Measurement of oxygen concentration for detection of subclinical mastitis

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Summary

Subclinical mastitis is a frequent and economically important disease in cattle. Since the milk appears macroscopically normal the diagnosis depends on indirect parameters. In automatic milking systems ideally the measurements have to be automatized and completed during milking process. Oxygen concentration of the milk is a parameter which has not been used widely to date. Oxygen is consumed by cells in the milk, hence an increased number of cells results in decreased oxygen concentration.

The main objectives of the study were to study the association of milk oxygen concentration with the number of cells and the electric conductivity and to assess the feasibility of oxygen concentration for detection of subclinical mastitis.

The study was performed on five dairy farms using 690 macroscopically normal quarter milk samples. Oxygen concentrations (OC), somatic cell count (SCC) and electric conductivity (EC) were measured. The associations between the parameters were calculated and the diagnostic value of OC for detection of subclinical mastitis was estimated.

Significant correlations were found between OC and SCC (rs =-0.28) and between OC and EC (rs = -0.20). SCC of the samples varied between 1,000 and 21,602,000 cells/ml. Out of the 690 milk samples 404 had SCC < 100,000 cells/ml; 95 had an SCC between 100,00 and 200,000 cells/ml and 191 had SCC >200,000 cells/ml (subclinical mastitis). OC decreased whereas EC increased significantly with increasing SCC ($P < 0.001$). The threshold for OC to diagnose subclinical mastitis was 5.22% (sensitivity 84%, specificity 46%). The area under the curve describing the diagnostic value of OC was 0.72 (confidence interval 0.68-0.76).

In conclusion OC has potential to be used as parameter to detect subclinical mastitis in dairy cattle. Currently the application is not suitable for routine use. Further research is necessary to improve measurement technology and diagnostic value of the parameter.

Keywords: mastitis diagnosis, oxygen concentration, subclinical mastitis

Messung der Sauerstoffkonzentration der Milch zur Diagnostik von subklinischen Mastitiden

Subklinische Mastitiden sind beim Rind häufig auftretende und ökonomisch bedeutende Erkrankungen. Da die Milch makroskopisch unverändert ist, muss die Diagnostik über indirekte Parameter erfolgen. Für automatische Melksysteme ist dabei eine automatisierbare Messung erforderlich. Ein bisher wenig beachteter Parameter stellt die Sauerstoffkonzentration in der Milch dar. Sauerstoff wird durch die ansteigende Anzahl an Zellen bei Mastitiden verbraucht, die Konzentration sinkt ab. Ziel der Studie war es, den Zusammenhang zwischen Sauerstoffkonzentration und Zellzahlgehalt sowie Leitfähigkeit der Milch zu untersuchen und die Eignung des Parameters zur Detektion von subklinischen Mastitiden zu überprüfen.

Die Untersuchungen wurden an fünf landwirtschaftlichen Betrieben an 690 klinisch unveränderten Viertelgemelksproben durchgeführt. Es wurden die Sauerstoffkonzentration (oxygen concentration, OC), die Anzahl somatischer Zellen (somatic cell count, SCC) und die elektrische Leitfähigkeit (electric conductivity, EC) gemessen. Die Assoziationen zwischen den Parametern und die diagnostische Wertigkeit der Sauerstoffkonzentration zur Diagnose von subklinischen Mastitiden wurden berechnet.

Signifikante Korrelationen lagen zwischen OC und SCC (rs =-0,28) sowie OC zur EC (rs = -0,20) vor. Der SCC lag zwischen 1.000 und 21.602.000 Zellen/ml. Von den 690 Viertelgemelksproben wiesen 404 SCC < 100.000 Zellen/ml auf, 95 hatten SCC zwischen 100.000 und 200.000 Zellen pro ml, während 191 Proben SCC > 200.000 Zellen/ml hatten (subklinische Mastitis). Die OC sank mit dem Anstieg der Zellzahl signifikant ab ($P < 0,001$) während die EC anstieg. Der cut-off Wert für die OC beträgt 5,22 %, die Sensitivität dieses Grenzwertes beträgt 84 %, die Spezifität 46 %. Die Fläche unter der ROC Kurve zur Beschreibung des diagnostischen Wertes der OC beträgt 0,72 (Konfidenzintervall 0,68 - 0,76).

