Estimation of Nitrogen Maintenance Requirements and Potential for Nitrogen Deposition in Fast-Growing Chickens Depending on Age and Sex

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ABSTRACT

Experiments were conducted to estimate daily N maintenance requirements (NMR) and the genetic potential for daily N deposition (ND_{max}T) in fast-growing chickens depending on age and sex. In N-balance studies, 144 male and 144 female chickens (Cobb 500) were utilized in 4 consecutive age periods (I: 10 to 25 d; II: 30 to 45 d; III: 50 to 65 d; and IV: 70 to 85 d). The experimental diets contained high-protein soybean meal and crystalline amino acids as protein sources and 6 graded levels of protein supply (N1 = 6.6%; N2 = 13.0%; N3 = 19.6%; N4 = 25.1%; N5 = 31.8%; and N6 = 37.6% CP in DM). The connection between N intake and total N excretion was fitted for NMR determination by an exponential function. The average NMR value (252 mg of N/BW_{kg}^{0.67} per d) was applied for further calculation of ND_{max}T as the threshold value of the function between N intake and daily N balance. For estimating the threshold value, the principle of the Levenberg-Marquardt algorithm within the SPSS program (Version 11.5) was applied. As a theoretical maximum for ND_{max}T, 3,592, 2,723, 1,702, and 1,386 mg of N/BW_{kg}^{0.67} per d for male and 3,452, 2,604, 1,501, and 1,286 mg of N/BW_{kg}^{0.67} per d for female fast-growing chickens (corresponding to age periods I to IV) were obtained. The determined model parameters were the precondition for modeling of the amino acid requirement based on an exponential N-utilization model and depended on performance and dietary amino acid efficiency. This procedure will be further developed and applied in the subsequent paper.

Key words: nitrogen maintenance requirement, growth potential, protein deposition, growing chicken, modeling protein metabolism

INTRODUCTION

Accurate estimation of the amino acid requirements in growing animals is of fundamental importance for diet formulation. Physiological-based models for simulating growth have been partly used to predict these requirements (Smith, 1978; Hurwitz et al., 1983; Alleman et al., 1999). Genetically, growing animals have a finite potential to deposit protein (Leclercq, 1983; Renden et al., 1992; Smith et al., 1998). In addition, sex (Rosa et al., 2001; Chamruspollert et al., 2002) and age (Zuprizal et al., 1992; Rimbach and Liebert, 1999) are important factors of influence, and it has been demonstrated that, related to the metabolic body mass, the potential of the growing chicken to deposit protein decreases with increased age (Liebert et al., 2000).

In addition, the ongoing improvement of the genetic potential for protein deposition (PD_{max}T) by breeding success has to be taken into account for qualified and physiologically based amino acid requirement data, according to a targeted amount of daily protein deposition. The purpose of the experiments was to estimate the genetic potential for daily N deposition (ND_{max}T) of fast-growing chickens (Cobb genotype) depending on age and sex. This estimation was based on results of N-rise experiments with graded dietary protein supplies and its use within an exponential model for evaluating the N-use process of growing animals.

MATERIALS AND METHODS

Birds and Housing

The experiments were conducted at the facilities of the Institute for Animal Physiology and Animal Nutrition and were approved by the Animal Welfare Committee of the Agricultural Faculty. Four hundred 1-d-old chickens (Cobb 500) were prepared for the experiments by being fed a commercial starter diet with 25.3% CP in DM up to the beginning of the trial period. A total of 288 chickens (144 male, 144 female) were used in 4 N-balance studies according to age periods I to IV (I: 10 to 25 d; II: 30 to 45 d; III: 50 to 65 d; and IV: 70 to 85 d). Each N-balance experiment utilized 72 chickens (36 males, 36 females), randomly allotted to 6 diets with graded protein contents (Table 1). Birds were individually housed in metabolism
cages with wire floors, equipped with individual feeders and self-drinking systems. Mean BW within the age periods was 289 ± 31 and 270 ± 29 g (I), 1,514 ± 62 and 1,430 ± 69 g (II), 3,212 ± 83 and 2,901 ± 68 g (III), and 4,517 ± 59 and 3,885 ± 44 g (IV) for males and females, respectively. Housing temperature was maintained at 32°C (1-d-old chicken) and was continuously decreased to 24°C up to the end of the experimental period (70 to 85 d). Warm red light was provided for 24 h/d.

