

Correlation between total bacterial and somatic cell counts in bulk tank ewes' milk

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Summary

Total counts of microorganisms (*TCM*) and somatic cell counts (*SCC*) were determined in raw ewes' milk samples ($n = 1256$). Statistical parameters, namely, median, arithmetic mean, geometric mean, mean of logarithmic values to base 10, standard deviation, Spearman correlation coefficient (*SP*) and Pearson correlation coefficient (*PE*) were calculated. Geometric mean and median for *TCM* were 205×10^3 CFU·ml⁻¹ and 154×10^3 CFU·ml⁻¹, for *SCC* were 967×10^3 ml⁻¹ and 987×10^3 ml⁻¹, respectively. Tightness of relationship between *TCM* and *SCC* was similar according to correlation transformed values (mean of logarithmic values to base 10 and *PE*) and to *SP*, and varied through lactation. This variation range was from 0.06 to 0.26 ($p < 0.001$) for *PE* and from 0.05 to 0.33 ($p < 0.001$) for *SP*. The explanation of variability in *SCC* values by variations in *TCM* values could be up to 20.3 % as maximum on the *PE* basis. Development curves of relations between *TCM* and *SCC* in bulk ewes' milk along months of lactation (*PE*: $y = 0.0031x^4 - 0.1037x^3 + 1.2181x^2 - 6.0068x + 10.640$; *SP*: $y = 0.0054x^4 - 0.1623x^3 + 1.7685x^2 - 8.2647x + 14.065$) may be associated with the dynamics of lactation and secretion disorders.

Keywords

ewes' milk; bulk; somatic cell; bacterial count; correlation coefficient

The microbiological-hygiene parameters of total counts of microorganisms (*TCM*) and somatic cell counts (*SCC*) represent the main qualitative parameters of raw milk. *TCM* is generally considered to be an indicator of milk contamination and *SCC* reflects the animal health condition. Their values depend on the farm environment hygiene, which means hygiene of feeding, housing and milking, and on animal health [1]. Therefore, they are influenced by many practical factors and are subject of standard legislative limitation for a good quality of milk food chain and for the safety of milk products and their consumers [2, 3]. *TCM* is a basic and mandatory parameter for evaluation of raw ewes' milk quality [4]. According to regula-

tion, raw milk shall not exceed the limit of *TCM* for small ruminants, 1500000 per millilitre of ewes' or goats' milk, and shall not exceed the limit 500000 in 1 ml if the milk is intended for direct consumption or processing without heat treatment. The next basic parameter *SCC* is defined in Regulation (EC) No 853/2004 only for raw cows' milk [4].

The microbiological-hygienic and health qualitative milk indicators (*TCM* and *SCC*) generally lack the normal frequency data distribution (*NFDD*), as it is known from the statistical point of view, and this data frequency distribution is usually evaluated as lognormal [5–13]. This is valid often in bulk milk [10–13] but more often in indi-

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vidual milk samples in dairy cows [5–9]. In small ruminants, it is valid regularly for both bulk and individual milk samples for *TCM* but especially *SCC* with generally higher mean values and higher variability. This fact is reflected by *TCM* limits ($< 1\,500\,000$ per millilitre, Regulation EC [4]) and by a proposal for *SCC* limits for payment for raw small ruminants' milk, e.g. elite class in Norway $\leq 1\,500\,000$ per millilitre [3, 4]. Therefore, the arithmetic means are not suitable representative (relevant) middle indicators of data files, and the parametric statistical testing methods are not suitable methods for evaluating these files. For this reason, appropriate statistical assessment methods must be used for evaluation of the relationship. In this case, it is mainly the regression evaluation of the correlation relationship of logarithmic values *TCM* and *SCC* (Pearson correlation coefficient (*PE*) between transformed values of *TCM* and *SCC*) and also the expression of the same relationship using Spearman rank correlation coefficient (*SP*, Pearson correlation coefficient between the ranked variables). The expression of geometric means and medians is also important, in addition to arithmetic means, since these, including the standard deviations, are less suitable representatives of the relevant data files, as already mentioned.

At evaluating the results of bulk milk samples, it may be beneficial to compare and interpret seasonal development dynamics of *PE* and *SP* relationships with *TCM* and *SCC* over calendar months, to the overall assessment of the season or lactation (season phase means the lactation stage in the physiological context).

The aim of the evaluation was to examine the possible causal relationship between milk hygienic

indicators (*TCM* and *SCC*), its variability and its interpretation for practical prediction of some hygienic and safety risks in the milk sheep rearing. These interpretations and predictions could contribute to the support of rearing technology efficiency, and they could also contribute to the improvement of the quality of produced milk.

