

Quality of raw cow's milk intended for processing and retail sale from Eastern Poland

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Summary

The study assessed the quality of raw cow's milk intended for processing and retail sale ($n = 595$ samples and $n = 19$ production batches, respectively) and the relationship between the number of somatic cell and the fat and protein contents. All milk samples were tested for total bacteria count, somatic cell count, antibiotic residues, and fat and protein contents. Additionally, raw drinking milk samples were tested for counts of *Enterobacteriaceae*, *Escherichia coli*, coagulase-positive staphylococci, enterococci, and the presence of *Listeria monocytogenes* and *Salmonella* spp. In raw milk samples, TBC, SCC, and fat and protein contents ranged from 4.9×10^3 to 2.3×10^5 CFU mL⁻¹, from 9.9 to 407.8 thou. mL⁻¹, from 3.2% to 5.9% and from 3.0% to 4.8%, respectively, while TBC, SCC, and fat and protein contents in raw drinking milk samples ranged from 1.8×10^6 to 3.3×10^6 CFU mL⁻¹, from 454 to 696.9 thou. mL⁻¹, from 1.7% to 2.9% and from 3.1% to 3.4%, respectively. The mean counts of *Enterobacteriaceae*, *E. coli*, coagulase-positive staphylococci, enterococci in raw drinking milk were 4.0×10^5 CFU mL⁻¹, 1.4×10^2 CFU mL⁻¹, 2.4×10^4 CFU mL⁻¹, and 2.2×10^5 CFU mL⁻¹, respectively. *L. monocytogenes*, *Salmonella* spp., and antibiotic residues were not detected in any of the samples tested. The study showed that 95% of the raw milk samples were of satisfactory quality, while 100% of the raw drinking milk samples were of unsatisfactory quality in relation to the regulatory thresholds. Additionally, raw drinking milk samples were a vehicle of potentially pathogenic bacteria. The somatic cell count did not affect the fat and protein content in the analysed milk.

Keywords: raw cow's milk, raw drinking milk, milk quality, antibiotic residues, pathogens

Milk production is a significant component of agricultural market output in Poland (53). With a production level of over 15 million tonnes, Poland ranks 12th in the World and 6th on the European list of producers, with a share of 2% in global and 6.7% in European milk production (9, 46, 47, 53). Poland is the fifth largest milk producer (over 12.8 mln tonnes of milk collected in 2022 by dairies) in the European Union (EU) after Germany (31.9 mln tonnes), France (24.1 mln tonnes), the Netherlands (13.9 mln tonnes), and Italy (13 mln tonnes) (53). Poland also maintains a strong export orientation, an important element of the Polish dairy industry. From 2005-2022, the export of milk and milk products from Poland in raw milk equivalent increased almost twice (by 96.4%), rising from 2435.4 thousand tonnes to 4785 thousand tonnes (53). Milk products produced in Poland are exported to many countries, including the EU member states,

Algeria, Great Britain, China, Ukraine, and Saudi Arabia (28). In Poland, the raw material base of the dairy industry relies on individual suppliers, with the majority (60-70%) of milk produced and delivered to the market by larger farms (> 30 cows). Small farms (< 10 cows) have around a 10% share of milk production in Poland, while their share in milk deliveries to the dairy industry is marginal (3-5%) (53).

"Raw milk" is defined as milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment that has an equivalent effect (37). Raw cow's milk intended for processing plants should meet requirements for total bacteria count (TBC), somatic cell count (SCC) and antibiotic residues (37). The limits for TBC and SCC have been set at $\leq 100,000$ CFU mL⁻¹ as a rolling geometric average over two months, with at least two samples per month and $\leq 400,000$

cells mL⁻¹ as a rolling geometric average over three months, with at least one sample per month, respectively (37). TBC values reflect hygiene practices on milk production holdings, while SCC reflect the health status of dairy cows, especially regarding subclinical mastitis (51). Measurements of TBC and SCC in the raw material, and the relationship between SCC and milk components, are considered to potentially affect technological suitability and milk product quality (27, 51). Control of antibiotic residues in raw milk is important from the perspective of the food safety and a technological suitability. The presence of antibiotic residues in milk has been linked to the development of antibiotic resistance in humans, as well as the onset of allergies and other serious pathologies including cancer, anaphylactic shock, nephropathy, bone marrow toxicity, mutagenic effects and reproductive disorders (1). In turn, antibiotic residues in the raw material may interfere with the fermentation process during cheese and fermented drinks production by inhibiting starter cultures (42). The European Union (EU) has adopted a zero-tolerance policy concerning foodstuffs antibiotic residues (37). Literature data indicate that raw cow's milk did not meet the accepted criteria in terms of total bacterial count (41, 51, 52), somatic cell count (51) and antibiotic residues (26, 36, 44, 50).