**Diets, Feeding, and Sampling**

Experimental diets (Table 1) were formulated for graded CP levels (N1 = 6.6%; N2 = 13.0%; N3 = 19.6%; N4 = 25.1%; N5 = 31.8%; and N6 = 37.6% CP in DM) based on high-protein soybean meal as the single protein source. Crystalline amino acids (L-Lys, based on high-protein soybean meal as the single protein source) replaced the protein source to achieve a graded dietary protein level and age period. Wheat starch and dietary amino acid patterns were unchanged, independent of dietary protein level and age period. Wheat starch replaced the protein source to achieve a graded dietary protein supply with a constant amino acid ratio (Table 2), indicating threonine as the first-limiting amino acid in all diets and age periods, according to the recommendations of the NRC (1994). Consequently, the dietary amino acid patterns were unchanged, independent of dietary protein level and age period. Wheat starch replaced the protein source to achieve a graded dietary protein supply with a constant amino acid ratio (Table 2), indicating threonine as the first-limiting amino acid.

The energy content of the pelleted diets was kept nearly constant and was calculated in terms of ME according to the World’s Poultry Science Association (1984).

The individual experimental period was divided into an adaptation period (5 d) and 2 consecutive collecting periods (each 5 d). At the beginning of the adaptation period, the feed was given ad libitum to estimate the proper level of individual feed intake under housing conditions in metabolic cages. The individual feed supply was kept constant beginning at the d 3 of the adaptation period, slightly adapted during the first 2 d of the collecting period, and kept constant again up to the end of the collecting period. By this procedure, the daily feed consumption was close to the free choice level. Excreta collection was conducted 3 times a day to prevent ammonia losses from unacidified excreta. Excreta samples were immediately frozen and stored at −20°C until further analysis.

### Chemical Analyses

Dietary ingredients, mixed diets, and excreta were analyzed according to the German Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten standards (Naumann and Bassler, 1976-1997). The N content was quantified due to the Dumas method (Leco LP-2000, Leco Instrument GmbH, Kirchheim, Germany) and CP was calculated (factor 6.25). Amino acids of the protein source were analyzed by ion-exchange chromatography (LC 3000, Biotronik, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) following acid hydrolysis with and without an oxidation step for quantitative determination of sulfur-containing amino acids. Ether extract was analyzed following HCl hydrolysis of the feed samples.

### Statistical Analyses

Results are presented as mean values ± SEM. Statistical analyses were run with SPSS software package (Version 12.0 for Windows; SPSS Inc., Chicago, IL). The N maintenance requirement (NMR) was determined using the exponential regression between N intake (NI; mg/BW<sub>kg</sub>−0.67 per d) and total N excretion (NEX; mg/BW<sub>kg</sub>−0.67 per d) for NI = 0, simulating N-free feeding. This NMR value was set as point of intersection with the y-axis when estimating the threshold value of the function between NI and N balance or deposition (ND) for each age period and gender. The statistical procedure followed several iteration steps by the Levenberg-Marquardt algorithm within the SPSS statistical package. The applied N-use model (Gebhardt, 1966; Liebert, 1995; Thong and Liebert, 2004a,b,c) is described as
Table 2. Analyzed nutrient content of the experimental diets (percentage of DM)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
<th>N6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>6.6</td>
<td>13.0</td>
<td>19.6</td>
<td>25.1</td>
<td>31.8</td>
<td>37.6</td>
</tr>
<tr>
<td>Ether extract</td>
<td>2.4</td>
<td>4.7</td>
<td>6.6</td>
<td>9.2</td>
<td>11.5</td>
<td>13.6</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>1.71</td>
<td>1.9</td>
<td>1.9</td>
<td>2.2</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Crude ash</td>
<td>5.14</td>
<td>6.2</td>
<td>6.7</td>
<td>7.5</td>
<td>8.3</td>
<td>8.7</td>
</tr>
<tr>
<td>N-free extract</td>
<td>84.1</td>
<td>74.2</td>
<td>65.2</td>
<td>56.0</td>
<td>45.9</td>
<td>37.1</td>
</tr>
<tr>
<td>Starch</td>
<td>78.2</td>
<td>65.8</td>
<td>52.7</td>
<td>40.8</td>
<td>28.9</td>
<td>17.1</td>
</tr>
<tr>
<td>Sugar</td>
<td>4.18</td>
<td>5.0</td>
<td>5.1</td>
<td>7.3</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
<td>AMEn (MJ/kg of DM)</td>
<td>15.5</td>
<td>15.3</td>
<td>14.8</td>
<td>14.8</td>
<td>14.8</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Amino acid composition (g/100 g of CP) (Lys = 100)