MATERIALS AND METHODS

Samples were analysed in the testing laboratory Examinála (Dairy Research Institute, Žilina, Slovakia), which is responsible for milk quality measurement in Slovakia. Samples were taken from 90 bulk milk samples of breeds Improved Valachian sheep, Tsigai, Lacaune, East Friesian sheep and Slovak milk sheep. The monthly file results from 2015 were included in the evaluation of *TCM* and *SCC*. Total number of samples was $n = 1\,256$ (Tab. 1–3).

TCM was determined by a method based on laser flow cytometry, using BactoScan FC (Foss, Hillerød, Denmark). *SCC* was determined by fluoro-opto-electronic method by Fossomatic 5000 (Foss) [14, 15].

The final results on *TCM* and *SCC* were logarithmically transformed, due to the absence of normal frequency data distribution (*NFDD*), and in order to express the results as geometric means [5–13]. The basic statistical parameters, namely, median, arithmetic mean, geometric mean, mean of logarithmic values to base 10, standard deviation (*SD*), Spearman rank correlation coefficient (*SP*) and Pearson correlation coefficient (*PE*) were used to evaluate sample files. Interrelationships between *TCM* and *SCC* were assessed using

Tab. 1. Basic statistical parameters of total count of microorganisms.

	April	May	June	July	August	September	Total
Number of samples (<i>n</i>)	236	208	230	218	189	175	1 256
Arithmetic mean [CFU·ml ⁻¹]	398×10^3	798×10^3	487×10^3	589×10^3	759×10^3	707×10^3	611×10^3
Standard deviation [CFU·ml ⁻¹]	$1\,104 \times 10^3$	$1\,582 \times 10^3$	$1\,032 \times 10^3$	$1\,332 \times 10^3$	$1\,497 \times 10^3$	$1\,669 \times 10^3$	$1\,376 \times 10^3$
Coefficient of variation [%]	277.4	198.2	211.9	226.1	197.2	236.1	225.2
Geometric mean [CFU·ml ⁻¹]	136×10^3	259×10^3	198×10^3	210×10^3	261×10^3	214×10^3	205×10^3
Median [CFU·ml ⁻¹]	113×10^3	175×10^3	158×10^3	157×10^3	176×10^3	155×10^3	154×10^3
Minimum [CFU·ml ⁻¹]	24×10^3	24×10^3	24×10^3	27×10^3	24×10^3	24×10^3	24×10^3
Maximum [CFU·ml ⁻¹]	$7\,208 \times 10^3$	$7\,208 \times 10^3$	$7\,208 \times 10^3$	$7\,208 \times 10^3$	$7\,208 \times 10^3$	$7\,208 \times 10^3$	$7\,208 \times 10^3$
Mean logarithm	2.1341	2.4134	2.2958	2.3212	2.4162	2.3311	2.3123
Standard deviation of mean logarithm	0.5114	0.5892	0.5129	0.5229	0.5756	0.5582	0.5522

Tab. 2. Basic statistical parameters of somatic cell count.

	April	May	June	July	August	September	Total
Number of samples (<i>n</i>)	236	208	230	218	189	175	1 256
Arithmetic mean [ml^{-1}]	$1\,074 \times 10^3$	$1\,107 \times 10^3$	$1\,041 \times 10^3$	$1\,112 \times 10^3$	$1\,165 \times 10^3$	$1\,036 \times 10^3$	$1\,088 \times 10^3$
Standard deviation [ml^{-1}]	463×10^3	663×10^3	477×10^3	712×10^3	834×10^3	470×10^3	616×10^3
Coefficient of variation [%]	43.1	59.9	45.8	64.0	71.6	45.4	56.6
Geometric mean [ml^{-1}]	969×10^3	986×10^3	926×10^3	978×10^3	$1\,001 \times 10^3$	949×10^3	967×10^3
Median [ml^{-1}]	$1\,013 \times 10^3$	984×10^3	955×10^3	$1\,009 \times 10^3$	$1\,020 \times 10^3$	960×10^3	987×10^3
Minimum [ml^{-1}]	55×10^3	158×10^3	34×10^3	86×10^3	164×10^3	232×10^3	34×10^3
Maximum [ml^{-1}]	$3\,338 \times 10^3$	$5\,110 \times 10^3$	$3\,160 \times 10^3$	$7\,031 \times 10^3$	$9\,568 \times 10^3$	$3\,331 \times 10^3$	$9\,568 \times 10^3$
Mean logarithm	2.9863	2.9937	2.9667	2.9904	3.0004	2.9772	2.9855
Standard deviation of mean logarithm	0.2135	0.2034	0.2366	0.2138	0.2363	0.1808	0.2160

Tab. 3. Correlations between total count of microorganisms and somatic cell count.

