD | 1.7 | 40.0 | 5.8 | 1.8 | 0.7 | 0.3 | 1.6 | 2.3 | 27.3 | 16.5 |
| **Overall** | | | | | | | | | | |
| No. farms | 27 | 27 | 27 | 27 | 27 | 27 | 27 | 27 | 27 | 27 |
| No. cows | 178 | | | 47 | 20 | 12 | 99 | 79 | | |
| Average | 6.6 | 157.0 | 23.0 | 1.7 | 0.7 | 0.4 | 3.7 | 2.9 | 43.2 | 18.8 |
| Min | 6.0 | 32.8 | 14.5 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| Max | 11.0 | 300.0 | 32.8 | 7.0 | 3.0 | 2.0 | 6.0 | 9.0 | 83.3 | 83.3 |
| SD | 1.4 | 87.1 | 5.3 | 1.9 | 0.9 | 0.7 | 1.8 | 2.2 | 28.3 | 26.1 |

[a] *Cows were classified by udder health as follows: "0" = negative, "1+" = weakly positive, "2+" = positive, and "3+" = strong positive.*
[b] *The total number of infected cows (≥1+) includes all animals classified as weakly positive, positive, or strongly positive on the CMT, corresponding to scores of 1+, 2+, and 3+. These findings are indicative of the presence of subclinical mastitis;*
[c] *The prevalence of infected cows (≥1+) represents the percentage of individuals with a CMT score ≥1+ within the total cow population tested;*
[d] *The prevalence of severe subclinical mastitis (≥2+) corresponds to the proportion of animals demonstrating California Mastitis Test (CMT) scores of 2+ and 3+, expressed in percentage to the total population tested.*

The descriptive data on SCM prevalence—i.e., the proportion of cows with a CMT score ≥1+ relative to the total number of cows tested per farm/herd—presented in the above table indicate a mean prevalence of 43.23%, ranging from 0% (observed in a herd of first-lactation cows) to 83.3% in several farms. The highest prevalence (55.6%) was clearly observed in the first testing, corresponding to the early lactation phase, with a range of 16.7% to 83.3%, and an average proportion of severely affected cows (<2+) of 31.5%, reaching up to 66.7%. Progression of lactation appears to reduce prevalence, which declined to 42% in the second testing—corresponding to mid-lactation—and decreased further in the third testing, corresponding to late lactation, reaching 32.1%. The SCM prevalence data reported here are intended only to support the analysis of associations with the nutritional supply parameters under investigation. Other authors (Bouchoucha et al., 2024; Ararsa et al., 2014) have reported comparable results for medium- and small-scale dairy farms in countries with a level of dairy sector development similar to that of Albania.

**Table 6.** Results of the regression analysis on the association between the prevalence of subclinical mastitis (score ≥ 1+), supply of energy and organic nutrients, and dry matter intake (n = 27).

| Nutrients | Adjusted $R^2$ |
|---|---|
| NEL, MJ/kg DM | 0.2061* |
| Crude protein (XP), g/d | 0.1958 |
| Crude fiber (XF), g/d | 0.1162 |
| Structural fiber (Str-XF), g/d | 0.116 |
| Starch (XS), g/d | 0.1159 |
| Starch + Sugar (XS_XZ), g/d | 0.116 |
| Roughage NDF (rNDF), g/d | 0.1159 |
| Roughage DM intake, kg/d | 0.1082 |
| Concentrate DM intake, g/d | 0.004 |

*$p<0,05$*

The regression analysis of the nutritional parameters of the rations—specifically the intake of energy and organic matter—revealed no significant association with SCM prevalence, except for one indicator: the concentration of net energy for lactation (NEL) per kilogram of dry matter in the ration. However, this indicator is de facto a derivative of other parameters, since energy is indirectly attributed to the content of organic matter, particularly carbohydrates and fat as energy sources in the ration, and to some extent also protein.

