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Milk electrical conductivity in Manchega ewes: Variation throughout milking and relation with mammary gland health status

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Abstract: The aim of this work was to study the effect of milking fraction and mammary gland health status on the electrical conductivity (EC) of milk from Manchega ewes, considering also the lactation number. To this end, we also studied the relationship of EC with milk macrocomposition, and the relation existing between EC and somatic cell count (SCC). Finally, the use of EC thresholds as a mastitis detection method (sensitivity, specificity, positive (PPV) and negative predictive value (NPV)) was assessed in each of the three fractions: first streams (F1), machine milk (F2) and stripping milk (F3). Milking fraction, mammary gland health status and lactation number had a significant effect on EC and SCC. In the case of EC, the milking fraction caused a more pronounced effect than health status of the glands or number of lactation (F = 19.95, 15.88 and 6.55, respectively; $P < 0.5$). In SCC, the gland health status caused the most pronounced effect followed by milking fraction and lactation number (F = 112.02, 6.89, and 5.28, respectively; $P < 0.05$). Changes in the milk composition, especially fat and lactose contents, explained the EC variation to a great extent. For the same EC threshold, specificity and sensitivity varied slightly depending on the milking fraction. NPV above 80% was obtained in the three milking fractions and at all EC thresholds tested, but PPV was only higher than 20% as of the threshold of 4.5, 4.4, and 4.2 mS/cm in F1, F2 and F3, respectively. From the results obtained, we concluded that the algorithm design for mastitis detection in sheep should include those factors affecting the composition and which therefore cause variations in EC, such as milking fraction, individual differences, lactation stage or lactation number.

Keywords: mastitis detection; milking fraction; sheep; sensitivity; specificity

The main advantage of measuring electrical conductivity (EC) as a mastitis detection method is the ease with which it can be automated by installing EC meters in the short milk tube. This allows us to record and analyse data from each gland as it is milked. Depending on the algorithm used, the data are compared with previous findings from the same gland or animal, allowing early detection of the disease (Romero et al. 2012). For this reason, the EC of milk has been widely studied in cattle as a mastitis detection method, and to a lesser extent in sheep.

Hamann and Zecconi (1998) found that 60% of EC variations in cow's milk were due to its chlorine, sodium and potassium content, with results similar to those obtained by Diaz et al. (2011) in goats. In sheep, Romero et al. (2017) reported that the fat content is largely responsible for variations in milk

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EC, followed by sodium and chloride content. In all three works, it was observed that milk EC not only varied due to the mammary gland health status, but also it was affected by other physiological factors such as lactation number, lactation stage and (morning or evening) milking session in ewes.

In bovines, it was observed that the milking fraction affected the EC of milk and its capacity for mastitis detection. Bansal et al. (2005) revealed that the EC decreases as milking progresses, while the difference in the EC of healthy and mastitic glands increases, concluding that the best results for mastitis would be achieved in stripping milk. In contrast, Bruckmaier et al. (2004b) and Lien et al. (2005) reported that the biggest difference in the EC of healthy and mastitic glands occurred in the first streams, stating that this fraction would be better for detecting the disease.

In small ruminants, there are few studies on the effect of milking fraction on EC and its mastitis detection capacity. Romero et al. (2012) reported in goat that EC decreased as milking progressed both in healthy and mastitic glands, although this did not affect the potential for mastitis detection as the same sensitivity was observed in the three fractions studied.

In ewes, the early detection of mastitis becomes really important as most of the milk obtained is destined for cheese production. Leitner et al. (2004) stated that curd production is lower in milk from infected glands than from healthy ones as a longer time is needed for its formation, in results coinciding with those reported by Bianchi et al. (2004). There are a few works available in this species on factors affecting EC in milk and its ability to detect mastitis. Peris et al. (1998) reported that mastitis causes an increase in the EC of milk, proposing two thresholds for mastitis detection. An absolute threshold of 5 mS/cm, whereby 87.9% of samples were correctly classified, and another one consisting in the difference in EC between the two glands, which was used to correctly classify 89.1% of samples, when the difference was greater than 0.3 mS/cm.

