Laboratory diagnostics in the urine of young and pregnant ewes

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Key words
Net acid base excretion, urine, ewes

Summary
Objective: The purpose of this study was to identify values for net acid base excretion (NABE) which are significant indicators of the acid-base equilibrium in pregnant and young ewes and to show its relationship with other parameters (base, acid, ammonium [NH₄⁺], base-acid quotient, sodium [Na], potassium [K], calcium [Ca]) in ovine urine. In contrast to dairy cows, data are rare on these parameters in ewes.

Material and methods: A total of 99 animals were used in the study, consisting of 56 young (average of 5.6 ± 1.1 months) and 43 pregnant ewes (average of 35.2 ± 18.8 months). Measurement of fractional NABE in urine samples was carried out according to the method reported by Kutas. The pH value of the urine was measured with a laboratory pH meter. Na, K and total Ca were measured with a flame photometer.

Results: For all values except Na significant differences occurred between urine samples of pregnant ewes and young ewes (p < 0.001). Base, acid, NH₄⁺, NABE, K and Ca values were significantly higher in the urine of the youngs than in pregnant ewes. In young ewes, a strong correlation was found between NABE and base values while a weak correlation could be observed between pH and base values. In pregnant ewes, strong NABE-base, NABE-K and K-base correlations were found as well as weak NH₄⁺-base, NH₄⁺-NABE and NH₂-K correlations. There was a strongly positive correlation between NABE and NH₂ in pregnant ewes, while a weak negative correlation between those values was observed in young ewes.

Conclusion: For the first time, we established values for NABE and certain other parameters in urine of pregnant ewes and young ewes. It was shown that the acid-base balance in pregnant ewes and young ewes can be evaluated by measuring NABE and certain trace elements in urine like in cattle.

Zusammenfassung
Ziel der Studie war, Orientierungswerte für die Netto-Säuren-Basenausscheidung (NSBA) zu ermitteln, da sie ein bedeutender Indikator des Säure-Basen-Haushalts bei trächtigen Schafen und Jungschafen ist. Ferner sollten mögliche Korrelationen zu anderen Parametern (Base, Säure, Ammonium [NH₄⁺], Basen-Säure-Quotient, Natrium [Na], Kalium [K], Kalzium [Ca]) im Schafrin nachgewiesen werden. Im Gegensatz zur Milchkuh gibt es dazu nur vereinzelte Untersuchungen beim Schaf.


Introduction

The urine pH level represents a measurement of the free (non-buffered) hydrogen ions in urine, while net acid base excretion (NABE) indicates the measurement of all free and buffered hydrogen ions in urine. NABE in urine can be measured in two ways: fractional and basic. The same values can be obtained with both methods of measurement (1). The measurement of NABE is based on the principle of titration (13). It has been proven that the measurement of NABE in urine is an important tool in determining the acid-base equilibrium in dairy cows (2, 3). Disruption of the acid-base equilibrium in an organism can cause acute, subacute or chronic acidosis or alkalosis. Chronic acidosis or alkalosis usually occurs with non-specific symptoms, causing significant loss in production and leading to secondary diseases (1).

In order to keep the acid-base equilibrium within normal physiological limits, it is necessary that the animals excrete excess cations and anions that are consumed in the diet. Excessive consumption of anions or cations can also upset this physiological equilibrium. Monovalent macromineral ions such as Na, K and Cl are reported to be elements that have an effect on the acid-base equilibrium. Studies indicate that acidic diets increase the concentration of calcium in the blood (11, 17). This development has led to the practical use of rations that contain more anions than cations in order to prevent hypocalcemia. Research shows that the incidence of hypocalcemia is reduced in prepartum cows fed a diet with negative dietary cation-anion difference (DCAD). When these animals give birth, the blood calcium level increases; this increase is probably either due to an increase in the mobilization of bone calcium or to an increase in the absorption of calcium from the digestive system. The increased calcium level prevents hypocalcemia and also reduces problems like retained placenta and displaced abomasum (17).

Consumption of a diet supplemented with anionic salt causes a change in urine pH levels within 2–4 days (10). Monitoring the level of urine pH can be a useful tool in determining whether or not the rations have caused the desired changes. Monitoring the urine pH level is more useful than controlling the blood pH level for detecting changes occurring in the body’s acid-base equilibrium (1, 21). It has been shown that there is a close relationship between DCAD and urine pH levels in periparturient cows (15).

