

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS

Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2024, 372(71)3, 1–16

Received 22 Mar 2024

Revised 7 May 2024

Accepted 16 May 2024

Przemysław DUDKO¹ , Stanisław WINIARCZYK² , Jacek MIEDZOBRODZKI³ ,
Piotr WÓJCIK⁴ , Agata PRUCIAK⁵ , Maciej KURPISZ⁶ 

QUANTITATIVE ANALYSIS IN MILK CYTOLOGICAL TESTS BASED ON ARITHMETIC AND GEOMETRIC MEANS

¹ Department of Animal Reproduction, Poznań University of Life Sciences, Poznań, Poland

² Department of Epizootiology and Infectious Diseases, University of Life Sciences in Lublin, Lublin Poland

³ Department of Microbiology, Jagiellonian University, Kraków, Poland

⁴ The National Research Institute of Animal Production, Balice near Kraków, Poland

⁵ Department of Computer Science and Statistics, University of Medical Sciences in Poznań, Poznań, Poland

⁶ Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland

Abstract. The aim of this study was to evaluate mathematical tests allowing a more precise statistical analysis of the SCC values contained in a millilitre of milked milk and measured with an electronic counter. The study was conducted on a herd of 800 hf cows (100%) with an average yield of ~9,500 kg of milk, which were kept in cubicles in a free-stall system. The analyses carried out showed that a comparison of the Nelson Philpot coefficient column values with the values of the other parameters and with the course of their curves suggests that the course of the geometric mean and natural logarithm curves was the most similar – but not identical – to the fluctuations in the height of the linear index columns. It should be pointed out, however, that the values of this logarithm turned out to be ~3.7 times greater than the linear coefficient, and the first of these averages was as much as ~50 times greater than the aforementioned coefficient. Despite the ever-increasing number of electronic counters used for quantitative cytological milk tests, the use of field cellular reaction tests (TOK or CMT) will not decrease at all. It is necessary, though, to sample more precisely and to harmonise the data of the linear Philpot index and the interpretation of the former Drury–Reed index. At the same time, since the dairy industry has already accepted the natural logarithm and the geometric mean in milk assessment, threshold values for SCC should also be established for these by means of an appropriate field trial. As it was noted in the study, each of the averages had its advantages and disadvantages; it would be worthwhile to assess the harmonic and quadratic averages similarly (observation under natural conditions).

Key words: SCC, milk, cattle.

Corresponding author: Przemysław Dudko, Department of Animal Reproduction, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Wojska Polskiego 52, 60-625 Poznań, Poland, e-mail: pdudko@wp.pl.

INTRODUCTION

Progress in veterinary science has been modifying both laboratory techniques and methods for the control of mastitis in cows. All instructions and procedures in this field are modified on an ongoing basis in Europe, by the International Dairy Federation and promulgated in the form of bulletins or other documents (FIL-IDF 1980, 1981, 1991, 1999, 2021). An indicator that reflects the severity of infection and inflammation of this gland is the somatic cell count (SCC) per millilitre of milk milked. This is already a routinely used parameter for assessing the severity of the inflammatory response in the udder. Previously, subclinical mastitis (SCM) was diagnosed on its basis by indirect measurement of the SCC value, i.e. individual cows were examined by field cell reaction (TOK in Poland and CMT in the USA), in order to then calculate the Drury–Reed index (Drury and Reed 1961) for the entire herd from the data obtained. It is important to emphasise, however, that this test currently still provides a number of advantages, as it is performed directly on the cows by examining milk from all four quarters (lobes) of the udder. The low price of this test is also important. However, the widespread availability of electronic counters now makes it possible to carry out quantitative cytological tests on milk on a mass scale, but this requires time, higher costs due to the greater labour involved, and the parameter measured by them changes over time.

The benefits of electronic counters, however, lie in the more accurate measurement of the SCC values contained in a millilitre of milk tested and the fact that in dairy herds covered by the Polish Federation of Cattle Breeders and Milk Producers (PFHBiPM) yield assessment almost every month, more accurate cow data is already available. With these, the veterinarian examining the herd can see the SCC values of the milk from each cow milked throughout the year even before the barn survey. The downside, however, is that at the time of the survey, this is already historical data, which refers to an average milk sample taken during the milking of the cow from all four quarters (lobes) of the udder. The herd doctor who implements the mastitis control scheme (MCS) in the herd can analyse the data of each cow even before the barn survey, in order to update and verify them during the survey by examining all the lactating cows and taking their milk from all 4 lobes of the udder. However, such an analysis requires harmonisation of the calculation methods hitherto used in the treatment of intermediate tests for SCC with those useful for more precise statistical calculations. In an earlier study, Dudko (1986), Dudko et al. (2010), Lisowska-Łysiak et al. (2018) used statistical tests recommended by the National Mastitis Council (NMC 1987) in the analysis of milk cytological examinations as part of mastitis monitoring to compare the udder condition of cows from 2 large dairy herds and those kept on 1 small farm where a full mastitis control scheme (MCS) was not implemented. The aim of this study was to evaluate mathematical tests allowing a more precise statistical analysis of the SCC values contained in a millilitre of milked milk and measured with an electronic counter.

MATERIAL AND METHODS

The evaluated G herd from the Agricultural and Livestock Farming Enterprise with an area of 3460 ha of cropland, including 2901.93 ha of arable land. It consisted of 800 hf cows (100%) with an average yield of ~9,500 kg of milk, which were kept in cubicles in a free-stall system. Their first calving age was 740 days. In contrast, the inter-calving period was 399 days. They were milked in a side-by-side milking parlour (2 × 18) implementing quarter milking (as in robot milking). Their locomotor activity and rumination were monitored by a system – SCR

Heatime HR. Mastitis prevention included the so-called Data Flow 2 and dipping before and after milking. The Data Flow 2 system allows rapid detection of cows whose milk quality suggests an incipient inflammatory process in the udder. As part of the yield evaluation of these cows conducted by PFHBiPM zotechnicians in herd G, 11 so-called average (AT4 system) milk samples (23) were taken at ~4-week intervals during the year. The milk samples thus collected were sent to the PFHBiPM laboratory in Krotoszyn, where their SCC value was measured with an electronic Fossomatic 5,000 FC counter. The research involved 12 investigations (all year round) carried out monthly in herd G by PFHBiPM employees as part of performance assessment. In herd G, ~232 lactating cows were milked monthly. The results obtained are given in thousands cells/ml.