In the "Feeding Level and Diet Characteristics" subsection, it was noted that the average coverage of energy and organic nutrient requirements was close to 100%, except for crude fat (72%). The impact of energy and organic matter levels in the ration on mastitis is primarily indirect, primarily through their role in developing digestive and metabolic disorders. These disorders can impair immunity and increase the animal's predisposition to mammary infections (Weiss et al., 1997; O'Rourke, 2009; Sordillo, 2016; GfE, 2023). The high degree to which energy and organic nutrient requirements are covered, along with the primarily indirect mode of action, likely explains why associations between subclinical mastitis prevalence and energy or organic matter are difficult to detect. The relatively small number of observations (n = 27) included in the regression analysis may have further limited the ability to identify these relationships. However, it is widely recognized that mastitis is more strongly influenced by the level of mineral and vitamin nutrition than by energy or other macronutrients (e.g., protein, carbohydrates, and fat) (Weiss et al., 1997; Wilde, 2006; Libera et al., 2021; GfE, 2023; Khan et al., 2024). Therefore, the following subsection of this paper will focus on the relationship between mineral nutrition levels and subclinical mastitis prevalence in more detail.

### *3.2. Associations between mineral–vitamin nutrition and the prevalence of subclinical mastitis*

To account for potential confounding by seasonal variability, the factor "season" was included as a fixed effect in the two-way analysis of variance alongside the primary fixed factor "nutrient". The results of the two-way ANOVA are presented in the table 6.

**Table 7.** Results of the analysis of variance assessing the effects of micronutrient concentration in dietary DM and seasonal variation on the prevalence of subclinical mastitis (score ≥1+) (n=27).

| Nutrient concentration in ration DM | Adjusted $R^2$ of the model | Significance level (p value =) of different sources of the model |
|---|---|---|

| | | Corrected model | Intercept | Nutrient | Season |
|---|---|---|---|---|---|
| *Total supply in the diet* | | | | | |
| Ca, g | -0.439 | 0.890 | 0.000 | 0.832 | 0.936 |
| P, g | -0.171 | 0.793 | 0.000 | 0.617 | 0.826 |
| Zn, mg | 0.886 | 0.018 | 0.000 | 0.015 | 1.000 |
| Se, mg | 0.061 | 0.310 | 0.000 | 0.146 | 0.544 |
| *Supply only by feed** | | | | | |
| Ca, g | 0.725 | 0.009 | 0.000 | 0.006 | 0.887 |
| P, g | 0.426 | 0.045 | 0.000 | 0.025 | 0.152 |
| Zn, mg | 0.883 | 0.000 | 0.000 | 0.000 | 0.775 |
| *Supply only by supplement*** | | | | | |
| Ca, g | 0.463 | 0.032 | 0.000 | 0.018 | 0.030 |
| P, g | 0.353 | 0.018 | 0.000 | 0.006 | 0.175 |
| Zn, mg | 0.668 | 0.040 | 0.000 | 0.031 | 0.642 |
| Se, mg | 0.060 | 0.288 | 0.000 | 0.115 | 0.367 |
| Vitamin E, mg | 0.636 | 0.035 | 0.000 | 0.023 | 0.624 |
| Vitamin A, IU | 0.607 | 0.034 | 0.000 | 0.024 | 0.169 |
| Vitamin D, IU | -0.022 | 0.514 | 0.000 | 0.311 | 0.423 |

** The nutrient amount, expressed as its concentration per kilogram of dry matter in the ration, provided exclusively by the feed, that is, excluding the quantity supplied by the mineral-vitamin supplement.*
*** The nutrient quantity, expressed as its concentration per kilogram of dry matter in the ration, supplied by the mineral-vitamin supplement.*

As shown in Table 7, the "season" factor only had a statistically significant effect on the amount of calcium supplied through supplementation via the mineral-vitamin premix ($p = 0.030$). In all other cases, no significant influence of the "season" factor was detected. In contrast, the "nutrient" factor, defined as the nutrient concentration per kilogram of dry matter in the ration, had a statistically significant effect on multiple parameters. These analyses were conducted separately to distinguish between quantities derived exclusively from the feed component of the ration and those provided solely through the mineral-vitamin premix. Considering mineral and vitamin concentrations separately from the feed component and the mineral-vitamin premix improves the understanding of farm advisers, zootechnicians, veterinarians, and farmers of the status of mineral and vitamin requirements and supply coverage (e.g., how many milligrams of zinc are supplied per kilogram of dry matter from the feed and from the premix alone).

