

Descriptive Analysis of Somatic Cell Count Using Statistical Tools

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Abstract

Somatic cell count (SCC) can be used as an indicator of subclinical mastitis and its analysis in relation with the milk composition can provide useful information on the existence of some correlations or patterns. Based on milk production data recorded during 5 years (2012-2015, 2017) at the Research and Development Station for Bovine Arad we conducted a statistical analysis aiming to identify correlations between SCC and milk characteristics (protein and fat content, lactose, non-fat solids, milk quantity, pH, casein) and to find potential profiles of SCC evolution. The correlation analysis was based on 226 lactating cows for which at least 20 measurements were available. Both classical correlation coefficient (i.e. Pearson) and correlation coefficient for repeated measurements (i.e. Bland-Altman) have been computed. In both cases, a moderate negative correlation between SCC and the lactose level has been identified while no significant correlation between SCC and the other milk characteristics has been detected. However, a more accurate description of the relation between SCC and lactose was obtained using a linear mixed model. Aiming to analyse SCC profiles, an additional attribute has been added to the data based on the following encoding rule: the attribute has value 0 if SCC is smaller than 2×10^5 cells/mL, 1 if it is larger than 2×10^5 cells/mL and 2 if the value is missing. In this way, data vectors containing 13 values per year have been constructed for 175 cows and a dissimilarity matrix has been constructed as a first step for cluster analysis. Overall, the results have shown that lactose and SCC were negatively correlated.

Keywords: clustering, correlation analysis, mastitis, similarity measures, somatic cell count.

1. Introduction

Mastitis is one of the most frequently occurring and costly diseases in dairy industry worldwide [1, 2] with an incidence ranging from 12% to 40%. The disease represents one of the major reasons for involuntary culling and productive loss. Economical estimations indicated an average cost per mastitis case ranging from 95 to 211 USD, depending on the pathogen and status of the cow

[3]. According to [4], the average cost of a clinical mastitis case was 179 USD (composed of 115 USD for milk yield losses, 14 USD for increased mortality and 50 USD for treatment-associated costs). Mastitis can have detrimental animal health and economic consequences and therefore it is essential to monitor intramammary infections in dairy cows. Various methods for intramammary infections diagnosis are available. The diagnosis of mastitis is usually based on clinical observations and measures of the inflammatory response to infection, whereas the diagnosis of an intramammary infection is based on the identification of the infectious agent [5]. However,

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somatic cell count (SCC) is frequently utilized to control the intramammary infections status at both herd and cow levels. The SCC measurements are extensively available to dairy farmers and are used more often as a result of low cost comparing to microbiological culture [6].

Milk somatic cells are secreted in milk during the normal course of milking and are a mixture of milk-producing cells and immune cells [7]. The SCC represents the cell count of somatic cells in milk and is used as an indicator for estimating mammary health and milk quality of dairy animals. The SCC is quantified as the number of somatic cells per millilitre and within Europe, the limit is 4×10^5 cells/ml according to Council Directive 92/46/EEC of 1992. In the USA, the legal maximum SCC is 7.5×10^5 cells/ml and Canada has a limit of 5×10^5 cells/ml of raw milk [8]. Generally, a level of SCC below 10^5 cells/ml represents a healthy quarter and a level larger than 2×10^5 cells/ml indicates mastitis [8, 9]. Many countries employ SCC as an indicator in order to monitor the mastitis frequency in dairy cows and also as a marker of raw milk quality because of wide availability and use throughout the dairy industry in the world. The number of somatic cells in milk can be significantly increased by poor management practices, stressful conditions and any environmental conditions changes [7]. However, the number of somatic cells in milk is influenced also by other factors such as parity, lactation stage, cow productivity, health and breed. Increased SCC is correlated with decreases in casein, milk fat, and lactose; increased enzymatic activity; and implicitly reduced quality and yield of milk [2, 10]. The inflammatory process in the mammary gland was observed at cow and herd level using the SCC as diagnostic tool.

The aim of the current research was to analyse the applicability of several statistical approaches (correlation analysis, mixed linear models, cluster analysis) in the identification of correlations between SCC and milk characteristics (protein and fat content, lactose, non-fat solids, milk quantity, pH) and of some potential profiles of SCC evolution.