Month	Number of samples (<i>n</i>)	Regression equation	Determination coefficient (R^2)	Correlation coefficient (<i>r</i>)	Relationship significance (<i>s</i>)
Relationship between TCM and SCC on the basis of PE					
April	236	$y = 0.0111x + 1\,069.2$	0.0007	0.03	> 0.05
May	208	$y = 0.0438x + 1\,072.3$	0.0109	0.10	> 0.05
June	230	$y = 0.0168x + 1\,049.4$	0.0013	0.04	> 0.05
July	218	$y = 0.2407x + 970.4$	0.2028	0.45	< 0.001
August	189	$y = 0.0063x + 1\,160.5$	0.0001	0.01	> 0.05
September	175	$y = 0.0015x + 1\,034.7$	0.00003	0.01	> 0.05
Total	1 256	$y = 0.515x + 1\,057$	0.0132	0.11	< 0.001
Relationship between log TCM and log SCC on the basis of PE					
April	236	$y = 0.1084x + 2.755$	0.0674	0.26	< 0.001
May	208	$y = 0.0191x + 2.948$	0.0031	0.06	> 0.05
June	230	$y = 0.0282x + 2.902$	0.0037	0.06	> 0.05
July	218	$y = 0.093x + 2.775$	0.0517	0.23	< 0.001
August	189	$y = 0.0851x + 2.795$	0.0430	0.21	< 0.001
September	175	$y = 0.0395x + 2.885$	0.0148	0.12	> 0.05
Total	1 256	$y = 0.061x + 2.845$	0.0242	0.16	< 0.001
Relationship between TCM and SCC on the basis of SP					
April	236	$y = 0.3256x + 79.915$	0.1060	0.33	< 0.001
May	208	$y = 0.0549x + 98.758$	0.0030	0.05	> 0.05
June	230	$y = 0.0974x + 104.250$	0.0095	0.10	> 0.05
July	218	$y = 0.2049x + 87.067$	0.0420	0.20	< 0.01
August	189	$y = 0.2102x + 75.035$	0.0442	0.21	< 0.001
September	175	$y = 0.1445x + 75.285$	0.0209	0.14	> 0.05
Total	1 256	$y = 0.170x + 521.510$	0.0290	0.17	< 0.001

Value of relationship significance represents the probability of null hypothesis *P*.

TCM – total count of microorganisms, *SCC* – somatic cell count, *log TCM* – logarithm of total count of microorganisms values, *log SCC* – logarithm of somatic cell count values, *PE* – Pearson correlation coefficient, *SP* – Spearman rank correlation coefficient.

linear regression (MS Excel, Microsoft, Redmond, Washington, USA) by monthly correlation coefficients and correlation in total, using *PE* and *SP*.

RESULTS AND DISCUSSION

The arithmetic means of *TCM* and *SCC* of bulk raw ewes' milk amounted $(611 \pm 1376) \times 10^3 \text{ CFU} \cdot \text{ml}^{-1}$ and $(1088 \pm 616) \times 10^3 \text{ ml}^{-1}$, respectively (Tab. 1, 2). The relevant values for the geometric mean and median were $205 \times 10^3 \text{ CFU} \cdot \text{ml}^{-1}$ and $967 \times 10^3 \text{ ml}^{-1}$, and $154 \times 10^3 \text{ CFU} \cdot \text{ml}^{-1}$ and $987 \times 10^3 \text{ ml}^{-1}$, respectively. Statistical parameters were expressed also on monthly basis. Large differences regarding

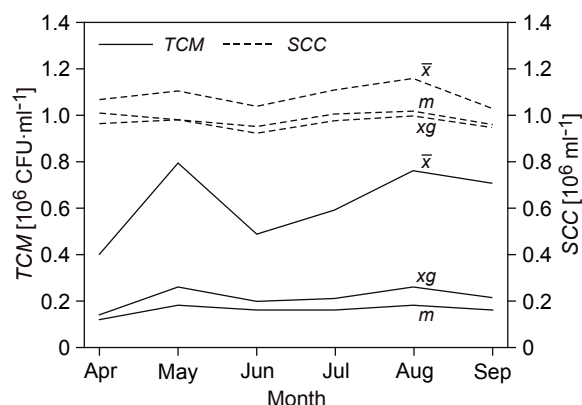


Fig. 1. Seasonal evolution of total count of microorganisms and somatic cell count in bulk sheep milk.