The quantities of Ca, P, and Zn supplied through plant feeds (roughage and concentrate) in the daily ration significantly impact the prevalence of subclinical mastitis (SCM). This emphasizes the importance of considering the levels of these trace minerals in the roughage and concentrate fed to dairy cows (DLG, 2023), especially in relation to mastitis (Libera et al., 2021). The situation was quite different for selenium (Se). As previously mentioned, the Se supply in dairy rations primarily depends on the Se concentration in mineral-vitamin premixes and the amount present in the diet (DLG, 2023). Clearly, supplementing with mineral nutrients formulated specifically for dairy cows has a stronger effect on SCM prevalence.

To gain a deeper understanding of how the intake of fat-soluble vitamins, particularly vitamins E, A, and D, influences subclinical mastitis, Table 4 shows the amounts of these vitamins supplied exclusively through the mineral-vitamin premixes, as declared by the premix provider. There was clear evidence that supplementing with vitamins E and A via mineral-vitamin premixes significantly impacts the prevalence of subclinical mastitis (SCM) in dairy cows (see Table 7). This emphasizes the practical recommendation (DLG, 2023) to evaluate the nutritional status of fat-soluble vitamins and calculate their supply to dairy cows based exclusively on vitamin supplements in their daily rations, since extremely low concentrations of these vitamins in the roughage and concentrate used to feed dairy cows can be ignored. The variance analysis results in Table 6 show a substantially higher coefficient of determination ($R^2 = 0.668$; $p = 0.0301$) for zinc concentration in relation to SCM than for selenium ($R^2 = 0.060$; $p = 0.115$) in the premix used in the investigated farms' rations. The influence of vitamins E and A on SCM, supplied through the mineral–vitamin supplement, was comparable to zinc's influence, though slightly lower ($R^2 = 0.607–0.636$; p

= 0.023–0.024). These results differ from those of Khan et al. (2022), who reported that selenium has a stronger effect on SCM than dietary vitamin E.

This finding underscores the importance of raising awareness among Albanian dairy farmers about the critical need to use mineral-vitamin premixes to increase resistance to SCM. As previously mentioned, four of the 27 analyzed rations from the included dairy farms did not contain mineral-vitamin premixes. This issue is particularly prevalent among small-scale dairy farms in Albania.

To explain the relationship between the supply of mineral nutrients and the prevalence of SCM at a severity threshold of ≥1+, the data were analyzed using regression models with three curve-fitting approaches: linear, quadratic, and combined.

**Table 8.** Results of the regression analysis on the association between the prevalence of subclinical mastitis (score ≥1+) and the supply of mineral nutrients provided as a concentration in the dry matter ration (n = 27).