Mathematical analysis of the data

From the SCC values obtained by this method, arithmetic means (\bar{x}), geometric means (\bar{y}) and standard deviations (SD) were calculated, after which exponential series from 1 to 9 were compiled according to the scheme given in Table 1. In order that, according to Table 2, these data could be interpreted by the method proposed by Nelson Philpot, the obtained series linearised by summing the scores obtained in series from 1 to 9 (NMC 1987; Malinowski 2006).

Table 1. Method of compilation of exponential series and their linear evaluation

	Exponential series	Number of elements and their evaluation	
1	0–25 000 cell/ml	a, a, a..... a	a_1/n
2	25 001–50 000 cell/ml	a, a, a..... a	$2 \times a_2/n$
3	50 001–100 000 cell/ml	a, a, a..... a	$3 \times a_3/n$
4	100 001–200 000 cell/ml	a, a, a..... a	$4 \times a_4/n$
5	200 001–400 000 cell/ml	a, a, a..... a	$5 \times a_5/n$
6	400 001–800 000 cell/ml	a, a, a..... a	$6 \times a_6/n$
7	800 001–1600 000 cell/ml	a, a, a..... a	$7 \times a_7/n$
8	1600 001–3200 000 cell/ml	a, a, a..... a	$8 \times a_8/n$
9	>3200 000 cell/ml	a, a, a..... a	$9 \times a_9/n$

Linear evaluation of an exponential series is σ of the evaluation values for the interval from 1 to 8

Table 2. Method of interpreting the results of the linear exponential series evaluation according to Nelson Philpot

Acc. to National Mastitis Council (NMC)	Acc. to Malinowski (DHI)
(I) 85% of cows with DHI SCC (linear support) <3.2 (II) 15% of cows with DHI SCC > 5.2	(I) The average value of the linear assessment of pooled milk from a herd of cows should decrease from year to year until (i) the linear index reaches a value of 3.3, and (ii) for 85% of the cows it will fall below 4.0. (II) The linear index for primiparous cows should be: (i) ± 3.0 , and (ii) in >90% of cows it should be <4.0. (III) Linear value (i) = 4.5, which corresponds to 280 000 cells/ml means that (ii) the udders of 60–70% of cows are infected with infectious pathogens.

Thus, the data obtained in each month of the year and from consecutive months of lactation were summarised in three-panel (a, b and c) Tables 5, 6 and 7. Mathematical classification of mastitis cases was carried out using the Gauss test. Significant differences ($p < 0.01$, $p < 0.02$, $p < 0.05$, $p < 0.001$ and $p < 0.0001$) were assessed at different p levels. The FREQ procedure of the SAS® 9.4 statistical package (SAS 2019) was used to assess the association between the analysed factors and the distribution of SCC values. The statistical significance of this association was assessed using Fisher's exact test. Detailed comparisons of subject averages were made using the Duncan test. Multivariate analysis of variance (GLM-SAS procedure) according to the following linear model was used to assess the effect of experimental factors (month of calendar year, consecutive lactation, phase and number of current lactation) on the SCC content of cows' milk:

$$y_{ijklm} = \mu + m_i + p_j + l_k + f_l + e_{ijklm}$$

where:

y_{ijklm} – observed somatic cell count in milk of cows,

μ – population average,

m_i – fixed effect of the month of the calendar year ($i = 1, 2, \dots, 11$),

p_j – lactation sample fixed effect ($j = 1, 2, \dots, 12$),

l_k – lactation fixed effect ($k = 1, 2, \dots, 8$),

f_l – lactation phase fixed effect ($l = 1, 2, \dots, 5$),

e_{ijklm} – random error.

RESULTS AND DISCUSSION

Table 5 summarises the milk SCC values for cows of herd G measured in consecutive postpartum months and irrespective of the season. In order to facilitate the evaluation of the value of the Philpot index analysed – which, according to the formula in Table 1, was calculated by summing up the numbers of cows requiring inclusion in one of the 8 separable DHI series based on the milk SCC value – the numbers of these cows (n) and their percentages in the herd (%) are summarised in numbered rows 1–8 at the top of Table 5. However, it turned out that in the evaluation of the herd (Table 2) by this indicator, the number of cows classified from row 5 to row 8 and their fraction were important, so they were given in row 9. In addition to the data already mentioned, the lower part of Table 5 contains the values of: linear Philpot index, arithmetic means, geometric means and natural logarithms, which were calculated from the SCC values of the milk of these cows milked during the 11 postpartum tests. In addition, the values of these parameters were graphically presented as curves in Fig. 2. It turned out that, according to the NMC criteria (Table 2), the udder condition of these cows, should already be considered unacceptable and the quality of the milk milked from them should be considered as poor or even unsuitable for dairying, as the average value of the linear index was 3.27, which should not exceed 3.20. Objectively, however, it must be acknowledged that such a deviation from the norm is not too great – since out of 11 results evaluated in successive surveys, 5 were within the norm and only 6 exceeded it. In this respect, the animals classified in groups 5–8 were much worse, whose percentage should have been $< 15\%$, but was $\sim 24\%$. Notably, it exceeded 20% in each of the 11 monthly surveys. A comparison of the column values of the Nelson Philpot index with the values of the other parameters and the course of their curves suggests that the geometric mean and natural

logarithm curves appeared to be most similar – but not identical – to the fluctuations in the height of the linear index columns. It should be emphasised, however, that the values of this logarithm were ~3.7 times higher than the linear coefficient, and the first of these averages was as much as ~50 times higher than this coefficient. For udder healthiness, the period from calving of cows to the peak of lactation (~100 days) is usually the most difficult. Only the first postpartum month was the worst in the evaluated herd; however, none of the SCC values measured in the following months fell within the limits of the norms, neither the norms recommended <200,000 cells/ml by the IDF/NMC (Table 3), let alone the <100,000 cells/ml proposed by the DVG (Table 4). Similar fluctuations are also seen in the geometric mean and natural logarithm curves, but the peaks of increasing values or decreasing values are less pronounced. On the other hand, statistically significant are the differences recorded in successive months between the arithmetic means and the natural logarithm values. In one study after calving, the differences in the arithmetic mean values recorded in the 5th, 8th, 9th, 10th and 12th month were significant. However, against the natural logarithm values from one postpartum survey, the data obtained at 5, 8, 9, 10 and 12 months were statistically significant. Similar statistically significant relationships were found against the value of the natural logarithm from the postpartum examination. The values of geometric means, which by definition should give identical results to the natural logarithm, were not mathematically tested in this study. Unfortunately – as a peculiar herd average – the value of the linear Nelson Philpot index is not subjected to this analysis, since the values forming these averages, and not the averages themselves, are subjected to statistical calculations.