It has been observed that the milking fraction has an effect on milk EC, but it is necessary to know how it affects its usefulness for detecting mastitis in sheep. To this end, we proposed this experiment to study the effect of milking fraction on EC, as well as the potential for mastitis detection depending on the milking fraction in which the variable is measured.

MATERIAL AND METHODS

Location and animals used. The animals, Manchega sheep, were kept on an intensive dairy farm. The reproductive rate was one parturition per year, with lambs weaned at birth and reared by artificial feeding. Postpartum, the ewes were milked twice daily (at 8:00 and 16:00 h) in a Casse type milking parlour 1 × 12 × 12 (number of platforms × number of places/platform × number of milking units/platform) with the following milking parameters: 36 kPa vacuum level, 180 pulsations per min and 50% pulsation ratio.

A diet, consisting of 2.5 kg per day of a complete feed (unifeed type) and straw *ad libitum*, was maintained constant throughout lactation.

Experimental design. To avoid variations in EC due to environmental factors, sampling was performed in a single session. Prior to sampling, three checks were performed at weekly intervals to determine the mammary gland health status, starting two months postpartum. At each control, samples were taken for bacteriological analysis of each gland and subsequently to analyse somatic cell count (SCC) in the glands of 103 lactating ewes present on the farm (86 multiparous and 17 primiparous). On the second day of sampling, in addition to the previous ones, three 100 ml samples were collected from each gland of the ewes whose production was higher than 500 ml (178 glands): first 100 ml, hand milked (F1); machine milk, collected using volumetric meters during mechanical milking (F2); stripping milk, collected by hand after machine milking (F3).

Variables analysed. For bacteriological analysis immediately after aseptic collection of 5 ml milk prior to milking, 20 µl of milk was seeded onto sheep blood agar plates (Biomerieux, France) which were then incubated at 37°C, performing bacterial growth counts at 24, 48 and 72 h after seeding. For classification into positive or negative culture, we followed the National Mastitis Council recommendations (Harmon et al. 1990). The culture was considered positive when at least five identical growth colonies were observed, and negative if there was no growth at 72 h after seeding.

EC (mS/cm) was measured immediately after collection using a laboratory conductivity meter GLP 32 (Crison, Spain) with automatic temperature compensation to 25°C.

After measuring the EC, two aliquots of 30 ml were separated from each of the three fractions.

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Azidiol was added to one of them for preservation and subsequent delivery to the Inter-Professional Milk Laboratory of the Valencia Community (Spain), where the SCC analysis (× 10^3 cells/ml) was performed by fluorometric method (Fossomatic 5000; Foss, Denmark). The other aliquot was used for determination of macroscopic composition (fat, casein (Cas), whey protein (WP), lactose monohydrate (Lac) and ash). Milk composition was analysed using Fourier Transform Infrared Spectrophotometry (FT120 Milko-Scan; Foss) with a commercial calibration (Improved Milk Calibration) from Foss Electric validated according to ISO 21543:2006. The milk samples were previously heated to 40°C. The results were expressed as a wet matter percentage.

Gland health status definition. The glands were classified by health status, according to the bacteriological analysis. Glands with a positive bacteriological reading or SCC higher than 400 × 10^3 cells/ml were classified as mastitic. Glands in which the bacteriological analysis was negative or SSC lower than 400 × 10^3 cells/ml were classified as healthy glands.

Statistical analysis. To normalize the data distribution and apply statistical analyses, we transformed the EC and SCC variables into base-10 logarithm (LEC, LSCC). Means and standard deviations of milk composition, LSCC and LEC at every milking fraction were calculated (Proc Means, SAS Version 9.2, 2012).