Die OC kann als Parameter zur Detektion von subklinischen Mastitiden in Betracht gezogen werden. Die An-

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wendung ist gegenwärtig jedoch nicht praxisreif, die diagnostische Wertigkeit noch zu gering, so dass weitere Untersuchungen und Verbesserungen notwendig sind.

Schlüsselwörter: Mastitisdiagnostik, Sauerstoffkonzentration, subklinische Mastitis

Introduction

The number of somatic cells in milk (somatic cell count, SCC) is used as parameter describing udder health in cattle. SCC below 100,000 cells per ml stands for physiological conditions.[6] An increase of SCC typically indicates udder inflammation; however SCC can be additionally influenced by other parameters like age of the animal, days in milk, milk yield or feeding.[2,3,4,5] Unless the cows are immediately after calving, a SCC in excess of 200,000 cells/ml without macroscopic changes of the milk is indicating subclinical mastitis. The SCC is measured routinely in the bulk milk (BTSCC) at the dairy; on a cow level, it is often also regularly measured at monthly milk yield recordings. The BTSCC is used as one of the parameters for milk quality and payment. In cases of an overall geometric mean figure in excess of 400,000 over 3 months milk processers penalize the farm or indeed refuse to buy such milk. Additionally the number of somatic cells can be estimated using the California Mastitis Test (CMT) or measured using portable cell counters.

With the advent of an increasing number of automatic milking systems (AMS) automatic point of care measurement systems are required for mastitis detection during milking. To date that has not been possible for SCC (ideally in this case it could be a quarter SCC (QSCC) but a variety of other possibilities (again ideally at a quarter level) have been suggested though unfortunately most investigations have been at a cow level) for example electric conductivity, haptoglobin, L-lactate-dehydrogenase, N-Acetyl-ß-D glucosaminidase or milk amyloid A.[7,13] However, currently these parameters have only low to moderate diagnostic values.[12]

To our knowledge the concentration of oxygen (OC) in milk is a parameter which has got only little attention so far, it has been used only in an experimental study including a small number of cows. OC in milk samples taken before milking from healthy and from *Staphylococcus aureus* infected udders has been measured.[8] While OC in milk from healthy udders was similar to that in venous blood, the concentration in milk from the infected udders was substantially decreased (in some cases as low as 10% of the normal concentration). The decrease of OC is the result of the activation and increased number of neutrophil granulocytes during inflammation. Phagocytic activity requires an accelerated metabolism and the production of oxygen radicals consuming oxygen solubilized in the milk.[8] A sensor has been developed and patented, which is designed to measure OC in milk and can potentially be used for mastitis detection.[11]

The objectives of the study were first to measure the OC in quarter macroscopically unchanged milk samples taken from cows with physiologically normal and increased SCC; second to study the association between OC and QSCC and then quarter electric conductivity (EC) were calculated. Additionally, the study aimed to estimate the diagnostic value of OC for detection of subclinical mastitis and to suggest a cut-off value.

We hypothesized that OC is significantly decreased in milk samples with increased SCC and that OC is a parameter having sufficient diagnostic value to detection of subclinical mastitis.

Material and Methods

Animals

For the present study 690 quarter milk samples taken from 180 cows in five dairy farms have been used. All available cows, which were lactating and did not show clinical mastitis, were included in the study on farms 1, 2, 3 and 4 during the herd visit. On farm 5, a large dairy farm (>2,000 cows), 90 cows which were milked at the time of the herd visit were coincidentally chosen. The cows were 155 days in milk (DIM) on average with a standard deviation of 105 d. There were no differences between the herds in DIM.

- Farm 1: 34 cows (29 Brown Swiss and 5 Simmental), bulk milk SCC 91,000 cells/ml, average daily milk yield 23.9 kg per cow
- Farm 2: 13 cows (Holstein Friesian), bulk milk SCC 244,000 cells/ml, average daily milk yield 25.9 kg per cow
- Farm 3: 23 cows (12 Brown Swiss and 11 Holstein Friesian), bulk milk SCC 120,000 cells/ml, average daily milk yield 30.8 kg per cow
- Farm 4: 20 cows (Simmental) bulk milk SCC 102,000 cells/ml, average daily milk yield 31.6 kg per cow
- Farm 5: 90 cows (Holstein Friesian); bulk milk SCC 119,000 cells/ml, average daily milk yield 31.8 kg per cow

Samples

After discarding fore milk samples, macroscopic assessment of the milk and CMT milk samples were obtained from all udder quarters. The samples were milked in sample tubes (10 ml volume), which were closed immediately. The tubes were filled with approximately 7 ml to allow a complete immersion of the sensor probe. The samples were stored in a cool place (8–12 °C) till analyzed. All measurements were performed at the farm within less than 30 minutes after taking milk samples.