In EU countries, including Poland, selling raw cow's milk (raw drinking milk) does not contravene the food law. Nevertheless, a Member State may, on its own initiative, maintain or enact national food laws restricting or prohibiting the sale of raw drinking milk on its territory. Raw drinking milk intended for retail sale should meet the same quality requirements as raw milk intended for processing plants (37, 40). However, the adopted limit of hygiene indicator bacteria may be insufficient to ensure the safety of raw drinking milk (52). Literature data showed that raw drinking milk was a carrier of bacteria pathogenic to humans as well as the cause of disease outbreaks in many countries (12, 52). According to Regulation (EU) No 1308/2013 (39), the non-standardised milk's fat and protein contents should not be lower than 3.5% and 2.9%, respectively. Tests of raw drinking milk showed different levels of these components, including fat contents outside of the adopted specification (21, 29).

The study aimed to assess the quality of raw cow's milk intended for processing and retail sale produced in Eastern Poland and the relationship between the somatic cell count and the fat and protein contents.

Material and methods

Sampling. The study was carried out in 2022 and 2023 in Eastern Poland. The study material consisted of samples of raw cow's milk intended for processing plants (raw milk) and retail sale (raw drinking milk). Milk producers were not notified in advance about the planned milk control. The raw milk samples (n = 595; 104 milk production holdings) were aseptically taken from bulk tanks, transferred to

sterile containers (50 mL) and transported to the laboratory at 0-4°C. The samples of raw drinking milk (n = 19 production batches) were purchased from a retail store and transported to the laboratory in the original packaging (one-liter plastic bottle) at 0-4°C. The analyses were undertaken within 24 hours of milk sample collection/purchase and continued following the microbiological standards listed below.

Analysis. All samples were tested for TBC, SCC, fat and protein contents, and antibiotic residues. TBC, SCC and fat and protein content were determined using the Bactoscan FC, Fossomatic 5000 FC and MilkoScan 4000 (Foss Analytical, Hillerød, Denmark), respectively. Antibiotic residues were assessed using the Polutest® (Polutest Barbara Kawiorska, Olsztyn, Poland) and TwinSensor (Unisensor Diagnostic Engineering, Belgium). The samples of raw drinking milk were also tested for the counts of *Enterobacteriaceae*, coagulase-positive staphylococci, *E. coli*, enterococci, and the presence of *Salmonella* spp. and *L. monocytogenes*. The counts of *Enterobacteriaceae*, coagulase-positive staphylococci, *E. coli* and enterococci were determined in accordance with the standards: PN-EN ISO 21528-2:2017-08 (32), PN-EN ISO 6888-1:2022-03 (34), PN-ISO 16649-2:2004 (35) and PN-A-82055-7:1997 (30), respectively. *Salmonella* spp. and *L. monocytogenes* were detected according to PN-EN ISO 6579-1:2017-04 (33) and PN-EN ISO 11290-1:2017-07 (31), respectively. *Enterococcus faecalis*, *Enterococcus faecium* and *Staphylococcus aureus* were identified using the STREPTOtest 24 kit and the STAPHYtest 24 kit (Erba Lachema, Czech Republic), respectively.

The TBCs and SSCs are presented as a rolling geometric average over a two-month period, with at least two samples per month (CFU mL⁻¹) and as a rolling geometric average over a three-month period, with at least one sample per month (thou. mL⁻¹), respectively (37).

Statistical analysis. The statistical analysis of the results was performed using Statistica software formulas, version 13.3 for Windows, StatSoft Inc., Tulsa, OK, USA. The data distribution was analysed using the Shapiro-Wilk test. The Pearson correlation coefficient (r) was determined for the selected parameters of raw milk samples. A p < 0.05 was assumed to be statistically significant for all correlations.