<table>
<thead>
<tr>
<th>Amino acid composite</th>
<th>Lys</th>
<th>Met</th>
<th>Met + Cys</th>
<th>Thr</th>
<th>Arg</th>
<th>His</th>
<th>Leu</th>
<th>Ile</th>
<th>Val</th>
<th>Phe</th>
<th>Tyr</th>
<th>Gly</th>
<th>Ser</th>
<th>Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.21</td>
<td>4.74</td>
<td>6.10</td>
<td>3.87</td>
<td>6.75</td>
<td>3.39</td>
<td>7.58</td>
<td>3.99</td>
<td>4.46</td>
<td>5.71</td>
<td>4.38</td>
<td>3.91</td>
<td>5.05</td>
<td>3.42</td>
</tr>
<tr>
<td>Amino acid ratio</td>
<td>100</td>
<td>66</td>
<td>85</td>
<td>54</td>
<td>94</td>
<td>47</td>
<td>105</td>
<td>106</td>
<td>62</td>
<td>79</td>
<td>54</td>
<td>61</td>
<td>70</td>
<td>47</td>
</tr>
</tbody>
</table>

1N1 = 6.6%; N2 = 13.0%; N3 = 19.6%; N4 = 25.1%; N5 = 31.8%; and N6 = 37.6% CP in DM.
2Calculated according to equation of the World’s Poultry Science Association (1984).

NR = NRmaxT (1 − e −b NI) [1]
ND = NRmaxT (1 − e −b NI) − NMR [2]

where NR = daily N retention (ND + NMR; mg/BWkg0.67); NRmaxT = theoretical maximum for NR (mg/BWkg0.67); b = slope of the N-retention curve (indicating the feed protein quality independent of N intake); and e = basic number of natural logarithm (ln). Consequently, PDmaxT is derived from NDmaxT and describes the theoretical maximum for daily protein deposition. The attribute “theoretical” indicates that the estimated threshold value (NDmaxT and NRmaxT, respectively), as well as the derived PDmaxT, are not in the area of practical growth data, but they characterize the estimated genetic potential that is not attainable by dietary factors. Consequently, the defined genetic potential can not be utilized completely by optimizing feeding strategies. But, it is possible to make use of this potential, defined as a percentage of PDmaxT or deduced daily protein gain as a performance parameter. Therefore, amino acid requirement data depending on levels of daily protein deposition and concerning the amino acid efficiency can be estimated (Thong and Liebert, 2004b,c).

RESULTS AND DISCUSSION

The data analysis was based on an exponential function between NI or limiting amino acid and ND, determined by N-balance studies. Ongoing experiments quantify the effects of several genotypes, including slow-growing chicken, on these parameters. Finally, the results of the study are an important tool for further model applications, mainly to obtain amino acid requirement data depending on performance and dietary amino acid efficiency (Liebert et al., 2000; Thong and Liebert, 2004a,b,c).