The relationship of the dependent variables LEC and LSCC with the fixed effects studied was analysed using a linear mixed model (Proc Mixed, SAS Version 9.2, 2012). The following fixed effects were considered: gland health status (INFi, with 2 levels: healthy or mastitic), lactation number of the ewe (NPj, with 2 levels: primiparous or multiparous), milking fraction (FRAk, with three levels: first 100 ml (F1), machine milk (F2) or stripping milk (F3)) and the interaction between gland health status and milking fraction. The interactions of the lactation number with gland health status or with milking fraction were not significant, so they were ruled out of the model. The random effect considered was the gland (right or left) nested to the ewe, to model the covariance between observations of the glands within each ewe (Barkema et al. 1997). We used a "compound symmetry" type model of fit for the correlation of variance among repeated measurements from the same animal. The model using this hierarchical structure provided the best fit for the data at every studied variable when compared with different models considering other covariance and hierarchical structures (as assessed using Bayesian and Akaike's information criteria). The correlation of EC with SCC (Proc Corr, SAS Version 9.2, 2012) was analysed globally and in each of the three fractions studied.

The relation between milk composition (Fat, Cas, WP, Lac, Ash) and EC (Proc Reg, SAS Version 9.2, 2012) was analysed. Some samples were damaged during transport to the laboratory for the analysis of macroscopic milk composition, so those glands for which there were no composition data in their three fractions were not taken into account for the overall study of the relation of EC with milk composition.

The evolution of the sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) for detecting mastitis was calculated based on different EC thresholds for each of the three milking fractions. We defined sensitivity as the percentage of positive cases which the method is able to detect, or the probability that a positive sample would be classified as such (true positives over the sum of true positives and false negatives). Specificity was defined as the percentage of negative cases that the method is able to detect or the probability that a negative sample will be classified as such (true negatives over the sum of true negatives and false positives). The positive predictive value was defined as the probability that a gland is mastitic when the sample is classified as positive (true positives over the sum of true positives and false positives). Finally, the negative predictive value was defined as the probability that a gland is not mastitic when the sample is classified as negative (true negatives compared to the sum of true negatives and false negatives).

All experiments were performed in compliance with the Spanish and European Union laws on animal care in experimentation (Spanish Real Decreto 53/2013 and European Directive 2010/63 EU) and have been analysed and approved by the Animal Experimentation Ethics Committee of our Institution and by the Competent Authority.

RESULTS AND DISCUSSION

The means of milk components (Table 1) in F2 (machine milk) were similar to those obtained

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Table 1. Means and standard deviations (SD) of milk components by fractions

Milk component	Fraction 1 (n = 137)	Fraction 2 (n = 142)	Fraction 3 (n = 129)
LEC	0.62 ± 0.07	0.61 ± 0.07	0.58 ± 0.06
(Antilog, mS/cm)	4.17 ± 1.17	4.07 ±1.17	3.80 ± 1.15
LSCC	2.04 ± 0.61	2.05 ± 0.58	2.21 ± 0.54
(Antilog, × 10^3 cells/ml)	109.65 ± 4.08	112.20 ± 3.80	162.18 ± 3.47
Fat (%)[1]	5.73 ± 1.99	7.02 ± 1.94	9.13 ± 2.47
Casein (%)[1]	5.02 ± 0.90	4.89 ± 0.83	4.94 ± 0.80
Serum protein (%)[1]	0.82 ± 0.23	0.75 ± 0.21	0.66 ± 0.17
Lactose (%)[1]	4.59 ± 0.57	4.56 ± 0.55	4.56 ± 0.32

LEC = electrical conductivity logarithm, LSCC = somatic cell count logarithm, n = number of observations
[1]wet matter

by Marti de Olives et al. (2015) in a study carried out in Manchega sheep. Significant effects of the milking fraction, mammary gland health status and lactation number of ewes were observed in the two variables studied. In the case of LEC, the milking fraction caused a more pronounced effect than health status of the glands or number of lactation (F = 19.95, 15.88 and 6.55, respectively). In LSCC, the gland health status caused the most pronounced effect (F = 112.02), followed by milking fraction (F = 6.89) and lactation number (F = 5.28). The interaction of health status with milking fraction had a significant effect only in LSCC, whereas in EC it was not significant (Table 2).