TCM – total count of microorganisms, *SCC* – somatic cell count, \bar{x} – arithmetic mean, *m* – median, *xg* – geometric mean.

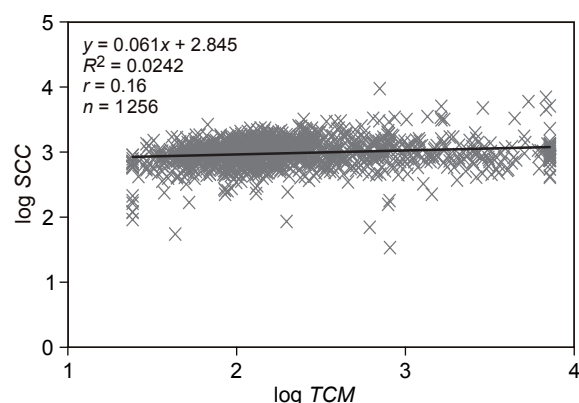


Fig. 2. Pearson correlation coefficient of total count of microorganisms and somatic cell count during the entire season.

log *TCM* – logarithm of total count of microorganisms values, log *SCC* – logarithm of somatic cell count values.

TCM and *SCC* were evident between medians and geometric means on one hand, and between arithmetic means on the other hand, both monthly and in total (Tab. 1, 2, Fig. 1). The arithmetic means are logically mostly significantly higher than the geometric means and medians. On the contrary, the geometric means and the medians are relatively close, which confirms the absence of *NFDD* and the correct use of the transformed values at calculating mutual interaction (*PE*) and Spearman coefficient (*SP*). The arithmetic mean, geometric mean and median correspond approximately to each other only in case of *NFDD*. In other cases (*SCC*), they require data transformation e.g. exponentiation [16], rooting, calculating logarithm on the base of 2 [5–9] or a non-parametric test (e.g. calculation of *SP*). For similar evidence, lack of *NFDD* may be indicated by variability of *TCM* and *SCC* significantly greater than 33% (Tab. 1, 2). The variability of *TCM* and *SCC*, significantly above 33%, can also be considered as an indirect evidence of *NFDD* absence, besides the direct evidence by the test for normality or skewness, and by kurtosis tests. The stated fact, assuming the theory of *NFDD* (Gauss law, 95% percentile includes $\pm 3 \text{ SD}$, the whole variation range of 6 *SD*, where the coefficient of variation is the relative proportion of *SD* in the arithmetic mean), hypothetically (only ostensibly) indicates the significant existence of negative values of *TCM* and *SCC*. Of course, this is not realistic in practice.

The same trends as those mentioned above between mean values of *TCM* and *SCC* in bulk milk could be seen at monthly (season of the year and stage of lactation) evaluation of the dataset (Tab. 1, 2, Fig. 1). The geometric mean and the median had, during lactation, relatively balanced course of both indicators (*TCM* and *SCC*), without any significant seasonal variations and some more obvious trends of milk quality. GONZALO et al. [17] presented relatively higher influence of months on *SCC* (factor of variability being 16.1%).

The tightness of relationship between *TCM* and *SCC* was similar in the whole set of data regarding the correlation of transformed values (mean of logarithmic values to base 10 and *PE*) and regarding *SP* (Tab. 3, Fig. 2; correlation coefficient $r = 0.16$ and $r = 0.17$, respectively; $P < 0.001$). HANUŠ et al. [18] found a correlation between *TCM* and *SCC* in the bulk milk in Holstein cows ($r = 0.31$; $n = 57$, $P < 0.05$; Fig. 3) and between log *TCM* and log *SCC* ($r = 0.29$; $n = 57$, $P < 0.05$; Fig. 3). GONZALO et al. [19] found a statistically significant relationship between log *TCM* and log *SCC* (the value of r was 0.23). *SP* proved to be statistically more effective for the purpose of evaluation (more