It has been reported that urine pH values and NABE can be used to monitor the acid-base equilibrium in rations for cattle (3). Significant changes in urine also occur during many diseases. However, there is very little information available about the determination of NABE in small ruminants and its relationship with other variables in urine. The purpose of this study was to identify values for NABE, which is a significant indicator of acid-base equilibrium, in young and pregnant ewes and to show its relationship with other parameters in urine.

Materials and methods

Animals

The 99 Merino sheep used in this study were obtained from the Department of Animal Breeding and Genetics at Justus Liebig-University. They consisted of 56 young ewes (4–9 months old, average age 5.6 ± 1.1 months) and 43 pregnant ewes in the first trimester of gestation (1.5–6 years old, average age 35.2 ± 18.8 months). The young ewes were fed a free amount of dry grass in addition to 0.8 kg of concentrated feed. The concentrated feed consisted of 18% raw protein, 9.5% raw cellulose, 8.4% raw ashes, 3.2% raw fat, 1.35% calcium, 0.55% phosphorus, 0.20% sodium and 0.20% magnesium. The pregnant ewes were fed colza, barley and 20 g of a commercial mineral feed in addition to being grazed freely in pasture.

Measurements

Urine was taken from all animals during spontaneous urination by completely filling sterile tubes. The specimens were stored for 1 day at –20 °C and then left to thawing at room temperature. After the tubes had been shaken well by hand, the urine was analyzed for pH, NABE, NH₄, acid, base, base acid ratio (B/A), total calcium, sodium and potassium levels.

Measurement of fractional NABE in urine samples was carried out according to the method reported by Kutas (13). In short, a 10 mL urine sample was diluted with 1 M HCl until its pH was 3.5 (V_{HCl}). After it had boiled for 30 seconds, it was left to cool at room temperature. 0.1 M NaOH was added to the solution, which was titrated until a pH value of 7.4 (V_{NaOH}) was reached. 10 mL of 20% formaldehyde was added and the solution was titrated again with 0.1 M NaOH until the pH was 7.4 (V_{NaOH2}).

\[
\text{Base (mmol/L)} = V_{\text{HCl}} \times 100 \\
\text{Acid (mmol/L)} = V_{\text{NaOH1}} \times 10 \\
\text{NH}_4 \text{ (mmol/L)} = V_{\text{NaOH2}} \times 10 \\
\text{B/A} = \text{base} / \text{acid} \\
\text{NABE (mmol/L)} = (V_{\text{HCl}} \times 10) - (V_{\text{NaOH1}} \times V_{\text{NaOH2}}/10) \\
\]

The pH value of the urine was measured with a laboratory pH meter (pH Level1, Inolab). Na, K and total Ca were measured with a flame photometer (Efox 5053, Eppendorf).

Statistical analysis

Basic descriptive statistics were carried out for all urine parameters in young and pregnant ewes. If the values were nearly normally distributed, mean ± standard deviation (x ± s) were given. Sometimes the statistical distribution of the values within young ewes and/or pregnant ewes was skewed to the right. In these cases the data was transformed logarithmically to get a normal distribution in approximation, and according to this the description was done by geometric mean and dispersion factor ( = logarithmic standard deviation) (XG; DF). In addition, a 95% normal range was computed according to the formula...
Results

The results of analyzed values and measurements in urine samples are given in Table 1. Significant differences were found between the urine samples of pregnant ewes and young ewes for all values except Na (p < 0.0001 resp. p < 0.001). Base, acid, NH₄, NABE, K and Ca values were significantly higher in young ewes’ urine than in pregnant ewes’ urine.

Correlation analysis was carried out according to the Pearson correlation analysis (Table 2 and 3). For all calculations the statistical program package BMDP/Dynamic, release 7.0 was used (4).