Table 3. Hillerton's method of interpreting SCC values

	SCC thresholds (in thousands of cells/ml of milk)			
	<100	150–250	200–400	>400
Lobes milk (quarter milk)	healthy lobe	unsettled secretion	increases % infection rate	inflammation
Udder milk or pooled milk	to be accepted		questionable usefulness	useless
	useful for dairy farming		min. 10% infected	>25% infected

Table 4. Variability in the SCC values of milk taken from cows in successive lactations (acc. to. DVG)

In normal milk from healthy cows (<100,000 cells/ml)
in milk collected in 1 lactation <35.000 cells/ml
in milk collected in 2 lactation <50.000 cells/ml
in milk collected in 3 lactation <60.000 cells/ml
in milk collected in 4 lactation <85.000 cells/ml
in subclinical mastitis conditions (SCM) from 400.000 to <1 mln cells/ml
in clinical conditions (CM) from 400,000 to several million cells/ml

The evaluation of the data in Table 6 (month of the calendar year) proved even weaker, as the health criteria (Philpot index <3.20 – Table 2) were only met by the udders of these cows in: May, June, September and October, and yet the percentage of animals classified in ranks 5–8 throughout the year was clearly higher than 15%. The differences between

arithmetic means and natural logarithm values were also statistically significant. Compared to the January survey, differences in arithmetic mean values were significant against those shown in: May, June, September and October. The course of the curves (Fig. 3) is essentially similar to the previous Fig. 2, while the values of \ln SCC are ~ 3.7 times higher than the linear coefficient and the geometric mean even ~ 50 times higher than its value. Similarly, as in the previous figure, the fluctuations in the curve of the arithmetic means were significantly greater than those of the other parameters. Moreover, analysis of their course showed again that upward and downward trends apply to all analysed parameters, but that the peaks in the curves illustrating the arithmetic means are clearly higher, and their increases and decreases more dynamic.

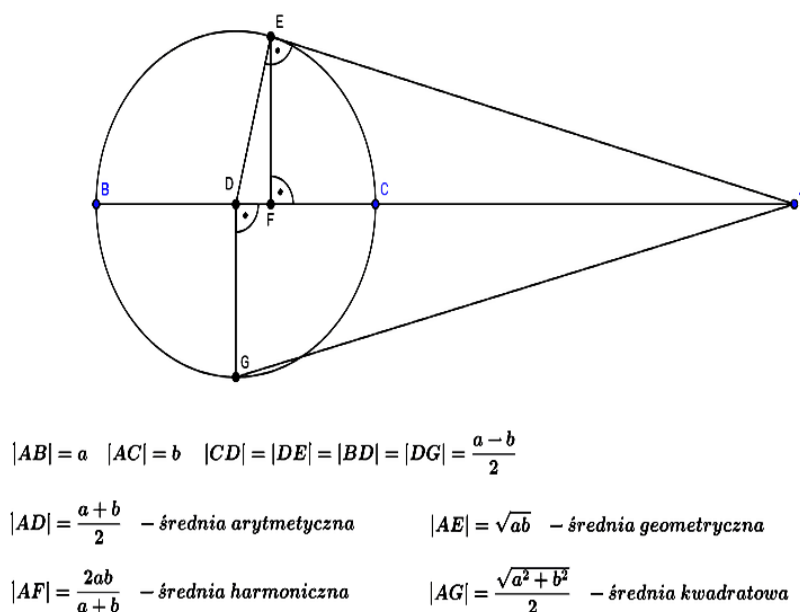


Fig. 1. The relationship between the values of the different averages (acc. to Jerzy Mil [22])

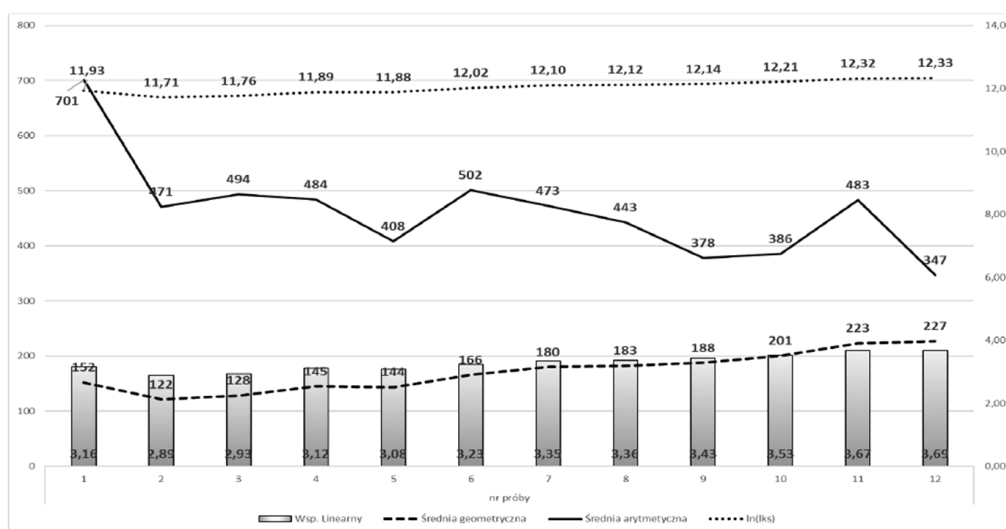


Fig. 2. The curves of linear coefficient values, arithmetic means, geometric means and natural logarithm of SCC in milk of cows from herd G in the following postpartum months

Table 5. Fluctuation of SCC values in milk from herd G cows in successive monthly postpartum surveys but regardless of season – comparison of linear coefficient values, arithmetic means, geometric means and their natural logarithm

