Nitrogen Sources of Urinary Nitrogenous Compounds
Stimulated by Infusion of Glutamine or Ammonia in the Chicken

Yutaka Karasawa
Laboratory of Animal Nutrition and Feed Science, Faculty of Agriculture,
Shinshu University, Minamiminowa-mura, Nagano-ken 399-45, Japan

Introduction

It has been reported that glutamine amide-N and ammonia-N are useful nitrogen sources for uric acid synthesis in the chicken. Ammonia-N is possibly incorporated into uric acid by way of glutamine formation, because the intravenously administered ammonia most largely appeared in the amide of glutamine of all nitrogenous compounds in chicken blood and tissues. Ammonia-N is also possible to be incorporated into uric acid directly or by way of glycine. Urinary ammonia, the second major nitrogenous compound in chicken urine, can be derived from blood glutamine amide-N and ammonia-N. Little information is available for comparison of ammonia-N and glutamine amide-N as nitrogen sources for urinary uric acid and ammonia in the chicken.

Our previous study has shown that the infused ammonia has more stimulatory effect on uric acid production than the infused glutamine at a relatively low infusion rate in the chicken. Not only the incorporation of the exogenous nitrogen into uric acid but also the contribution of endogenous N to uric acid are possibly involved in the stimulation of uric acid production by the infusion of ammonia or glutamine. However, the contribution of the infused and endogenous nitrogen to the stimulated nitrogenous compounds has not been compared between ammonia and glutamine infusions.

The present study was carried out to compare the incorporation of the infused ammonia-N and glutamine amide-N into urinary nitrogenous compounds and its contribution to the stimulation of these urinary compounds by the infusions of glutamine and ammonia in the chicken.

Materials and Methods

Animals and diets

The experimental birds used were 12-month-old dwarf single comb White Leghorn male chickens from Nagano prefectural Animal Industry Experiment Station and weighed 1.2 kg on the average. They were housed in individual cages in a light-controlled room (12 L: 12 D), fed 35 g of an experimental diet per kg body wt once a day (9:00 a.m.) for 5 days and allowed to drink water freely. The chickens consumed the experimental diet within 40 min. The protein level of the diet containing egg albumen as the sole source of protein was 5% or 20%. The composition of the diet was similar to as described earlier.

Catheterization and infusion

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The chickens were operated 6 hr after the meal on day 5 for the catheterization of heart, portal vein and ureter, including 1.5-1.8 hr of time to complete the operation and a 200 U per kg body wt of heparin was injected into circulatory blood through the cardiac catheter as an anticoagulant. Just after the catheterization, $^{15}$N labeled ammonium acetate or glutamine with $^{15}$N labeled amide (25.35 atom % excess, Shokoh Tsusho Co., Ltd, Tokyo) dissolved in 5% glucose solution was continuously infused into the portal vein at a rate of 0.05 mmole/kg body wt/min for 6 hr. The ammonia or glutamine concentration of the infusate was 180 mM or 171 mM, respectively, and 20 ml of the infusate was infused in 1 hr. The infusates were adjusted to pH 7.4.

Collection of samples and chemical analysis

Total urine naturally excreted through the ureter catheter was collected at 20-min intervals for the first hour of glutamine or ammonia infusion and thereafter at 30-min intervals. Urine samples were diluted with lithium carbonate solution (0.5%) and the diluted urine was used for determinations of uric acid, ammonia, urea and total nitrogen and of their $^{15}$N enrichments.

Uric acid was determined by the uricase method, ammonia by the spectrophotometric method, urea by the urease method and total nitrogen by the Kjeldahl method. Samples for $^{15}$N determinations of uric acid, urea, ammonia and total nitrogen were processed as reported previously. $^{15}$N-Enrichments of these samples were determined by the emission spectroscopy with an N-150 $^{15}$N-analyzer (Japan Spectroscopic Co., Ltd, Tokyo).

Calculation and statistical analysis

Other urinary nitrogen than uric acid and ammonia was obtained by subtracting uric acid-N and ammonia-N from total nitrogen, since urinary urea was detected in a small amount. Each of urinary total nitrogen, uric acid-N, ammonia-N and other nitrogen than uric acid and ammonia was divided into endogenous and exogenous components according to the nitrogen origin by the previously reported equation. The exogenous component is derived from the infused glutamine amide-N or ammonia-N and the endogenous one from other nitrogen than the infused N present in the body before the infusion starts.

Data were statistically analyzed by Student's $t$ test.