2. Materials and methods

Animals

All research activities involved in the present study were performed in accordance with the European Union's Directive for animal

experimentation (Directive 2010/63/EU). The study was carried out in a farm situated in the West part of Romania at the Research and Development Station for Bovine Arad, where Romanian Spotted and Romanian Brown cows, managed under identical conditions, were included in the research herd. The cattle involved in the study were primiparous and multiparous (between 1st and 11th lactation) and were included in the Official Performance and Recording Scheme. A dataset of 264 cows (215 Romanian Spotted and 49 Romanian Brown) was analysed.

All cattle were milked twice per day in a 'herringbone' milking parlour (2 sides x 14 units). The milking parlour was equipped with AfiMilk 3.076 A-DU® software. Furthermore, all cattle were fitted with AfiTag® pedometers. During the study, cattle were kept on deep straw bedding, with a space allowance of 9 m² in the resting area and free access to water and outside paddocks. Cattle were housed in groups of 40 to 50 animals, according to lactation stage and productivity, regardless of their breed.

Data collection

Milk yield per milking session and milk conductivity were recorded and collected daily using AfiMilk 3.076 A-DU® software and hardware fitted in the milking parlour during 5 years (2012-2015, 2017). Production and milk quality data (milk production, protein and fat content, lactose, non-fat solids, pH and the somatic cell count) were taken from the results of the official performance recordings

Statistical analysis

Aiming to identify statistical methods appropriate for the analysis of the collected data, characterized by a temporal but also hierarchical structure due to repeated measurements, several methods have been investigated. The analysed statistical techniques are shortly described in the following.

a) Pearson correlation analysis. Primary statistical tools to study correlations between two quantitative variables X and Y are the Pearson correlation coefficient r (quantifying linear association) and the Spearman correlation coefficient ρ (used to investigate monotonic associations that may not necessarily be linear).

(b) Intra-class correlation coefficient. An important characteristic of nested data is that

observations on the same subject tend to be more alike than observations on different subjects. The extent of this homogeneity is quantified by the intra-class correlation coefficient (ICC), defined, for a given quantitative variable, as the ratio of the between-subject variance and the total variance. A high ICC indicates that groups of observations are homogeneous and/or very different from each other. An ICC equal to 0 means that there is no between-subject variance. In this case, in the context of a correlation analysis, the grouping of observations has no impact on the relation between the variables of interest and can be ignored in further analyses.

(c) *Correlation coefficient for repeated measurements.* An alternative that can be used when there are several measurements on the same variable for each subject is the *repeated measurements correlation coefficient (rmcorr)* proposed by Bland and Altman [11, 12], which quantifies the common within-individual linear association for paired measures assessed on two or more occasions for multiple subjects. Namely, it can be used to analyse if, for a subject, an increase in the values of the first variable is associated with an increase or decrease in values of the second variable. The determination of *rmcorr* between X and Y involves defining a linear model with Y as the response variable and X and the subject as predictors by which the variation of Y is partitioned in the variation explained by X (SS_X), the variation explained by subjects (SS_B), and the residual variation (SS_{res}). Removing the variation due to subjects, *rmcorr* is defined as

$$rmcorr = \sqrt{\frac{SS_X}{SS_X + SS_{res}}}$$

having the same sign as X in the regression model. The value of *rmcorr* remains the same if X and Y are interchanged. A limitation of *rmcorr* is that, as one can see from the definition, it assumes that the impact of X on Y is the same for all individuals (the slopes of the regression lines for all subjects are the same). More details about this statistical analysis technique can be found in [13].

(d) *Linear mixed models.* A more general framework which allows the modelling of differing within-subject behaviours is represented by linear mixed models. Here the slopes of individual regression lines are allowed to vary, as

well as the intercepts. They are treated as random coefficients and we may be interested in their mean, their variance and their covariance. Namely, in our case the model can be stated as

$$y_{ij} = \beta_{0j} + \beta_{1j}x_{ij} + \varepsilon_{ij},$$

where x_{ij} and y_{ij} denote the values of X and Y for observation i on subject j , β_{0j} and β_{1j} are subject – specific intercepts and slopes, and ε_{ij} represents residual random error, assumed to be normally distributed, with homogeneous variance across subjects. For a detailed technical discussion on linear mixed models we refer the reader to [14] and [15].