Exponential series cells/ml	Entirety		Classifying factor – sample month																								
			v		2		3		4		5		6		7		8		9		10		11		12		
			n	%	n	%	n	%	n	%	n	%	N	%	N	%	n	%	n	%	n	%	n	%	n	%	
1	0–50 000	635	18.79	73	25.17	128	35.56	108	29.92	79	23.80	68	21.45	53	17.21	37	12.98	35	12.82	25	10.64	13	6.77	9	6.98	3	3.23
2	50 001–100 000	740	21.90	75	25.86	71	19.72	87	24.10	82	24.70	74	23.34	72	23.38	67	23.51	60	21.98	42	17.87	35	18.23	23	17.83	17	18.28
3	100 001–200 000	711	21.04	53	18.28	46	12.78	52	14.40	65	19.58	70	22.08	79	25.65	64	22.46	64	23.44	70	29.79	57	29.69	33	25.58	21	22.58
4	200 001–400 000	503	14.89	24	8.28	37	10.28	41	11.36	26	7.83	39	12.30	35	11.36	54	18.95	48	17.58	44	18.72	43	22.40	28	21.71	28	30.11
5	400 001–800 000	328	9.71	11	3.79	28	7.78	25	6.93	27	8.13	31	9.78	27	8.77	28	9.82	38	13.92	24	10.21	23	11.98	17	13.18	16	17.20
6	800 001–1 600 000	210	6.21	18	6.21	20	5.56	20	5.54	22	6.63	15	4.73	18	5.84	16	5.61	14	5.13	20	8.51	13	6.77	12	9.30	6	6.45
7	1 600 001–3 200 000	137	4.05	13	4.48	16	4.44	12	3.32	22	6.63	12	3.79	14	4.55	12	4.21	7	2.56	8	3.40	5	2.60	5	3.88	1	1.08
8	>3 200 000	115	3.40	23	7.93	14	3.89	16	4.43	9	2.71	8	2.52	10	3.25	7	2.46	7	2.56	2	0.85	3	1.56	2	1.55	1	1.08
Sum of 5 to 8 rows		790	23.38	65	22.41	78	21.67	73	20.22	80	24.10	66	20.82	69	22.40	63	22.11	66	24.18	54	22.98	44	22.92	36	27.91	24	25.81
Accurate Fisher test												P = 0.0001															
Linear coefficient		3.27		3.16		2.89		2.93		3.12		3.08		3.23		3.35		3.36		3.43		3.53		3.67		3.69	
Geometric mean		166		152		122		128		145		144		166		180		183		188		201		223		227	
Arithmetic mean (p = 0.0095)		493		702 ^A		471 ^{AB}		494 ^{AB}		484 ^{AB}		408 ^B		502 ^{AB}		473 ^{AB}		443 ^B		378 ^B		386 ^B		484 ^{AB}		347 ^B	
ln(lks) (p = 0.0001)		12.02		11.93 ^{ABC}		11.71 ^A		11.76 ^A		11.89 ^{ABC}		11.88 ^{AB}		12.02 ^{BCD}		12.10 ^{BCDE}		12.12 ^{BCDE}		12.14 ^{CDE}		12.21 ^{DE}		12.32 ^E		12.33 ^E	
SD		1124		1612		1100		1233		1036		958		1193		1093		992		626		647		1028		440	
Number of observations (n)		3379		290		360		361		332		317		308		285		273		235		192		129		93	

Average values marked with the same letters are not statistically significantly different (P ≤ 0.05).

Table 6. Fluctuation of SCC values in milk of cows of herd G in successive months of the year – comparison of linear coefficient values, arithmetic means, geometric means and their natural logarithm in months of the calendar year

Exponential series	Entirety		Classifying factor – sample month																							
			1		2		3		4		5		6		8		9		10		11		12			
From-to	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Sum of 5 to 8 rows	790	23.38	87	27.71	91	26.07	89	24.32	138	23.39	43	19.91	38	18.10	56	22.67	56	21.29	48	18.97	67	24.54	77	25.84		
Accurate Fisher test	P = 0.0001																									
Linear coefficient	3.27		3.50		3.45		3.45		3.32		2.91		3.03		3.28		2.98		2.89		3.38		3.37			
Geometric mean	166		200		184		189		179		129		140		164		129		117		178		184			
Arithmetic mean (p = 0.0095)	492		657 ^A		571 ^A		532 ^{AB}		494 ^{AB}		359 ^B		458 ^{AB}		611 ^A		394 ^B		324 ^B		485 ^{AB}		459 ^{AB}			
ln(lks) (p = 0.0001)	12.02		12.20 ^A		12.12 ^A		12.15 ^A		12.09 ^A		11.77 ^{BC}		11.85 ^{BC}		12.01 ^{AB}		11.77 ^C		11.67 ^C		12.09 ^A		12.12 ^A			
SD	1134		1460		1254		1151		1123		1025		1197		1496		949		713		910		840			
Number of observations (n)	3379		314		349		366		590		216		210		247		263		253		273		298			

Average values denoted by the same letters are not statistically significantly different ($P \leq 0.05$), a/g~3, g/ln~13.6, a/g3, g/ln~13.6

Table 7. Variation of SCC values in milk from herd G cows in 5 main phases of 305-day lactation – comparison of linear coefficient values, arithmetic means, geometric means and natural logarithm over the course of 4 lactation phases

Exponential series	Entirety		Classifying factor – lactation									
			(1) <30 days		(2) 31–60 days		(3) 61–100 days		(4) 101–200 days		(5) >200 days	
From–to	n	%	n	%	n	%	n	%	n	%	n	%
Sum of 5 to 8 rows	790	23.38	63	22.26	77	22.19	92	20.44	226	22.14	332	25.98
Accurate Fisher test	P = 0.0001											
Linear coefficient	3.27		3.15		2.92		2.96		3.16		3.58	
Geometric mean	166		152		124		129		155		210	
Arithmetic mean (p = 0.0095)	493		704A		487B		507B		455B		473B	
ln(lks) (p = 0.0001)	12.02		11.93AB		11.73C		11.77BC		11.95A		12.25D	
SD	1124.28		1621		1127		1259		1047		987	
Number of observations (n)	3379		283		347		450		1021		1278	

Average values denoted by the same letters are not statistically significantly different ($P \leq 0.05$), a/g~3, g/ln~13.6, a/g3, g/ln~13.6.

