THE DETERMINATION OF AMMONIA NITROGEN IN STEER'S URINE.

BY DONALD C. COCHRANE.

(From the Institute of Animal Nutrition of the Pennsylvania State College, in cooperation with the Bureau of Animal Industry of the United States Department of Agriculture.)

(Received for publication, September 27, 1915.)

In connection with certain metabolism experiments now in progress in this Institute, the determination of the nitrogen present in the urine of a steer as ammonium compounds and ammonia has become of considerable importance. Recurring peculiarities in the results obtained in some earlier work involving ammonia determinations led to the belief that the chloroform used in the urine as a preservative was inefficient, since in every case urine so treated showed an increase in the ammonia from day to day. Therefore it was planned to test out several preservatives especially as to their ability to prevent the breaking up of nitrogenous substances.

Numerous attempts have been made in this laboratory to differentiate between the nitrogen present in steer's urine as ammonium compounds and that present as ammonia. Braman,¹ however, has shown that practically all the ammonia nitrogen present is in the form of ammonium carbonate with possibly small quantities of ammonia. Simple aeration causes decomposition of ammonium carbonate, and while the addition of sodium chloride prevents a complete breaking up of the carbonate it has been found impossible to obtain a satisfactory separation.

The total quantity of urine excreted by a steer at one voiding was collected, immediately divided into four approximately equal parts, and preserved at about 10°C. during the period covered by analysis. The four samples were treated as follows:

I No preservative added.
II Chloroform added to saturation.
III Toluene added.
IV Sufficient N sulphuric acid added to render the sample slightly acid, using cochineal as an indicator.

The total nitrogen per cc. of urine was determined in all four samples using the Kjeldahl method. It was necessary, because of the dilution incident to the addition of the sulphuric acid to Sample IV, to compute the results obtained for ammonia nitrogen in terms of the original urine. In doing this, use was made of the total nitrogen in the original urine and the total nitrogen in the acid sample since these determinations were much more accurate than any measurement of volume available.

**TABLE I.**

*Experiment 220, Period I, Steer K, Preliminary.*

*Ration 7.0 Kg. Clover Hay.*

Nitrogen as ammonium compounds expressed as mg. nitrogen per cc. of original urine.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1st day*</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>7th day</th>
<th>8th day</th>
<th>9th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.44</td>
<td>0.98</td>
<td>1.93</td>
<td>4.02</td>
<td>4.86</td>
<td>5.74</td>
<td>6.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0.96</td>
<td>1.66</td>
<td>2.43</td>
<td>2.51</td>
<td>2.61</td>
<td>2.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1.39</td>
<td>2.27</td>
<td>2.87</td>
<td>3.44</td>
<td>3.59</td>
<td>3.75</td>
<td>3.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0.44</td>
<td>0.40</td>
<td>0.44</td>
<td>0.52</td>
<td>0.57</td>
<td>0.59</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Total nitrogen 8.48 mg. per cc.

Nitrogen as ammonium compounds was determined by Steel's modification of Folin's method. This method was decided upon because of the heavy precipitates of phosphates so often encountered in steer's urine. Duplicate determinations of ammonia nitrogen were made daily using the apparatus described by Folin, except that aeration was obtained through the use of a Crowell blower delivering about 7 liters per minute. Three hours were found to be sufficient for the recovery of the ammonia in all cases.

---

Table I shows the comparative results obtained upon each sample during nine successive days.

An examination of the table reveals rather astonishing results especially in view of the wide use of chloroform as a urinary preservative. The progressive and rapid increase in the ammonia nitrogen in Samples II and III, in which chloroform and toluene were used, would tend to cast doubt on the accuracy of ammonia determinations in the urine of herbivora reported by previous investigators.

The inhibiting action in the case of the sulphuric acid may be ascribed to the bactericidal action of the slight excess of acid over that necessary to neutralize the titratable alkalinity, or possibly to the formation of acid phosphates.6

To confirm the conclusions drawn from this experiment a further investigation of the action of sulphuric acid as a retardant of ammoniacal decomposition was undertaken. This covered a ten day period during which the total quantity of urine excreted each day was collected and sampled. Two samples of each daily urine were taken. One sample was treated with N H₂SO₄ as described above; the other was untreated. Both samples were kept in the ice chest until the analyses were made. In every case ammonia determinations were made as soon as possible after the close of the experimental day (6.00 p.m.) and in only two instances was the urine as much as forty hours old at the time of analysis. In the majority of cases fifteen hours, and in one case only two hours elapsed before the determinations were made.