(e) *Cluster analysis.* This technique relies on the usage of the dissimilarity between data instances (e.g. consisting of values recorded for one subject) in order to identify natural groups (clusters) in the data. In the context of this paper both hierarchical agglomerative algorithms and partitional algorithms (PAM – partitioning around medoids) have been used to identify atypical behaviour (outliers) and prototypes (e.g. behaviour profiles), respectively.

Software tools

The statistical analyses were carried out in R, version 3.5.2. The computation of *rmcorr* was performed using the package *rmcorr* [16]. The linear mixed model was fit using the facilities of the package *multilevel* [17]. The cluster analysis is based on *clust* and *TSclust* packages [18].

3. Results and discussion

(a) *Results of the analysis based on Pearson correlation coefficient.* By computing the Pearson correlation coefficient between SCC and the other milk characteristics (see Table 1), it follows that the strongest association with SCC was found for lactose ($r=-0.30$). This would imply that an increase of SCC (i.e. a potential infection) is associated with a significant decrease of lactose. However, the definition of the correlation coefficient assumes that the observations are independent. Therefore, the Pearson correlation coefficient may not be appropriate in the analysis of our data because of their inherent nested structure: there are multiple observations for the same subject (cow), and as such, the assumption

of independence is violated, making the conclusions obtained by using this coefficient unreliable. Same remark is true for the Spearman correlation coefficient. In the following, we will

analyse the association between SCC and lactose by using approaches which take into account the nested structure of data.

Table 1. Pearson Correlation Coefficient between SCC and milk characteristics

Characteristic	C1	C2	C3	C4	C5	C6	C7	C8	C9
r	-0.08	-0.01	0.05	-0.30	-0.14	0.06	0.02	-0.09	0.01

Milk characteristics: C1 – quantity, C2 – casein, C3 – fat level, C4 – lactose, C5 – pH, C6 – protein level, C7 – fat per protein level, C8 – non-fat solids, C9 – urea

Table 2. Intraclass Correlation Coefficient for SCC and milk characteristics

Characteristic	SCC	C1	C2	C3	C4	C5	C6	C7	C8	C9
ICC	0.17	0.13	0.01	0.15	0.34	0.23	0.15	0.11	0.24	0.05

Milk characteristics: C1 – quantity, C2 – casein, C3 – fat level, C4 – lactose, C5 – pH, C6 – protein level, C7 – fat per protein level, C8 – non-fat solids, C9 – urea

(b) *Results of the analysis based on ICC.* Table 2 shows the intra-class correlation coefficients for the investigated variables. The highest ICC was found for lactose. This is a strong argument against the validity of the Pearson correlation coefficient when analysing the relation between lactose and SCC suggesting that other techniques should be investigated.

(c) *Results of the analysis based on the correlation coefficient for repeated measurements.* The value of *rmcorr* between SCC and lactose for our data set is -0.26, which is only slightly different from the Pearson correlation coefficient found earlier. However, this result should be treated with caution as *rmcorr* assumes, as mentioned before, that the impact of *X* on *Y* is the same for all individuals. When this is not the case, i.e. when we suspect that different subjects exhibit different relations between *X* and *Y*, *rmcorr* may no longer be appropriate. To investigate this possibility, we computed the Pearson correlation coefficient between SCC and lactose for each subject. These coefficients were found to have a large variability, ranging between -0.85 and 0.22, with a mean of -0.30. The distribution of the correlation coefficients is presented in (Figure 1.(a)). There are subjects displaying a positive association between SCC and lactose and subjects for which the association is negative, pointing to a clear difference in individual responses. This could be explained by the existence of various individual traits, such as age, breed, season, diet, environment, which have an influence on SCC

and lactose and which are not taken into account in this model.