Discussion

There is almost no research available on the levels of NABE in urine samples of ewes, especially when compared with studies in cattle. The most likely reason is that less importance is given to ewes’ ration than to cattle feed and that ewes are less valuable. For this reason we were usually forced to make comparisons with studies of cattle.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Animals</th>
<th>Min</th>
<th>Max</th>
<th>Mean ± SD or XG; DF</th>
<th>95% normal ranges*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH[^a]</td>
<td>Young</td>
<td>7.5</td>
<td>8.7</td>
<td>8.4 ± 0.3</td>
<td>7.9; 8.9</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>7.6</td>
<td>8.5</td>
<td>8.2 ± 0.2</td>
<td>7.7; 8.6</td>
</tr>
<tr>
<td>Base[^b] (mmol/L)</td>
<td>Young</td>
<td>140.0</td>
<td>570.0</td>
<td>376.1 ± 116.7</td>
<td>140.1; 612.0</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>15.0</td>
<td>305.0</td>
<td>68.6; 2.2[^c]</td>
<td>13.5; 349.1</td>
</tr>
<tr>
<td>Acid[^d] (mmol/L)</td>
<td>Young</td>
<td>11.0</td>
<td>150.5</td>
<td>96.8 ± 21.6</td>
<td>53.2; 140.4</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>3.0</td>
<td>75.5</td>
<td>9.2; 2.3[^c]</td>
<td>1.7; 50.7</td>
</tr>
<tr>
<td>NH₄[^e] (mmol/L)</td>
<td>Young</td>
<td>5.0</td>
<td>22.5</td>
<td>11.4; 1.4[^d]</td>
<td>6.1; 21.2</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>1.0</td>
<td>13.5</td>
<td>3.4; 1.7[^c]</td>
<td>1.1; 10.5</td>
</tr>
<tr>
<td>B/A[^f]</td>
<td>Young</td>
<td>0.4</td>
<td>27.7</td>
<td>3.7; 1.7[^c]</td>
<td>1.3; 10.3</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>3.3</td>
<td>17.0</td>
<td>7.5; 1.6[^c]</td>
<td>3.1; 18.6</td>
</tr>
<tr>
<td>NABE[^g] (mmol/L)</td>
<td>Young</td>
<td>5.0</td>
<td>473.8</td>
<td>261.4 ± 122.4</td>
<td>14.0; 508.9</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>14.1</td>
<td>276.7</td>
<td>64.6; 2.2[^c]</td>
<td>13.5; 310.1</td>
</tr>
<tr>
<td>Na[^h] (mmol/L)</td>
<td>Young</td>
<td>0.4</td>
<td>358.8</td>
<td>3.9; 11.1[^d]</td>
<td>0.03; 511.1</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>0.1</td>
<td>204.1</td>
<td>3.3; 6.7[^c]</td>
<td>0.07; 159.2</td>
</tr>
<tr>
<td>K[^i] (mmol/L)</td>
<td>Young</td>
<td>123.3</td>
<td>854.0</td>
<td>485.3 ± 173.1</td>
<td>135.3; 835.3</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>27.2</td>
<td>584.2</td>
<td>104.1; 2.3[^c]</td>
<td>18.8; 576.4</td>
</tr>
<tr>
<td>Ca[^j] (mmol/L)</td>
<td>Young</td>
<td>0.08</td>
<td>12.4</td>
<td>0.9; 3.0[^d]</td>
<td>0.1; 8.1</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>0.01</td>
<td>3.7</td>
<td>0.3; 3.9[^c]</td>
<td>0.02; 5.3</td>
</tr>
</tbody>
</table>

Variables marked with the following superscripts are significantly different in mean between young ewes and pregnant ewes:[^a] p < 0.0001;[^b] p < 0.001
[^a] XG = geometric mean, DF = dispersion factor (= logarithmic standard deviation)
[^b] Computed according to the formula XG ± t₀.975ₙ⁻¹ · sqrt((n + 1) / n) · s

XG ± t₀.975ₙ⁻¹ · sqrt((n + 1) / n) · s

for all parameters, where t₀.975ₙ⁻¹ denotes the one-sided t-value with n-1 degrees of freedom and with a cumulative probability of 0.975. The formula was applied to original data or logarithms depending on the form of distribution described above. The Wilcoxon-Mann-Whitney test was used to compare the average values between young ewes and pregnant ewes independent of the form of distribution because the statistical distribution in the groups could not be assumed to be normal with equal variances for all variables.

