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The relationship between the occurrence of subclinical mastitis and milk quality in medium-sized Holstein cow farms in Albania

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Abstract

Subclinical mastitis (SCM) reduces both milk production and milk quality. In this study, the prevalence of SCM, as determined by the California Mastitis Test (CMT), was analyzed in relation to lactation year, milk density, lactose content, electrical conductivity, and fat content at both the individual cow level and the farm level. The focus was on the overall prevalence of CMT-positive cases within the farms and the mean values of physicochemical changes in milk for each farm. A total of 711 udder quarters from 178 Holstein cows across nine dairy farms were sampled at three intervals during lactation (*i.e.*, in three lactation periods). The number of cows per farm ranged from 21 to 140, covering various lactation stages. A CMT score of 1+ in any quarter was considered SCM-positive. The significance and correlation of SCM's impact on changes in physicochemical milk parameters were analyzed using a robust compound regression. Prevalence of SCM was found to be between 0.14 and 0.63. High prevalence of SCM showed a significant increase in milk conductivity and a significant decrease in milk lactose, protein, solid non-fat (SNF) content, and density. The prevalence of mild and severe SCM varied across different seasons and lactation stages ($p<0.05$). Significant correlations ($p<0.05$) between lactation year, lactose content, electrical conductivity, SNF content, and protein were measured at the farm level using a milk analyzer and compared with SCM ($R^2=0.28$; $R^2=0.41$; $R^2=0.26$; $R^2=0.36$; $R^2=0.39$). These findings suggest that physicochemical milk parameters, which are routinely measured in Albanian dairies, can serve as an effective early-warning indicator for dairy farms to detect potential cases of SCM.

Introduction

Subclinical mastitis (SCM) is a prevalent, asymptomatic form of mastitis in dairy cows, characterized by an elevated somatic cell count (SCC) in milk, indicating an inflammatory response (Dohoo and Leslie, 1991; Barkema, 1999; Ruegg, 2011; Romero *et al.*, 2018; Fernandes *et al.*, 2021; Lisuzzo *et al.*, 2024). Despite its lack of visible symptoms, SCM significantly reduces milk quality and yield, impacting dairy production and economic efficiency (Pankey *et al.*, 1987; Sharma *et al.*, 2011; Gleeson *et al.*, 2018; Liu *et al.*, 2023; Paramasivam *et al.*, 2023; Goto *et al.*, 2024). It often remains undiagnosed, particularly in regions lacking veterinary care and diagnostic tools (Krishnamoorthy *et al.*, 2021; Tanni *et al.*, 2021; Liu *et al.*, 2023). In developing countries like Albania, SCM presents a major challenge, particularly for small and medium-sized farms, where inadequate housing, poor hygiene, unbalanced nutrition, and limited veterinary services heighten the risk (Romero *et al.*, 2018; Borena *et al.*, 2023; Iraguha, 2023). Improper udder hygiene and inadequate cleaning of milking equipment facilitate bacterial transmission, while unsanitary milking

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Contributions: ReM, conceptualized the methodology for the physicochemical evaluation of milk, statistically processed data, and wrote the manuscript. RoM, MS, FP, ES, XK, identified the farms, collected milk samples and performed CMT, gathered farm data, and provided guidance during the manuscript preparation process; AX, organized farmer contacts and dissemination seminars to discuss the study's findings with farmers in the included regions; MT, supervised the comprehensive experimental design and execution of the project, and collaborated with the primary author to analyze the statistics and develop the manuscript.

Conflict of interest: the authors declare no conflicts of interest

Ethics approval and consent to participate: participation was voluntary after farmers were informed about the aims of the project, which align with their interests in improving the mastitis situation on their farms. These conditions were communicated to the Albanian National Agency for Scientific Research and Innovation (NASRI).

Availability of data and materials: the data that support the findings of this study are available from the corresponding author upon request.

