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RESEARCH ARTICLE
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The use of Na^+ and K^+ ion concentrations as potential diagnostic indicators of subclinical mastitis in dairy cows

Abdul Wahid Haron[1,3], Faez Firdaus Jesse Abdullah[1,3], Abdulnasir Tijjani[2], Yusuf Abba[2], Lawan Adamu[1], Konto Mohammed[1], Aisyah Munira Mohammed Amir[1], Mohammad Abubakar Sadiq[1] and Mohd Azmi Mohd Lila[2]

1. Department of Veterinary Clinical Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; 2. Department of Veterinary Pathology and Microbiology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; 3. Research Centre for Ruminant Disease, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Corresponding author: Abdul Wahid Haron, e-mail: wahidharon@gmail.com, FFJA: jesseariasamy@gmail.co, AT: nasirvet69@gmail.com, YA: yabbavet@gmail.com, LA: drlawan3758@yahoo.com, KM: kontomohammed@yahoo.com, AMMA: aisyahmunira@gmail.com, MAS: masadiqvet@gmail.com, MAML: azmi@upm.edu.my

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Abstract

Aim: This study was conducted to evaluate the concentrations of sodium (Na^+) and potassium (K^+) ions in milk of lactating dairy cows with and without subclinical mastitis as putative indicators for detecting subclinical mastitis in dairy cows.

Materials and Methods: Thirty seven lactating dairy cows were screened for the evidence of subclinical mastitis using California mastitis test (CMT). The lactating dairy cows were categorized as CMT-Positive (CMT-P; n=20) and CMT-Negative (CMT-N; n=17) based on whether they were positive or negative for CMT using a standard kit. The CMT-P lactating dairy cows were further sub divided into subclinical 1+ (S1+; n=6), subclinical 2+ (S2+; n=9), and subclinical 3+(S3+; n=5). Direct microscopy somatic cell count (SCC) was used to determine the SCC using Wright's stain. The samples were filtered and diluted at 1:100 dilutions before being measured for the concentrations of Na^+ and K^+ using atomic absorption spectrophotometer.

Results: There was a significant increase ($p<0.05$) in SCCs and Na^+ concentration in the milk of CMT-P dairy cows, with a mean Log_{10} SCC score of 5.35±0.06 cells/ml and mean Na^+ concentration of 232±19.1 mg/dL. However, there was a significant reduction ($p<0.05$) in the concentration of K^+ (123±7.6 mg/dL) in the milk samples of the CMT-P cows. There were significant differences ($p<0.05$) in SCC, Na^+ and K^+ concentrations between milk samples from the CMT-N dairy cows and CMT-P subgroups; S1+, S2+, and S3+ respectively. Potassium (K^+) concentration had a significant strong negative correlation with sodium (Na^+) concentration ($r=-0.688$; $p<0.01$) and weak positive correlation with SCC ($r=-0.436$; $p<0.01$). The sensitivity of using Na^+ and K^+ concentrations as detection indices for sub-clinical mastitis is 40% and 90%, respectively, while the specificity of each was 100%.

Conclusion: This study thus shows that evaluation of Na^+ and K^+ concentrations from milk samples of dairy cows with sub clinical mastitis can be of immense diagnostic benefit and will compliments detection using CMT alone.

Keywords: potassium ion, sodium ion, somatic cell count, subclinical mastitis.

Introduction

Mastitis is defined as an inflammation of the mammary gland. Mastitis can be divided into two broad categories: Clinical; where changes in the milk and the udder can be visually detected, and subclinical; where the changes can be more subtle and require the use of laboratory tests to detect it [1]. The somatic cell count (SCC) of milk is widely used to monitor udder health and milk quality. The SCC is a count used to screen epithelial cells that have been shed from the lining of the gland and white blood cells (leucocytes) that have entered the mammary glands in response to injury or infection [2]. Lymphocytes, macrophages and polymorphonuclear cell counts can identify inflammatory processes in quarters with low SCC that are otherwise considered healthy [3]. During the inflammatory process, a significant increase in SCC is due to the influx of neutrophils into milk from the infected tissue [4]. Changes in the permeability of the membranes would lead to increased leakage of blood components into the udder and changes in the composition of milk [5].