Correlation analysis was carried out according to the Pearson correlation analysis (Table 2 and 3). For all calculations the statistical program package BMDP/Dynamic, release 7.0 was used (4).
Kraft and Dürr (12) list the following reference values for certain parameters in sheep urine: NABE: 10–190 mmol/L; base: 120–550 mmol/L; acid: 20–80 mmol/L; NH₄: < 20 mmol/L; and B/A: 2.0–5.0. However, these researchers do not mention how many sheep were used to determine these reference values and they do not describe the feeding regime of the animals, but state that these values are very sensitive to differences in feeding. Kraft and Dürr (12) emphasize that in general, measuring NABE in urine is ideal for identifying chronic feeding problems (without specifying the animal type). The same researchers proved in a study of five sheep that in case of anorexia a drop in urine pH as well as in NABE values occurs.

It has been reported that significant changes occur in NABE values when acidic salts or sodium bicarbonate are added to cow rations (1). The use of acidic salts in order to prevent hypocalcemia causes a reduction in urine NABE values. It is even possible to observe negative NABE values based on the amount of acidic salt added to cow rations. According to Hu et al. (8), net acid excretion is a more reliable indicator of acid load in animals than blood acid-base parameters. Our study showed that NABE values in young ewes and pregnant ewes differ significantly. While NABE is below 100 mmol/L in pregnant ewes, the limit is much higher in young ewes. Although rations might be considered to be the primary cause of this difference, it could also be caused by the differences in metabolism in older (pregnant) ewes and young ewes as well as the fact that the ewes were pregnant.

The evaluation of the correlation of NABE with acid and base in pregnant ewes and young ewes showed a significant positive correlation with base. No correlation with acid could be determined in young ewes, while a moderate degree of correlation between NABE and acid was found in pregnant ewes. In cows it has been reported that NABE has a positive correlation with base as well as with acid (2).

The base/acid ratio was significantly higher in pregnant ewes than in young ewes. In other words, young ewes excrete more acid in their urine than pregnant ewes do. In spite of this, young ewes have higher base and pH values in their urine than pregnant ewes. The most important reason may be that they have larger amounts of base elements in their urine than pregnant ewes.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Base</th>
<th>Acid</th>
<th>NH₄</th>
<th>NABE</th>
<th>B/A</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.72**</td>
<td>0.23†</td>
<td>−0.19†</td>
<td>0.69**</td>
<td>0.19†</td>
<td>−0.47**</td>
<td>0.66**</td>
<td>−0.35**</td>
</tr>
<tr>
<td>Base</td>
<td>0.40**</td>
<td>0.18†</td>
<td>0.93**</td>
<td>0.20†</td>
<td>−0.68**</td>
<td>0.74**</td>
<td>−0.21†</td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>−0.06†</td>
<td>0.27*</td>
<td>−0.52**</td>
<td>−0.52**</td>
<td>0.23†</td>
<td>−0.03†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄</td>
<td>−0.43**</td>
<td>0.09†</td>
<td>−0.14†</td>
<td>0.08†</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NABE</td>
<td>0.31†</td>
<td>−0.57**</td>
<td>0.69**</td>
<td>−0.21†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/A</td>
<td>0.01†</td>
<td>0.11†</td>
<td>0.08†</td>
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<td></td>
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</tr>
<tr>
<td>Na</td>
<td>−0.67**</td>
<td>0.25†</td>
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</tr>
<tr>
<td>K</td>
<td>−0.15†</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

** Correlation is significant at the p < 0.01 level.
* Correlation is significant at the p < 0.05 level.
† Correlation is not significant (p > 0.05).

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Base</th>
<th>Acid</th>
<th>NH₄</th>
<th>NABE</th>
<th>B/A</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.75**</td>
<td>0.60**</td>
<td>0.57**</td>
<td>0.76**</td>
<td>0.29†</td>
<td>0.15†</td>
<td>0.71**</td>
<td>0.37*</td>
</tr>
<tr>
<td>Base</td>
<td>0.78**</td>
<td>0.84**</td>
<td>0.99**</td>
<td>0.15†</td>
<td>0.39**</td>
<td>0.93**</td>
<td>0.40**</td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>0.64**</td>
<td>0.71**</td>
<td>−0.33*</td>
<td>0.03†</td>
<td>0.90**</td>
<td>0.44**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄</td>
<td>0.82**</td>
<td>0.15†</td>
<td>0.37*</td>
<td>0.83**</td>
<td>0.37*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NABE</td>
<td>0.21†</td>
<td>0.43**</td>
<td>0.90**</td>
<td>0.39**</td>
<td></td>
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<tr>
<td>B/A</td>
<td>0.33*</td>
<td>0.07†</td>
<td>−0.17†</td>
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<tr>
<td>Na</td>
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<td>−0.04†</td>
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</tr>
</tbody>
</table>

