Association between electrical conductivity and milk production traits in Dairy Gyr cows

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ABSTRACT
This study investigated the association of electrical conductivity (EC) of milk with milk production and composition traits in Dairy Gyr cows. Monthly milk samples were collected on the official milk recording day for laboratory analysis of somatic cell count, EC and milk composition. A total of 680 samples from 268 cows belonging to eight herds in the southeastern region of Brazil were collected between January and July 2012. Traits were analysed by the restricted maximum likelihood method using mixed models in order to associate EC and somatic cell score (SCS) with the other traits. Mean EC and SCS were 4.90 mS/cm and 4.66, respectively, and the correlation between these traits was 0.41. Linear regression of EC on SCS indicated an increase in EC of 0.094 mS/cm per SCS. Despite the relationship between EC and SCS, the former analysed only on the official recording day may not be a reliable method for the diagnosis of mastitis. However, EC could be used as an auxiliary trait in the analysis of mastitis resistance simultaneously with somatic cell count since, in addition to the association between these two traits, the measurement of EC is an inexpensive, simple and rapid method.

1. Introduction
Selection practiced continuously on production traits has caused udder problems, increasing the occurrence of mastitis in dairy herds. As a consequence, great economic losses were incurred due to decreasing in milk production and quality, and increasing the disposal rates of animals, antibiotics and labour costs.

Somatic cell count (SCC) is the trait most commonly used in breeding programmes for the analysis of mastitis resistance. However, the heritabilities of this trait are of low to moderate magnitude, ranging from 0.04 to 0.17, a fact making selection for mastitis resistance difficult (Mrode & Swanson 2002; Andrade et al. 2007). In large countries such as Brazil, routine collection of samples for SCC is very difficult, a fact impairing control of the disease in the herds and the inclusion of this trait in genetic evaluation programmes of dairy cattle. As an alternative, the measurement of electrical conductivity (EC) of milk has been studied as a routine method to be used on dairy farms, which is relatively simple and inexpensive. The method consists of determining the concentration of ions, particularly sodium, potassium and chloride, in milk. According to Nielen et al. (1992), the concentration of potassium decreases in milk in the presence of mastitis, whereas the concentrations of sodium and chloride increase, with a consequent increase in EC.

The Dairy Gyr breed plays an important role in Brazil due to its importance for crossings with specialized taurre breeds, and is the first Zebu breed in the world to participate in an animal-breeding programme. The breed has been selected for increased milk production, but studies evaluating parameters and risk factors for mastitis in Dairy Gyr females are sparse. However, some authors recently reported a high prevalence of subclinical mastitis in herds of this breed (Porcionato et al. 2010; Malek dos Reis et al. 2011), possibly as a consequence of exclusive selection for increased milk production. In this respect, studies investigating indicator traits of mastitis such as SCC and EC, as well as their association with milk production and composition, are important.

The objective of this study was to evaluate EC as an indicator trait for udder health and its association with milk production and composition traits in Dairy Gyr cows.

2. Material and methods
The milk samples used in this study were obtained from eight Dairy Gyr herds in the southeastern region of Brazil between January and July 2012. The samples were collected monthly by the official milk recording service of the Brazilian Association of Zebu Breeders (ABCZ). A total of 680 samples were obtained from 268 cows of different ages, calving orders, lactation stages and submitted to different types of milking (manual or mechanical). The number of samples per cow varied from one (28.73% animals) to two to six (71.27% animals) in this period. In all herds, the cows were milked in the presence of the calf. Pooled milk samples were collected, that is, samples representing all...
four quarters of the udder. Samples were collected from mammary quarters that showed no evident clinical alteration in milk.

The samples were collected in duplicate, one destined for the measurement of EC and the other, containing the preservative bronopol (2-bromo-2-nitropropane-1,3-diol) and natamycin, was sent to the laboratory to determine percentages of fat (%F), protein (%P), lactose (%L) and total solids (%TS) content and SCC.