Table 8. Variation in SCC values in milk from herd G cows over successive lactations – comparison of linear coefficient values, arithmetic means, geometric means and their natural logarithm in successive lactations

Exponential series	Entirety		Classifying factor – lactation count															
			1		2		3		4		5		6		7		8	
From–to	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Sum of 5 to 8 rows	790	23.38	134	10.24	259	24.43	210	33.49	120	50.63	37	44.05	13	50.00	9	36.0	8	66.67
Accurate Fisher test	P = 0.0001																	
Linear coefficient	3.27		2.57		3.38		3.85		4.58		3.88		4.08		4.32		4.92	
Geometric mean	166		100		180		252		416		252		314		349		535	
Arithmetic mean (p = 0.0095)	493		260A		461AB		744ABC		1054CD		672ABC		931BCD		1279D		714ABC	
ln(lks) (p = 0.0001)	12.02		11.51A		12.10B		12.44BC		12.94CD		12.44BC		12.66BCD		12.76CD		13,19D	
SD	1124		754		961		1456		1692		1213		1732		2389		548	
Number of observations (n)	3379		1308		1060		627		237		84		26		25		12	

Average values denoted by the same letters are not statistically significantly different ($P \leq 0.05$), a/g~3, g/ln~13.6.

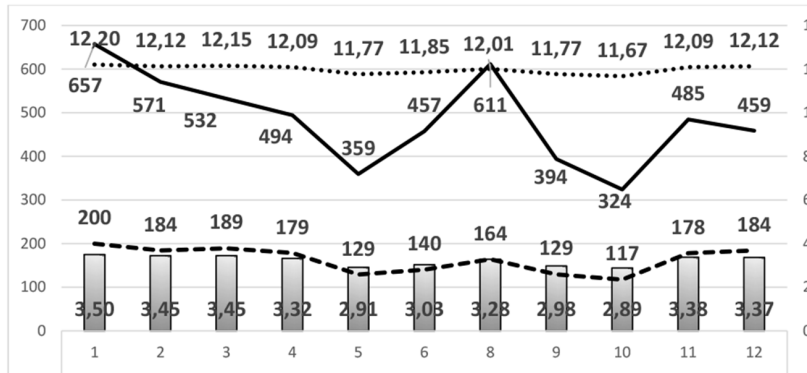


Fig. 3. The curves of the linear, arithmetic, geometric mean and natural logarithm SCC values in the milk of cows of herd G in the following months of the calendar year

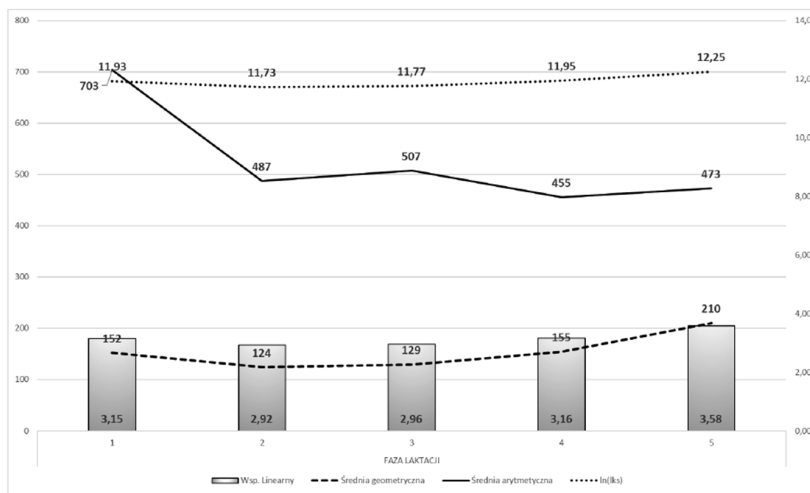


Fig. 4. Curves of linear coefficient values, arithmetic means, geometric means and natural logarithm of SCC in milk of cows from herd G during 5 main phases of their lactation

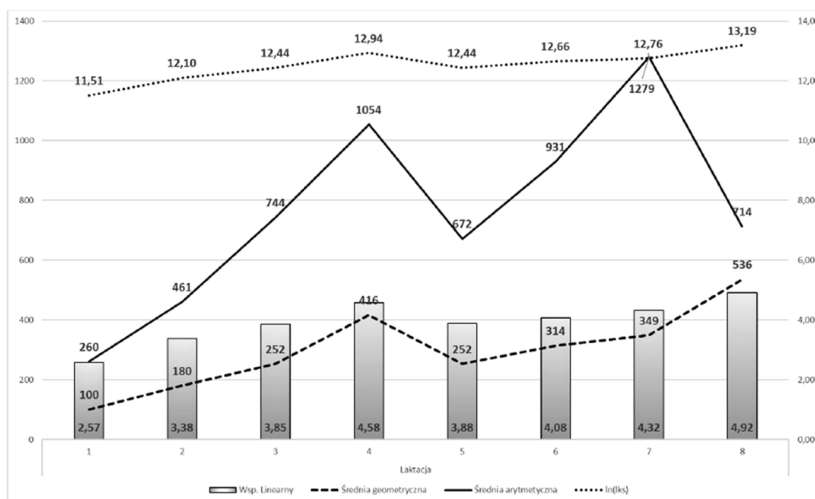


Fig. 5. The course of the curves of the linear coefficient values, arithmetic means, geometric means and natural logarithm of SCC in the milk of cows of herd G during successive months of lactation

Despite the same evaluation of the total as in previous studies, the situation is slightly better for the five main phases of lactation (Table 7), because in the four initial phases of lactation, the linear index (<3.20) is normal, which is not confirmed by the percentage of cows classified in rows 5–8, which exceeds 20%. On the other hand, the higher-than-normal value of the Nelson Philpot index in the last phase before drying off is not necessarily related to mastitis. On the other hand, the course of the curves (Fig. 4) confirms the worst udder condition in the first 30 days after parturition, but according to the threshold values (Table 3), the milk from these cows in the four remaining phases of lactation had to be considered unsuitable for dairying, as theoretically each of these cows had one quarter (lobe) of the udder infected.

A little different, is the analysis of the data of Table 8, which analysed the SCC value of the milk milked from cows in 8 consecutive lactations (from Table 7). Admittedly, the linear coefficient for the total was – 3.27, which was the same as in the previous tables, but the sum of cows classified in ranks 5–8 was also above 15%. The difference was that only in primiparous cows (I lactation) the value of the Nelson Philpot index was 2.57, thus proving to be lower than 3.20 and accompanied by a lower percentage of cows classified in these ranks than 15%. However, in successive lactations, the value of the analysed parameter strongly increased, with two unexpected decreases in the fifth and eighth lactations. The differences between the arithmetic means and the natural logarithm values were also statistically significant in this case. Compared to lactation 1, the values of the natural logarithm of SCC recorded in lactations 4, 6 and 7 were significant. And when comparing to the natural logarithm values of SCC recorded in lactation I, they proved statistically significant in the other seven lactations.