Results

The exogenous and endogenous components of urinary nitrogenous compounds excreted during the infusion of glutamine or ammonia in chickens fed a 5% protein diet are shown in Table 1. The exogenous components of urinary uric acid-N, ammonia-N and other nitrogen were not significantly different between the chickens infused with ammonia and glutamine, but a mean value for the exogenous component of the other nitrogen was about 6-times higher in chickens infused with glutamine than in those infused with ammonia, explaining the larger exogenous component of urinary total N in the glutamine infusion. No significant differences were also observed in endogenous components of uric acid and ammonia between glutamine and ammonia infusions, although the endogenous uric acid tended to become larger in the glutamine infusion. Like the exogenous component of other nitrogen than uric acid-N and ammonia-N, the endogenous one of the other nitrogen was 6-times larger in glutamine infusion than in ammonia infusion ($P < 0.01$), which almost accounted for all the
Table 1. The exogenous and endogenous components of urinary nitrogenous compounds excreted during the infusion of ammonia or glutamine in chickens fed a 5% protein diet

<table>
<thead>
<tr>
<th>Urinary nitrogen</th>
<th>Infusion¹</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ammonia</td>
<td>Glutamine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg/kg body wt/6 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N</td>
<td>77.4±9.1²</td>
<td>172.9±6.9**</td>
<td></td>
</tr>
<tr>
<td>Exogenous</td>
<td>27.1±3.9</td>
<td>44.0±10.1</td>
<td></td>
</tr>
<tr>
<td>Endogenous</td>
<td>50.3±5.2</td>
<td>128.9±14.7*</td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>45.5±9.2</td>
<td>55.0±8.0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14.3±3.9</td>
<td>10.0±2.7</td>
<td></td>
</tr>
<tr>
<td>Exogenous</td>
<td>31.1±5.3</td>
<td>45.0±7.8</td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td>15.2±3.1</td>
<td>21.5±3.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8.9±1.2</td>
<td>10.8±3.2</td>
<td></td>
</tr>
<tr>
<td>Exogenous</td>
<td>6.3±0.2</td>
<td>10.7±3.8</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>16.6±1.8</td>
<td>96.3±3.4**</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.8±1.1</td>
<td>23.1±6.2</td>
<td></td>
</tr>
<tr>
<td>Exogenous</td>
<td>12.7±0.7</td>
<td>73.1±4.8**</td>
<td></td>
</tr>
</tbody>
</table>

¹ Ammonium acetate or L-glutamine was continuously infused into the portal vein at a rate of 0.05 mmole/kg body wt/min for 6 hr.
² Values are means±SEM of three chickens.
³ Exogenous N=¹⁵N x 100/Atom % excess of infused ammonia or glutamine amide-N, Endogenous N=N-Exogenous N.
*,** Significantly different from a corresponding value for ammonia-infused group at P<0.05 and P<0.01, respectively.

larger endogenous component of total N in glutamine infusion than in ammonia infusion (P<0.05). As a result total other nitrogen composed of endogenous and exogenous other nitrogen was significantly larger in glutamine infusion than in ammonia infusion (P<0.01), mostly accounting for the larger total urinary nitrogen in the glutamine infusion (P<0.01). Neither urinary excretion of total uric acid nor total ammonia was significantly different between glutamine and ammonia infusions.

The exogenous and endogenous components of urinary nitrogenous compounds excreted during the infusion of ammonia or glutamine in chickens fed a 20% protein diet are shown in Table 2. Although no significant differences were observed in exogenous components of uric acid and ammonia between ammonia and glutamine infusions, the exogenous component of urinary uric acid tended to be larger in the ammonia infusion. On the other hand, exogenous other nitrogen was almost zero in ammonia infusion, but nearly the same as exogenous ammonia-N when glutamine was infused. Owing to the small contribution of ex-
Table 2. The exogenous and endogenous components of urinary nitrogenous compounds excreted during the infusion of ammonia or glutamine in chickens fed a 20% protein diet