A portable EC meter (Akso – model AK 83 – Brazil) was used for the measurement of EC. The meter measures the EC, on a scale up to 200 mS/cm (milliSiemens per centimetre), with a resolution of 0.5% of full scale. The metre was calibrated with a specific standard solution before measuring the EC of milk samples. The metre electrode of the meter was washed with distilled water and dried with paper towels after each measurement. In the credentialed laboratory, milk composition was analysed with a rapid infrared analyser (Barbano & Clark 1989) and SCC was determined by flow cytometry in a Somacount 300® instrument (Bentley Instruments 1995).

Since the SCC data were not normally distributed, they were log-transformed into somatic cell score (SCS) using the formula proposed by Ali and Shook (1980):

\[
SCS = \log_2(\text{SCC}/100,000) + 3
\]

2.1. Statistical analysis

For statistical analysis, test-day milk yield (TDMY) and milk components (%F, %P, %L, %TS) were analysed as dependent variables (Model 1) using mixed models that included SCS or EC (udder health) as the covariate (linear regression). In addition to the effects of these variables related to udder health, the fixed effects of the herd, contemporary group (CG – year and month of calving), test month and the classificatory effect of the number of days in milk (stage of lactation) were also included in the model. Days in milk (stage of lactation) was divided into three periods (1–3): early lactation (up to 100 days), mid-lactation (100–200 days) and late lactation (more than 200 days). The age of the cow at calving was included as covariate (linear effect) and the minimum age of cow was 25.5 months and the maximum age was 146 months. Cows were milked twice a day, by mechanical or manual milking. The fixed effect of mechanical or manual milking was not significant in preliminary analyses and was excluded in the statistical model. The model employed in the statistical analysis for each trait was:

\[
y_{ijkl} = \mu + H_i + YM_j + S_k + M_l + a(\text{Age} - \bar{\text{Age}}) + b(\text{health} - \bar{\text{health}}) + e_{ijkl},
\]

(Model 1);

and

\[
y_{ijkl} = \text{response variable of day in milk (TDMY, %F, %P, %L, or %TS); } \mu = \text{general mean; } H_i = \text{fixed effect of herd; } YM_j = \text{fixed effect of year–month of calving; } S_k = \text{fixed effect of stage of lactation (k = 1, 2 and 3); } M_l = \text{fixed effect of month of test (l = 1, ..., 6); } a = \text{Linear regression coefficient of age of cow at calving; } b = \text{Linear regression coefficient of variables related to udder health, EC (Model 1a) and SCS (Model 1b); } e_{ijkl} = \text{random error.}
\]

In an additional analysis for TDMY (Model 2), the model included the nested effect EC as a covariate according to SCC classes, establishing limits of 250,000; 400,000; 600,000 and 750,000 cells/mL (1–4 SCC classes). The classes of SCC were defined according to the National Mastitis Council (NMC 1997) and Normative Instruction 62 (Brasil 2011). Model 2 can be described as:

\[
y_{ijkl} = \mu + H_i + YM_j + S_k + M_l + SCC_m + a(\text{Age} - \bar{\text{Age}}) + b_m(\text{EC} - \bar{\text{EC}}) + e_{ijkl},
\]

(Model 2); and

\[
y_{ijkl} = \text{response variable of day in milk (TDMY, %F, %P, %L, or %TS); } \mu = \text{general mean; } H_i = \text{fixed effect of herd; } YM_j = \text{fixed effect of year–month of calving; } S_k = \text{fixed effect of stage of lactation (k = 1, 2 and 3); } M_l = \text{fixed effect of month of test (l = 1, ..., 6); } SCC_m = \text{fixed effect of SCC class (m = 1, ..., 4)}; a = \text{Linear regression coefficient of age of cow at calving; } b_m = \text{Linear regression coefficient of EC nested by SCC class (1–4); } e_{ijkl} = \text{random error.}
\]