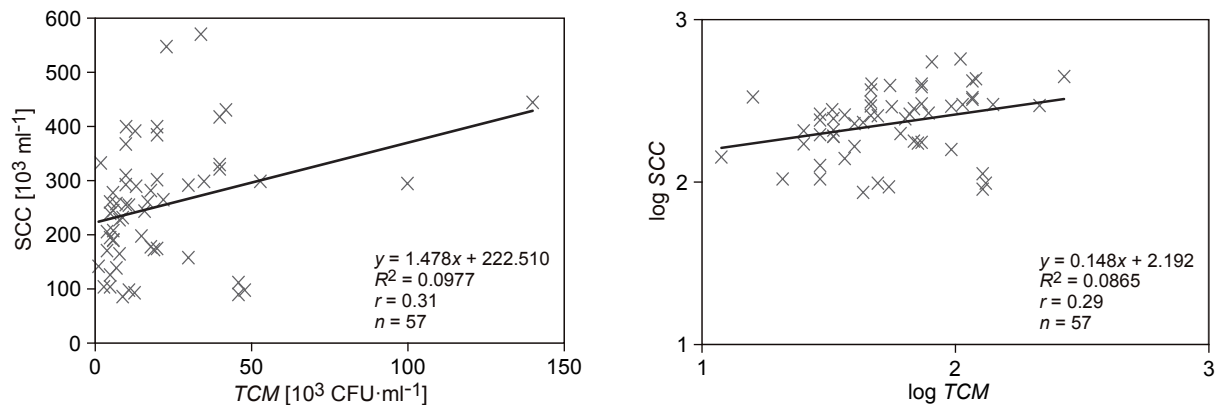


Fig. 3. Relationship between total count of microorganisms and somatic cell count and their logarithmic values in bulk milk of Holstein cows [19].

TCM – total count of microorganisms, *SCC* – somatic cell count, *log TCM* – logarithm of total count of microorganisms values, *log SCC* – logarithm of somatic cell count values.

favourable and therefore preferable) compared to *PE* for a slightly higher yield of the obtained information (Tab. 3) in this case. *PE* and *SP* correlations were similar in evaluated months except for July. In both (*PE* and *SP*), a trend of gradual elimination of relationship tightness (*r* from 0.05 to 0.1; *P* > 0.05 i.e. insignificant) could be observed after months from the beginning (*r* from 0.26 to 0.33; *P* < 0.001) and its subsequent increase (*r* from 0.12 to 0.23, *P* < 0.001; Tab. 3, Fig. 4, 5). The closest (*r* = 0.26 and *r* = 0.33, respectively), and thus the most significant relationship (*P* < 0.001) was in the first month of sampling (April) and in the third month of lactation, i.e. during early lactation. Another closer relationship was observed since 6 months of lactation (July) till its end, with the

exception of September. There, the highest values were in July and August (*r* = 0.23 and *r* = 0.21, respectively; *P* < 0.001; Tab. 3).

The proof of correctness of the procedure for evaluation of the relationship between *TCM* and *SCC* might be the comparison of three correlation coefficients for April, and of correlations in total (Tab. 3). The correlation coefficients for April were as follows: *r* = 0.03 (*P* > 0.05) for relationship between *TCM* and *SCC* on the basis of *PE*; *r* = 0.26 (*P* < 0.001) for relationship between *log TCM* and *log SCC* on the basis of *PE*; *r* = 0.33 (*P* < 0.001) for relationship between *TCM* and *SCC* on the basis of *SP*. The recovery efficiency of statistical testing grew with transformation of the original values because of absence of *NFDD*.

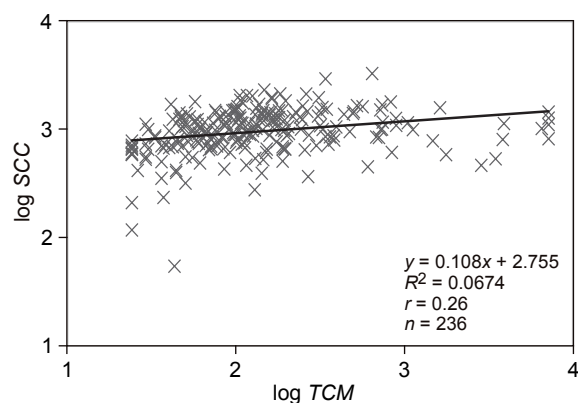


Fig. 4. Pearson correlation coefficient between total count of microorganisms and somatic cell count in April.

log TCM – logarithm of total count of microorganisms values, *log SCC* – logarithm of somatic cell count values.

