Variations in Vaginal pH in Dairy Cattle Associated with Parity and the Periparturient Period

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ABSTRACT

The objective of this study was to determine a baseline vaginal pH value for clinically healthy dairy cattle and to examine whether this value varies as a function of days in milk (DIM) or parity. One hundred and seventy one healthy Israeli Holstein-Friesian breed cows, heifers and first calf heifers, located on one farm were screened on the same day for vaginal pH values by intravaginal insertion of a pH strip. The mean pH value for cows, heifers and first calf heifers was 7.35, 7.58 and 7.48 respectively; the median value was 7.50 for all groups. DIM did not significantly influence vaginal pH values in the examined population. In addition, vaginal pH values were measured in 18 healthy cows and nine healthy first calf heifers of Israeli Holstein-Friesian breed, on another farm, during the periparturient period. While the mean pH values remained stable for cows before and after parturition at around 7.50, in the 1st calf heifer group, the mean pH was 7.25 one week before parturition; the value increased to 7.75 the week following parturition and finally stabilized at about 7.50. Results show that regardless of the periparturient period, cows and heifers tend to have more variable vaginal pH than first calf heifers and that vaginal pH values tend to be more acidic in cows. These differences may contribute to the increased susceptibility of first calf heifers to vaginal infections such as bovine necrotic vulvovaginitis.

Keywords: cow; heifer; calf; vaginal pH; periparturient

INTRODUCTION

The pH of bodily secretions has long been recognized as one of the body's primary innate defense mechanisms, preventing microbial infections through skin, mucous membranes and the alimentary tract (1). In humans, vaginal pH changes have been linked to bacterial vaginosis and other types of microbial vaginitis (2). A change of one pH unit may determine the difference between healthy vaginal flora and heavily infected vaginal flora (3). Although the pH value of human vaginal secretions is not routinely used in the diagnosis of vaginal infections, it is a reliable tool for the screening of bacterial vaginosis (4). Notwithstanding, it is difficult to determine whether bacteria cause the increase in the vaginal pH or whether the latter facilitates the proliferation of the microorganisms. Probiotics have been used in attempts to reestablish physiologic pH environments in humans (5).

While not examined as thoroughly as in humans, it may be assumed that vaginal acidity-alkalinity in animals, in general, and in cattle in particular, fulfill the same protective function. Thus pathological variations of vaginal pH values may act as a predisposing factor for infections of the bovine genital tract resulting in conditions such as bovine necrotic vulvovaginitis (BNVV), a syndrome causing a major problem in Israeli dairy herds (6). Since postparturient first calf heifers are the main risk population, vaginal pH value differences between these animals and cows in second and higher lactation are of interest.

The majority of previous studies involving genital pH of

cattle have examined the relationship between pH and the estrus cycle, pregnancy status or conception rate. Many of these studies have been performed post mortem (7, 8), on a small group, (9-11) or provided non-numeric test results (8, 12). According to a review of the existing literature, the measured range of vaginal pH is 5.52 to 8.60. In 400 cows the vaginal pH near the cervix was between 5.52 and 8, while nearly 50% of these measurements were in the range of 6.51 to 7 and 93.5% were in the range of 6.01 to 7.50 (13), without any correlation between vaginal pH and breeding efficiency. In 54 cows examined at different periods of the estrous cycle, results showed a decrease in pH (an increase in acidity) during estrus (14). These results correspond to those found in women, whereby estrogen acidifies the vaginal environment by up-regulating the proton secretion from vaginal epithelial cells (15). A decrease in pH values has also been reported in measurements of uterine pH during the luteal phase in cows receiving a high degradable protein diet (16). Variations in vaginal pH in dairy cattle associated with parity and the periparturient period have, to the best of our knowledge, not been reported

The objectives of this survey were:

1. To establish a baseline average pH value in healthy cows and first calf heifers.
2. To examine the relationship between pH values and days in milk (DIM) for dairy cows in general and also to examine any difference in this relationship between cows and first calf heifers.
3. To compare pH value variations during the periparturient period in cows and first calf heifers.

MATERIALS AND METHODS

Animals

Healthy female cows, heifers and first calf heifers of the high yielding Israeli Holstein-Friesian breed from two medium sized commercial dairy cattle farms in central Israel, which implement intensive husbandry methods, were examined in this study. The dietary intake varied according to age groups: heifers received the poorest ration including 12% protein, mostly of poor quality (non-protein nitrogen), dry cows and first calf heifers received a ration including 12.5% of high quality protein and milking primiparous and multiparous cows received the richest ration including 16.3% of high quality protein. Starting about one month prior to the expected parturition date heifers were fed with the same formulation as the cows. Measurements of pH were made between January and March 2008.

Cross-sectional study

Vaginal pH values of 171 healthy animals, comprising 62 cows, 73 1st calf heifers and 32 heifers from a kibbutz dairy farm were measured on the same day. One third of the heifer group and the dry cow group were selected randomly and all first calf heifers and cows available for examination were included (eight first calf heifers and 14 cows were excluded since they were not available at the time of measurements). This data was used to assess variations in pH values as a function of DIM.