There is already a wealth of literature on the use of mathematical statistics to analyse cytological milk test data, both in the FIL-IDF and NMC recommendations (FIL-IDF 1980, 1981, 1991, 1999, 2021; NMC 1987) and in the work carried out in Poland by Kurek (1986, 2005), Kurek and Roczniak (1987), Kurek and Szwabe (1987) or other authors (Dudko 1994; Lisowska-Łysiak et al. 2018; Dudko and Zawadzki 2015; Dudko et al. 2015a, 2015b). In the meantime, the values of SCC in milk (quantitative cytology) measured with electronic counters by the PFHBiPM as part of the evaluation of the dairy management of cows are widely available for dairy producers (PFHBiPM 2023). Zadoks (2002) was the first to report that the proportion of infectious forms of mastitis had been decreasing over the last 20 years, accompanied by an expansion of infections with environmental and opportunistic pathogens. She also implemented 2 mathematical models, of which Reed–Frost describes a situation where mastitis spreads in the herd through cow contact and the vector of infection is most often the mechanical milking machine. The probability of new infections (IMI) in the herd depends, in this model (infectious course of mastitis), on the number of animals already infected in the herd. In contrast, the situation typical of other germs is described by the Greenwood model, in which the probability of infection depends on the equilibrium between the cows' susceptibility and the pressure of their environment to become infected. Juxtaposing these mathematical models with the molecular diagnosis of mastitis, the author (Zadoks 2002) concluded that even once the germplasm species has been established, it is sometimes difficult to decide whether it is infectious or not, and so it must be determined for the specific strain in each case. Philpot and Nickerson in the NMC manual (NMC 1987) considered forms of mastitis caused by bacteria such as *Streptococcus agalactiae* and *Str. dysgalactiae*, coagulase-positive staphylococci (including *S. aureus*), *Mycoplasma bovis* and *Truepella pyogenes* to be infectious. While they considered infections by *Streptococcus*

uberis, aerobic Gram-negative bacilli, other streptococci and enterococci, e.g. *Enterococcus faecalis* and *Ent. faecium*, and other microorganisms (e.g. *Mycobacterium* sp., *Nocardia* sp., yeast- and mould-like fungi and even some algae) as environmental. Recognising the greater experience of MNC experts in the fight against environmental and opportunistic pathogens and the better equipment of their laboratories in Poland, the manual and recommendations of the NMC (1987), in which the Nelson Philpot linear index is the basis for the evaluation of dairy herds, were relied upon at this time.

Such an unambiguous orientation to the NMC has led to the overlooked change in our country in the threshold values of SCC per millilitre of milk tested, and this applies both to secretions taken directly from the diseased lobe, as well as to average samples from the udder of the cow tested or the pooled milk of all cows in the herd tested. In Table 3, they are given after FIL-IDF (FIL-IDF 1999; Schallibaum 1993), whose expert Hillerton believes that the advantage of this indicator (SCC value in ml of milk) is that it can be used not only for mastitis diagnosis, but also in the evaluation of delivered milk. The current document No. 132 (FIL-IDF 1981) in our country on this subject already needs to be modified according to the findings of bulletin No. 345 (FIL-IDF 1999), where the experts of the A2 Group and the National Mastitis Council (NMC from the USA) jointly changed the limit thresholds for SCC values, which are already accepted by the Committee and written into the Codex Alimentarius (2010). In contrast, Eberhart et al. (1982) described a linear relationship between the SCC value of pooled milk and the percentage in a given herd of cows with infected udders. According to them, at 200 000 cell/ml of milk, udder infections should be reckoned with in 6.2% of cows, while 400 000 cell/ml already suggests 12.8% of such infections; at 750 000 cell/ml, this percentage increases to 24.3%, and a value of 1 000 000 cell/ml is equivalent to ~32.6% of infected mammary glands in that herd.

The Codex Alimentarius (2010), which is the Food Code (literal translation from Latin), contains general and specific standards (norms) to ensure food safety and, above all, to protect the health of consumers and ensure fair practices for the food industry. Food marketed locally or shipped for export must be safe and of adequate, quality. In 1960, two UN agencies – the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) – created this system to implement and disseminate definitions and to facilitate the harmonisation of food requirements in international trade. There are 166 countries participating in the Code, but the standards contained therein are voluntary. It may be this is why Poland has not ratified the Code. However, it is of great importance and is considered to be in line with the current state of the art. Moreover, an important advantage of these standards is their worldwide coverage and the fact that the Codex Alimentarius Commission (2010) keeps them up to date. This is particularly important for our country, where every change of ruling party is accompanied by an amendment of the legislation. And the success of the Swiss programmes described in a previous paper (Dudko 1994) was due to the fact that the way in which milk quality is assessed, in this country, is regulated by federal decrees, which have been implemented for more than 40 years by the 15 cantonal or regional dairy control and advisory centres (MKBDs), and each of these MKBDs works with the Healthy Exchange Service (UHS), which helps farmers manage their dairy herds.

Since the beginning of the FAO/WHO Food Code Commission, there has been a FAO/WHO Codex Contact Point in Poland, organised in 1963 at the Polish Committee for Standardisation. By (Decree 1964) Order No. 91 of the Minister of Foreign Trade of 28 October 1964 (Official Gazette of MHZ No. 26, item 171), the running of the Polish Secretariat (contact point) and cooperation with the FAO/WHO Food Code Commission was entrusted to the

Central Inspectorate for Standardization (CIS), operating at the Ministry of Foreign Trade (1964). Since 1 January 2003, by virtue of the Act of 21 December 2000 on commercial quality of agri-food articles and the statute of the Main Inspectorate of Commercial Quality of Agricultural and Food Articles conferred by Order No. 15 of the Minister of Agriculture and Rural Development of 21 July 2005 (Official Journal of the Ministry of Agriculture No. 8, item 9) (Decree 2005), the operation of the FAO/WHO Codex Contact Point was entrusted to the Main Inspector of Commercial Quality of Agricultural and Food Articles. However, the problem is that no changes to this Code have been noticed in our country after the elaboration of document 345 (FIL-IDF 1999) jointly by IDF and NMC experts. This is especially the case since a comparison of the SCC threshold values according to the IDF (Table 3) and according to the DVG (Table 4) shows that the quality criteria for milk assessment (SCC threshold values) are increasing over time both in Europe and worldwide.