Results and discussion

TBC and SCC are useful indicators of raw milk quality from management and hygiene practices perspectives on milk production holdings and for animal health surveillance. In the present study, the TBCs and SCCs in raw milk ranged from 4.9×10^3 to 2.3×10^5 CFU mL⁻¹ and from 9.9 to 407.8 thou. mL⁻¹, respectively (Tab. 1). The regulatory limits for SCC and TBC were exceeded in only 1/104 (0.96%) and 4/104 (3.84%) of milk production holdings, respectively. The results of subsequent routine monitoring samples taken from these holdings were satisfactory, indicating that the cause had been identified and corrective action had been implemented. Overall, 99 out of 104 (95%) holdings showed satisfactory results for both parameters (TBC and SCC), indicating that these farms maintain

strict controls including hygiene practices and health surveillance of dairy cows (Fig. 1). According to the EFSA report, the mean TBCs ranged from 3.6×10^3 to 7.3×10^4 CFU mL⁻¹ in most EU countries (12). TBCs in milk from the analysed milk production holdings were comparable to those reported from EU countries, except for four samples with the counts of bacteria exceeding the regulatory limits (37). Authors from other countries also reported non-compliance of raw milk with TBC and SCC standards, with the percentage of positive samples ranging from 15.4% to 87.5%

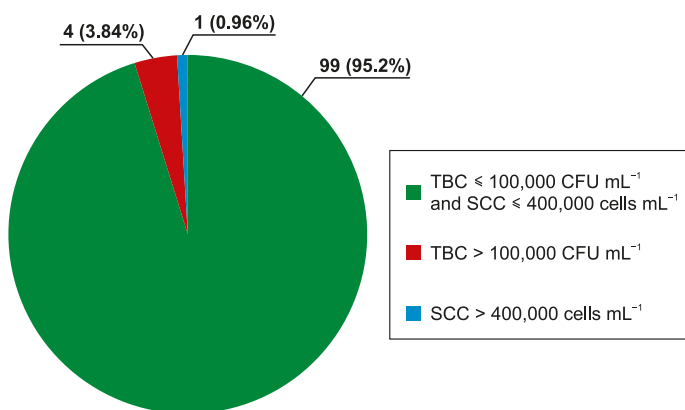


Fig. 1. TBC (the rolling geometric average over a two-months period, with at least two samples per month, CFU mL⁻¹) and SCC (the rolling geometric average over a three-months period, with at least one sample per month) values in the analysed raw cow's milk samples (n = 104)

Tab. 1. TBC (CFU mL⁻¹) and SCC (thou. mL⁻¹) in the samples of raw cow's milk

Raw cow's milk	Microbiological criteria	Mean value	Minimum value	Maximum value
Raw drinking milk intended for retail sale	TBC ^a	2.6×10^6	1.8×10^6	3.3×10^6
	SCC ^b	527.1	454	696.9
Raw milk intended for processing	TBC ^a	4.1×10^4	4.9×10^3	2.3×10^5
	SCC ^b	173	9.9	407.8

Explanations: a – the rolling geometric average over a two-months period, with at least two samples per month; b – the rolling geometric average over a three-months period, with at least one sample per month

Tab. 2. Fat and protein contents in the samples of raw cow's milk

Raw cow's milk	Nutrients	Mean value (%)	Minimum value (%)	Maximum value (%)
Raw drinking milk intended for retail sale	Fat	2.2	1.7	2.9
	Protein	3.3	3.1	3.4
Raw milk intended for processing	Fat	4.3	3.2	5.9
	Protein	3.5	3.0	4.8

Tab. 3. Correlation coefficients (r) between SCC, fat and protein content of raw cow's milk (n = 595)

Parameters*	SCC	Fat	Protein
SCC	–	0.07	0.04

Explanation: * p < 0.05

and from 9.6% to 29%, respectively (3, 4, 41, 51). Although regulatory requirements play a key role in ensuring the quality of raw milk, most segments of the dairy industry consider that more stringent standards should be maintained (6). In the present study, 76/104 (73.07%) and 55/104 (52.8%) holdings produced milk with lower bacterial and somatic cell counts than the accepted limits, i.e. $\leq 50,000$ CFU mL⁻¹ and $\leq 200,000$ cells mL⁻¹, respectively. In summary, the hygienic quality of raw milk intended for processing was satisfactory in the context of regulatory requirements. Additionally, the content of fat (3.2-5.9%) and protein (3.0-4.8%) in the samples of the analysed milk (Tab. 2) were higher than the minimum requirements set by processing plants, i.e. 3.0% for fat and 2.8% for protein. The results of this study clearly showed that raw cow's milk produced in 104 milk production holdings in Eastern Poland was a valuable raw material for the production of milk products.