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practices further increase SCM prevalence (Neculai-Valeanu and Ariton, 2022). Nutritional deficiencies also compromise immune function, making cows more susceptible to infection (Sordillo, 2016). SCM adversely affects milk composition, leading to increased SCC, altered fat and protein levels, reduced lactose content, and higher bacterial loads, thereby compromising milk quality, taste, shelf life, and processing properties (Miglior *et al*., 2007; Hagnestam-Nielsen *et al*., 2009; Malek dos Reis *et al*., 2013; Bludau *et al*., 2014; Costa *et al*., 2025; Zalewska *et al*., 2025). These changes can impair dairy product production, such as yogurt and cheese (McCain *et al*., 2018). Early detection is crucial to mitigate economic losses, with the California Mastitis Test (CMT) providing a cost-effective, accessible diagnostic tool (Antanaitis *et al*., 2021). In this context, the question arises: how can farms with limited access to technology, resources, and investment capacities detect and prevent SCM in a timely manner? The CMT is a simple, cost-effective tool that can be easily learned and applied by farms with basic training and practical instruction. In Albania, dairy companies now have the necessary laboratory infrastructure to perform rapid tests for milk quality and safety, including SCC and various physical and chemical properties [*e.g*., conductivity, density, freezing point, pH, lactose content, protein, solid non-fat (SNF), fat content]. The results of these tests are often provided to farms shortly after milk delivery. Based on the data available in the literature, the hypothesis of this study is that alterations in milk constituents, as determined by rapid testing, may serve for farms as an early indicator of the presence of SCM in dairy farms.

The objective of this study is to investigate the relationship between the physicochemical properties of milk, as determined through rapid testing, and the prevalence of SCM, with the aim of enabling early detection of SCM by farms through observed changes in milk parameters.

Materials and Methods

Study area and experimental design

The study was conducted using an experimental design with measurements at different time intervals during lactation. The study was carried out on 9 dairy farms of Holstein cattle in the lowland regions (Shijak/Durres 41°20’44”N 19°34’01”E 42 m above sea level; Bushat/Shkoder 42°00’36”N 20°23’47”E 40 m above sea level; Valias/Tirana 41°23’30”N 19°44’20”E 45 m above sea level) and the hilly areas of Maliq/Korce (40°42’12”N 20°43’54”E 810 m above sea level). The measurement period spanned the winter, spring, and summer months and lasted for approximately 9 months. The first measurements were conducted in November 2023, followed by the second measurement in April 2024 and the third measurement in July 2024. On each farm, 6-10 cows in lactations 1 to 3 were randomly identified, and at the start of the first measurement, they were in either the first phase or at the beginning of the second phase of lactation.

Sample collection and testing

CMT was performed individually for each quarter, involving 6 to 10 animals per farm, at midday. For each cow, 2 mL of milk was obtained from each udder quarter, following the standard cleaning and drying of the teats. The physicochemical properties of the milk were also analyzed in the same cows that underwent the CMT. Subsequent to the CMT, 100 mL of milk was collected from each cow, proportionately from each udder quarter, into sterile bottles. Samples were stored at 4°C and analyzed within 4 to 6 hours (ISO, 2008).

California Mastitis Test

The prevalence of SCM on each farm was determined by the SCC in the milk from clinically healthy cows, which was evaluated using the CMT (KEPRO, Kuipersweg 93449 JA, Woerden, The Netherlands). The milk samples were taken into a clean CMT plate, which had four small wells (A, B, C, and D) to identify the respective udder parts. An equal amount of CMT reagent was added, and the plate was mixed in circular motions until the mixture was thoroughly mixed. The reaction should not last longer than 10 seconds, as it rapidly dissolves afterward. Results were visually assessed based on the amount of gel formed. The more gel formed, the higher the result, rated on a scale from negative, traces, 1+, 2+, to 3+. CMT results directly correlated with the average SCC (Jasper, 1975).

Physicochemical testing in bovine milk

The composition of the milk was measured using the Lactoscan MCCW v1 (Milkotronic Ltd., Nova Zagora, Bulgaria) (Nakov *et al*., 2023). This device was calibrated for cow milk. The density was measured using an aerometer, while the SNF, lactose, salts, total protein content, freezing point, and added water content were determined using the formulas provided by the manufacturer.