For further emphasis of this behaviour, the two cows with the most extreme values of the Pearson correlation coefficients, -0.85 and 0.22, respectively, were considered separately. In (Figure 1.(b)) is illustrated that the relation between SCC and lactose is very different for these two subjects, the correlation coefficient of $r=-0.65$ found for their joint data being representative for neither subject. For the first one (dashed line), there is a clear decrease in lactose as SCC increases, whereas for the second one (dotted line), lactose remains elevated even as SCC increases. Consequently, the determination of *rmcorr* is not a suitable analysis technique in this case.

(d) *Results of the analysis based on linear mixed models.* We considered a linear mixed model with lactose as the response variable and SCC and subject as the random effects. The estimated model coefficients can be found in Table 3 and the regression lines per subject are displayed in (Figure 2). A likelihood ratio test confirmed that the model with random slopes and intercepts is significantly better than one with random intercepts alone ($p<0.0001$). It can be seen that, on average, lactose decreases as SCC increases, but significant variability exists between subjects. This variability can also be seen in (Figure 3), which displays the estimated regression lines for 10 arbitrarily selected cows. Moreover, the slopes and intercepts appear to be uncorrelated, therefore

a greater base lactose value does not imply a stronger relation with SCC.

(e) *Results of the cluster analysis.* The aim of the cluster analysis was to investigate the time series of events corresponding to subclinical mastitis occurrence (SCC value larger than 2×10^5

cells/ml). Therefore, in a first approach, an additional attribute has been added to the data, based on the following encoding rule: the attribute has value 0 if SCC is smaller than 2×10^5 cells/ml, 1 if it is larger than 2×10^5 cells/mL and 2 if the value is missing.

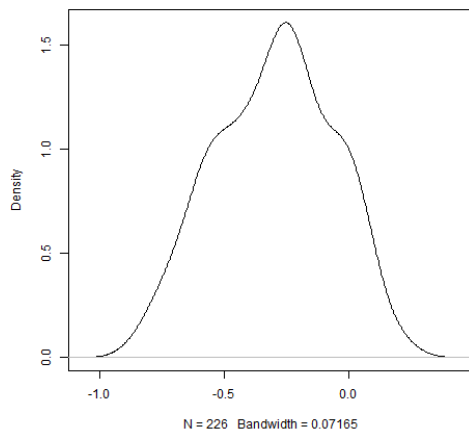


Figure 1. (a) The distribution of individual Pearson correlation coefficients for the 226 cows

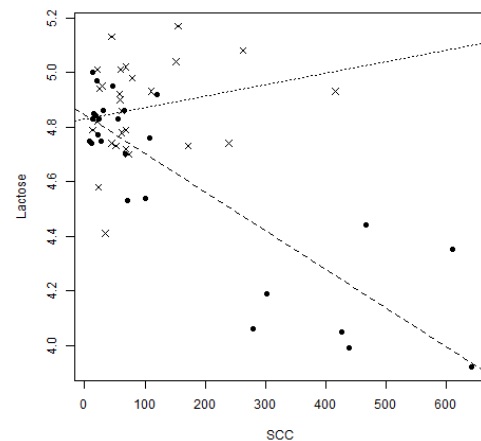


Figure 1. (b) Scatterplot of SCC vs lactose for two cows (dashed line: cow 1 and dotted line: cow 2) and corresponding regression lines

Table 3. Coefficients of the linear mixed model describing the relation between lactose and SCC, accounting for differences between cows

Fixed effects: lactose ~ SCC	Value	Std. Error	DF	t-value	p-value
Intercept	4.747	0.009	5619	549.372	0
SCC	-0.087	0.007	5619	-12.384	0
Random effects: ~ SCC subject	Std. Dev.	Correlation			
Intercept	0.124	Intercept			
SCC	0.065	0.051			
Residual	0.175				

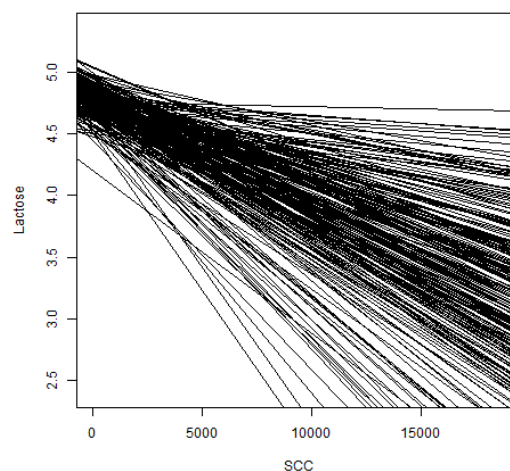


Figure 2. Individual regression lines for the 226 animals, estimated by the linear mixed model