EC was also analysed as a dependent variable (Model 3). The model included the same fixed effects as described above, in addition to the effect of SCC, age of cow at calving and fat content as covariates (linear effect). For this analysis, milk fat content was divided into six classes: less than 2%, 2.1–3%, 3.1–4%, 4.1–5%, 5.1–6%, and >6%. Model 3 is described below:

\[
y_{ijkl} = \mu + H_i + YM_j + S_k + M_l + a(\text{Age} - \bar{\text{Age}}) + b(\text{SCS} - \bar{\text{SCS}}) + c(F - \bar{F}) + e_{ijkl},
\]

(Model 3); and

\[
y_{ijkl} = \text{response variable of electrical conductivity (EC); } \mu = \text{general mean; } H_i = \text{fixed effect of herd; } YM_j = \text{fixed effect of year–month of calving; } S_k = \text{fixed effect of stage of lactation (k = 1, 2 and 3); } M_l = \text{fixed effect of month of test (l = 1, ..., 6); } a = \text{Linear regression coefficient of age of cow at calving; } b = \text{Linear regression coefficient of SCS; } c = \text{Linear regression coefficient of class of fat content (F); } e_{ijkl} = \text{random error.}
\]

Traits were analysed by the restricted maximum likelihood method using the MIXED procedure, of the Statistical Analysis System (SAS 2003). The random effect of repeated measures per cow was considered based on a compound symmetric covariance structure. This structure was chosen according to the magnitude of Schwarz’s Bayesian information criterion (BIC). The model showing the lowest BIC value was selected (Wolfinger 1993).

3. Results

The mean EC obtained was 4.90 mS/cm and SCS mean was 4.66, which corresponds to SCC of 316,017 (+66,896) cells/mL. The mean for milk yield (14.12 kg) was superior to those reported in literature for Gyr cattle (Costa et al. 2005; Pereira et al. 2010). The means for %F, %P, %L and %TS contents were 3.84 (+1.53), 3.63 (+0.41), 4.60 (+0.35) and 9.20 (+0.48), respectively.

Table 1 shows the Pearson’s correlation coefficient (above the diagonal) and the estimated linear regression coefficients
(below the diagonal) obtained for EC of milk, SCS, TDMY and fat (%F), protein (%P), lactose (%L) and total solids (%TS) content in Dairy Gyr cows.

All Pearson’s linear correlation coefficients between SCS or EC and the other production traits were significant, but of low to medium magnitude. The correlation between EC and SCS was positive and moderate (0.41).

The records frequency and EC, SCC, %Lactose and TDMY means by SCS class are shown in Table 2. The % Lactose and TDMY were included in this analysis due to the important association of these traits with the EC and SCS, which was observed in Pearson’s correlation and linear regression coefficients in Table 1. Increasing the SCS and the SCC, the EC has an increasing trend from score 4. In scores 1, 2 and 3 progressive increase of EC did not occur. Probably, as there is no disease in lower SCC, the EC tends to a constant value, that is, without an increasing trend from score 4. In scores 1, 2 and 3 progressive increase of EC did not occur. Probably, as there is no disease to medium magnitude. The correlation between EC and SCS was positive and moderate (0.41).

The correlation and linear regression coefficients estimated for EC were relatively higher compared to those obtained when SCS was used as the covariate, according to Table 1. This finding can be explained by the fact that SCS ranged from 1 to 10 units, whereas the range of EC was only from 3.67 to 7.03 mS/cm.

Analysis including EC as covariate in the model within SCC range (Model 2) were performed to identify the association between mastitis and EC (Table 3). The SCC class were defined 250, 400, 600 and 750,000 somatic cells/mL, according to the National Mastitis Council – NMC (1997) and the Department of Inspection of Animal-Origin Products (DIPOA), through the Normative Instruction no. 62/2011 (IN 62). The NMC (1997) quotes as a reference value 250,000 somatic cells/mL to indicate that the milk is obtained from cows with mastitis (above 250,000) or physiologically healthy (below 250,000). The SCC acceptable value (NMC 1997) for bulk tank is 400,000 and 750,000 somatic cells/mL for the European Union and the USA, respectively. In Brazil, according to Normative Instruction no. 62, the acceptable value is 600,000 somatic cells/mL for Southeast, South and Midwest regions, and 750,000 somatic cells/mL for North and Northeast regions. (BRASIL 2011).