NMR

Tables 3 and 4 summarize the results of N-balance studies with male and female growing chickens. Nitrogen-balance data for 288 chickens were part of the study and are included in the estimation of NMR. Due to the 2 consecutive N-balance periods with fast-growing chickens, the SEM of the N-balance data was increased. The regression between daily NI and total N excretion, depending on age, period, and sex, was fitted by exponential functions (Figures 1 and 2). The results of breakpoint analysis with the y-axis were very similar for the different age periods and both sexes (Table 5). In conclusion, the average of NMR = 252 mg/BWkg0.67 per day can be proposed as a “working value” for the daily NMR of male and female growing chickens within the age periods under study.

Protein deposition in growing animals is the balance between synthesis and degradation of protein. The rate of protein synthesis and protein degradation as well as the surplus between synthesis and degradation in farm animals are influenced by many factors, such as age (Moehn and De Lange, 1998; Rimbach and Liebert, 1999), gender (Proudfoot and Hulan, 1978; Ajang et al., 1993; Sebastian et al., 1997), genotype (Barbato, 1992; Renden...
### Table 3. Summarized results of the N-balance experiments in age periods I (10 to 25 d) and II (30 to 45 d)\(^1\)

<table>
<thead>
<tr>
<th>Diet (% CP)</th>
<th>N1 (6.6)</th>
<th>N2 (13.0)</th>
<th>N3 (19.6)</th>
<th>N4 (25.1)</th>
<th>N5 (31.8)</th>
<th>N6 (37.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Mean BW (g)</td>
<td>358 ± 93</td>
<td>337 ± 91</td>
<td>488 ± 113</td>
<td>441 ± 101</td>
<td>525 ± 109</td>
<td>519 ± 107</td>
</tr>
<tr>
<td>DM intake (g/d)</td>
<td>46.8 ± 10.8</td>
<td>45.7 ± 9.8</td>
<td>57.9 ± 8.1</td>
<td>569 ± 8.2</td>
<td>51.6 ± 7.6</td>
<td>58.4 ± 7.6</td>
</tr>
<tr>
<td>N intake (mg/BW(_{kg^{0.67}}) per d)</td>
<td>939 ± 69</td>
<td>984 ± 44</td>
<td>2,023 ± 57</td>
<td>2,109 ± 46</td>
<td>2,605 ± 20</td>
<td>2,933 ± 52</td>
</tr>
<tr>
<td>N excretion (mg/BW(_{kg^{0.67}}) per d)</td>
<td>362 ± 13</td>
<td>342 ± 17</td>
<td>517 ± 14</td>
<td>675 ± 36</td>
<td>765 ± 73</td>
<td>893 ± 35</td>
</tr>
<tr>
<td>N balance (mg/BW(_{kg^{0.67}}) per d)</td>
<td>577 ± 59</td>
<td>641 ± 42</td>
<td>1,505 ± 56</td>
<td>1,433 ± 72</td>
<td>1,840 ± 14</td>
<td>2,040 ± 43</td>
</tr>
</tbody>
</table>

\(^1\)Mean ± SEM.