An undeniable advantage of the Nelson Philpot index used in the study by Lisowska-Łysiak et al. (2018) was the ability to compare udder healthiness in cows from 2 large dairy herds (C and G) and in cows from a small farm (W). It turned out that the highest SCC values were initially found in the milk of cows from herd C, slightly lower from herd G and the lowest from herd W. However, with the passage of time (months), the udder condition deteriorated in all 3 herds, so that while only ~26% of cows from the best herd W had clinical or subclinical mastitis at the start of the study, by the end of the observation >50% of the animals had already developed it. The effect of season on the SCC value and the quality of milk milked from these cows was also analysed in all three herds. Furthermore, it was noted that the SCC value increased in the milk of these cows in successive lactations. However, the law of large numbers means that comparisons of statistical tests in the herd with the highest number of cows should be more reliable, which was realised in the study. This report also adds to the fact that, according to IDF-NMC criteria (FIL-IDF 1999), only milk from primiparous cows proves useful for dairying, while milk from older cows in principle no longer meets the standards in force both in Europe and worldwide (FIL-IDF 1999; DVG 2002). In the analytical systems used in our study (Table 5–8), the milk obtained from cows just after calving proved to be of the worst quality. This shows, however, the possible management of milk quality in herds. For, primiparous cows are basically just learning to produce milk, but through insemination or embryo transfer they guarantee a much higher milk yield than older cows. However, it should be remembered that there is more water in their milk. Consequently, the correct composition will only be ensured by the addition of milk from older cows, but this of course increases the SCC value in it. In this situation, only the right proportions of milk obtained from primiparous, and multiparous cows will determine its suitability for dairying.

The curves of the geometric mean and the natural logarithm, where the peaks of increase or decrease were much smaller than those of the arithmetic mean, appeared to be the most similar – but not identical – to the fluctuations in the height of the linear indicator bars. This is consistent with Fig. 1, which graphically compares the four averages, including arithmetic and geometric. It appears that the extremes have a much smaller effect on the geometric mean than on the arithmetic mean. For this reason, it is more useful for assessing, for example, the quality of milk in a dairy area, a district or a country. For the veterinary surgeon evaluating a herd, the arithmetic mean is better, because it highlights the problem in the herd. Even if, when managing milk quality, it is found that the problem is in a herd of three cows, out of 100, which are the easiest to exclude from milk yield. However, this problem can recur on a regular basis and an appropriate solution must then be found (Mil 2024). On

the other hand, the problem of differences between average values is that the arithmetic mean value is ~3.7 times greater than the geometric mean, while the difference between the threshold value of SCC (<100,000 cell/ml) for good milk and completely unsuitable milk (>400,000 cell/ml) is also ~4 (FIL-IDF 1999). The aforementioned thresholds were therefore adapted – to arithmetic mean values, and, as the work presented here shows, the differences between these averages concern not only their values, but also the significance in subsequent studies of the differences between these values.

CONCLUSION

To summarise, it is worth noting that, despite the increasing number of electronic counters for quantitative milk cytology in our country, the use of field cell reaction tests (TOK or CMT) will not diminish at all, but in the new situation, care should be taken both (a) to make the results obtained by this means more precise, and (b) to harmonise the data of the linear Philpot index and the interpretation of the former Drury–Reed index. Although the values of the linear index are not subjected to statistical tests, the curves of the natural logarithm and the geometric mean were similar to this parameter in all the figures in this work. Therefore, a more serious problem is posed by the large spread between the values of these three parameters (3.7× and 50.7×) and the fact that the already established threshold values for SCC are adapted to arithmetic mean values. However, the dairy industry has already accepted the natural logarithm and the geometric mean in milk assessment, so threshold values for SCC should also be established for these by means of a suitable experiment under natural conditions (field trial). According to IDF and NMC experts, experimentation will be necessary to establish them, but simulation calculations will certainly be helpful in developing an appropriate methodology. In the analysis presented in this paper, it was noted that each of the averages had its advantages and disadvantages, so similarly (observation under natural conditions) it would be worth evaluating the harmonic and quadratic averages.

REFERENCES

- Codex Alimentarius Commission.** 2010. Procedural manual. Nineteenth edition. Rome: WHO/FAO.
- Decree No 15** Of The Minister Of Agriculture And Rural Development Of 21 July 2005 On Granting The Status Of The Central Inspectorate For Agricultural And Food Quality.
- Decree No 91** of the Ministry of Foreign Trade of 28 October 1964 on entrusting the Central Inspectorate for Standardisation (CIS), under the Ministry of Foreign Trade, with the running of the Polish Secretariat and cooperation with the FAO/WHO Food Code Commission.
- Drury A.R., Reed S.W.** 1961. A herd irritation index using the California Mastitis test. *Vet. Med. Small Anim. Clin.* 50(147), 16–20.
- Dudko P.** 1986. Badania nad czynnikami etiologicznymi, leczeniem i zwalczaniem zapaleń gruczołu mlekowego u krów [Research on the aetiological factors, treatment and control of mastitis in cows]. Lublin: Akademia Rolnicza [in Polish].
- Dudko P.** 1994. Niekonwencjonalne metody eliminacji stanów podklinicznych mastitis u bydła/ I. Biostymulacja krów szczepem *Corynebacterium uberis* 22 [Unconventional methods to eliminate subclinical mastitis conditions in cattle. I. Biostimulation of cows with *Corynebacterium uberis* strain 22]. *Med. Wet.* 4, 170–174 [in Polish].

