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Original article

# Validation of relationship between milk resistance and daily yield of dairy cows

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## Abstract

**Purpose:** The reduced value of resistance (R) of milk ( $<167\Omega$ ) or increased conductivity (EC)  $>6.0$  mS/cm indicate the growing number of ions in milk during the initial phase of mastitis. The aim of this study is to demonstrate a linear (verified by a validation procedure) dependency between R and mean daily yield (MDY), which could be used for current monitoring and forecasting of milk yield in dairy cow herds. Although the topic has frequently been examined, a validated model for prediction of MDY based on R has still not been presented.

**Methods:** Data from 118 dairy cows were analyzed in the study. The validation of model dependency  $R \leftrightarrow MDY$  was performed by the live-one-out method (LOO).

**Results:** The minimum geometrical/arithmetical mean of R milk was observed during the 1st month of lactation and was  $53.40/254.86 \Omega$ . However, the maximum geometrical/arithmetical mean of R milk was observed during the 7th month of lactation and has  $189.62/574.51 \Omega$ . The final model was described by the curve equation  $MDY = -04461 \times R\% + 51.58$  where R% – percentage share of cows in a herd whose R oscillated within the limits  $49.38-154.32 \Omega$ .

**Conclusions:** Complete predictivity of the model within the above mentioned limits (“prognostic range”) was confirmed by the results of validation of the model. The developed model enables the efficiency of a herd at specified percentage share of cows of defined milk R value to be determined.

**Key words:** resistance, milk, conductivity, yield, dairy

## Introduction

The maintenance of milk production at a stable and predictable level is the main aim of dairy cow breeders. Early identification of potential threats such as pre-clinical or clinical mastitis enables this aim to be achieved. One of the basic devices used by people supervising breeding to quickly determine health con-

ditions is the analysis of resistance (R) or analysis of electrical conductivity (EC) of milk (Zecconi et al. 2004, Ferrero et al. 2014, Caria et al. 2016, Zaninelli et al. 2016). Even though these analyses do not correlate with the number of somatic cells (SCC), they can allow simple supervision of a herd on a constant basis (Hamann and Gyodi 1994, Norberg et al. 2004, Juozaitiene et al. 2010). The dependencies between

the state of mastitis advancement and EC or R are most commonly evaluated with the use of classification models or mixed linear models, due to the lack of linear dependency with SCC (Wilson et al. 2004, Cavero et al. 2006).

EC of milk within the limits  $\approx 4\text{-}6$  mS/cm or R within the range  $R \approx 167\text{-}250 \Omega$  (in scale of DRAMINSKI Mastitis Detector  $\approx 324\text{-}487$  units) are regarded as the level corresponding to the physiological condition of the mammary gland (Ferrero et al. 2014). The reduced value of R ( $<167 \Omega$ ) or increased EC ( $>6.0$  mS/cm) indicate the growing number of ions in the milk (Ferrero et al. 2014). This phenomenon is associated with greater permeability of intercellular spaces in the mammary gland during the initial phase of mastitis. This in turn results in greater permeability of  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Na}^+$  ions present in the intercellular fluid (Ferrero et al. 2014). Up to date R or EC of milk have not been directly correlated with the average daily yield in a herd (MDY) (Wilson et al. 2004, Lukas et al. 2009, Vilas Boas et al. 2016). Modeling of such a dependency at a high level of significance has not yet been presented (Vilas Boas et al. 2016). There were attempts to analyze the dependency between milk production losses and clinical mastitis using very sophisticated models (mixed linear models) (Wilson et al. 2004). However, one of the main obstacles to defining the dependency model between R and MDY is the lack of linear dependency in on appropriately wide range as well as great inter-individual variability in the case of R and MDY. No easy device in application, which allows the supervision of herd productivity only on the basis of R milk measurement, has been presented so far. The impact of MDY losses as a result of mastitis is common knowledge (Wilson et al. 2004). Nevertheless, the relationship between R and MDY within the frames of a linear model has not yet been precisely defined.

The aim of this study is to demonstrate a linear (verified by a validation procedure), dependency between R and MDY ( $R \leftrightarrow \text{MDY}$ ), which could be used for current monitoring and forecasting of milk yield in dairy cow herds.