### Table 4. Summarized results of the N-balance experiments in age periods III (50 to 65 d) and IV (70 to 85 d)\(^1\)

<table>
<thead>
<tr>
<th>Diet (% CP)</th>
<th>N1 (6.6)</th>
<th>N2 (13.0)</th>
<th>N3 (19.6)</th>
<th>N4 (25.2)</th>
<th>N5 (31.8)</th>
<th>N6 (37.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Mean BW (g)</td>
<td>1,682 ± 162</td>
<td>1,553 ± 188</td>
<td>1,896 ± 201</td>
<td>1,779 ± 193</td>
<td>1,953 ± 173</td>
<td>1,805 ± 224</td>
</tr>
<tr>
<td>DM intake (g/d)</td>
<td>120 ± 4.0</td>
<td>115 ± 8.2</td>
<td>127 ± 5.0</td>
<td>124 ± 6.5</td>
<td>127 ± 4.7</td>
<td>124 ± 4.2</td>
</tr>
<tr>
<td>N intake (mg/BW(_{kg^{0.67}}) per d)</td>
<td>879 ± 37</td>
<td>888 ± 35</td>
<td>1,728 ± 57</td>
<td>1,759 ± 67</td>
<td>2,567 ± 57</td>
<td>2,698 ± 149</td>
</tr>
<tr>
<td>N excretion (mg/BW(_{kg^{0.67}}) per d)</td>
<td>362 ± 10</td>
<td>375 ± 5.6</td>
<td>725 ± 31</td>
<td>751 ± 66</td>
<td>1,207 ± 70</td>
<td>1,092 ± 87</td>
</tr>
<tr>
<td>N balance (mg/BW(_{kg^{0.67}}) per d)</td>
<td>516 ± 30</td>
<td>513 ± 34</td>
<td>1,002 ± 47</td>
<td>1,008 ± 33</td>
<td>1,359 ± 52</td>
<td>1,605 ± 77</td>
</tr>
</tbody>
</table>

\(^1\)Mean ± SEM.
et al., 1992, 1994; Pesti et al., 1996; Fatufe et al., 2004), and the plane of nutrition (Alleman et al., 2000; Sterling et al., 2003; Çiftci and Ceylan, 2004). In addition, the breeding process influences the surplus between synthesis and degradation (Schadereit et al., 1998). The approach used in the study was only focused on resulting protein deposition. Consequently, no conclusion was possible relating to the development of protein synthesis in chickens. But, for practical application of amino acid requirement data in relation to growth performance, the definition of the genetic growth potential depending on age, gender, and genotype was very important. Due to the applied model, the potential was estimated as a theoretical threshold value for daily NR, and the bird’s performance was expressed as the percentage to make use of these threshold values. Additionally, the results of estimating the threshold values ($NR_{\text{maxT}}$, $ND_{\text{maxT}}$) were a reflection of the breeding progress. Ongoing studies with slow-growing chickens, as well as with another genotype of fast-growing chickens, will further demonstrate this type of application of the exponential model. The approach for estimating NMR by regression analysis based on N-rise experiments was described by Thong and Liebert (2004a), using a quadratic function. However, the physiological interpretation of such a type of function may become difficult, especially if the peak of the fitted function is

Figure 1. Estimation of N maintenance requirement (NMR) by fitting an exponential function between daily N intake (NI) and N excretion (NEX) following graded protein supply in male growing chickens. e = basic number of natural logarithm (ln).

Figure 2. Estimation of N maintenance requirement (NMR) by fitting an exponential function between daily N intake (NI) and N excretion (NEX) following graded protein supply in female growing chickens. ND = daily N deposition or N balance; $NR_{\text{maxT}}$ = theoretical maximum for daily N retention; e = basic number of natural logarithm (ln).
detected for NI > 0. Consequently, the exponential function is preferred (Stunder and Liebert, 2005; Wecke and Liebert, 2005). The calculated mean value (NMR = 252 mg/BW_{kg}^{0.67} per d) was in agreement with data (NMR = 264 mg/BW_{kg}^{0.67} per d) summarized within the German Recommendations for Nutrient and Energy Requirement Standards (1999) and according to earlier observations (Velu et al., 1971; Scott et al., 1982). Furthermore, similar to German Recommendations for Nutrient and Energy Requirement Standards (1999), no significant effect of the age period on NMR was observed. Other experiments are more focused on amino acid maintenance requirement data (Edwards et al., 1999b; Edwards and Baker, 1999a; Sklan and Noy, 2004), using a factorial approach for evaluating total amino acid requirements. Consequently, actual N-maintenance requirement studies are scarce. Leeson and Summers (2001) summarized data from protein-free feeding of adult birds and concluded 200 to 300 mg of N/BW_{kg} per day (180 mg of N/BW_{kg}^{0.75} per d) as the NMR. Our NMR data per kilogram of BW per day were 30 to 50% higher, indicating that nonphysiological protein-free feeding tends to underestimate endogenous losses. The principle for evaluating amino acid requirements due to our procedure is the reliance of protein deposition on intake of the limiting amino acid (Liebert and Gebhardt, 1986a,b; Liebert et al., 2000; Thong and Liebert, 2004a,b,c). Consequently, the NMR is utilized as an approximated and generalized average quantity of N, which is taken into account for replacing endogenous losses via feces and urine.