- Dudko P., Jędrus A., Grzelak M., Stanisławski D., Zawadzki W.** 2015a. Próba oceny nierównomierności rozkładu mleka w poszczególnych ćwiartkach wymion krów [A trial above the irregularity of milk arrangement assessment among a cows' quarters]. *Acta Sci. Pol. Med. Vet.* 14(1-4), 19–34.
- Dudko P., Jędrus A., Grzelak M., Stanisławski D., Zawadzki W.** 2015b. Wpływ zmiany systemu doju na wartość liczby komórek somatycznych (LKS) w mleku krów [An exchange of milking systems from an alternating to a simultaneous in small animal farms and the value of somatic cells count (SCC) in milk collected from quarters of their udders]. *Acta Sci. Pol. Med. Vet.* 14(1-4), 35–52.
- Dudko P., Kostro K., Kurpisz M.** 2010. Adaptation of Microstix®-Candida Slide-test for diagnosis of bovine mastitis due to anascogenic yeasts. *Acta Vet. Brno* 79(1), 113–120. DOI: 10.2754/avb201079010113.
- Dudko P., Zawadzki W.** 2015. Badania nad efektywnością preparatu Biomast w zwalczaniu mastitis u krów [The study in effectiveness the immune-modulation of res in bovine udders by Biomast]. *Acta Sci. Pol. Med. Vet.* 14(1-4), 5–18 [in Polish].
- DVG.** 2002. German Veterinary Medical Society. Relevant aspects of combating bovine mastitis as a herd problem. Giessen: GVA Publication.
- Eberhart R.J., Hutchinson L.J., Spencer S.B.** 1982. Relationships of bulk tank somatic cell counts to prevalence of intramammary infection and to indices of herd production. *J. Food Prod.* 45, 11–25.
- FIL-IDF.** 1980. Facteurs affectant la qualite bacteriologique du lait cru. Document No. 120. Brussels: International Dairy Federation.
- FIL-IDF.** 1981. Laboratory methods for use in mastitis work. Document No. 132. Brussels: International Dairy Federation.
- FIL-IDF.** 1991. Payment systems for ex-farm milk. Document No. 262.
- FIL-IDF.** 1999. Quality and safety of raw milk and its impact on milk and milk products. Bulletin No. 345.
- FIL-IDF.** 2021. Milk – Quantitative determination of microbiological quality – Guidance for establishing and verifying a conversion relationship between results of an alternative method and anchor method for results.
- Kurek C.** 1986. Biologiczna metoda zwalczania podklinicznych gronkowcowych stanów zapalnych wymienia krów w okresie laktacyjnym i w zasuszeniu [Biological methods of the control of subclinic staphylococcal mastitis in lactating cows and in a dry period]. *Med. Wet.* 42(6), 338–341 [in Polish].
- Kurek C.** 2005. Bio-chemotherapy of subclinical *Staphylococcal mastitis* in lactating cows. The monograph contains the lectures, and the papers are presented during VI Middle-European Buiatrics Congress in Cracow, 467–471.
- Kurek C., Rocznik W.** 1987. Biologiczna metoda zwalczania mastitis u krów. I. Odczyn komórkowe i indeks według Drury–Reed'a [A biological method for mastitis control in cows. I. Cellular field reaction results and Drury–Reed index]. *Med. Wet.* 12, 740–743 [in Polish].
- Kurek C., Szwab E.** 1987. Biologiczna metoda zwalczania mastitis u krów. II. Dynamika zmian częstości występowania ujemnych wyników Terenowego Odczynu Komórkowego a indeks według Drury–Reed'a [A biological method for mastitis control in cows. II. Dynamics of change in the prevalence of negative cellular field reaction results versus the Drury–Reed index]. *Med. Wet.* 12, 740–743 [in Polish].
- Lisowska Łysiak K., Dudko P., Kosecka-Strojek M., Walczak J., Wójcik P., Międzobrodzki J.** 2018. Characteristics of advanced methods used for typing bacterial isolates from

mastitis with particular reference to Staphylococci. Pol. J. Vet. Sci. 21(1), 229–239. DOI: 10.24425/119041.

Malinowski E. 2006. Zwyciężyć w walce z mastitis [Winning the battle against mastitis]. Bydgoszcz: Westfalia Surge.

Mil J. GeoGebra 2024, <https://www.geogebra.org/m/y2YSgsRE>.

NMC 1987. National Mastitis Council. Laboratory and Fidel handbook on bovine mastitis. Arlington: NMC.

PFHBiPM. 2023, Ocena i hodowla bydła. Dane za rok 2023.

SAS. 2019. User's guide. Statistics version 9.4 edition. Cary: SAS Institute.

Schallibaum M. 1993. Proceedings of the IDF Seminar held in Cork (Ireland), in April 1993.

Zadoks R. 2002. Molecular and mathematical epidemiology of Staphylococcus aureus and Streptococcus uberis mastitis in dairy herds. Utrecht: Utrecht University.

ANALIZA ILOŚCIOWA W BADANIACH CYTOLOGICZNYCH MLEKA OPARTA NA ŚREDNICH ARYTMETYCZNYCH I GEOMETRYCZNYCH

Streszczenie. Celem pracy była ocena matematycznych testów pozwalających na precyzyjniejszą analizę statystyczną wartości SCC zawartych w mililitrze dojonego mleka, a zmierzonych licznikiem elektronicznym. Porównanie wartości kolumn współczynnika Nelsona Philpota z wartościami pozostałych parametrów oraz z przebiegiem ich krzywych sugeruje, że najbardziej zbliżony – ale nie identyczny – do wahań wysokości słupków linearnego wskaźnika jest przebieg krzywych średniej geometrycznej i naturalnego logarytmu. Podkreślenia wymaga jednak, że wartości tego logarytmu okazały się ~3,7-krotnie większe od współczynnika linearnego, a pierwszej z tych średnich były aż ~50-krotnie większe od wspomnianego współczynnika. Mimo coraz większej liczby stosowanych elektronicznych liczników przeznaczonych do ilościowych badań cytologicznych mleka zastosowanie badań terenowym odczytem komórkowym (TOK lub CMT) wcale nie zmniejszy się. Koniecznie jest jednak precyzyjniejsze pobieranie prób oraz zharmonizowanie danych linearnego współczynnika Philpota i sposobu interpretacji dawnego indeksu Drury'ego–Reeda. Ponieważ przemysł mleczarski zaakceptował już logarytm naturalny oraz średnią geometryczną w ocenie mleka, należałoby ustalić też i dla nich progowe wartości SCC przez odpowiedni eksperyment w naturalnych warunkach (*field trial*). W prezentowanej pracy zauważono, że każda ze średnich miała swe zalety i wady, dlatego podobnie (obserwacja w warunkach naturalnych) warto by ocenić średnie harmoniczne i kwadratowe.

Słowa kluczowe: komórki somatyczne, mleko, bydło.