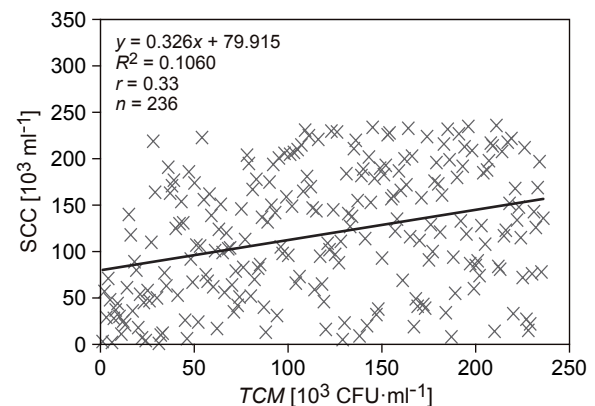


Fig. 5. Spearman correlation coefficient between total count of microorganisms and somatic cell count in April.

TCM – total count of microorganisms, *SCC* – somatic cell count.

This trend could be observed in most months and, of course, also in the overall evaluation. Nevertheless, a different trend was observed in July: $r = 0.45$ (on the basis of PE ; $P < 0.001$) for the relationship between TCM and SCC ; $r = 0.23$ (on the basis of PE ; $P < 0.001$) for relationship between $\log TCM$ and $\log SCC$; $r = 0.2$ (on the basis of SP ; $P < 0.01$) for relationship between TCM and SCC (Tab. 3; Fig. 6). This rare opposite trend was probably caused by the influence of random frequency distribution in given month. However, this result was in line with the logical trend of the analysed dependence of SCC on TCM in the bulk raw ewes' milk.

Regarding PE and SP values and their coefficients of determination for the relationship between TCM and SCC , it should be mentioned that 2.4 % of the variability in $\log SCC$ was explainable by variance in $\log TCM$, and 2.9 % in the SCC value sequence was determined by the order of TCM values (Tab. 3; Fig. 2). Although these relative values are not high, they may explain a causal dependence. Nevertheless, similar to the evaluation by month (Tab. 3), it can be stated that in selected cases (April, the third month lactation) that up to 6.7 % of variability in $\log SCC$ was determined by variability in $\log TCM$ ($P < 0.001$; Fig. 5), and up to 10.6 % in the ordering of SCC values was determined by ordering in TCM values ($P < 0.001$; Fig. 4). Explanation of the variability origin in the mentioned relationship could be up to 20.3 % even regarding original TCM and SCC values in July (Tab. 3, Fig. 6).

It is logical that the higher microbial pressure (higher TCM values) in the sheep flock probably

involves a higher quantity of mastitis pathogens, which may be reflected by a higher value of SCC as a health indicator. However, this relationship need not apply necessarily each time, i.e. individually on the farm or in short-time investigations, but it can be expected in larger files and in longer monitoring periods. The basic function of white blood cells (95 % of SCC) in both species (cows, sheep) as components of the defense immune system of mammary gland would remain the same, maybe with minor differences in efficiency, although SCC values may be different in different animal species. Of course, variability of SCC within species could be large and even greater than that between species. Therefore, SCC will generally react by an increase to inflammatory process in both species.

Then, when 2.4 % (Fig. 3) and 20.3 % (July, Tab. 3, Fig. 2, 6) of variability in $\log SCC$ values and in their order, respectively, is explainable by variations in $\log TCM$ values and in TCM values order, respectively, it is possible to state that better care of stable and milking hygiene could help to reduce the mastitis incidence in healthy and hygienically consolidated herds. Limitation of this expectation to hygienically consolidated herds can be explained by a hypothetical calculation based on qualified estimation and on our previous results. The resource-technological specification of TCM during production of raw cows' milk under conditions of poor and good rearing and milking hygiene, respectively, is known [18]. Considering this specification generally valid, it can be assumed that pathogens from the mammary gland (mainly in conditions of subclinical mastitis) can form approximately 2–5 % of TCM at a good milking hygiene (TCM up to $100 \times 10^3 \text{ CFU} \cdot \text{ml}^{-1}$ in cows and, for instance, up to $300 \times 10^3 \text{ CFU} \cdot \text{ml}^{-1}$ in small ruminants). This can be taken as a qualified estimate according to previous results [18, 20, 21]. Further, taking the lower value of 2 % to consideration, these represent $2 \times 10^3 \text{ CFU} \cdot \text{ml}^{-1}$ in cows and $6 \times 10^3 \text{ CFU} \cdot \text{ml}^{-1}$ in small ruminants at a consolidated housing hygiene.