Measurements

The measurement of OC was performed applying the fluorescence quenching method.[1] The method does not consume oxygen and therefore it can be applied repeatedly. The oxygen sensor TecSence-Micro (Fig. 1) was immersed in the milk sample for 30 seconds and measured OC every 0.8 seconds resulting in 37 or 38 measurements during that time. Data was transferred to a laptop computer; arithmetic mean was calculated for each sample and used for further statistical analyses. The oxygen sensor is able to measure the OC with an accuracy of 0.1%. The sensor is applied with a temperature adjustment compensating changes caused by different sample temperatures. The coefficient of variation was calculated at 0.01% (0.006 to 0.016%, calculated from the raw data).

SCC was measured using the Laval Cell Counters DCC cell counter; the measurement is based on an optical measurement principle. The cell counter was used following the manufacturer's instructions. The coefficient of variation is provided by the manufacturer at 12% in samples with SCC of 100,000 cells/ml, 8% in samples with SCC of 400,000 cells /ml and 7% in samples with SCC of 1 million cells/ml.

Electric conductivity has been measured using a hand held conductivity meter (Greisinger GMH3431) which is also temperature adjusted. According to the manufacturer the accuracy of measurement is <0.5%; the coefficient of variation is <1%.

The institutional ethic committee of the Vetmeduni Vienna has approved the study. The input on the animals was low (the study only included milking which is part of the daily routine), therefore approval of governmental bodies was not required.

Statistical analyses

All statistical analyses were performed using the software package SPSS Version 24.0. Since there were only space information on OC measurement in milk available a sample size calculation was impossible. Normal distribution has been tested applying Kolmogorov-Smirnov-Test. The QSCC figures were not normal distributed; hence all parameters are presented as median and 1st/3rd quartiles. Spearman Rank correlation coefficients (rs) between SCC, OC and EC were calculated. Data were log-transformed before further calculation to facilitate normal distribution. For the parameter OC and EC analyses of variance (mixed linear model) was calculated using farm, breed, cow, DIM and udder quarter as fixed effects. Bonferoni Test was used as *post hoc* test. The diagnostic value of OC and EC for detection of subclinical mastitis (SCC > 200,000 cells/ml) were calculated using a receiver operating characteristic curve analysis (ROC-curve) and cut off value for these parameters were calculated applying Youden Index calculation.

Figure 1: Opto-chemical oxygen sensor sensor (TecMicro, Tecsense); the sensor is placed in the tip of the device and is immersed in the fluid for measurement

Results

A total of 720 quarter milk samples were taken from 180 animals originated from 5 farms. Due to technical problems (accidental failures of the measurement devices) and incomplete data transfer some samples could not be measured and some values are missing. For all 3 parameters the results are available for 690 quarters and are included in the analysis.

The SCC ranged between 1,000 and 21,602,000 cells/ml. Of the 690 quarter milk samples, 404 SCC showed < 100,000 cells/ml (physiological secretion), 95 SCC had between 100,000 and 200,000 cells/ml (disturbed secretion), while 191 samples had SCC > 200,000 cells/ml (subclinical mastitis). The measurements for OC and

Table 1: Oxygen concentration and electric conductivity in quarter milk samples with different somatic cell counts (SCC <100,000 cells/ml, from 100,000 to 200,000 cells/ml, and >200,000 cells/ml), presented as Median/1st quartile/ 3rd quartile. Significant differences are indicated by different indices a b & c