An increase in the SCC is used as an indicator of udder health for management and selection purposes, as well as for certification of milk quality [6,7] reported a significant alteration with an increase in Na^+, Cl^- and a decrease in K^+ from milk of Holstein Haryana Cross cows with subclinical mastitis. In another study by Gera *et al.* [8], a significant increase in iron, zinc and cobalt were observed in milk from a cow with subclinical mastitis [9] reported that amino acid levels significantly increases in cows with subclinical mastitis, with a measured value 20 times higher than in normal cow milk. A study by Ambade *et al.* [10] also stated that there was a significant increase in the pH and lactate dehydrogenase content from milk of cows with clinical and subclinical mastitis. Several studies have been done on the effects of SCM on electrolyte concentration in cow milk. In Malaysia, there is a paucity of information on evaluation sodium and potassium ions as putative diagnostic indicators for detection of subclinical mastitis.

Therefore, the aim of this study is to evaluate sodium (Na^+) and potassium ion (K^+) concentrations from milk samples of cows with sub clinical mastitis and to use them as putative indicators for the detection of subclinical mastitis in dairy cows.

Materials and Methods

Ethical approval

The study protocol followed the ethical guidelines on the proper care and use of animals and had been approved by the Institutional Animal Ethics Committee Universiti Putra Malaysia. Reference number: PM/IACUC/FYP-2013/FPV.043 and FPV/FYP/2013/059

Animal selection and detection of subclinical mastitis

37 lactating dairy cows from five dairy herds in Selangor were randomly selected for this study. Milk samples (5 ml) were collected from each lactating cow and screened for subclinical mastitis using the California mastitis test (CMT) kit. Negative milk samples (CMT-N) showed no or trace reaction, while the positives samples (CMT-P) were rated as 1+, 2+ and 3+ based on whether the reaction was weak, distinct or strong respectively. The positive animals were then subsequently divided into three groups as subclinical 1 (S1+); subclinical 2 (S2+) and subclinical 3 (S3+) for ease of categorization [10].

Direct microscopy SCC

10 µl of milk sample was pipetted onto a clean glass slide. The milk was then spread using a spreader to form a circular smear of about ±1.5 cm in diameter. The slides were air dried overnight and then stained with Wright's stain. Somatic cells were counted from 30 microscopic fields using 40×10 magnifications. The SCC per ml was calculated by multiplying the mean SCC per microscopic field with the dilution factor.

Electrolyte evaluation

20 ml milk solution with dilution of 1:100 was prepared, filtered using filter paper and then left to recondition at room temperature before being analyzed using atomic absorption spectrophotometer for electrolyte content in mg/dL.

Statistical analysis

The SCC was reduced to Log_{10}SCC and used as an independent t-test and also ANOVA was used with Tukey Kramer to test for the significant differences in means of SCC, Na^+ and K^+ concentrations between milk samples from the CMT-P and CMT-N dairy cows while, pairwise correlation coefficient was used to test for the linear relationship between SCC, Na^+ and K^+ concentrations using the statistical software package (JMP® 11. NC: SAS Institute Inc. USA) Analyses were considered as significant at $p<0.05$. Receiver operating characteristic curve was used to test for the sensitivity and specificity of Na^+ and K^+ as potential markers using MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014).

Results

Following CMT, it was observed that 17 cows were CMT-N, 20 were CMT-P. The CMT-P dairy cows comprised of S1+=7, S2+=9 and S3+=4.

The means of the variables SCC, Na^+ concentration and K^+ concentration between the CMT-P and CMT-N lactating dairy cows are shown in Table-1. There was a significant increase ($p<0.05$) in the SCC and Na^+ concentration in the CMT-P dairy cows compared to SCC and Na^+ concentration in the CMT-N dairy cows.