EC in mastitic glands was higher than that found in healthy glands (4.64 vs 3.82 mS/cm) (Table 3). As for milking fraction, the highest EC value was recorded in F1 (4.27 mS/cm), and fell off significantly as milking progressed, being 4.14 and 4.06 mS/cm in F2 and F3, respectively (Table 4), which also agrees with several studies carried out in cattle (Bruckmaier et al. 2004a; Bansal et al. 2005; Lien et al. 2005) and goat (Romero et al. 2012). When distinguishing between healthy and mastitic glands, a similar evolution was observed (Table 5), with the EC of healthy glands (4.02, 3.94, 3.81 mS/cm for F1, F2 and F3, respectively) significantly lower than the EC in mastitic glands (4.54, 4.34, 4.32 mS/cm for F1, F2 and F3, respectively) in the three milking fractions. The difference in EC between healthy and mastitic glands was slightly higher in F1 (0.52, 0.40, 0.41 mS/cm for F1, F2 and F3, respectively), in agreement with the studies performed in cattle by Bruckmaier et al. (2004b) and Lien et al. (2005), who observed that the greatest difference between the EC of healthy and infected glands occurred in the first streams, stating that this fraction would be better for detecting the disease. In contrast, Bansal et al. (2005), also in cattle, stated that it would be better to use stripping milk than the first streams in

Table 2. Statistical analysis result (F value and significance level) of LEC and LSCC variables

Effect	LEC (n = 534)	LSCC (n = 534)
Fraction	19.95***	6.89***
Health status	15.88***	112.02***
Lactation number	6.55*	5.28*
Fraction × infective status	1.98[ns]	8.82***

LEC = electrical conductivity logarithm, LSCC = somatic cell count logarithm, n = number of observations
*P < 0.05, **P < 0.01, ***P < 0.001, ns = non-significant

Table 3. LEC and LSCC means comparison results according to health status of glands

Health status	Mean LEC (Antilog, mS/cm) (n = 534)	SE	Mean LSCC (Antilog, × 10^3 cells/ml) (n = 534)	SE
Healthy	0.5936 (3.92)	0.0063	1.9849 (96)	0.0419
Mastitic	0.6432 (4.39)	0.0122	2.8663 (735)	0.0814
P-value	***		***	

LEC = electrical conductivity logarithm, LSCC = somatic cell count logarithm, n = number of observations, SE = standard error
***P < 0.001

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Table 4. LEC and LSCC means comparison results according to milking fraction

Fraction	Mean LEC (Antilog, mS/cm) (n = 534)	SE	Mean LSCC (Antilog, × 10^3 cells/ml) (n = 534)	SE
First streams (F1)	0.6304[a] (4.27)	0.0077	2.3954[a] (248)	0.0522
Machine milk (F2)	0.6169[b] (4.14)	0.0077	2.3952[a] (248)	0.0522
Stripping milk (F3)	0.6080[c] (4.06)	0.0077	2.4862[b] (306)	0.0522

LEC = electrical conductivity logarithm, LSCC = somatic cell count logarithm, n = number of observations, SE = standard error
[a–c]different superscripts in the same column indicate significant differences

order to discriminate between healthy and mastitic glands, as at the end of milking the differences between the studied variables increased.

The SCC of mastitic glands (735 × 10^3 cells ml) was higher than 96 × 10^3 cells/ml recorded in healthy glands (Table 3), an increase being observed as milking progressed, in contrast to what happened in EC, where the highest value (306 × 10^3 cells/ml) was in F3, while only 248 × 10^3 cells/ml were recorded in F2 and F1. This is consistent with what was found by Bansal et al. (2005) in cattle, who as a general observation noted that the SCC of the stripping milk fraction was significantly higher than that recorded in the machine milk and the initial streams. Differentiating by health status and fraction (Table 6), in all fractions SCC was significantly higher in the mastitic glands compared to the healthy ones. Regarding the evolution during milking, the same behaviour was observed in healthy glands when analysing all the data together. The SCC increased in line with milking, with the SCC in F3 significantly higher than that recorded in F1 and F2. However, no significant differences were observed between the SCC of the different fractions in mastitic glands, which shows that the interaction of health status with milking fraction was significant. The outcomes obtained in the mastitic glands contradict the results reported by Bansal et al. (2005) in cattle, and Romero et al. (2012) in goat, who also found high SCC in the stripping milk fraction, even in mastitic glands. The correlation coefficients between the EC and SCC obtained were significant but moderate when the data were studied both jointly (r = 0.47) and separately (r = 0.49, 0.50, and 0.45 for F1, F2 and F3, respectively). In all cases the scores were higher than those recorded by Caria et al. (2016) in Sarda sheep (r = 0.31). McDougall et al. (2001) found a correlation of –0.37 between SCC and impedance (inverse of EC), stating that the impedance would not be a good indicator of mastitis in sheep according to the results obtained in that work.