## Materials and Methods

One hundred and eighteen cows, meeting the requirements for the analysis, were selected from a group of over 928 cows (Agromarina Sp. z o. o., Poland). The key criterion was the time of the last calving at the turn of December and January 2015/2016. A subsequent criterion referred to the monitoring of R performed during the period of 9 months from the last calving. The cows were Polish

Holstein-Friesian Black-White strain, weighing about 700 kg each at the age of 3-8 years. The R analysis was conducted during the morning milk yield (the average for milk from all teats). Milk from the initial/final phase of milking was used for the analyses. Nine hundred and thirteen R measurements and nine hundred and twenty-nine data representing MDY were applied to the analyses. Data were obtained within the period of 9 months of herd monitoring between January and September 2016. For technological reasons, the R analysis was not performed in August (the eighth month of this herd supervision).

The study was carried out on the basis of the data obtained during routine inspection of R milk. To this purpose a DRAMINSKI Mastitis Detector 4x4Q was used, an analyzer of electrical resistance of milk (Dramiński®, Poland), (Hamann et al. 1995, Ferrero et al. 2014, Iraguha et al. 2015, Preethirani et al. 2015). Since routine inspection was the data resource for the analyses, the research did not require permission from the Bioethics Committee.

The analysis of raw data from 118 cows was conducted with the use of GraphPad Prism® v. 6.01 (GraphPad Software Inc.). The validation of model dependency  $R \leftrightarrow \text{MDY}$  was performed using the live-one-out method (LOO) (Todeschini et al. 2004, Grabowski et al. 2012). The final model was verified by the highest value of Fisher's test (confidence interval 95%) and  $p < 0.05$  was taken as statistically significant. Additionally, for the LOO procedure, the coefficient of determination ( $R^2$ ) of the observed (Draminski mastitis detector) versus predicted MDY (based on the model equation) data was determined, a squared cross-validated correlation coefficient ( $Q^2$ ) parameter, and differences between  $Q^2$  and  $R^2$  were calculated as the measure of internal performance and model predictive ability. The difference between fitting and predictive ability of the model was analyzed using the difference between an asymptotic squared cross-validated correlation coefficient ( $Q^2_{\text{asym}}$ ) and  $Q^2$ . Validation acceptance criteria which have to be fulfilled by the optimized model were assumed on the level:  $Q^2 \geq 0.65$ ,  $R^2 \geq 0.85$ ,  $Q^2 - R^2 < 0.3$ ,  $Q^2_{\text{asym}} - Q^2 >$  between  $-0.005$  and  $0.005$  (Todeschini et al. 2004, Pratim Roy et al. 2009).

## Results

The monitored herd during the months in which the milk production and R milk were analyzed, produced 1100 tonnes of milk. The minimum MDY value was  $30.16 \pm 7.74$  L/24h, observed during the 9<sup>th</sup> month of lactation. The maximum MDY value was  $45.24 \pm 9.05$  L/24h, observed during the 2<sup>nd</sup> month of

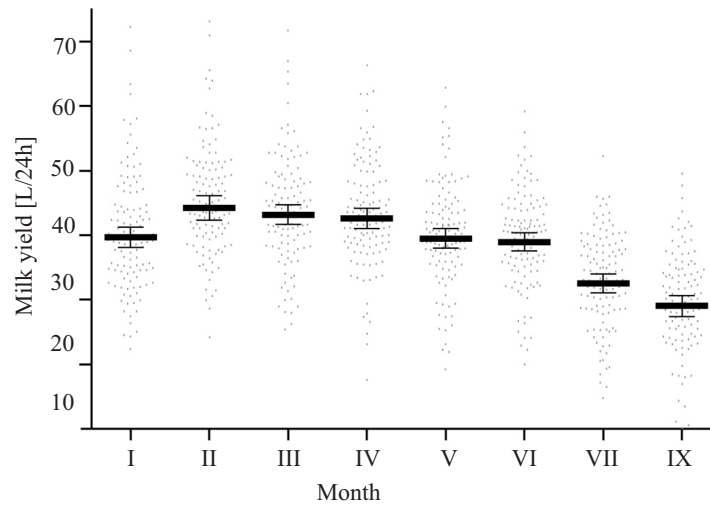


Fig. 1. Geometric mean (confidence interval 95%) illustrating milk yield in a herd (n=118) within 9 months from calving. dots – milk yield of specific animals; bold line – geometric mean; error bars – 95% confidence interval.