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. One-way analysis of variance (ANOVA) was used to test the impact of specific factors on SCM. Robust compound regression examined SCM indicators and converted ln values of physicochemical milk parameters. A p-value <0.05 indicated significance. To determine the prevalence of SCM on the farm, the ratio of cows with grade ≥1 SCM to the total number of cows tested for CMT was calculated.

Results

Occurrence of subclinical mastitis

Table 1 shows the CMT results for 178 cows from 9 Albanian farms, for a total of 712 observations. Farm 5 had the highest percentage of score 1+ results at 0.72, while Farms 7 and 8 had the lowest at 0.11 and 0.06, respectively, with most cows having negative or trace reactions at 0.89 and 0.94. Moderate positive reactions (score 2+) were less common than scores 1 and 2, with percentages ranging from 0.04 (Farm 2) to 0.33 (Farm 5). Most farms exhibited score N and trace, with Farm 8 (0.94) having the highest percentage. Minimum prevalence for CMT score 1+ was 0.06, maximum 0.72, and average 0.37. CMT score 2+ prevalence ranged from 0.0 to 0.33, averaging 0.14. The average negative (N) and trace CMT score was 0.63, ranging from 0.28 to 0.94. In this study, the mean prevalence of bovine SCM was found to be 0.37.

Infection in the different quarters of the udder

The study covered 711 single observations with measurements recorded for each quarter of dairy cows. We have used classification of CMT scores based on four tested categorical levels [0, 0.5 (T), 1, and 2]. ANOVA statistical testing revealed a significant difference among the groups, with a p-value below 0.05 of mean values (0.26, 0.19, 0.10, 0.23) to each quarter, indicating that the Right Front Quarter (RFQ) and left back quarter (LBQ) have the highest-level SCC compared to quarter left front quarter (LFQ) and right back quarter (RBQ). The prevalence of SCM was RFQ (0.26/177), LFQ (0.19/178), RBQ (0.10/178), and LBQ (0.23/178) at the cow and quarter levels, respectively.

Occurrence of subclinical mastitis across lactation stages and seasons

The occurrence of SCM exhibited changes across different seasons and lactation stages ($p<0.05$). During early lactation, the SCC percentage peaked in summer, averaging 0.61 (±0.22) with a range of 0.17 to 0.83. In the spring, we had the lowest SCM at this phase, with a mean of 0.30 (±0.02) and a range of 0.25 to 0.50. Conversely, winter exhibited an intermediate prevalence, with a mean of 0.46 (±0.03) and a range from 0.17 to 0.83. Additionally, during mid-lactation, the peak SCM was observed in winter, averaging 0.67 (±0.11) with a range of 0.17 to 0.83. In spring, it was found a mean of 0.51 (±0.04) was found with a range of 0.27 to 0.83. Conversely, summer demonstrated the minimal SCM during this phase, with a mean of 0.21 (±0.02) and a range from 0.13 to 0.33. During late lactation, SCM data were gathered solely in spring and summer. The average for spring was 0.42 (±0.05), with a range of 0.27 to 0.83. During summer, the average was marginally reduced to 0.36 (±0.04), with a range spanning from 0.17 to 0.83.

Occurrence of subclinical mastitis across lactation year

In Figure 1A, a moderate positive association between the year of lactation and the SCM prevalence is shown. The regression analysis indicates that the lactation year significantly affects the incidence of SCM in cows. The model accounts for 28% of the variance in the dependent variable, and the positive coefficient for the year of lactation suggests that the prevalence of SCM rises with the length of lactation.