In studying the relationship of EC with the chemical composition, a high coefficient of determination

Table 5. LEC according to health status of the glands and the fraction studied

Fraction	Healthy glands		Mastitic glands		P
	mean LEC (Antilog, mS/cm) (n = 534)	SE	mean LEC (Antilog, mS/cm) (n = 534)	SE	
First streams (F1)	0.6042[a] (4.02)	0.0065	0.6566[a] (4.54)	0.0127	***
Machine milk (F2)	0.5961[b] (3.94)	0.0065	0.6376[b] (4.34)	0.0127	***
Stripping milk (F3)	0.5806[c] (3.81)	0.0065	0.6354[b] (4.32)	0.0127	***

LEC = electrical conductivity logarithm, n = number of observations, SE = standard error
[a–c]different superscripts in the same column indicate significant differences
***P < 0.001

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Table 6. LSCC according to health status of the glands and the fraction studied

Fraction	Healthy glands		Mastitic glands		*P*
	mean LSCC (Antilog, × 10^3 cells/ml) (n = 534)	SE	mean LSCC(Antilog, × 10^3 cells/ml) (n = 534)	SE	
First streams (F1)	1.9044[a] (80)	0.0439	2.8864 (770)	0.0868	***
Machine milk (F2)	1.9393[a] (87)	0.0439	2.8511 (710)	0.0868	***
Stripping milk (F3)	2.1111[b] (129)	0.0439	2.8613 (727)	0.0868	***

LSCC = somatic cell count logarithm, n = number of observations, SE = standard error

[a,b]different superscripts in the same column indicate significant differences

***$P < 0.001$

was obtained by studying the data both jointly (R^2 = 0.89) and in each of the milking fractions (R^2 = 0.94, 0.94 and 0.86 for F1, F2 and F3, respectively) (Table 7). Overall, the component that explained the greatest variance in EC was fat, followed by whey protein and lactose; all three variables presented a negative relation with EC. In fractions 2 and 3, it was also fat which scored a higher R^2, followed by whey protein and lactose. This is in agreement with Prentice (1962) and Mucchetti et al. (1994), who observed that fat globules increased the actual distance in the migration of ions which tended to cross over between the two conductivity meter electrodes. In sheep, McKusick et al. (2002) found that the fat content of alveolar milk was significantly higher than that measured in cisternal milk. Furthermore, and according to the above-mentioned, in cattle Nielsen et al. (2005) and Bansal et al. (2005) observed that the fat content of milk tended to increase during milking. This, along with the fact that in F3 the greatest EC variance was due to the fat percentage in milk, would

Table 7. Relation between EC and macro composition of milk

	Variables	Statistics			Model	
		parameter	SE	partial R^2		
Global	intercept	14.2502	0.2406		n	297
	fat (%)[1]	–0.1846	0.0048	0.4495	R^2	0.8908
	lactose (%)[1]	–0.1703	0.0400	0.3048	F-value	780.35
	serum protein (%)[1]	–1.4056	0.7422	0.1365	P-value	< 0.0001
Fraction 1	intercept	12.5426	0.1726		n	137
	lactose (%)[1]	–0.1637	0.0098	0.6066	R^2	0.9468
	fat (%)[1]	–0.2825	0.0197	0.2582	F-value	788.32
	casein (%)[1]	–1.2962	0.0321	0.0802	P-value	< 0.0001
Fraction 2	intercept	14.5675	0.3909		n	142
	lactose (%)[1]	–1.7620	0.0613	0.7145	R^2	0.9423
	fat (%)[1]	–0.1984	0.0092	0.1987	F-value	750.96
	serum protein (%)[1]	–1.3229	0.1587	0.0291	P-value	< 0.0001
Fraction 3	intercept	14.2988	0.4421		n	129
	fat (%)[1]	–1.4643	0.1394	0.3886	R^2	0.8630
	lactose (%)[1]	–0.1658	0.0078	0.3535	F-value	262.48
	serum protein (%)[1]	–1.7460	0.0753	0.1208	P-value	< 0.0001