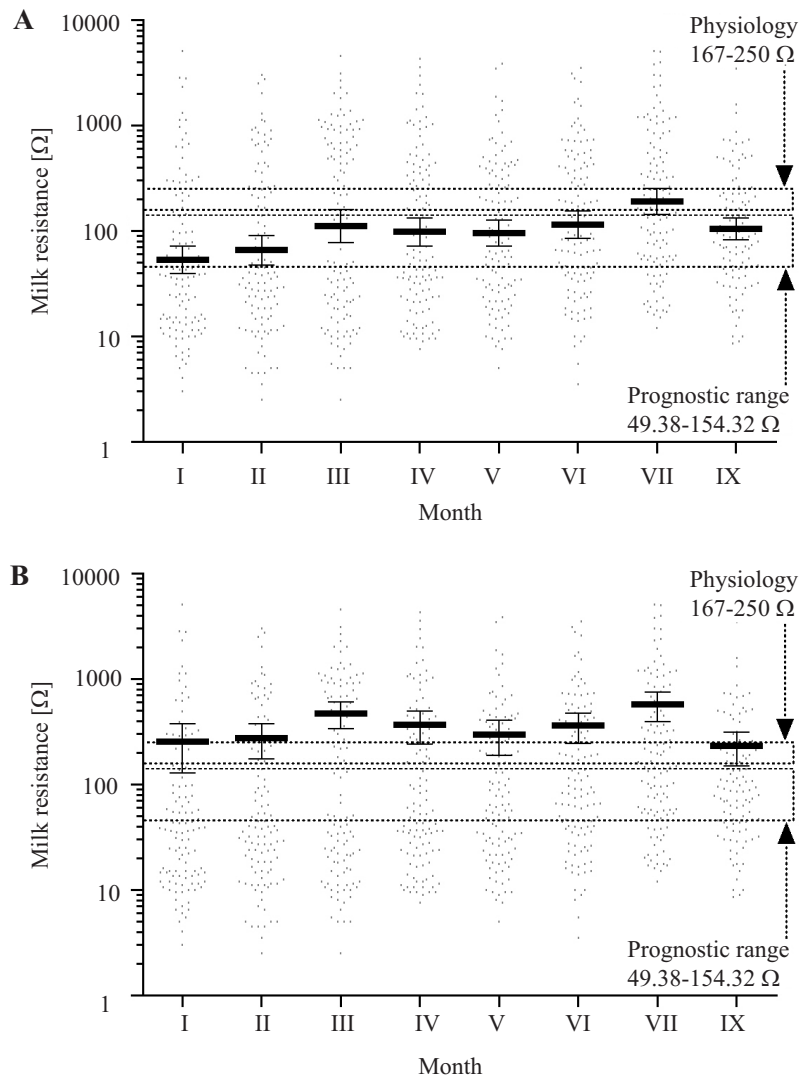


Fig. 2. Geometric (A) and arithmetical mean (B), and their confidence interval of 95% illustrating milk resistance in a herd (n=118) within the period of 9 months from calving. dots – milk resistance of specific animals; bold line – geometric mean; error bars – confidence interval 95%; Ω – Ohm.