The influence of subclinical mastitis infection on the milk quality in the studied farms

In Table 2, we have presented descriptive data for physiochemical parameters at the farm levels. Farm 1 had the lowest mean fat content (2.39%), but the highest SNF (9.65%) and lactose content (5.30%). Farm 8 had the highest average fat content (4.59%) and fat/protein ratio (1.46) but the lowest SNF (8.64%), protein (3.15%), and lactose content (4.75%). Farm 2 had the highest density (34.20 kg/m³), and Farm 8 had the lowest (28.70 kg/m³). The freezing points ranged from -0.629°C (Farm 1) to -0.559°C (Farm 8). The findings in Figure 1B point to a statistically significant positive correlation between the logarithm of milk conductivity and the prevalence of SCM in both mild and severe cases. About 26% of the variance in the dependent variable, the logarithm of milk conductivity, is explained by the predictor, the prevalence of SCM. The model demonstrated a moderate correlation ($R=0.51$), with an R^2 value of 0.260. The results demonstrate a statistically significant and moderately strong relationship between the prevalence of.SCM and milk electrical conductivity.

Figure 1C indicates a significant positive correlation between the prevalence of SCM and the logarithm of lactose content in milk. SCM prevalence greatly affects the logarithm of lactose content, accounting for 41.2% of variability. The model fits well, and the predictor variable explains lactose content variation, as shown by the significant F-statistic and p-value. The individual effect of lactation year on lactose content was also tested. Lactose content decreased with lactation year ($R^2=0.41$ for $n=178$, $p<0.0001$). This relationship with conductivity at the individual level was weaker compared with lactose ($R^2=0.21$, $p<0.05$ ($n=178$).

In Figure 1D and E, our findings demonstrate a statistically significant and strong relationship between the prevalence of SCM and milk SNF and protein. The positive association suggests that higher SCM prevalence is associated with increased SNF and protein content. The combined model displays the regression analysis results of $R^2=0.36$ and $R^2=0.34$, respectively ($p<0.05$). However, a weak positive correlation was found between the prevalence of SCM and the logarithm of fat content ($R^2=0.049$).

Of the total 132 observations, a statistically significant positive correlation between the incidence of SCM and the logarithm of milk density was found (Figure 1F). The results demonstrate a statistically significant and moderate relationship ($R^2=0.19$) between the prevalence of SCM and milk density.

Discussion

The results of this study show a strong link between SCM and milk quality measures in medium-sized Holstein dairy farms in Albania. The total prevalence of SCM varied among farms, with some having a significantly higher rate. This variety shows that

Table 1. Results of the occurrence of subclinical mastitis grouped by farm presented as the mean value for each level.

Farms	Region	Number of cows	Average daily milk	CMT score 1	CMT score 2	CMT score N and trace
Farm 1	Maliq/Korce	22	30.3	0.36	0.23	0.64
Farm 2	Maliq/Korce	28	31.7	0.25	0.04	0.75
Farm 3	Maliq/Korce	20	23.6	0.35	0.10	0.65
Farm 4	Bushat/Shkoder	18	18.4	0.39	0.17	0.61
Farm 5	Bushat/Shkoder	18	23.6	0.72	0.33	0.28
Farm 6	Bushat/Shkoder	18	16.8	0.61	0.28	0.39
Farm 7	Shijak/Durres	18	23.8	0.11	0.00	0.89
Farm 8	Shijak/Durres	18	20.8	0.06	0.00	0.94
Farm 9	Tirana	18	18.4	0.44	0.11	0.56
Minimum				0.06	0.00	0.28
Maximum				0.72	0.33	0.94
Mean				0.37	0.14	0.63

CMT, California Mastitis Test.

managerial strategies, husbandry conditions, and milking hygiene are critical in preventing SCM. The average prevalence at the farm level, as determined by SCC measurement, was consistent with the findings reported by Bludau *et al*. (2014) and Chen *et al*. (2022).

This study found a significant link between SCM prevalence and milk physicochemical parameters. Milk from SCM-affected cows had increased electrical conductivity but decreased lactose, protein, SNF, and density values. These findings are consistent with previous research (Pyörälä, 2002; Forsbäck *et al*., 2010; Gillon *et al*., 2010), which demonstrated changes in milk composition owing to SCM. Another author investigated the use of electrical conductivity as an indicator of SCM in dairy cows, finding a strong positive correlation between conductivity and SCC (Kitchen, 1981; Maatje *et al*., 1992; Norberg *et al*., 2004). Additionally, the substantial association between electrical conductivity and SCM prevalence implies that this parameter could serve as a rapid, cost-effective diagnostic tool for early diagnosis, as indicated by Kitchen (1981) and Norberg *et al*. (2004).