	SCC <100,000/ml	SCC 100,000 to 200,000/ml	SCC >200,000/ml
Udder quarters (number)	404	95	191
Oxygen concentration (%)	7.34/5.79/7.84 [a]	6.55/5.08/7.20 [b]	5.14/4.23/6.88 [c]
Electric conductivity (mS/cm)	4.67/4.48/4.99 [a]	4.97/4.64/5.28 [b]	5.30/4.96/5.88 [c]

Table 2: Cut off values (Youden Index), sensitivity, specificity, area under the receiver operating characteristic curve analysis (ROC curve) and confidence intervals for oxygen concentration and electric conductivity

	Cut-off value	Sensitivity	Specificity	area under ROC curve	confidence intervals
Oxygen concentration	5.22%	84%	46%	0.72	0.68 – 0.76
Electric conductivity	5.04 mS/cm	78%	67%	0.78	0.74 – 0.82

EC are assigned to these cell number classes (physiological, secretion disorder, subclinical mastitis) (Table 1).

The oxygen concentration showed a weak but highly significant ($P < 0.01$) negative correlation ($rs = -0.28$) while the conductivity was only weakly correlated to the QSCC, ($rs = 0.46$, $P < 0.01$). The correlation between oxygen concentration and conductivity was $rs = -0.20$ ($P < 0.01$). The farm of origin, breed, the cow or the quarter examined had no significant influence on the parameters

However OC decreased significantly with the increase of the cell count in the three classes and the difference was highly significant ($P < 0.001$) between these with the physiological cell counts and the class >200,000 cells/ml while the significance level ($P = 0.046$) was just reached between the group with the mean cell counts between 100,000 and 200,000 cells/ml and the high cell count Quarters. EC increased significantly between all groups, also in this parameter the difference between the group with physiological cell counts and the subclinical mastitis group was highly significant. The optimized cut-off values calculated by Youden Index are 5.22% for OC and 5.04 mS/cm for EC. The sensitivity, specificity, area under the ROC curve and confidence intervals are shown in table 2, figures 2 and 3.

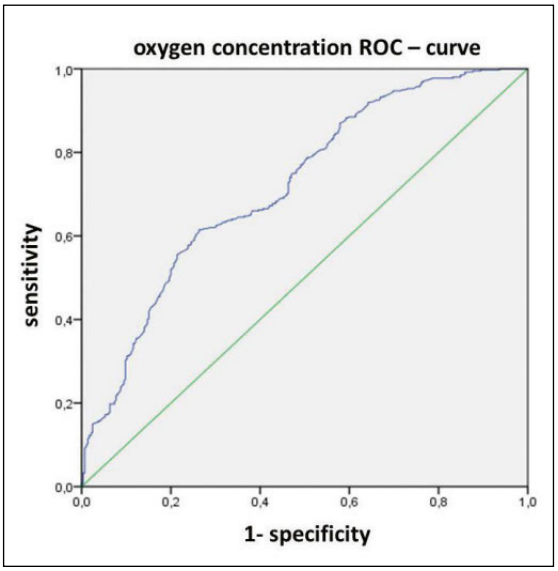

Figure 2: Receiver operating characteristic curve analysis (ROC curve) for the oxygen concentration of milk to estimate the diagnostic value for detection of subclinical mastitis (somatic cell counts > 200,000/ml), the area under the curve (blue line) is 0.72, the green line represents the line of identity

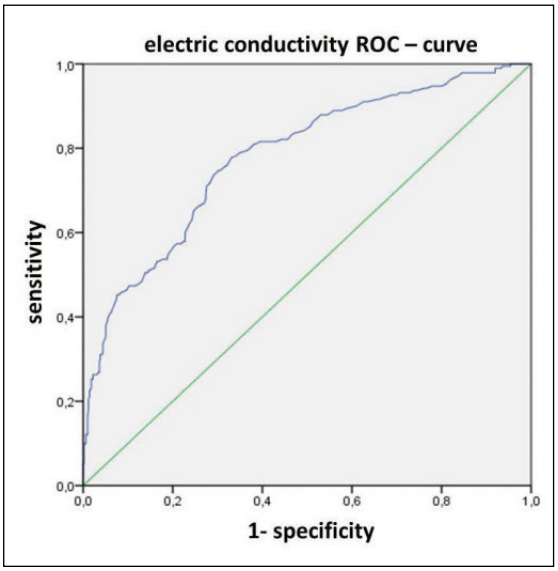

Figure 3: Receiver operating characteristic curve analysis (ROC curve) for the electric conductivity of milk to estimate the diagnostic value for detection of subclinical mastitis (somatic cell counts > 200,000/ml), the area under the curve (blue line) is 0.78, the green line represents the line of identity