Understanding the relationship between somatic cell count (SCC) and the percentage of milk components is important for increasing the efficiency of the whole production chain. Some studies indicated a variable relationship between SCC and the percentage of various milk components, including fat and protein (13, 43, 51). Garcia et al. (13) reported that the increased SCC in milk was associated with lower fat and higher protein content. Conversely, Safak et al. (43) reported that the protein content in milk decreased with increasing somatic cell count, while no statistical difference was observed between the fat content of milk and the level of somatic cell count.

In contrast to these data, Visciano and Schirone (51) observed a positive correlation of SCC with the content of fat and protein, which resulted in increased percentages of protein and fat contents in milk with high SCC values. In present study, no effect of the count of somatic cells on the fat and protein contents in the analysed milk was observed, as evidenced by very low values of correlation coefficients (Tab. 3). These results suggest that protein and fat contents in raw milk do not seem to be significantly affected by $SCC \leq 400,000$. This is also confirmed by the results of studies by other authors (10, 15, 16). Data reported in the literature on the relationship between SCC and fat and protein contents are contradictory, and no trends can be determined for these parameters (23): "A clear and noncontroversial effect is a change in the protein profile:

a rise in the level of whey proteins (with a change in whey protein composition) and changes in the case in profile" (23). Considering that milk with various SCCs and compositions, including different profiles of endogenous enzymes, leads to different patterns of proteolysis and lipolysis and finally offers different

characteristics of final dairy products (25), future studies in this field are needed.

There is a growing global interest in consuming unprocessed, natural food commodities, including raw drinking milk (2, 20). Consumers actively seek out raw drinking milk due to its perceived better organoleptic properties than heat-treated milk and the belief in its higher nutritional value and health benefits (2, 20). According to the literature, raw drinking milk introduced to the market did not meet basic regulatory requirements (5, 19, 29, 52). In the present study, the values of TBC and SCC in raw drinking milk samples ranged from 1.8×10^6 CFU mL⁻¹ to 3.3×10^6 CFU mL⁻¹ and from 454 to 696.9 thou. mL⁻¹, respectively (Tab. 1). Consequently, none of the production batches of the analysed product were within the permissible limits for both of these parameters. The values of both metrics revealed, on the one hand, non-compliance with the principles of Good Hygienic Practice (GHP) and Good Manufacturing Practice (GMP) and, on the other hand, ineffective supervision over the health of dairy cows on the farm. Regardless of the results of these studies, it is worth noting that compliance of raw drinking milk with accepted limits does not ensure consumer safety regarding potential risks from pathogenic bacteria (52). Therefore, additional microbiological monitoring parameters should be considered based on the risk analysis assigned to the milk production holding. In addition, training for dairy farmers in hygienic and safe milk production can help ensure high-quality raw drinking milk. Bacteria of the *Enterobacteriaceae* family, *E. coli*, coagulase-positive staphylococci, and enterococci are considered hygiene indicators. In this study, the samples of raw drinking milk were analysed for these bacteria, even though their evaluation is not required by food law (Tab. 4). The count of *Enterobacteriaceae* ranged from 2.2×10^3 to 2.5×10^6 CFU mL⁻¹. According to the UK Health Protection Agency's guidance on interpreting results for hygiene indicator organisms in ready-to-eat foods placed on the market, a count of *Enterobacteriaceae* $> 10^4$ CFU g⁻¹ indicates an overall low general hygiene standard of a food product (17). Most raw drinking milk samples (78.9%) exceeded this limit. Literature data showed that the counts of *Enterobacteriaceae* in raw drinking milk were within a wide range of values, i.e. from $< 10^2$ to 8.0×10^7 CFU mL⁻¹, and contamination also affected a significant percentage