As SCC classes increased, there was also a larger distance between the estimated regression coefficients of EC within each defined class, indicating that a more antagonistic relationship between TDMY and EC tends to occur at higher SCC levels (Table 3). However, it was not possible to predict the loss in milk production due to this increased EC, since the determination coefficients for different models provided with the different classes of SCC were low, all being less than 0.19. Thus, the model was not a good indicator of the loss of milk yield due to the increase in EC, probably because the EC variable does not show a linear trend. The average TDMY by EC in the SCC

### Table 1

| Trait        | EC | SCS | TDMY | %F | %P | %L | %TS |
|--------------|----|-----|------|----|----|----|-----|
| EC           | 1  | 0.41*| -0.32*| -0.18*| 0.14*| -0.65*| -0.40*|
| SCS          | 0.094| 1   | -0.22*| 0.36*| 0.31| -0.66*| -0.19*|
| TDMY         | -2.01*| -0.26*| 1    | -0.02 NS| -0.31*| 0.30*| -0.03 NS|
| %F           | -0.54*| 0.18*| -    | 1   | 0.25*| -0.32*| 0.03 NS|
| %P           | 0.07*| 0.01 NS| -| -| -1| -0.26*| 0.63*|
| %L           | -0.36*| -0.09*| -| -| -| 1| 0.45*|
| %TS          | -0.32*| -0.08*| -| -| -| -| 1|

NS, not significant.
*Significant at the 1% probability level.
*Linear regression coefficients (Model 1a).
*Linear regression coefficients (Model 1b).

### Table 2

| SCC | N   | Frequency of records (%) | SCC | EC (mS/cm) | Lactose (%) | TDMY (kg) |
|-----|-----|-------------------------|-----|------------|-------------|-----------|
| 1   | 47  | 6.91                     | 16.43 (±5.64) | 4.69 (±0.42) | 4.93 (±0.15) | 14.56 (±4.38) |
| 2   | 53  | 7.79                     | 37.62 (±7.06) | 4.68 (±0.29) | 4.87 (±0.14) | 15.55 (±4.39) |
| 3   | 90  | 13.24                    | 71.23 (±14.50) | 4.59 (±0.34) | 4.83 (±0.18) | 16.26 (±6.04) |
| 4   | 98  | 14.41                    | 143.77 (±28.03) | 4.78 (±0.45) | 4.72 (±0.18) | 15.14 (±5.72) |
| 5   | 89  | 13.09                    | 287.37 (±56.42) | 4.86 (±0.48) | 4.67 (±0.24) | 14.13 (±5.20) |
| 6   | 91  | 13.38                    | 593.57 (±116.28) | 4.97 (±0.50) | 4.53 (±0.30) | 13.42 (±7.22) |
| 7   | 81  | 11.91                    | 1122.46 (±219.29) | 5.16 (±0.62) | 4.47 (±0.31) | 13.37 (±6.37) |
| 8   | 57  | 8.38                     | 2252.04 (±485.08) | 5.08 (±0.62) | 4.35 (±0.32) | 12.89 (±6.43) |
| 9   | 59  | 8.68                     | 4702.10 (±890.70) | 5.40 (±0.77) | 4.10 (±0.42) | 11.81 (±6.61) |
| 10  | 15  | 2.21                     | 8601.93 (±1200.97) | 5.32 (±0.46) | 4.07 (±0.28) | 10.27 (±5.58) |

*Values of SCC were divided by 1000.
4. Discussion

Mean EC values of normal milk reported in the literature for taurine breeds, ranging from 0.27 to 0.46 (Janzekovic et al. 2009; Tavares 2010). The correlation between TDMY and EC was higher than between TDMY and SCS, but both correlations were negative, suggesting milk production losses with increasing SCS or EC. The positive correlation estimated among %F and SCS (Table 1) suggests that the increase in SCS could be responsible for an increase in fat percentage. However, %F was negatively correlated with the EC, therefore an increase in the %F is related to the decrease in milk ability to conduct electrical current. This negative association is probably due to the fact that fat is a poor conductor of electricity. Among all studied traits, %L showed the strongest correlation with SCS (−0.66) and EC (−0.65) (Table 1), indicating that an increase in these traits leads to a reduction in %L, probably as a result of a decline in lactose synthesis in infected mammary glands.