It is known that about 80 species of microorganisms in cattle are able to cause mastitis, but only 8 species are economically important, causing loss of milk in the given etiology; the most important is *Staphylococcus aureus*. BENDA et al. [22] and TICHÁČEK and BENDA [23] reported an increase in mastitis incidence in a herd, and estimated a relevant SCC increase for milk, using irrational functions. The authors associated prediction equation with presence of pathogens *Staphylococcus aureus* and *Streptococcus agalactiae* in bulk samples of cows' milk. They reported *Staph. aureus* at $>200 \text{ CFU} \cdot \text{ml}^{-1}$ and *S. agalactiae* at

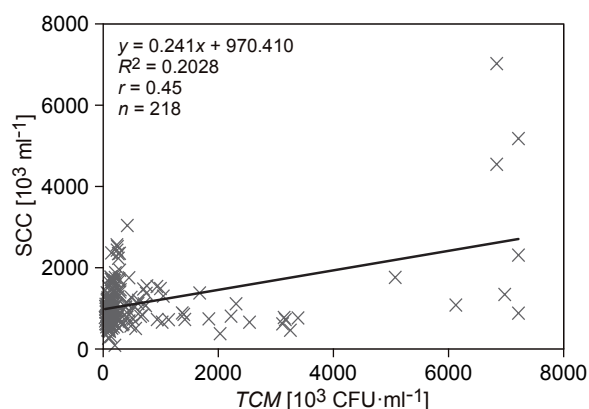


Fig. 6. Pearson correlation coefficient between total count of microorganisms and somatic cell count in July.

TCM – total count of microorganisms, SCC – somatic cell count.

$> 100 \text{ CFU} \cdot \text{ml}^{-1}$ as the limits for suspicion of mastitis in a herd. However, *TCM* may increase up to $500 \times 10^3 \text{ CFU} \cdot \text{ml}^{-1}$ in cows or $1500 \times 10^3 \text{ CFU} \cdot \text{ml}^{-1}$ in small ruminants under worsened technological and hygienic conditions of rearing and milking [2, 18]. These values correspond with 1.2% of pathogens in bulk milk of cows and small ruminants. The absolute slight increase in pathogens, at a massive increase in *TCM*, results in their relative decrease [18]. This may mask the actual relationship between *TCM* and *SCC* in some cases, at high *TCM* levels and base variability.

On the other hand, it is evident that the pathogenic microorganisms that cause mastitis create a very small part of *TCM* [21]. This fact impedes any larger increase in correlation coefficients (r) in the relationship between *TCM* and *SCC* in ewes' milk because this is dependent on the relationship between the pathogens and *SCC* rather than on the rest of *TCM* and *SCC*. This relationship (r) will be, from biological and pathological views, always weak, with the exception of random results [20, 22]. Above mentioned argument can be also documented by further calculations, as a qualified estimate in the form of statistical inference, referring to validity of Laplace-Gauss law of *NFDD*:

$$MT = (\bar{x} \pm SD) \times 1.96 \quad (1)$$

where *MT* is maximum tightness, \bar{x} is arithmetic mean of correlation coefficients r for relationship between *TCM* and *SCC* results on the basis of *SP* according to months of lactation, number 1.96 is coefficient of 95% probability of confidence interval by reciprocal restriction of data file.

In this case, *NFDD* can be expected with a high probability. The average of correlation coefficients r has a value of 0.1717 ± 0.0983 according to months of lactation ($n = 6$, Tab. 3). The suggested model calculation is as follows:

$$MT = (0.1717 \pm 0.0983) \times 1.96 \quad (2)$$

The maximum tightness of this relationship (r) can be estimated at 0.36 in absolute value. However, due to the fact that all r values for relationship between *TCM* and *SCC* of lactation (seasonal) months were regularly positive, it can be stated that the relevant r will mostly vary between -0.02 and 0.36 (Tab. 3). Eventually, tighter correlation (higher r values) of the relationship between *TCM* and *SCC* will likely occur randomly, in less representative data sets, probably with the lower frequency of cases.

The polynomic description of the development dynamics of the relationship between *TCM* and *SCC* regarding *PE* and *SP*, depending on months, is presented in Fig. 7. The progression of curves of

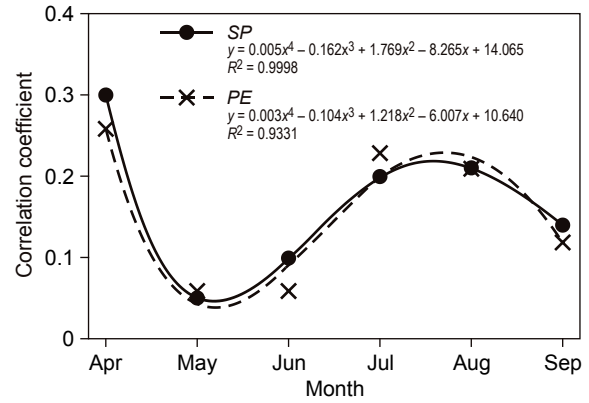


Fig. 7. Spearman and Pearson correlation coefficients of the relation between total count of microorganisms and somatic cell count along the season.