SE = standard error, R^2 = regression coefficient, n = number of observations

[1]wet matter %

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explain why the EC in that fraction was lower than that found in the other two fractions. In contrast, lactose is responsible for most of the EC variance in F1 and F2 (R^2 = 0.61 and 0.71, respectively), fat being relegated to the second place, and casein content to the third. In this fraction, whey protein showed no significant effect on EC variation, so it was removed from the model. In the three fractions, a negative and significant relationship between lactose content and milk EC was observed, in agreement with that reported by Fernando et al. (1985) in cattle and Caria et al. (2016) in sheep. According to Hamann and Zecconi (1998), lactose is the most important milk component in regulation of the osmotic pressure of milk. For the same osmotic pressure, the lactose content is inversely proportional to the Cl^- concentration. The passage of chloride ion to the alveolar lumen from the bloodstream is favoured by the deterioration of the epithelial cell membranes of the mammary gland, which is offset by a decrease in lactose. At the same time, the deterioration of lactocytes also produces a decrease in lactose synthesis (Shuster et al. 1991). According to the above-mentioned, an intramammary infection may cause a drop in the amount of lactose, ranging from 3% observed by Bianchi et al. (2004) to 25% obtained by Leitner et al. (2004). There are two reasons for the increased EC recorded in the three milking fractions from mastitic glands. On the one hand, it is explained by reduced lactose synthesis caused by an intramammary infection settling in, and on the other, by the reduction in fat synthesis observed by Albenzio et al. (2002), Bianchi et al. (2004), Leitner et al. (2004), and Santos et al. (2007) in milk from sheep mastitic glands.

Mastitis detection by absolute EC thresholds. Slight variations were observed in sensitivity and specificity recorded in the three fractions for the same EC threshold (Figure 1). Sensitivity decreased as the EC threshold increased, while a simultaneous increase in specificity was observed. This is so because at very low thresholds most mastitic glands will be classified as such, but many healthy glands may also be classified as mastitic; in other words, the false negative rate is very high (low PPV). As reported by Romero et al. (2017), the cut-off point of the curve traced by the sensitivity with that of the specificity varied with the milking fraction, occurring at 4.2 mS/cm in F1 and 4.1 and 4 mS/cm in F2 and F3, respectively. The sensitivity and specificity found at this point ranged around 50% in all three cases, those recorded in F3 being slightly higher, as observed by Bansal et al. (2005) in cattle. Contrarily, in goat Romero et al. (2012) found no differences in sensitivity and specificity in the three milking fractions, regardless of the EC threshold applied.

The recorded PPV was less than 20% in the three fractions with thresholds lower than 4.3 mS/cm (Figure 2). At higher thresholds, the PPV improved, but we must bear in mind that the sensitivity for these thresholds was below 50%. The NPV in the three fractions, and for all the thresholds proposed, was greater than 80%. Romero et al. (2017) also observed that the NPV of the method was higher than the PPV, concluding that the use of EC to detect mastitis would be more accurate when discarding healthy glands than for detecting mastitic glands.