<table>
<thead>
<tr>
<th>Urinary nitrogen</th>
<th>Infusion</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ammonia</td>
<td>Glutamine</td>
</tr>
<tr>
<td></td>
<td>mg/kg body wt/6 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N</td>
<td></td>
<td>134.7±13.4</td>
<td>234.8±32.7</td>
</tr>
<tr>
<td>Exogenous</td>
<td></td>
<td>52.0±4.8</td>
<td>49.2±6.7</td>
</tr>
<tr>
<td>Endogenous</td>
<td></td>
<td>82.6±9.3</td>
<td>185.5±37.3</td>
</tr>
<tr>
<td>Uric acid</td>
<td></td>
<td>111.5±9.4</td>
<td>155.3±19.9</td>
</tr>
<tr>
<td>Exogenous</td>
<td></td>
<td>48.0±3.7</td>
<td>33.3±5.4</td>
</tr>
<tr>
<td>Endogenous</td>
<td></td>
<td>63.5±6.3</td>
<td>122.0±22.9</td>
</tr>
<tr>
<td>Ammonia</td>
<td></td>
<td>13.4±1.3</td>
<td>22.4±2.3</td>
</tr>
<tr>
<td>Exogenous</td>
<td></td>
<td>5.8±0.9</td>
<td>7.5±1.1</td>
</tr>
<tr>
<td>Endogenous</td>
<td></td>
<td>7.5±1.0</td>
<td>14.9±3.4</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>9.7±4.5</td>
<td>56.9±12.2*</td>
</tr>
<tr>
<td>Exogenous</td>
<td></td>
<td>−1.8±1.6</td>
<td>8.4±0.4*</td>
</tr>
<tr>
<td>Endogenous</td>
<td></td>
<td>11.5±3.2</td>
<td>48.4±12.1</td>
</tr>
</tbody>
</table>

Explanations are given in footnote for Table 1.

Exogenous other nitrogen to total exogenous nitrogen compared to chickens fed a 5% protein diet, exogenous component of urinary total nitrogen was almost the same irrespective of the infused nitrogenous compounds. Endogenous components of uric acid-N, ammonia-N and other nitrogen in glutamine infusion were 2-, 2- and 4-times those in ammonia infusion, respectively, although not significant. Consequently the endogenous component of urinary total nitrogen in glutamine infusion was 2-times that in ammonia infusion. The total uric acid-N, ammonia-N and other nitrogen and total of these tended to be larger in glutamine infusion than in ammonia infusion (P<0.05, other nitrogen), which was almost accounted for by the differences in their endogenous components between ammonia and glutamine infusions.

Little or no incorporation of glutamine amide-N into urinary urea was observed (not shown) as reported previously.

Discussion

The present experiment demonstrated that the amide-N of the infused glutamine appeared in urine as nitrogenous compounds other than uric acid and ammonia in much larger amounts than did the infused ammonia-N, irrespective of dietary protein intake. According to O'Dell et al., the nitrogenous compounds other than uric acid and ammonia involve urea,
creatine, creatinine, amino acids, purines and undetermined nitrogen compounds. In the present experiment the amide-N of glutamine was not incorporated into urea as reported previously\(^1\) and nor did it appear in urine in intact form\(^2\). Consequently the other nitrogenous compounds in glutamine infusion in the present experiment seem to consist of creatine, creatinine, amino acids except glutamine, purines except uric acid and undetermined nitrogenous compounds. The conversion of glutamine amide-N to these compounds should be further studied.

It has been reported that both glutamine amide-N and ammonia-N are useful nitrogen sources for uric acid synthesis in the chicken\(^1\text{--}^5\). The larger stimulation of urinary uric acid excretion by ammonia infusion than by glutamine infusion in the chicken has been shown by our previous experiment\(^12\), but the comparative study on the incorporation of glutamine amide-N and ammonia-N into uric acid has not been reported. The present experiment indicated that ammonia-N tended to be more incorporated into urinary uric acid than glutamine amide-N at a high level of portein intake. This fact suggests that the infused ammonia-N is not incorporated into uric acid all by way of glutamine formation, although ammonia-N infused into the chicken is greatly incorporated into blood and tissue glutamine\(^6,7\).

This suggestion is supported by the fact that the avian liver has enzymes involved in a direct incorporation of ammonia into uric acid\(^8\text{--}^{10}\), ammonia infusion stimulates uric acid production in the chicken treated with a glutamine synthetase inhibitor\(^11\) and ammonia-N is incorporated into uric acid as the amino nitrogen of glycine in chicken liver\(^23\).

It has been shown that urinary ammonia is possibly liberated from glutamine\(^1\), asparagine\(^24\) and AMP\(^25\) in chicken kidney and derived from blood ammonia\(^6\). In acidotic dogs\(^26,27\) the amide nitrogen of circulating plasma glutamine, the arterial blood ammonia and the amino nitrogen of plasma glutamine are reported to contribute 33--50\%, 35\% and 16--25\% of the urinary ammonia, respectively. The present experiment did not indicate clearly that the amide-N of blood glutamine is a better source of urinary ammonia than blood ammonia. This may be due to the fact that blood ammonia-N can appear in urine by way of glutamine formation, since the infused ammonia is greatly incorporated into the amide of glutamine in the chicken\(^6,7\).