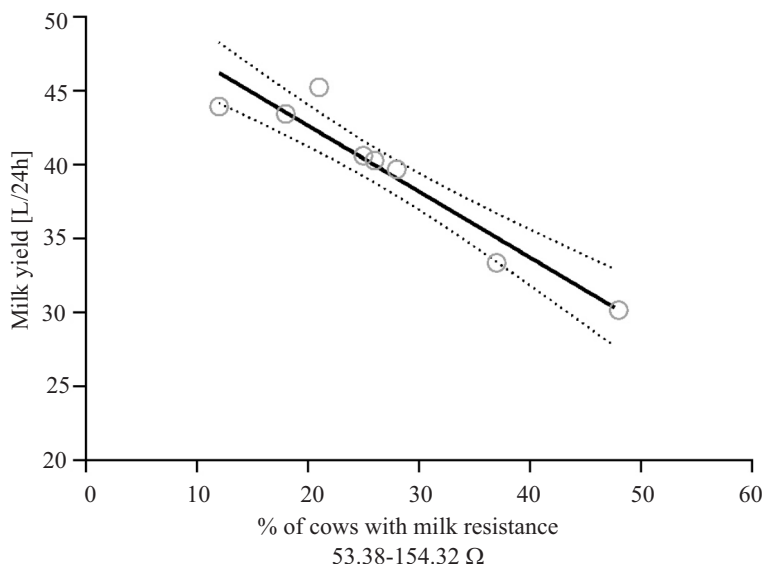


Fig. 3. Validated linear regression model (confidence interval 95%) illustrating the dependency between the percentage share in the herd of animals in which the resistance oscillates in the range of 43.38-154.32  $\Omega$  and the average daily milk yield (n=118). dotted line – confidence intervals 95%; grey circle – data per month; bold line – regression line;  $\Omega$  – Ohm.

lactation. The MDY arithmetical mean did not statistically differ in a substantial way ( $p>0.05$ ) during the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> months of lactation. A statistically significant increase of milk productivity was between the 1<sup>st</sup> and 2<sup>nd</sup> month of lactation ( $p<0.05$ ). A considerably higher milk production both in relation to the 7<sup>th</sup> and 9<sup>th</sup> month was correlated with the results of all the preceding months ( $p<0.05$ ). Detailed data illustrating changes in milk production in specific months are given in Fig. 1.

The minimum geometrical/arithmetical mean of R milk was observed during the 1<sup>st</sup> month of lactation and was 53.40/254.86  $\Omega$ . However, the maximum geometrical/arithmetical mean of R milk was observed during the 7<sup>th</sup> month of lactation and was 189.62/574.51  $\Omega$ . Both with reference to the arithmetic and geometrical mean, they were substantially different within the range of all analyzed months. The arithmetical means in every case had greater values than geometrical means. Detailed data illustrating the changes in milk production in particular months are shown in Figs. 2 A,B.

The analyses show no considerable dependency between R expressed by the average arithmetical or geometrical mean and the MDY arithmetical mean or MDY geometrical mean. Yet it is clear that there were considerable dependencies between the percentage number of cows in the herd of defined R value and MDY. The highest value of F test was assumed for the dependency between MDY and a percentage share of cows in a herd whose R oscillated within the limits of 49.38-154.32  $\Omega$ . The value of the F test was determined at 59.41 at CI 95% and  $p<0.002$ . For other studied ranges, the tested F value was lower. The as-

sumed correlation was statistically significant and predictive (Fig. 3). It was described by the curve equation  $MDY = -04461 \times R\% + 51.58$  where R% is the percentage share of cows in a herd whose R oscillated within the limits 49.38-154.32  $\Omega$  (indications Draminski Mastitis detector 96-300 or 6.48-20.25 mS/cm of conductivity). Complete predictivity of model in the above mentioned limits (:prognostic range;) was confirmed by the results of validation which were obtained at the level:  $Q^2=0.9286$ ,  $R^2=0.9305$ ,  $Q^2-R^2=0.002$ ,  $Q^2_{asym}-Q^2=0.0019$ .

## Discussion

In this study an analysis of the dependency between DMY and R of milk over 9 months was carried out. It is confirmed that analyses are enable herd be controlled health and MDY in a herd to be predicted. The dependency between R and MDY is verified on the basis of the analysis of a complete production cycle, from the beginning to the end of lactation. The profile of MDY changes during lactation has a typical course (Fig. 1). The analyses demonstrate that the R arithmetical mean in a herd does not precisely reflect the risks related to the potential influence of mastitis on herd efficiency. It is proven by comparison of the average arithmetical and geometrical R values (Figs. 2 A,B). In the analyzed case, the average arithmetical mean in particular months did not make it possible to find a relevant correlation with MDY ( $p>0.05$ ). The analyzed data, both in the case of R and MDY were characterized by high inter-individual variability. The issue of high variability of such data