The decrease in lactose content, in particular, is a good sign of udder health since it is directly related to the integrity of the mammary gland epithelium. Many studies have emphasized that lactose could be a potential indicator of intramammary infections in cows (Pyörälä, 2002; Reist *et al*., 2002; Forsbäck *et al*., 2010; Gillon *et al*., 2010). Costa *et al.* (2019) found that cows with milk lactose concentration less than or equal to 4.553% had a higher rate of udder impairment compared with cows with a lactose content of ≥5.045%; also, the authors discovered that milk lactose and SCM have a genetic correlation (r=0.518) and that more productive cows are genetically more prone to mastitis than less productive cows (Costa *et al*., 2019).

The study also discovered that SCM occurrence was impacted by lactation stage and year, as well as season, supporting findings by other authors (Biffa *et al*., 2005; Shittu *et al*., 2012). The largest occurrence was seen during the early lactation period, notably in the summer, while the lowest occurred in mid-lactation throughout the spring. These findings are congruent with those of Hagnestam-Nielsen *et al*. (2009), who found that seasonal differences in temperature, humidity, and farm management techniques influence SCM prevalence. Heat stress during the summer months may contribute to a higher prevalence of SCM since it weakens dairy cows' immune systems, leaving them more susceptible to infections (Sordillo, 2016).

Regarding infection distribution inside the udder, our findings show that RFQ and LBQ exhibited greater SCC than the other quarters. This pattern could be due to milking equipment inefficiencies, poor cluster attachment, or individual cow position dur-