** Correlation is significant at the p < 0.01 level.
* Correlation is significant at the p < 0.05 level.
† Correlation is not significant (p > 0.05).
pH value

Scott (20) reported that urine pH values in sheep vary between 8.2 and 5.6 depending on the rations they eat. Ewes and calves excrete more phosphorus and acid in urine when fed concentrate diets than when fed roughage diets. Similarly, it has been reported that the difference between cations and anions in the diet of cows is closely related to urine pH values (15). The addition of anions to the diet causes a decrease in urine pH values in male goats (22) and pregnant sheep (14). According to Hu et al. (7) the pH value of blood and urine increases when the cation-anion ratio in the diet changes in favor of cations. In sheep, the addition of gypsum to rations at a rate of 1% causes the urine pH value to drop as far as 6.5 (23). Jones et al. (10) reported that goats in the 0 mEq/kg DCAD group had a urine pH of 6.0 to 6.5 five days after initiation of feeding the diet, and that pH was maintained through day 7, without significant reduction in blood pH. Our research revealed alkaline pH values in the urine of pregnant ewes as well as young ewes.

Feeding acidogenic salts to cows significantly reduces the sodium concentration and NABE values in urine while increasing the excretion of calcium (2, 5). Feeding them sodium bicarbonate, on the other hand, leads to a reduction of the concentration of chloride while causing NABE and pH values to swing toward alkaline values. Because sodium bicarbonate increases alkaline urine production, it may not be possible to identify when the rations are acidogenic (2).

Ammonium

The kidneys release ammonium into the urine as the final product of nitrogen metabolism in the body. According to Scott (18) ammonium is the primary means by which acidic ions coming in through the digestive system are excreted into the urine in sheep. It has been reported that the amount of ammonium in cow urine increases in parallel with a change towards acidosis in the acid-base equilibrium (2). Bacteria in the urine can break down urea in the urine into ammonium; consequently, bacterial contamination during sample collection can affect the levels of ammonium in the urine (2, 23). In our study, the amount of ammonium in young ewes’ urine was significantly higher than in the urine of pregnant ewes. This may be due to the differences in rations fed to the two groups of animals. It was expected that there would be a correlation between the amount of ammonium in the ewes’ urine and the base and NABE values. However, the fact that these relationships were not observed in young ewes indicates that there are differences in the metabolism of young and adult animals.

Sodium, potassium and calcium

Ninety percent of sodium is eliminated through the urine. For this reason, examining the urine is important for identifying the level of sodium in a flock. Furthermore, it is reported that there is a strong relationship in ruminants between sodium concentration and sodium intake or need (2). When acidic salts are added to cow rations, the sodium concentration drops significantly and this drop can be serious enough to cause sodium deficiency in some animals. Conversely, adding sodium bicarbonate to the rations leads to a significant increase of the sodium concentration in the urine (2). Similarly, the addition of sodium bicarbonate to sheep rations increases the level of sodium in the urine (9). It has been shown that climate conditions also affect urinary sodium levels (6). Excretion of sodium via urine increases in sheep exposed to hot weather which compensates for a drop in potassium and chloride ions (6). In our study, a negative correlation was found between sodium and potassium levels in the urine of young ewes (p < 0.01), but no such correlation was found in pregnant ewes. However, a strongly positive correlation was found between NABE and potassium levels, particularly in pregnant ewes.

Sheep consume much more potassium in their diet than they need. Since unneeded potassium is excreted in the urine, this causes alkaline urine in sheep (19). Similarly, a significant positive correlation was found in our study between base and potassium levels in urine.

The addition of acidic salts to rations in goats and sheep causes a slight metabolic acidosis; this in turn increases the mobilization of calcium from the bones. Acidosis decreases the binding of proteins to calcium and consequently more free calcium is excreted by the kidneys into the urine (14). Organisms (especially cows in the dry period) maintain a balance by excreting excessive calcium in their urine (2). In our study, the calcium concentration in the urine of young ewes was lower than that in pregnant ewes. According to Nelson and Tillman (16) this difference is due to the fact that adult ewes are better at absorbing dietary calcium and young ewes have a higher need for calcium for bone formation.

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Conflict of interest

The authors confirm that they do not have any conflict of interest.

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