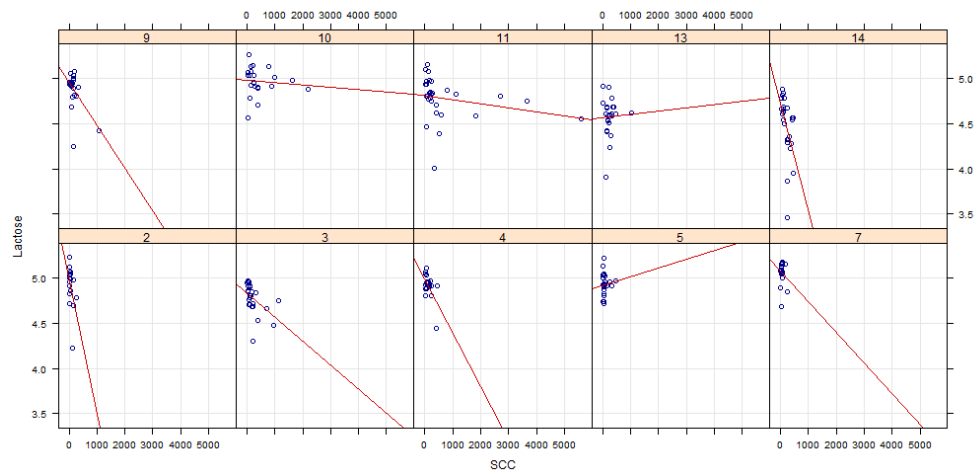


Figure 3. Individual regression lines estimated by the linear mixed model (for 10 arbitrarily selected animals)

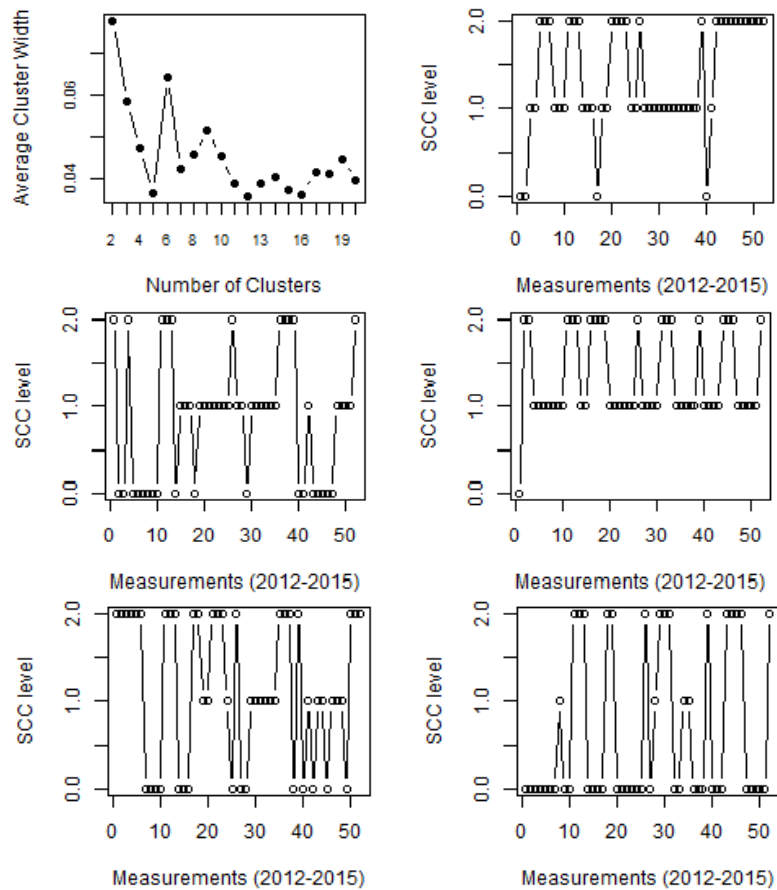


Figure 4. Influence of the number of clusters on the average cluster width and prototypes obtained by applying a PAM (Partitioning Around Medoids) algorithm