Our previous study\(^12\) has shown that the infused ammonia has more stimulatory effect on uric acid production than the infused glutamine at a relatively low infusion rate. In the present experiment, however, the larger stimulation of urinary excretion of uric acid, ammonia and total nitrogen was shown when glutamine was infused into chickens fed a 20\% protein diet. In addition, the data obtained here indicated that the stimulatory effects of infused ammonia and glutamine on these urinary nitrogenous compounds rather reflect the changes in the endogenous components of these compounds than those in the infused N-incorporated components. Since the endogenous components of urinary uric acid, ammonia, other nitrogenous compounds and total nitrogen are decreased by the infusion of ammonia\(^17,18\), the large effects of the infused ammonia in decreasing the endogenous components of urinary nitrogenous compounds are concluded to be one of reasons for the larger excretion of urinary total nitrogen, uric acid, ammonia and other nitrogenous compounds in the glutamine infusion than in the ammonia infusion.

It has been unknown through what mechanisms the endogenous components of urinary
uric acid, ammonia, other nitrogenous compounds and total nitrogen are decreased by the infusion of ammonia and increased by the infusion of glutamine. The stimulatory and depressive mechanisms should be studied further.

Summary

The present study was carried out to compare the incorporation of the infused ammonia-N and glutamine amide-N into urinary nitrogenous compounds and its contribution to the stimulation of these urinary compounds by the infusions of glutamine and ammonia in the chicken fed 5% or 20% protein diet.

There were no significant differences between incorporations of ammonia-N and glutamine amide-N into urinary uric acid, ammonia or total nitrogen, although ammonia-N tended to be more incorporated into uric acid than glutamine amide-N in chickens fed a 20% protein diet. The incorporation of glutamine amide-N into other nitrogenous compounds than ammonia and uric acid was more than 6-times that of ammonia-N (P<0.05 in chickens fed a 20% protein diet). No significant differences were observed in endogenous uric acid-N and ammonia-N between glutamine and ammonia infusions in chickens fed a 5% protein diet, but endogenous other nitrogen and total nitrogen in glutamine infusion were 6- and 2.5-times those in ammonia infusion, respectively (P<0.05). In chickens fed a 20% protein diet, endogenous uric acid-N, ammonia-N, other nitrogen and total nitrogen in glutamine infusion were 2-, 2-, 4- and 2-times those in ammonia infusion, respectively, although not significant. The stimulatory effects of the infused glutamine and ammonia on urinary uric acid, ammonia, other nitrogenous compounds and total nitrogen excretion rather reflected the changes in the endogenous components of these compounds than those in the infused N-incorporated components.

Acknowledgments

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22) KARASAWA, Y. Unpublished data.


アンモニアあるいはグルタミンを注入したニワトリで
増加する尿窒素化合物の窒素源

唐澤　豊

信州大学農学部，上伊那郡南箕輪村399–45

本研究は，5％あるいは20％蛋白質飼料を摂取した
ニワトリに注入したアンモニア-N とグルタミンアミド
-N の尿窒素化合物への取り込みと，その取り込みがアンモニアあるいはグルタミンの注入による尿窒素化合物
の変化にどの程度貢献するかを比較するために行った。

尿の尿酸，アンモニアあるいは総窒素へのアンモニア
-N とグルタミンアミド-N の取り込みの間には有意差
はなかった。しかし20％蛋白質飼料を摂取したニワト
リでは，アンモニア-N の方がグルタミンアミド-N よ
り多く尿酸へ取り込まれる傾向があった。アンモニア，
尿酸以外の尿窒素化合物へのグルタミンアミド-N の取
り込みは，アンモニア-N の取り込みの6倍以上であっ
た（5％蛋白質飼料摂与時に P < 0.05）。内因性尿酸と
アンモニアは，5％蛋白質飼料を摂与したニワトリでは
アンモニア注入とグルタミン注入で有意差が認められな
かったが，内因性の他の窒素化合物と総窒素はグルタミ
ン注入ではアンモニア注入のそれぞれ6倍と 2.5 倍の取
込みであった（P < 0.05）。20％蛋白質飼料を摂与した
ニワトリでは，内因性の尿酸，アンモニア，他の窒素化
合物および総窒素は，グルタミン注入時にアンモニア注
入時のそれぞれ2倍，2倍，4倍および2倍の取り込み
であった。グルタミンあるいはアンモニア注入時の尿尿
酸，アンモニア，他の窒素化合物および総窒素の排泄量
は，注入グルタミンアミド-N およびアンモニア-N 由
来のこれら尿窒素化合物の変化よりむしろ内因性N由来
のこれら尿窒素化合物の変化を反映していた。

（家禽会誌，24，8～15，1987）