**NR_{max} T**

The estimation of threshold values in male and female growing chickens is demonstrated in Figures 3 and 4, and...
related to the metabolic BW, the high capacity for protein deposition during early growth might be due to improved N utilization, as concluded by Zuprizal et al. (1992). Studies from Krogdahl and Sell (1988) give support to this conclusion by demonstrating increased protease activity in the gut of young chickens as compared with older ones. Due to morphological changes, the potential for nutrient digestion and absorption seems to increase during the starting phase (Sell et al., 1991; Uni et al., 1999; Geyra et al., 2001; Sklan, 2001). Additionally, lower protein catabolism in the early stage of growth (Sklan and Noy, 2004) is an important factor of influence. A declining fractional synthesis rate of protein in defined breast and leg muscles was observed with increasing age of chicken (Kang et al., 1985). In our study, the theoretical threshold value for daily ND was reduced from 3,592 mg/BW<sub>kg</sub><sup>0.67</sup> (10 to 25 d) to 1,386 mg/BW<sub>kg</sub><sup>0.67</sup> (70 to 85 d) for male and from 3,452 mg/BW<sub>kg</sub><sup>0.67</sup> (10 to 25 d) to 1,286 mg/BW<sub>kg</sub><sup>0.67</sup> (70 to 85 d) for female broilers, respectively. Similar trends were observed by Rimbach and Liebert (1999).

Calculating the protein deposition by different percentage of use of PD<sub>max</sub> and defined BW, increased daily protein deposition is observed between age period I and II for male and female chickens. Afterwards, the ability for daily protein deposition expressed as percentage of PD<sub>max</sub>T is reduced by about 10%. The trend was in general accordance with actual results from slow-growing broiler genotypes (Samadi et al., 2004).

Genotype is another important factor for protein deposition (Leclercq, 1983; Marks and Pesti, 1998; Smith and Pesti, 1998; Shelton et al., 2003), obviously with strong effects on the level of protein degradation (Simon, 1989).
Rimbach and Liebert (1999) observed superior potential of the genotype Cobb (6.2%) compared to the Ross genotype in the early growth period. In growth studies conducted by Han and Baker (1991), a fast-growing strain gained faster than a slow-growing strain due to higher voluntary feed intake and, consequently, improved feed efficiency in fast-growing chickens.

In our study, only a trend was observed for increased daily protein deposition potential in male growing chickens, compared with females. This observation was generally consistent with growth studies from Han and Baker (1994), indicating that male growing chickens gained faster (12%) than females due to higher daily feed intake in males. Zuprizal et al. (1992) and the NRC (1994) came to similar conclusions. More pronounced sex differences were also observed by Fanatico et al. (2005). Sex-dependent carcass composition is a further factor of influence (Hurwitz et al., 1980; Moran and Bilgili, 1990), but was not a focus in our study.

In conclusion, the metabolic BW-related potential of growing broilers to retain N is decreasing with increasing age. This is a biological growth mechanism and in agreement with other growing animals. The estimation of threshold values, based on N-rise experiments with both sexes, indicated that male broilers tended to retain more N than females. However, the difference was lower than expected and, in practice, more pronounced due to superior feed intake in male growing chickens. The applied procedure gives a reflection of changing the potential for protein deposition depending on sex and age. The determined N-metabolism data (NMR, NRmaxT) are extremely important as databases for further model applications, including the modeling of amino acid requirements depending on daily protein deposition and efficiency of the dietary limiting amino acid. Due to this model application, a connection has to be established between intake of the limiting amino acid and N retention.

REFERENCES