Effect of DIM on vaginal pH study

Data obtained from the cross-sectional study was used, including only animals between 70 days pre-partum and 320 days post-parturition, thus excluding 31 heifers or 1st calf heifers and ten cows.

Periparturient pH value variation study

Vaginal pH values of 27 healthy animals (18 cows and nine first calf heifers) on a research dairy cattle farm were measured every four days with the purpose of documenting fluctuations during the periparturient period, defined by preliminary results as one week prior and three weeks after parturition. Three cows were excluded from the initial study group of 30 (two due to abortion and one due to aggressive behavior).

pH measurements

pH values were measured using pH-Fix 4.5 to10.0 strips (Macherey-Nagel, Germany) having a range of 4.5 to 10.0 with intervals of 0.5. Consequently, results were treated as categorical rather than continuous. Each animal was measured once using a single pH test strip. Each pH strip was inserted approximately 5 cm into each cow's vagina and pressed against the left lateral vaginal wall. Strips were maintained in the vagina for approximately ten seconds before removal. Special attention was paid to avoid the urethral opening upon insertion. If the cow urinated, measurements were deferred for at least five minutes to avoid false positive alkaline results. The same team of tes-

ters read all results. Since the age group (cow, heifer, etc.) and status (before or after calving) of the animals could be easily deduced, this information was not blinded from the testers.

Statistical methods

Based on empirical data a 95% confidence interval (CI) for the pH values was calculated for the entire examined population and for cows, heifers and 1st calf heifers separately.

Several statistical tests were implemented using SPSS (IBM, USA) predictive analytic software. Since strips having a value interval of 0.5 units were used, pH values were treated as a categorical as well as a quantitative variable. The latter was used to calculate the descriptive statistics (mean, standard deviation and standard error of the mean) and the former was used for the remaining statistical tests. The ANOVA Model with Dunett's T3 *post hoc* test was applied in order to compare pH values in three different study groups. When comparing the mean for all three groups, the heterogeneity of variances found by the Levene Statistic Test was taken into account. The Pearson Chi-Square Test as well as the Fisher's Exact Test were applied in order to examine the association between pH values in the study groups. Spearman's Rank Correlation coefficient was used in order to assess the correlation between pH values and DIM. The Friedman's non-parametric test as well as the ANOVA model for repeated measures were applied in order to assess whether or not there was a trend in repeated measurements of pH values.

All tests applied were two-tailed and a p-value of five percent or less was considered statistically significant.

RESULTS

Cross-sectional study

The results of the cross-sectional study showed that the majority of the animals had a vaginal pH value between 7 and 8. Higher values were exceptional (Figure 1). The relationship between the groups and pH values as a discrete variable was examined by two methods. When using the Pearson Chi-Square Test, a significance level of p=0.01 was found. Since the reliability of the Pearson Chi-Square Test is influenced by groups of a small size, we also implemented Fisher's Exact Test, which exhibited a significance level of p=0.004.

Table 1 presents the descriptive statistics for the pH values of all three age groups. Mean pH values found for heifers, 1st calf heifers and cows were variable; median pH values of the groups, however, were identical.

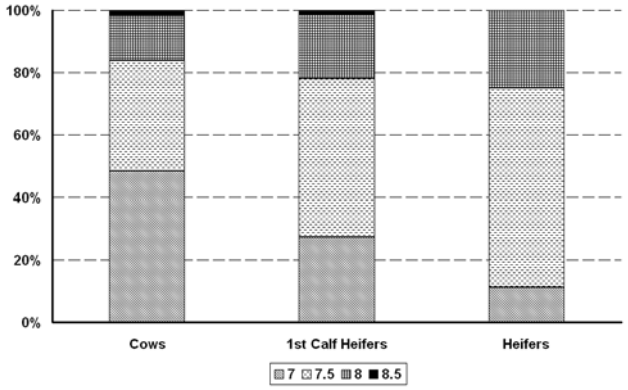

Figure 1: Distribution of pH values in the different age groups.

Table 1: Descriptive statistics of pH values in the various age groups.

	Cows	First calf heifers	Heifers
Mean	7.35	7.48	7.58
Median	7.5	7.5	7.5
Standard deviation	0.40	0.37	0.30
95% CI	7.25-7.45	7.40-7.56	7.47-7.67

ANOVA showed that the difference between the mean pH values among all three groups was statistically significant, giving a result of P=0.007.

Since three groups were examined in this study, we were interested in determining which coupled groups had provided the greatest difference. This was achieved using Dunett's T3 *post hoc* test (Table 2). The greatest, statistically significant, difference was due primarily to the extreme groups (i.e. cows and heifers). Although the difference was minor, it was statistically significant (p<0.05).