Analysis of variance for dependent variables TDMY, %F, %P, %L and %TS showed a significant effect of EC on all variables, whereas SCS was not significant only for %P (Table 1). The linear regression coefficients of TDMY on EC (−2.01) and SCS (−0.26) were in the same direction, indicating that an increase in either EC or SCS values during the lactation leads to a reduction in TDMY. An increase of 1 mS/cm EC might be responsible for a decrease of 2.01 kg in milk, while for the SCS, the increase in a unit was associated with a loss of 0.26 kg of milk. However, it should be observed that the range in classes of EC is much more discreet than the class variance for SCS (1–10).

The regression coefficients estimated for %F showed opposite directions depending on the covariate used, as well as the Pearson’s correlation between the traits (Table 1). When SCS was included in the model as covariate, the coefficient was positive (0.18), indicating a small increase in %F as a function of increasing SCS. In contrast, the regression coefficient of %F on EC was negative (−0.54), a finding that can be explained by the fact that fat molecules are poor conductors of electricity. As a consequence, the higher the %F, the lower will be EC.
An increase in the lipid fraction of milk exerts an inhibitory effect on EC not only due to the reduction in the total conducting medium, but also due to the physical obstacle that fat globules pose to the migration of ions (Nielen et al. 1992). The coefficients of linear regression for %P on EC showed a significant effect, but was close to zero (0.07). When SCS was the covariate, the regression coefficient (0.01) was not significant (Table 1). These results suggest that an increase in EC or SCS did not change the total protein content of milk. However, although total protein concentration in milk remains relatively stable in the presence of a high SCS, casein content decreases, whereas albumin and immunoglobulin concentrations increase (Brito & Brito 2001). This reduction in casein content results in losses to the dairy industry since it is associated with the yield of milk-based products (Emmons et al. 1990).

For %L, the regression coefficients were significant and negative, as were the correlation coefficients, indicating that an increase in the two covariates leads to a reduction in the lactose content of milk (Table 1). The reduction in %L is associated with decreased synthesis of this component in infected mammary glands due to the damage caused to secretory cells, the utilization of lactose by intramammary pathogens and also the loss of lactose from the gland to the bloodstream because of increased permeability of the membrane that separates milk from blood, leading to the excretion of lactose in urine (Kitchen 1981; Shuster et al. 1991; Auldist et al. 1995).

The regression coefficients of %TS on EC and SCS were significant and negative (Table 1). An increase in EC or SCS is related to lower TS content. This finding is expected since, in addition to protein and mineral salts, the %TS comprises the lactose fraction of milk, a component strongly affected by an increase in SCC.

The %L and TDMY decreased with the progressive increase in the EC, SCC and SCS (Table 2). Lactose is the main factor responsible for the osmolality of milk, influencing milk volume. According to Coldebella et al. (2004), studying Holstein cows in Brazil, the average losses of milk production can reach 2.40 kg of milk per cow per day, with losses of 0.90 kg of milk in primiparous and 3.64 kg in multiparous. The losses in milk yield range from 10% to 30% by lactation (Auldist & Hubble 1998).

The results obtained by analyses of Model 2 (Table 3), for TDMY with nested effect of EC on the SCC class, showed that when the SCC limit established increased from 250,000–750,000 cells, the distance between the regression coefficients estimated for EC within each defined class also increased, indicating a trend towards a more antagonistic relationship between TDMY and EC at higher levels of SCC. However, no statistically significant difference was observed to TDMY. The average TDMY according to EC within the SCC class tended to decrease for all classes above the SCC limits established. Thus, the higher EC and SCC, the greater will be the milk production losses. However, the losses in milk yield remained nearly constant according to the class of SCC, around 0.5 kg of milk. These results could suggest that for Gyr cattle although loss due to the SCC occurs, these are smaller than those observed for taurine breeds.