of samples (84-100%) (45, 48). The *E. coli* count (β -glucuronidase-positive) ranged from 0 to 5.4×10^2 CFU mL⁻¹, and 94.7% of the tested samples were contaminated with these bacteria. Comparison of the results presented in this study with the limit value for cow's milk given by Bogdanovičová et al. (3), i.e. 1.0×10^3 CFU mL⁻¹, indicated low *E. coli* contamination of the tested samples. Literature data showed that *E. coli* count in raw cow's milk ranged from 0 to 5.0×10^5 CFU mL⁻¹ and the percentage of positive samples ranged from 45% to 100% (3, 22, 41, 48). The coagulase-positive staphylococci were found in all tested samples, and their count ranged from 2.4×10^3 to 1.6×10^5 CFU mL⁻¹. *Staphylococcus aureus* was confirmed in one sample, indicating a potential risk to consumer health, as the bacteria cause a wide range of clinical infections (49). The count of coagulase-positive staphylococci $> 10^4$ in ready-to-eat foods placed on the market is categorised as having a high microbiological risk (17). A total of 36.8% of the tested samples also exceeded this limit. Literature data showed that the count of coagulase-positive staphylococci in raw cow's milk ranged from $< 10^2$ to 3.7×10^5 CFU mL⁻¹, with the presence of bacteria in 94-100% of the samples (8, 48). The enterococci were found in all tested samples, and their count ranged from 1.0×10^4 to 3.1×10^6 CFU mL⁻¹. *Enterococcus faecium* and *Enterococcus faecalis* were confirmed in one and eleven samples. *E. faecium* and *E. faecalis* indicated a health risk to consumers, as these bacteria have been linked to humans disease (14). In another study, the counts of enterococci in raw cow's milk were also within wide ranges of values, i.e. from $< 10^2$ to 6.6×10^6 CFU mL⁻¹, and the presence of the bacteria was confirmed in 100% of the samples (48). The presented data clearly showed that contamination with bacteria of the *Enterobacteriaceae* family (except for *E. coli*), coagulase-positive staphylococci, and enterococci counts were high in most samples of the analysed raw drinking milk. The present study also tested raw drinking milk for the presence of *Salmonella* spp. and *L. monocytogenes*. These pathogens were not detected in any of the tested samples. Other studies have shown the presence of *Salmonella* spp. and *L. monocytogenes* in raw cow's milk. The percentage of samples positive for *Salmonella* spp. and *L. monocytogenes* ranged from 12.3 to 50% (7, 11, 18) and from 0.1 to 28.6% (24), respectively. Raw drinking milk intended for retail

Tab. 4. *Enterobacteriaceae*, *Escherichia coli*, coagulase-positive staphylococci and enterococci count* in the samples of raw drinking milk

Bacteria	Mean value	Minimum value	Maximum value	% of positive samples
<i>Enterobacteriaceae</i>	5.60 (4.0×10^5)	3.34 (2.2×10^3)	6.40 (2.5×10^6)	100
<i>Escherichia coli</i>	2.15 (1.4×10^2)	0	2.73 (5.4×10^2)	94.7
Coagulase-positive staphylococci	4.38 (2.4×10^4)	3.38 (2.4×10^3)	5.20 (1.6×10^5)	100
Enterococci	5.34 (2.2×10^5)	4.00 (1.0×10^4)	6.49 (3.1×10^6)	100

Explanation: * log CFU mL⁻¹ (CFU mL⁻¹)

sale should also meet other quality standards, including fat and protein contents (at least 3.5% and 2.9%, respectively) and reliable information on the product composition (38, 39). In the present study, raw drinking milk's fat and protein contents ranged from 1.7% to 2.9% and from 3.1% to 3.4%, respectively (Tab. 2). Accordingly, 100% of the samples showed lower fat than the regulatory limit. Food law obliges the producer to provide the consumer with reliable information on the composition of the product (38). According to the information on the label on the packaging, the analysed raw drinking milk should have contained approximately 4% fat. However, no production batch of raw drinking milk corresponded to the declared fat content, which is tantamount to adulteration of the product. In summary, the quality of raw drinking milk was unsatisfactory regarding regulatory limits for TBC and SCC, food safety and fat content, including its content declared on the packaging.

Antibiotic residues were not detected in any of the tested samples, indicating that both raw milk intended for processing and raw drinking milk intended for retail sale complied with the requirements in this respect (37, 40). The presence of antibiotic residues in milk remains a current problem and a threat to food safety, as confirmed by data in the literature (26, 36, 42, 44, 50).

In conclusion, the quality of raw milk intended for processing was satisfactory in terms of the criteria set out in the European Union food law and the accepted standards by processing plants, while the quality of raw drinking milk intended for retail sale was unacceptable and posed a potential health risk. The results of the current study highlight the importance of maintaining good hygiene practices on milk production holdings and effective herd health surveillance. Maintaining rigorous, frequent inspections and monitoring additional microbiological parameters resulting from the individual development of risk analysis should be introduced on farms producing raw milk for direct sale. The support of competent services for raw drinking milk producers in understanding potential risks, identifying critical points in the production chain and taking preventive measures seems essential from a food safety perspective. Considering the possible relationship between the somatic cell count (SCC) and the fat and protein content in milk, the content of which is important for milk processing, it is justified to monitor these parameters in the raw material and to continue research in this area.

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