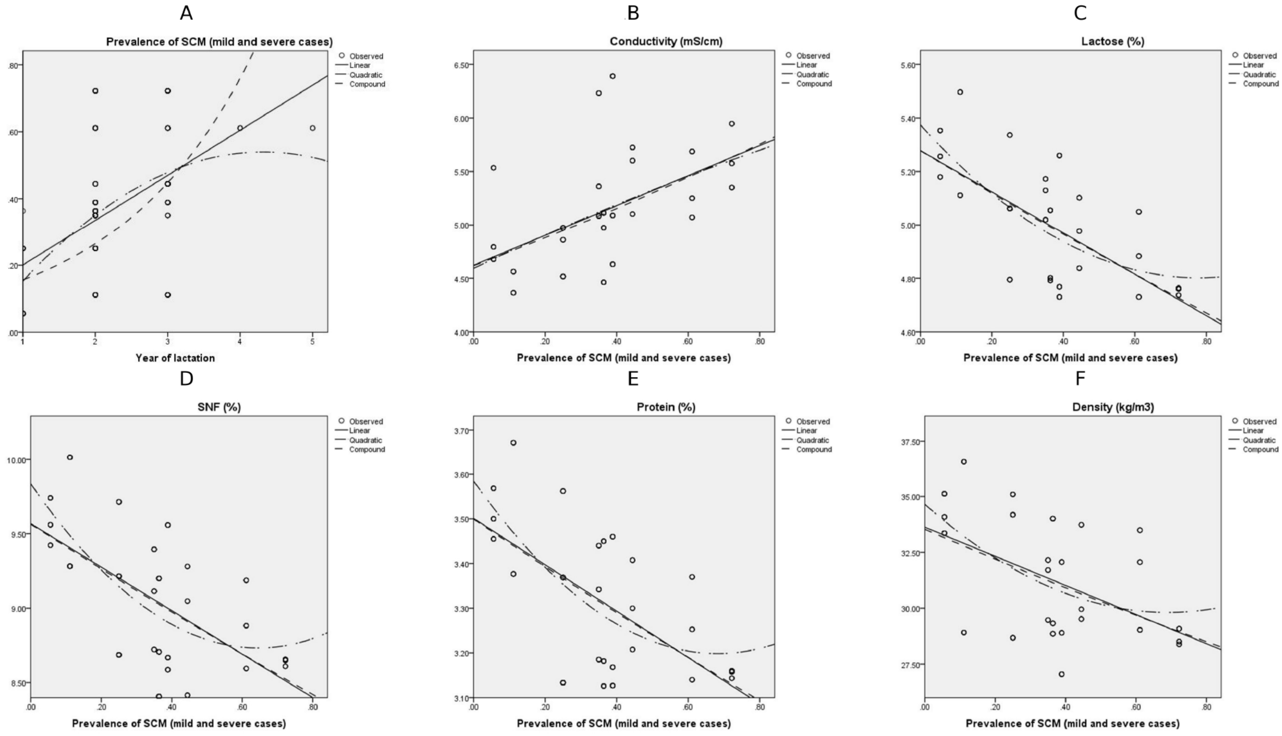

Figure 1. **A**) Influence of lactation year on the occurrence of subclinical mastitis (SCM) at farm Level. Sig. $p<0.05$, compound regression model for $R^2=0.28$; **B**) the influence of subclinical mastitis infection on the conductivity in the studied farms at the farm level. Sig. $p<0.05$. $R^2=0.26$; **C**) the influence of subclinical mastitis infection on the lactose in the studied farms. Sig. $p<0.05$. $R^2=0.41$; **D**) the influence of subclinical mastitis infection on the solid-non-fat in the study at the farm level. Sig. $p<0.05$, $R^2=0.36$; **E**) the influence of subclinical mastitis infection on protein content in the study at the farm level. Sig<0.05, $R^2=0.39$; **F**) the influence of subclinical mastitis infection on milk density in the study at the farm level. Sig. $p<0.05$; $R^2=0.19$.

ing milking, as documented in previous investigations (Reist *et al.*, 2002; Bludau *et al*., 2014).

Furthermore, the variation in physicochemical parameters among farms demonstrates the impact of SCM on milk yield and quality. Notably, farms with higher SCM prevalence showed bigger variances in milk composition. Farms that produce with the highest fat content in milk had the lowest SNF and lactose levels, confirming the findings of this study that milk composition changes in response to mastitis-induced inflammation. These findings emphasize the necessity for effective SCM management strategies, including routine monitoring, improved hygiene, and optimized feeding practices. Regular use of the CMT and monitoring of electrical conductivity could enable early detection and intervention, reducing the economic burden on farms. Additionally, further research should explore genetic predisposition to SCM and the long-term effects of SCM on milk production and reproductive performance in Albanian dairy farms. In conclusion, this study clarifies the presence and influence of SCM on milk quality in Albanian dairy farms. Dairy farmers can reduce the negative impacts of SCM and increase milk production efficiency by employing proactive measures such as increased hygiene, regular health checks, and early diagnosis tools.

Conclusions

In regions with limited veterinary services, such as Albania, physical and chemical milk tests enable the cost-effective and rapid detection of SCM. Mobile ultrasound milk devices provide on-site analysis, allowing farms to receive immediate feedback on milk quality and to initiate targeted follow-up tests, such as the CMT or microbiological analyses. For farms without regular CMT testing, monitoring milk parameters such as lactose content and conductivity by dairy processors can aid in early infection detection. The combined use of these parameters enhances early detection and reduces the risk of progression to clinical mastitis. Future studies should incorporate larger sample sizes and advanced diagnostic techniques to further validate the effectiveness of these methods under the real-world conditions of dairy farms in Albania.

Table 2. Physiochemical parameters across farms.