The K+ concentration was lower in the CMT-P group than observed in the CMT-N dairy cows.

Table-2 shows the SCC, Na^+ and K^+ concentrations of milk samples from the CMT-N group and the CMT-P sub divisions (S1+,S2+ and S3+). There were significant differences ($p<0.05$) in the SCC, Na^+ and K^+ concentrations between the CMT-N and the CMT-P sub-divisions (S1+, S2+ and S3+). The mean Log_{10} SCC from the CMT-N group is lower than those from the CMT-P sub-divisions; S1+, S2+ and S3+. The Na^+ concentrations from the CMT-N group is equally lower compared to those of the CMT-P sub-divisions S1+, S2+ and S3+. The K^+ concentration was found to be higher in the CMT-N group as compared to the CMT-P sub-divisions: S1+, S2+ and S3+. These results show a significant difference ($p<0.05$) between CMT-N and CMT-P with an increase in Na^+ content and decrease in K^+ content. There were significant differences ($p<0.05$) in SCC between the CMT-N group and those from the CMT-P sub-divisions.

The Pearson's correlation coefficient between the SCC, Na^+ and K^+ concentrations showed a weak positive correlation ($r=0.270$, $p<0.01$) between SCC and Na^+ concentration, while K^+ concentration had a significant weak negative correlation with SCC ($r=-0.436$, $p<0.01$).

The reference detection range of Na^+ concentration from CMT-N cows with 95% confidence interval was 75.4-91.6 mg/dL, while those of positive cows

Table-1: Mean±SEM of SCC, Na^+ and K^+ concentrations in milk from CMT-N and CMT-P dairy cows.

Parameters	CMT-N	CMT-P
Log_{10}SCC	4.72^a±0.10	5.35^b±0.06
Na^+ (mg/dL)	141^a±23.4	232^b±19.1
K^+ (mg/dL)	195^a±14.0	123^b±17.6

Mean±SEM with different superscripts a,b within rows differ significantly at P<0.05. SEM=Standard error of mean, SCC=Somatic cell count, CMT-N=California mastitis test-negative, CMT-P=California mastitis test-positive

Table-2: Mean±SEM of Log_{10} SCC, Na^+ and K^+ contents in milk from CMT-N and subclinical mastitis sub divisions (S1+, S2+, S3+).

Parameters	CMT-N	S1+	S2+	S3+
Log_{10}SCC	4.72a±0.10	5.24^{a}±0.09	5.30^{a}±0.11	5.57^{b}±0.09
Na+ (mg/dL)	141a±23.4	205^{ab}±25.6	213^{abc}±19.7	299^{bc}±23.7
K+ (mg/dL)	195a±14.0	122^{bc}±18.7	137^{b}±17.9	97.2^{c}±21.2

Mean±SEM with different superscripts ^{a,b,c}within rows differ significantly at $p<0.05$. SEM=Standard error of mean, SCC=Somatic cell count, CMT-N=California mastitis test-negative, CMT-P=California mastitis test-positive

ranged from 106.5 to 159.5 mg/dL. Furthermore, the 95% confidence interval detection range for K^+ concentration was 187-204 mg/dL in CMT-N cows and 106.7-138.5 mg/dL in CMT-P cows. The sensitivity and specificity of using Na^+ as a diagnostic indicator in CMT-P cows is 40% and 100%, respectively, while that of K^+ is remarkably high at 90% and 100% (Figures-1 and 2).