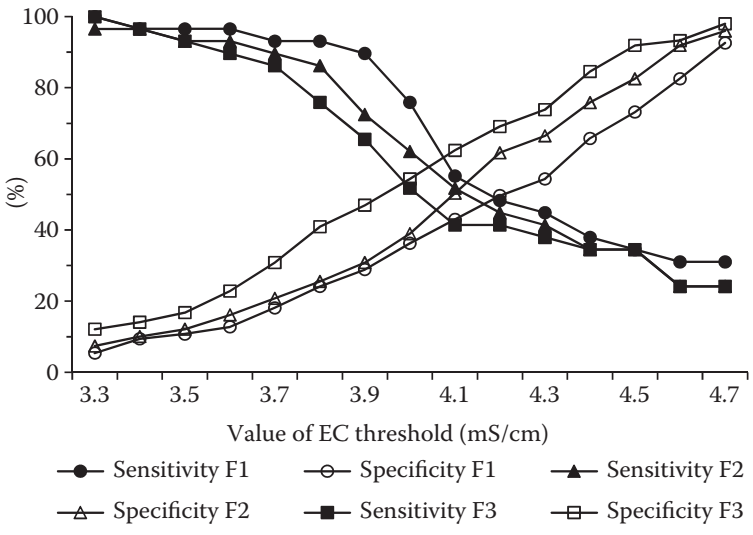

Figure 1. Sensitivity and specificity of electrical conductivity (EC) reading for mastitis detection depending on milking fraction

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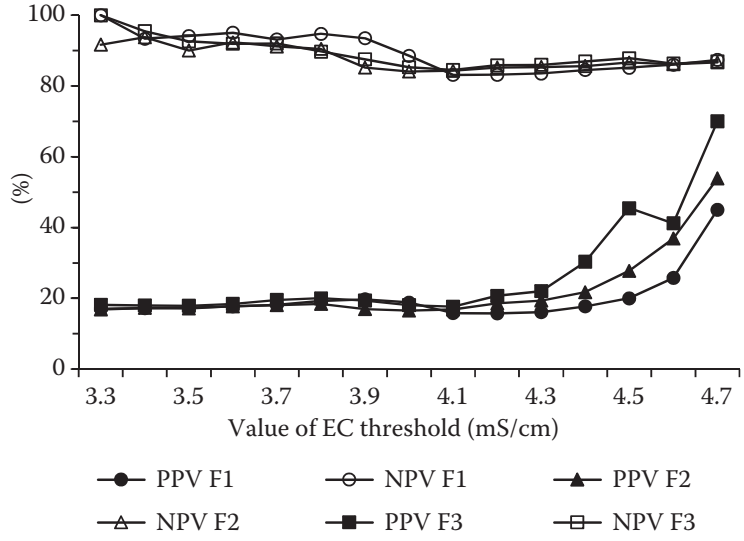

Figure 2. Positive predictive value (PPV) and negative predictive value (NPV) of electrical conductivity (EC) reading for mastitis detection depending on milking fraction

In glands causing concern, the use of other methods would be recommendable to confirm the disease and avoid unnecessary treatment costs.

The results reported by Peris et al. (1998) in sheep were better than those obtained in this work, being able to correctly classify 87.9% of samples, achieving 60.2% sensitivity and 91.4% specificity by setting a threshold of 5 mS/cm. These outcomes improved considerably when taking into account the difference in EC between both glands of the same animal (70% sensitivity, 93% specificity and 89.1% of samples classified correctly for a difference between glands greater than 0.3 mS/cm).

The results obtained corroborate previous studies in cattle (Bruckmaier et al. 2004a, b; Lien et al. 2005; Bansal et al. 2005) and goat (Romero et al. 2012) showing the variations that occur in EC and SCC according to the milking fraction, causing a variation in the mastitis detection capacity depending on the sampling fraction. Nevertheless, in our study this variation was not very pronounced.

CONCLUSION

The EC of milk varies depending on the milking fraction in which it is measured, both in healthy and mastitic glands, due to differences in the composition that take place, with a slightly higher sensitivity observed in the first 100 ml (hand milked), while specificity and PPV were slightly higher in stripping milk, collected by hand after machine milking.

This must be taken into account when using the EC variable as a method of mastitis detection, so that when it comes to designing algorithms for mastitis detection, those factors which affect the composition and therefore cause variations in EC should be included, such as milking fraction, individual differences, lactation status or lactation number.

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