Farm		Fat (%)	Density (kg/m³)	Conductivity (mS/cm)	SNF (%)	Protein (%)	Lactose (%)	Freezing point (°C)	Salinity (%)	pH	Fat/protein ratio (%)
Farm 1	Mean	2.39	32.74	4.46	9.65	3.52	5.3	-0.629	0.79	6.48	0.68
	Min	2.07	27.27	3.97	8.81	3.2	4.85	-0.703	0.74	6.32	0.59
	Max	2.71	9.5	5.06	10.64	3.91	5.85	-0.589	0.87	6.64	0.77
	SD	0.45	4.42	0.27	0.6	0.23	0.33	0.035	0.04	0.11	0.13
Farm 2	Mean	3.25	34.2	4.93	9.59	3.51	5.27	-0.611	0.78	6.53	0.91
	Min	2.22	30.78	4.28	8.79	3.22	4.83	-0.68	0.72	6.39	0.6
	Max	5.28	37.56	5.93	10.23	3.73	5.63	-0.558	0.84	6.86	1.42
	SD	1.19	1.7	0.47	0.43	0.16	0.24	0.035	0.04	0.14	0.32
Farm 3	Mean	3.78	31.17	5.51	8.9	3.31	4.97	-0.58	0.74	6.43	1.17
	Min	2.41	27.46	4.84	7.16	3.02	4.56	-0.648	0.69	6.22	0.68
	Max	4.54	34.53	6.73	9.88	3.61	5.44	-0.533	0.81	6.55	1.5
	SD	0.84	2.68	0.58	0.79	0.2	0.3	0.043	0.04	0.09	0.29
Farm 4	Mean	4.17	30.8	4.84	8.77	3.26	4.89	-0.567	0.73	6.51	1.3
	Min	2.46	24.1	4.21	6.72	2.5	4.4	-0.612	0.67	6.44	0.7
	Max	5.99	35.47	5.53	9.6	3.66	5.28	-0.526	0.79	6.66	1.93
	SD	0.99	2.98	0.42	0.65	0.24	0.22	0.022	0.03	0.06	0.33
Farm 5	Mean	3.91	32.14	4.76	9.11	3.32	5.02	-0.583	0.71	6.5	1.19
	Min	2.33	24.1	4.28	7.79	2.5	4.4	-0.639	0.08	6.36	0.67
	Max	5.26	36.39	6.18	10.05	3.69	5.52	-0.53	0.82	6.67	1.63
	SD	1.16	3.25	0.42	0.54	0.25	0.28	0.03	0.15	0.08	0.38
Farm 6	Mean	3.71	31.19	5.61	9.08	3.32	5.1	-0.585	0.75	6.48	1.12
	Min	2.26	26.05	4.58	8.39	3.06	4.62	-0.64	0.69	6.14	0.71
	Max	6.64	33.76	8.12	9.78	3.61	6.2	-0.541	0.81	7.05	2.17
	SD	1.27	2.06	1.18	0.43	0.17	0.41	0.032	0.04	0.24	0.41
Farm 7	Mean	4.15	29.19	5.35	8.92	3.24	4.91	-0.584	0.74	6.51	1.27
	Min	2.23	18.41	4.24	7.65	2.75	4.23	-0.66	0.65	6.38	0.61
	Max	5.76	37.15	7.28	10.14	3.72	5.58	-0.54	0.83	6.71	1.79
	SD	1.14	4.93	0.84	0.74	0.27	0.4	0.04	0.06	0.11	0.38
Farm 8	Mean	4.59	28.7	5.6	8.64	3.15	4.75	-0.559	0.71	6.6	1.46
	Min	2.81	26.11	4.61	8.21	2.99	4.52	-0.586	0.68	6.44	0.86
	Max	6.61	31.16	6.26	9.06	3.31	4.98	-0.529	0.74	6.72	2.13
	SD	1.07	1.79	0.53	0.28	0.11	0.15	0.017	0.02	0.09	0.37
Farm 9	Mean	3.42	31.53	5.34	8.89	3.25	4.89	-0.564	0.73	6.5	1.07
	Min	2	26.91	4.58	8.29	3.02	4.56	-0.616	0.68	6.29	0.56
	Max	5.17	35	6.19	9.7	3.56	5.34	-0.527	0.79	6.69	1.71
	SD	1.21	2.42	0.46	0.46	0.17	0.25	0.031	0.04	0.1	0.43

SNF, solid non-fat; SD, standard deviation.

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