was dealt with among others by the application of cumulative sum charts (Lukas et al. 2009). In this study R measured by the value of average arithmetical mean in the whole analyzed period falls within the R ranges and it is considered to be physiological (167-250  $\Omega$ ; Fig. 2B) (Ferrero et al. 2014). However, the same data illustrate a very different trend if an arithmetical mean is applied to their analysis. The R arithmetical means with reference to the analyzed herd fall within R ranges which are associated with growing mastitis (Fig. 2A). Thus, there arises the question of which value informs a breeder of possible risks in a precise and predictive way. The solution to this problem is a correlation demonstrating the dependency between the percentage share of animals in a herd whose R oscillates within the limits of 43.38-154.32  $\Omega$  a MDY.

The correlation which is determined in this study is relevant and predictive ( $p < 0.002$ ), and is confirmed by model verification using the LOO method widely applied to the dependency validation of a linear type (Todeschini et al. 2004, OECD 2007, Grabowski et al. 2012). On the basis of the results of model validation, it can be stated that the correlation is characterized by high internal performance and predictive ability ( $Q^2$ ,  $R^2$ ,  $Q^2-R^2$ ). The value of  $Q^2-R^2$  demonstrated that the current model was not overfitted. Yet the values  $Q^2$  and  $R^2$  confirm the high value of the linear fitting.  $R^2 > 0.85$  proves that the explained variance in the presented model is higher than the unexplained one. The statistical significance of the proposed regression model was positively verified by the highest F test value. The small difference between fitting and predictive ability (asymptotic  $Q^2$  rule) was proven by the acceptable low value of  $Q^2_{\text{asym}}-Q^2$ .

It should be stated that the range of R milk, which provided the highest value of F test during the search for the model characterized by the highest predictive ability ("prognostic range"), corresponds to the values of average R geometrical mean and not the arithmetical one (Fig. 2A). Only in the case of one lactation month (Viitanen and Annala 2001), the R geometrical mean in the herd is beyond the "prognostic range" in which the  $R \leftrightarrow \text{MDY}$  correlation was verified (43.38-154.32  $\Omega$ ). The average geometrical R values from all the other months fall within the range of R 43.38-154.32  $\Omega$ . The results of conducted analyses indicate that the obtained values of R milk at the level of 154.32  $\Omega$  (indications of Draminski detector  $\approx 300$ ) classify the animal to the "prognostic range" (43.38-154.32  $\Omega$ ). As demonstrated the growing percentage share of animals in the "prognostic range" is inversely proportional to MDY in a herd. The conclusions from the above presented works do not cover the results of some studies performed within this

range. The outcome of Juozaitiene team was a statistically insignificant correlation between milk yield and EC (Juozaitiene et al. 2010). Nevertheless, it shall be pointed out that the results of the presented research are frequently contradictory. Other authors report that a decrease in DMY is associated with a decrease in EC, which is consequent upon the influence of fat level on the results of analysis (Lukas et al. 2009). The relationship between MDY and mastitis seems to be clear (Wilson et al. 2004). However, depending on the mastitis progress, opinions on the impact of sub-clinical mastitis on MDY are still divided. Some works highlight a significant impact of sub-clinical mastitis on MDY, while others do not confirm these observations (Oshima et al. 1990, Koop et al. 2010).

In the case of the studies on the dependency between R and MDY, no model which would comprehensively evaluate the quality of the model and verify its predictive ability has been validated so far. The proposed model confirms that even a small excess of physiological R values of milk is a significant predictive factor with reference to MDY in a herd.

## Conclusions

The search for the dependency between milk efficiency and R value enabled to be development of a simple model described by on equation which makes it possible to predict MDY in a herd on the basis of animal inclusion in the "prognostic range". The prediction of the model was confirmed by the validation of the LOO model. The model enables the efficiency of a herd at specified percentage share of cows of defined milk R value to be determined. The obtained results also indicate that following the arithmetical R mean is not an appropriate way to monitor the risk of MDY decrease.

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