Two composite samples were also made up covering the ten day period. One composite sample was untreated while to the other was added the quantity of sulphuric acid necessary to make the daily aliquot slightly acid.

The acid composite was found to be alkaline at the end of the eleventh day showing that some decomposition had taken place. The quantity of ammonia nitrogen present confirms this, there being twice as much in the acid composite as the average of the acid daily samples.

Table II shows the comparative ammonia nitrogen in the ten different urines together with that in the corresponding composite sample.

**TABLE II.**

*Experiment 220, Period I, Steer K.*

*Ration 7.0 Kg. Clover Hay.*

Nitrogen in urine as ammonium compounds expressed as mg. nitrogen per cc. of original urine.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>7th day</th>
<th>8th day</th>
<th>9th day</th>
<th>10th day</th>
<th>Composite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>0.25</td>
<td>0.25</td>
<td>(0.22*)</td>
<td>0.19</td>
<td>0.19</td>
<td>(0.21§)</td>
<td>0.27</td>
<td>0.34</td>
<td>0.13</td>
<td>(0.19*)</td>
<td>0.39</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.45</td>
<td>0.46</td>
<td>(0.53)</td>
<td>0.32</td>
<td>0.32</td>
<td>(0.26)</td>
<td></td>
<td></td>
<td>0.19</td>
<td>(0.42)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

**TABLE III.**

*Experiment 220, Periods II and III, Steer K.*

*Rations 2.25 Kg. Clover Hay and 1.5 Kg. Clover Hay with 3.0 Kg. Maize Meal.*

Nitrogen in urine as ammonium compounds expressed as mg. nitrogen per cc. of original urine.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Period II</th>
<th>Period III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
<td>Composite</td>
</tr>
<tr>
<td>Acid</td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.21</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>1.71</td>
</tr>
</tbody>
</table>

* Urine about 40 hours old when determinations were made.
§ Determinations made immediately at close of experimental day.

A comparison of the ammonia nitrogen in the acid with that in the untreated urine furnishes a full confirmation of the results shown in Table I. In every case there is much more nitrogen present as ammonia in the untreated than in the corresponding acid sample.

In order to be sure that sufficient sodium hydroxide was added to neutralize the slight excess of sulphuric acid and also liberate all ammonia, the quantity added was varied with the results in Table IV.
A variation from 0.5 to 1.0 gram of NaOH makes no appreciable difference in the quantity of ammonia liberated as shown in Table IV.

### Table IV.
The Effect of Variations in the Quantity of NaOH on the Ammonia Determinations.

<table>
<thead>
<tr>
<th>NaOH (gm.)</th>
<th>Nitrogen (mg.)</th>
<th>Urine 1514</th>
<th>Urine 1516</th>
<th>Urine 1556</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.18</td>
<td>1.08</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>1.13</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td></td>
<td>1.12</td>
<td></td>
</tr>
</tbody>
</table>

An examination of the results obtained on the acid and on the untreated composite sample, Table II, shows the same relative increase in ammonia as was found in the case of the daily urines. That decomposition took place even in the acid composite is shown by comparing it with the average of the acid daily samples from which it was made.

A composite of a series of urines, Table III, Period II, containing a slightly greater quantity of ammonia, treated as above showed an even greater difference between the acid and the untreated sample and about the same increase over the average of the acid daily urines from which it was made.

### Conclusions.

1. Figures for nitrogen as free ammonia in the urine of cattle are unreliable because of the decomposition of ammonium carbonate.
2. Figures for total ammonia nitrogen are worthless unless special precautions are taken to overcome the rapid ammoniacal decomposition.
3. Chloroform and toluene fail to prevent the breaking up of the nitrogenous compounds.
4. Sulphuric acid when added to the urine of a steer in sufficient quantity to fix the ammonia present as carbonate and to slight
excess retards decomposition to such an extent as to allow time for analysis.

5. All ammonia determinations must be made on daily samples of urine because the sulphuric acid does not completely stop decomposition in a composite sample.
THE DETERMINATION OF AMMONIA NITROGEN IN STEER'S URINE
Donald C. Cochrane


Access the most updated version of this article at http://www.jbc.org/content/23/1/311.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/23/1/311.citation.full.html#ref-list-1