Development of relationship is described by a fourth-order polynomial equations.

SP – Spearman rank correlation coefficient, *PE* – Pearson correlation coefficient (logarithm of original microbiological values).

PE and *SP* ($r = 0.97$, $P < 0.001$, Eq. 3 and Eq. 4) could be explained on the basis of comparison and combination of specific selected results, and by deduction.

$$y = 0.0031x^4 - 0.1037x^3 + 1.2181x^2 - 6.0068x + 10.640 \quad (3)$$

$$y = 0.0054x^4 - 0.1623x^3 + 1.7685x^2 - 8.2647x + 14.065 \quad (4)$$

The beginning of the lactation, and also the period of its end at drying of the mammary gland, is the most risky period for the formation of new mastitis infections in cows [24, 25]. The physiological aspects of rebuilding of mammary gland play an important role with respect to immune defense mechanisms. The effect of release and subsequent increase of tightness of *SCC* dependence on *TCM* after months of lactation in sheep is similar to cows. The higher microbial pressure in early lactation (*TCM*) may increase the incidence of subclinical and clinical mastitis (and consequently also *SCC*) to a greater extent than during the period of continuous lactation.

The explanation of the periodicity could be different at the start and at the end of lactation.

At the start of lactation, it depends on the stress from birth and weakening of immunity, sometimes retention of placenta, immunosuppression from early lactation, given by the secretion of large Ca volumes and related to increased production of cortisol from the adrenal cortex as an immunosuppressive factor (related to the potential

course of postpartum paresis), milking of a larger milk volume by a longer milking process, and thus the longer period of teat closing as a mechanical barrier against pathogens in the period after milking, weakening tone of the teat sphincter as a mechanical barrier against pathogens due to deficiency of Ca in the body (in blood), lack of energy in early lactation (larger volume of nutrients leaving body by milking than coming by nutrition), which may result in ketosis and catabolism of fat body reserves, increase in concentration of ketones in body fluids and weakening of the immune competence of the relevant defense cells (leukocytes).

At the end of lactation, it depends on ending the milking season and, thereby, release of the teat sphincter after lactation when no subsequent milking takes place, short-term edema from the udder internal pressure due to absence of milking, residual milk in the mammary gland at this time, physiological regression of mammary gland tissue during the dry period etc.

In conclusion, the decreased tightness of the relationship (r) between *TCM* and *SCC* for bulk milk in the middle of lactation may be explained by increasing the ability of the immune responsiveness of animals to pathogens, e.g. from metabolic reasons, and vice versa. Another part of explanation can also be the increase in *TCM* and, eventually, its variability in this period. It can be also accepted that the relative stability of *TCM* and *SCC* means, at estimation of the relationship between *TCM* and *SCC*, depends more on the physiological aspects of lactation than on the calendar season. The monthly correlation dynamics results of the relationship between *TCM* and *SCC* during lactation suggest here that a similar trend in mastitis incidence in cows could be also in sheep because the tightness of the relationship between *TCM* and *SCC* is more significant at beginning of lactation than at its end. This similarity can be illustrated, based on our results, by a relatively good agreement of trends and shapes of the curves of the relationship between *TCM* and *SCC*.

While a significant relationship between *TCM* and *SCC* in bulk milk was determined in cows [18] and sheep [19], and was observed also in this study, it was not determined in goats [26, 27]. Other authors also reported on higher means and high variability of *TCM* and *SCC* values in bulk or individual milk of small ruminants [3, 17, 19, 28–39].

CONCLUSION

Development curves of relations between *TCM* and *SCC* in bulk milk along months may be associated with the dynamics of lactation during sheep seasonal reproduction cycle. This is similar to small ruminants, in case of which these dynamics show often simultaneous progress with calendar season, and different from cows reared mostly independently of the seasonal reproduction cycle. Therefore, the development of *TCM* and *SCC* relationships along calendar months can be related to the historically known curve of mastitis incidence in lactating cows, which indicates an increased risk in the beginning and in the end of lactation. Higher dependence of *SCC*, as an indicator inflammation, on the hygienic indicator *TCM* in milk may correspond to the higher incidence of mastitis (secretion disorders) in a sheep flock during lactation. The correct interpretation of relations between *TCM* and *SCC* in bulk milk during sheep lactation may contribute to improvement of the quality of milk.

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