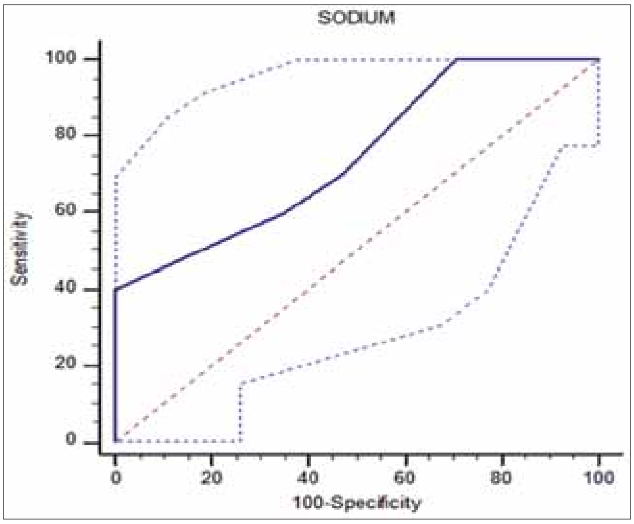

Figure-1: Sensitivity and specificity of sodium diagnostic test using receiver operating characteristic curve.

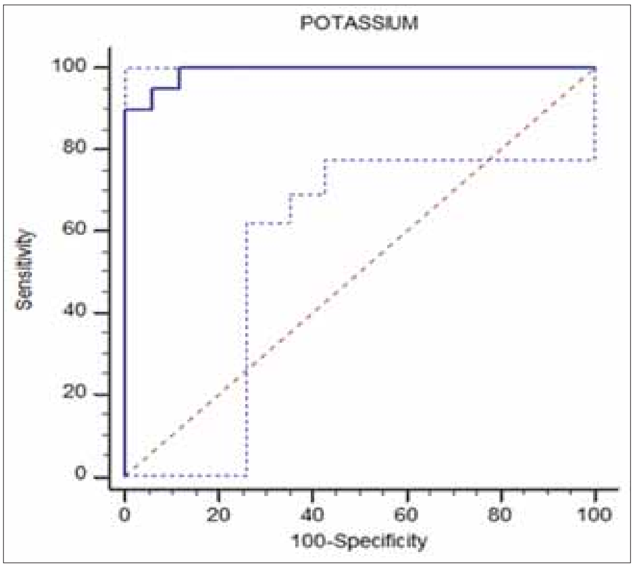

Figure-2: Sensitivity and specificity of potassium diagnostic test using receiver operating characteristic curve.

Discussion

The concentrations of many minerals are altered during mastitis, and these changes can play significant roles in determining the industrial quality of the milk and diagnosis of sub-clinical mastitis [11]. Potassium, the most abundant mineral in milk, leaks out of milk through the paracellular pathway. Consequently, its concentration decreases. Conversely, sodium found in high concentrations in the blood leaks into the milk and increases the concentration above normal [12,13]. The significant increase ($p<0.05$) observed in the mean SCC in the milk samples from CMT-P dairy cows as compared to those from CMT-N cows in this study may be due to inflammation associated with mastitis, which could result in the influx of neutrophils giving rise to increase SCC [4,5]. The increase in Na^+ concentrations and decrease in K^+ observed among the CMT-P dairy cows corroborates the reports of [14,15]. They reported that during a mastitic event, damage to the epithelial tissue, and, in particular, an increase in permeability of the "paracellular" junctions allows Na^+ to diffuse into the mammary gland, resulting in an increase in milk Na^+ concentration. At the same time, in an attempt to achieve osmotic equilibrium, milk potassium concentration declines, proportionally less than Na. The results of the Pearson's correlation coefficient between SCC, Na^+ and K^+ concentrations in the current study corresponds with previous study from [7], where the SCC was reported to have a positive correlation with Na^+ content and negative correlation with K^+ content. It was equally observed in this study that K^+ had a significant correlation with SCC and Na^+ content. This suggests that as SCC and Na+ content increases in subclinical mastitis, the K+ content decreases.

Conclusion

This study showed that SCC had an association with the level of Na^+ and K^+ in milk and these two electrolytes may serve as potential indicators for the detection of subclinical mastitis in dairy cows.

Authors' Contributions

AWH, FFJA and MAML conceptualized, and supervised the research. AMMA,,, KM,, and MAS collected and analysed the samples. AT, YA and LA drafted the manuscript and ran all statistical tests. All authors read and approved the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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