| Nutrient concentration in ration DM | Regression (Curve fit) parameters | | | | | Sig. (p value) |
|---|---|---|---|---|---|---|
| | R | $R^2$ | Constant | B | $B^2$ | |
| *Total supply in the diet* | | | | | | |
| Ca, g | 0.374 | 0.140 | | | | 0.165 |
| P, g | 0.067 | 0.005 | | | | 0.739 |
| Zn, mg | 0.359 | 0.129 | 0.665 | -0.007 | | 0.066 |
| Se, mg | 0.409 | 0.167 | 0.535 | -0.692 | | 0.034 |
| *Supply only by feed** | | | | | | |
| Ca, g | 0.035 | 0.001 | | | | 0.863 |
| P, g | 0.187 | 0.035 | | | | 0.350 |
| Zn, mg | 0.080 | 0.006 | | | | 0.693 |
| Se, mg | 0.149 | 0.022 | | | | 0.459 |
| *Supply only by supplement*** | | | | | | |
| Ca, g | 0.642 | 0.413 | 0.454 | 0.205 | -0.189 | 0.002 |
| P, g | 0.561 | 0.315 | 0.483 | -0.714 | | 0.002 |
| Zn, mg | 0.565 | 0.320 | 0.458 | 0.012 | -0.001 | 0.010 |
| Se, mg | 0.350 | 0.122 | 0.496 | -0.703 | | 0.074 |
| Vitamin E, mg | 0.371 | 0.138 | 0.502 | -0.007 | | 0.055 |
| Vitamin A, IU | 0.371 | 0.138 | 0.500 | -0.051 | | 0.057 |
| Vitamin D, IU | 0.380 | 0.144 | 0.500 | -0.294 | | 0.051 |

*The regression parameters are presented in the table exclusively for those nutrients that demonstrated a statistically significant association ($p < 0.05$) with the prevalence of subclinical mastitis.*

*R- Correlation; $R^2$ – R Square; Constant - the intercept of regression; B – Regression coefficient; $B^2$ - represents the squared coefficient B in cases where the relationship was quadratic.*

The results of the regression analysis (Table 8) indicate that the regression was statistically significant (p = 0.034) with respect to the influence of the total Se concentration per kilogram DM in the ration (including both feed and supplementation via premix). An association approaching statistical significance was observed for Zn (p = 0.066) in relation to SCM prevalence. However, the regression models for Ca and P concentrations per kg of ration DM were not statistically significant (p = 0.165 and p = 0.739, respectively). But, when the analysis focuses on the concentrations of Ca, P, Zn, and Se supplied exclusively via the mineral-vitamin supplement, all four elements demonstrate statistically significant effects on SCM prevalence. On the contrary, no significant associations were found when considering only the concentrations of these minerals derived from the basal feed, excluding the mineral-vitamin supplement.

In cases where significant regressions were identified, the observed correlations were weak (r = 0.359 and 0.409), and the respective coefficients of determination ($R^2$) were low (0.129 and 0.167) for total Zn and Se concentrations per kilogram of dry matter (DM) in the rations. The correlations increased (r = 0.350 to 0.642), and the $R^2$ values improved when focusing on the concentrations of Ca, P, Zn, and Se per kilogram of dry matter (DM) in the rations derived from the mineral-vitamin supplement, though they remained at an average level ($R^2$ = 0.320 and 0.413). These findings are supported by similar correlation coefficients (r = 0.37 and 0.38) and $R^2$ values (0.13–0.14) for vitamin E, vitamin A, and vitamin D concentrations per kilogram of dry matter in the rations, which were provided exclusively via the mineral-vitamin supplement.

The analysis of variance and regression above showed that the amounts of Ca, P, Zn and Se supplied via premixes have a much stronger impact on SCM prevalence than those obtained from the basal roughage and concentrate feeds in the dairy cows' rations. Among the trace mineral elements studied, Zn showed the strongest influence on SCM prevalence, both in absolute intake and concentration per dry matter. This influence was markedly more pronounced when Zn was supplied through supplementation rather than through the basal roughage and concentrate feeds in the dairy cows' diet.

Variance and regression analyses of the relationship between the supply of minerals and vitamins and the prevalence of SCM provided consistent evidence of the importance of key macro- and micronutrients, particularly Zn and Se, as well as vitamins E, A, and D, for maintaining udder health in Holstein cows throughout the lactation cycle under practical dairy farming conditions in Albania. These relationships have long been studied and documented in scientific literature (Smith et al., 1984; Weiss et al., 1997; O'Rourke, 2009; Sordillo, 2016; Libera et al., 2021; Khan et al., 2024). Some of the most significant findings are cited in the introduction to this article.