Table 4 presents the records frequencies by EC and SCC classes. For class of SCC below the established limit, there is a higher proportion of records for the lower EC class. Similarly for classes of SCC above the established limit, there is a higher proportion of records in the higher EC classes. Values for EC classes lower than 4.80 had higher proportions of records for lower limits of SCC (below 250,000 to below 750,000). However, when values of EC classes reached 5.00, there was a change in the proportion of records when the SCC limits were higher (above 250,000 to above 750,000). The point at which there was a change of behaviour, that is, when the EC was between 4.81 and 5.00 mS/cm, can suggest the occurrence of mastitis in Dairy Gyr cows.

Analysing EC as the dependent variable (Model 3), the regression of EC on SCC showed a positive trend (Figure 1), suggesting a progressive increase in EC with increasing SCS class. An increase in the SCC of milk is observed in mastitis, which is due to the attempt to defend the organism by sending leukocytes to the gland as well as to the desquamation of the glandular epithelium. Furthermore, a greater flow of ions from the bloodstream to milk occurs, thus increasing EC. All of these changes occur concomitantly when the gland is colonized by microorganisms. This positive relationship between these characteristics suggests the use of EC as an auxiliary method for the diagnosis of mastitis.

It should be noted that the present study evaluated udder health based on pooled samples of milk. This approach is more practical in terms of collection and analysis, but can lead to dilution of the infected milk derived from infected mammary quarters, thus reducing the sensitivity of the test.

Point measures of EC, that is, those obtained on the monthly day of official recording, may not be good indicators of udder health since, in addition to being influenced by factors other than mastitis, the variation in EC is very subtle and it is therefore difficult to diagnose animals as sick or healthy based on a single monthly measurement. The same statement is valid for SCC, explaining the low heritability estimates found in the literature for this trait. However, in view of the low acquisition cost of EC meters and the rapid results, as well as the fact that some electronic milking machines already measure this parameter daily, additional studies should be conducted to identify the detection limits for clinical or subclinical mastitis and the interaction of pathogenic agents with EC.

Measurements of EC might be efficient in breeding programmes seeking to identify animals that are more resistant to mastitis. Some advantages exist in obtaining EC on the official recording test day; for example, EC can be measured by
the technicians themselves, permitting its large-scale measurement. Furthermore, since many commercial herds already possess electronic milking systems, the measurement of EC can be done in a simple, inexpensive and large-scale manner to permit genetic evaluation of dairy cattle for mastitis.

In view of the changes caused in milk due to the occurrence of mastitis, it is important the study of traits that may be indicative of the disease, and thus can control this occurrence in herds. The Gyr breed, although resistant, may suffer in the future as a result of selection for increasing the milk production, as already observed for European breeds. The EC is a trait correlated with mastitis and SCC and has been studied. Besides being correlated with the SCC, EC has greater heritability estimates than those reported for SCC and clinical mastitis.

5. Conclusions

The measurement of EC as an indicator trait of mastitis, evaluated only in the official milking recording, may not be a useful method to determine udder health. However, this trait could be used as a selection criterion for mastitis resistance in conjunction with SCC, especially in the case of herds that do not have access to accredited milk analysis laboratories since, in addition to the association between these two traits, the measurement of EC is an inexpensive, simple and rapid method when compared to SCC.

Acknowledgements

Thanks are extended to the Brazilian Associations ABCZ and ABCGIL for providing the data and support, especially the official testers of ABCZ; to Antonio Carlos Godoy, Department of Agriculture, Mococa/SP; to CAPES for granting the Master’s fellowship; to APTA for research support and to Instituto de Zootecnia.

Disclosure statement

No potential conflict of interest was reported by the authors.

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