Table 2: Differences between age group means evaluated for statistical significance by Dunett's T3 *post hoc* Test; pH being the independent variable.

Groups compared	Difference
Cows/First calf heifers	0.1327
Heifers/Cows	0.2227*
Heifers/First calf heifers	0.0900

* Statistically significant: p<0.05.

Effect of DIM on vaginal pH

No correlation was found between DIM and pH values using Spearman's correlation coefficient (r= -0.092 and r= -0.100 in cows and in heifers and first calf heifers, respectively).

Periparturient pH value variation

When examining the entire study group of 27 animals the ANOVA model for repeated measures and the Friedman non-parametric test were applied in order to evaluate whether the pH value changes in time were statistically significant. Both tests showed a tendency to statistical significance p=0.065 and p=0.059, respectively. However, when examining first calf heifers and cows separately (Figure 2), using the Friedman non-parametric test, the pH variations were found to be significant for the heifer group and insignificant in the cow group: p=0.031 and p=0.670, respectively.

These findings are further underlined when taking into account the small group size of first calf heifers, and when regarding the median measurements for each week, which remained unchanged at pH=7.50 for the cow population, but exhibited a clear increase in the heifer group in the first week after parturition (changing from 7.25 to 7.75).

DISCUSSION

The importance of pH values in various body fluids and their normal range has long been established for human beings (2, 3). However, a comprehensive, relevant literature review indicated that these basic parameters have yet to be established for live healthy cows.

The first objective achieved in this study was to determine a pH baseline in healthy dairy cattle. The values found were 7.35, 7.48 and 7.58 for heifers, first calf heifers, and cows respectively, whereas the median for all examined groups was 7.50. When examining coupled groups, a small but statistically significant difference was found between the extreme groups, cows and heifers. The importance of these cross-sectional findings lies in their use as a reference value for further studies in dairy cattle of different parities and for various topics, including fertility or vaginal diseases, such as BNVV. It must be taken into account, however, that the pH values established in this study may be different for other breeds, climatic conditions, geographical location and dietary regimens. In particular, the quality and quantity of protein dietary intake, while uniform within all examined groups, varied among them with milking cows receiving the richest ration.

When examining the effect of DIM on pH values for the general cattle population, no significant fluctuations were observed; however, when examining pH values in a specific cattle population during the periparturient period, the difference between cows and first calf heifers was significant. First calf heifers showed a significant increase in pH value, starting at a median pH value of 7.25, rapidly becoming more alkaline during the first week post parturition and reaching a median pH value of 7.75, and then declining to an average value of 7.50. On the other hand, cows showed no significant digression, maintaining a steady median pH value of 7.50. Since at the time these measurements were made, first calf heifers and cows were fed the same feed formulation, the differences could not be attributed the animals' diet. This study's focus on repeated measures in a small group during a limited period of time may ex-

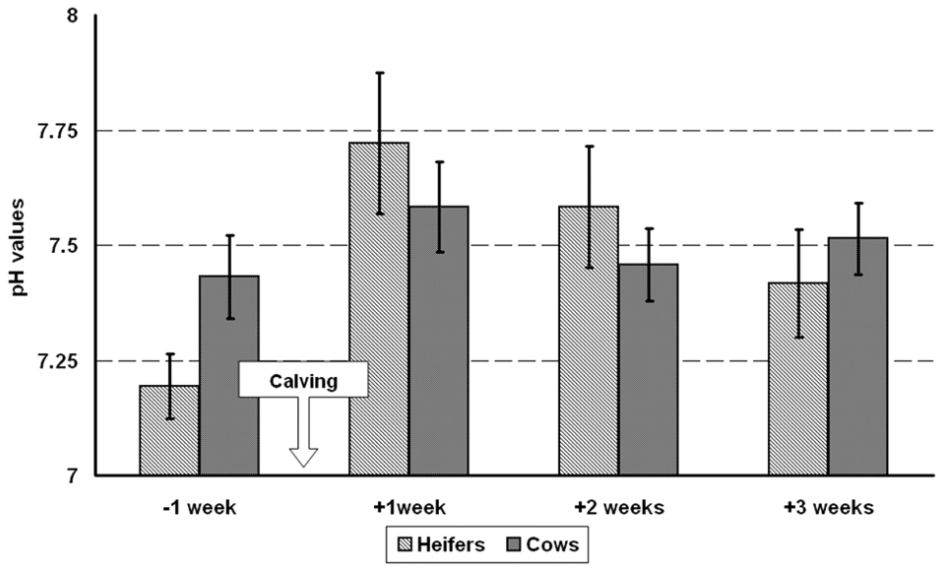

Fig. 2: Mean pH value of cows and first calf heifers during the periparturient period. Error Bars signify ± 1 Standard Error.

plain why no difference was observed in larger populations that were evaluated over a longer period of time. The variations observed in first calf heifers occurred rapidly and only during a very specific time period post parturition, while the average median for this time period was comparable with that observed in the general population.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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