The role of trace elements and fat-soluble vitamins in immune competence and udder health is well-known. However, this field continues to evolve dynamically. Recent studies and systematic reviews (Sordillo, 2016; Libera et al., 2021; Khan et al., 2024) have revealed that the chemical form and quantity of these nutrients are crucial for bioavailability and metabolic effects. Organic compounds of Zn and Se, as well as their complexes with essential amino acids (e.g., methionine), have been shown to have higher bioavailability and a greater impact on immunity to mastitis than their inorganic salts (Kellogg et al., 2004; Chen et al., 2020; Hachemi et al., 2023; Respati et al., 2023; Oconitrillo et al., 2024; Klein et al., 2025). Therefore, they exhibit stronger antioxidant and immune-protective effects against udder mastitis. The recommended amounts of Zn, Se, vitamins E, A, D, and folic acid have increased for high-yielding dairy cows due to an increasing number of supporting studies (NRC, 2005; GfE, 2023).

Research has focused on mineral and vitamin nutrition, especially essential micronutrients such as Zn and Se, as well as vitamin E (Wilde, 2006; Khan et al., 2020; Haga et al., 2021; Yu et al., 2025), during the transition or periparturient period of lactation in dairy cows. This period is defined as three weeks before and three to four weeks after calving. This phase is the most metabolically critical in the production cycle of dairy cows. During this time, cows undergo profound endocrine and metabolic changes. The late gestation phase is characterized by anabolic nutrient distribution for fetal and reproductive tissue development, as well as preparation for the next lactation (e.g., mammary gland and udder development, and synthesis of body reserves). The early postpartum phase is characterized by the extensive mobilization of fat reserves, mainly, to compensate for the negative energy and nutrient balance associated with the rapid onset of lactation and intensive milk secretion. This corresponds with insufficient energy and nutrient intake due to inhibited feed intake. These changes impair immune competence, increasing susceptibility to subclinical mastitis. Various metabolic and digestive disorders that occur during the transition period in dairy cows indirectly stimulate subclinical mastitis, especially during the first month of lactation, due to their strong negative effect on immunity.

It is widely accepted that diets for dairy cows containing only roughage and concentrate feeds cannot provide even the minimum amount of essential trace elements, such as Zn, Se, and Cu, nor fat-soluble vitamins, particularly vitamin E. This is because these feed components contain very low concentrations, or even negligible amounts, of these nutrients. Therefore, targeted supplementation with mineral-vitamin premixes has long been an integral part of modern feeding strategies for dairy cows. According to current best practice recommendations (DLG, 2023), such premixes must be formulated and dosed to meet the physiological requirements of important micronutrients, with a focus on Zn, Se and Cu, and vitamins E, A, and D. These nutrients play a crucial role in supporting immune competence, antioxidant defense mechanisms, and udder health in dairy cows during lactation and the dry period.

## 4. Conclusions and Implications

This investigation was not a classical experimental study because it was not conducted under controlled conditions at an experimental station. Rather, it was conducted under the practical field conditions prevailing on Albanian dairy farms. Subclinical mastitis remains prevalent in these dairy cow herds/farms, and awareness of its negative effects on animal health, milk yield, and milk quality is often insufficient. This limited awareness often results in neglecting targeted mineral and vitamin supplementation in dairy cow feeding altogether.

The findings of this study demonstrate the substantial impact of feeding practices, especially the provision of minerals and vitamins, on the prevalence of subclinical mastitis in Albanian dairy farms under practical management conditions. Further research is needed to evaluate the effects of different mineral and vitamin premix dosages on farms with diverse feeding, housing, and hygiene conditions. Small- and medium-sized dairy farms in Albania, which have limited resources, need more training that emphasizes the importance of nutritionally balanced, requirement-based feeding, especially with regard to minerals and vitamins.

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