Discussion

The hypothesis that the oxygen concentration decreases with increasing SCC was confirmed by the study. However, the low association also indicates that a large part of the variability is caused by other factors. One factor might be that SCC only estimates the number of cells but not their activity and thus their oxygen consumption. Further there might be a variability of oxygen consumption by neutrophil granulocytes during the inflammatory cycle contributing to the variability. Additionally, it seems possible that oxygen may also be consumed by bacteria which are possibly present in the milk from quarter with subclinical mastitis. Bacteria are not detected by measuring the SCC.

Sampling in the study was done by hand milking, which causes the milk to come into contact with oxygen in the air. However, this should have had little or no effect on the measurements as it takes much longer for oxygen to dissolve in liquids like milk.[10] It would also be a systematic error that would have influenced in all milk samples. This is a limitation of the study design using a non-customized sensor; the problem could be avoided by installing the sensors in the milking cluster.

Due to the fact that hardly any information was available on OC measurement in milk, it was not possible to estimate an exact sample size. However, we assume that the sample size with samples from 690 udder quarters is sufficiently large to support the statements made here. In consecutive studies on larger sample size and higher number of dairy farms the possible effect of additional factors (e.g. farm, season, breed, parity, days in milk and others) need to be evaluated. Additionally the influence of milk yield on SCC need to be considered in further studies since high yielding dairy cows tend to have lower SCC.[9]

The diagnostic value with an AUC of 0.72 is not yet sufficient, but it is only slightly below that of conductivity (AUC 0.78). The cut off for oxygen concentration calculated in the study cannot be regarded as a generally valid reference value, further investigations on larger samples and the improvement of the measurement methodology are necessary. It should be noted that the sensor is one for general applications and has not been optimized for this task. Furthermore, a definition of the cut-off value can be discussed which does not follow the optimization between sensitivity and specificity by Youden Index but favor sensitivity or specificity.

Moderate diagnostic values of different sensors were reported in dairy cattle farming.[12] The current results of the method investigated in the present pilot study confirm this. However, by improving the measurement technology and the customization of the sensors and above all the installation of the sensors in the cluster i.e. ideally measuring quarter OC as opposed to a cow level measurement, there seems to be potential which justifies further research. An advantage of the method of oxygen concentration measurement is the short measurement time (used sensor measured every 0.8 seconds). The optimal number of measurement repetitions however needs still to be specified. The measurement can be carried out by udder quarters as in-line measurement, whereas the current cell count measurement systems in the AMS measure in complete milk samples from all quarters. If a reduced oxygen concentration is detected, only the milk of the affected quarter and not all milk could be discharged after a few seconds. The data could be transmitted directly to a herd management program and thus also be used to monitor udder health in the herd and the success of the therapy. Furthermore, the parallel application of two or more parameters (e.g. OC and EC) could be reconsidered, since it seems quite possible that an improvement of the diagnostic value can be achieved by combination of measurements.

Conclusions

Concluding OC in milk may be considered a parameter potentially suitable for the detection of subclinical mastitis. However, the oxygen sensor system is currently not feasible for practical use; additionally the diagnostic value is still too low but can be potentially improved by optimization for this application. Further investigations on larger samples and adaption of the measurement technology are necessary.

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Mesure de la concentration en oxygène pour la détection de la mammite subclinique

La mammite subclinique est une maladie fréquente et économiquement importante chez les bovins. Puisque le lait apparaît macroscopiquement normal, le diagnostic dépend des paramètres indirects. Dans les systèmes de traite automatique, les mesures doivent idéalement être automatisées et effectuées pendant le processus de traite. La concentration en oxygène du lait est un paramètre qui n'a pas été largement utilisé à ce jour. L'oxygène est consommé par les cellules dans le lait, un nombre accru de cellules entraînant donc une diminution de la concentration en oxygène.

Les principaux objectifs de l'étude étaient d'étudier l'association de la concentration d'oxygène du lait avec le nombre de cellules et la conductivité électrique et d'évaluer la praticabilité de la mesure de la concentration en oxygène pour la détection de la mammite subclinique.