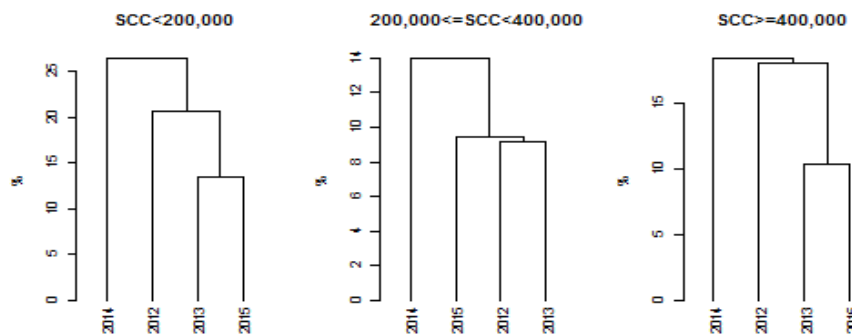


Figure 5. Results of agglomerative clustering applied to the 10-dimensional vectors containing the percentage of cases with SCC in the specified range

Only the animals for which there have been recorded values in 2012-2015 have been taken into account. In this way, data vectors containing 13 values per year have been constructed for 175 cows. Based on the dissimilarity values computed for these 52-dimensional data using the CORT measure (an adaptive dissimilarity measure for time series which quantifies both the difference on values and on the temporal correlation behaviour) provided in the TSclust R package [19], five clusters have been identified, as suggested by the intra-class variance illustrated in the first graph of (Figure 4) where a minimum is obtained for five clusters.

The five prototypes presented in the other graphs of (Figure 4) provide several evolution profiles which might be useful in identifying specific behaviours. For instance, the first and the third prototypes represent clusters where for most measurements the SCC level is either missing or larger than 2×10^5 , while the last prototype corresponds to a cluster for which in most cases SCC is either missing or smaller than 2×10^5 .

The second approach aimed to analyse patterns of behaviour based on the percentage of cases when SCC is smaller than 2×10^5 , when it is between 2×10^5 and 4×10^5 and when it is larger than 4×10^5 . The percentages have been computed using the values corresponding to 10 measurements in each year. By applying a single linkage agglomerative algorithm (based on the Euclidean distance), for 10-dimensional vectors corresponding to four years (2012-2015), the dendrograms illustrated in (Figure 5) have been obtained. Such dendrograms allow the identification of atypical cases, as is the case of values corresponding to 2014 year, which was consistently identified by clustering

algorithms as corresponding to a different behaviour. These results illustrate different types of information which can be extracted from data by applying clustering algorithms.

Studies conducted by other authors with respect to the relations between SCC and milk characteristics revealed a negative correlation between SCC and lactose. The authors of [20] found a negative correlation between SCC and milk yield and lactose. The results reported in [21] indicate that SCC was significantly correlated with a decrease in milk constituents only under conditions of average SCC in milk above 1×10^6 cells/ml.

However, in the same study [21] correlations obtained between SCC and lactose were not significant in one farm comparing with another farm where the author found a negative correlation between SCC and total solids and also lactose contents. The lactose is a sensitive parameter in milk that reports disorders in secretory tissues and biosynthesis of this component is decreased due to infection of mammary gland [22].

In the present study, the overall results have shown that lactose and SCC were negatively correlated which confirms the statements of other authors.

4. Conclusions

When using statistical tools to analyse somatic cell count and its relation with other milk characteristics based on multiple measurements obtained from the same lot of animals, it is important to acknowledge that these data are not independent, and there may be various characteristics of each individual that influence

the intensity and even the type of the relation. A high intra-class correlation coefficient indicates that appropriate measures must be taken to account for the lack of independence, and among these, the use of linear mixed models appears as most suitable. On the other hand, in the cluster analysis, if the dissimilarity measure is adequately chosen, then useful information on atypical behaviours or prototypes corresponding to clusters grouping common behaviours can be extracted from data which describe the evolution of SCC levels.

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