L'étude a été réalisée dans cinq fermes laitières à l'aide de 690 échantillons de lait de quartier macroscopiquement normaux. Des concentrations d'oxygène (OC), le nombre de cellules somatiques (SCC) et la conductivité électrique (EC) ont été mesurés. Les associations entre les paramètres ont été calculées et la valeur diagnostique de l'OC pour la détection de la mammite subclinique a été estimée.

Des corrélations significatives ont été trouvées entre OC et SCC (rs= -0,28) et entre OC et EC (rs = -0,20). Le SCC des échantillons variait entre 1'000 et 21'602'000 cellules/ml. Sur les 690 échantillons de lait, 404 avaient un SCC de <100 000 cellules/ml; 95 avaient un SCC entre 100 000 et 200 000 cellules/ml et 191 avaient un SCC de > 200 000 cellules/ml (mammite subclinique). L'OC a diminué alors que l'EC a augmenté de façon significative avec l'augmentation du SCC (P - 0,001). Le seuil pour que l'OC permette un diagnostic de mammite subclinique était de 5,22 % (sensibilité 84 %, spécificité 46 %). La zone sous la courbe décrivant la valeur diagnostique de CO était 0.72 (intervalle de confiance 0.68-0.76).

En conclusion, l'OC pourrait être utilisée comme paramètre pour détecter la mammite subclinique chez les bovins laitiers. Actuellement, l'application n'est pas adaptée à une utilisation de routine. D'autres recherches sont nécessaires pour améliorer la technologie de mesure et la valeur diagnostique du paramètre.

Mots-clés: Diagnostic de mammite, concentration en oxygène, mammite subclinique

Misura della concentrazione di ossigeno per la rilevazione della mastite subclinica

La mastite subclinica è una malattia frequente ed economicamente importante nei bovini. Poiché il latte appare macroscopicamente normale, la diagnosi dipende da parametri indiretti. Idealmente, nei sistemi di mungitura automatica, le misurazioni devono essere automatizzate e completate durante il processo di mungitura. La concentrazione di ossigeno nel latte è un parametro finora poco utilizzato. L'ossigeno viene consumato dalle cellule del latte, quindi un numero maggiore di cellule comporta una minore concentrazione di ossigeno.

Gli obiettivi principali dello studio erano di studiare l'associazione della concentrazione di ossigeno nel latte con il numero di cellule e la conduttività elettrica e valutare la fattibilità della concentrazione di ossigeno per la rilevazione della mastite subclinica.

Lo studio è stato condotto in cinque aziende di produzione di latte utilizzando 690 campioni di latte proveniente da dei quarti macroscopicamente normali. Sono state misurate le concentrazioni di ossigeno (OC), il conteggio delle cellule somatiche (SCC) e la conduttività elettrica (EC). Sono state calcolate le associazioni tra i parametri ed è stato stimato il valore diagnostico della OC per la rilevazione della mastite subclinica.

Sono state riscontrate correlazioni significative tra le OC, il SCC (rs = -0.28) e tra la OC e la EC (rs = -0,20). Il SCC dei campioni variava tra 1.000 e 21.602.000 cellule / ml. Dei 690 campioni di latte 404 avevano un SCC <100.000 cellule / ml; 95 avevano un SCC tra 100,00 e 200.000 cellule / ml e 191 avevano un SCC> 200.000 cellule / ml (mastite subclinica). La OC è diminuita mentre la EC è aumentata significativamente con l'incremento del SCC (P <0,001). La soglia per la diagnosi della mastite subclinica da parte della OC era del 5,22% (sensibilità 84%, specificità 46%). L'area sotto la curva che descrive il valore diagnostico della OC era 0,72 (intervallo di confidenza 0,68-0,76).

In conclusione, la OC potrebbe essere utilizzata come parametro per rilevare la mastite subclinica nei bovini da latte. Attualmente l'applicazione non è adatta per un uso di routine. Sono necessarie ulteriori ricerche per migliorare la tecnologia di misurazione e il valore diagnostico del parametro.

Parole chiave: Diagnosi di mastite, concentrazione di ossigeno, mastite subclinica

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