INTRODUCTION

The essential amino acid lysine is frequently the first- or second-limiting amino acid in poultry diets. Improving the efficiency of lysine use for protein synthesis would effectively lower the lysine requirement and decrease feed costs. Understanding how lysine is degraded and how the degradation is regulated would identify potential molecular targets for interventions to decrease lysine degradation. To better understand lysine degradation in poultry, 3 experiments were conducted. In experiment 1, one-day-old chicks were fed 1.07, 1.25, 1.73, or 3.28% dietary lysine for 2 wk. Measures of liver lysine catabolism including lysine α-ketoglutarate reductase (LKR) and lysine oxidation (LOX) were assessed. The α-aminoadipate δ-semialdehyde synthase (AASS) is a bifunctional enzyme composed of both LKR and saccharopine dehydrogenase activities, and the relative abundance of this protein and mRNA were likewise assessed. Moreover, potential alternative pathways of lysine catabolism that depend on L-amino acid oxidase (AAOX) and on lysyl oxidase (LYLOX) were considered. In experiment 1, chicks fed lysine-deficient diets had decreased \((P < 0.05)\) LKR activities compared with chicks fed at or above the requirement. However, the lowered LKR activities were not associated with a decreased \((P > 0.05)\) LOX as measured in vitro. In experiments 2 and 3, chicks 28 d of age did not decrease LKR activity \((P > 0.05)\) in response to a lysine-deficient diet. No changes in AASS protein abundance or mRNA were detected. Likewise, no differences in the mRNA abundances of AAOX or LYLOX were detected. The activity of AAOX did increase \((P < 0.05)\) in birds fed a lysine-adequate diets compared with those fed a lysine-deficient diet. Based on kinetic parameters and assumed concentrations, AAOX could account for about 20% of liver lysine oxidation in avians.

Key words: lysine, lysine α-ketoglutarate reductase, α-aminoadipate δ-semialdehyde synthase, lysyl oxidase, L-amino acid oxidase

Effect of dietary lysine on hepatic lysine catabolism in broilers\(^1,2\)

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ABSTRACT Lysine is frequently a first- or second-limiting amino acid in poultry diets. Improving the efficiency of lysine use for protein synthesis would effectively lower the lysine requirement and decrease feed costs. Understanding how lysine is degraded and how the degradation is regulated would identify potential molecular targets for interventions to decrease lysine degradation. To better understand lysine degradation in poultry, 3 experiments were conducted. In experiment 1, one-day-old chicks were fed 1.07, 1.25, 1.73, or 3.28% dietary lysine for 2 wk. Measures of liver lysine catabolism including lysine α-ketoglutarate reductase (LKR) and lysine oxidation (LOX) were assessed. The α-aminoadipate δ-semialdehyde synthase (AASS) is a bifunctional enzyme composed of both LKR and saccharopine dehydrogenase activities, and the relative abundance of this protein and mRNA were likewise assessed. Moreover, potential alternative pathways of lysine catabolism that depend on L-amino acid oxidase (AAOX) and on lysyl oxidase (LYLOX) were considered. In experiment 1, chicks fed lysine-deficient diets had decreased \((P < 0.05)\) LKR activities compared with chicks fed at or above the requirement. However, the lowered LKR activities were not associated with a decreased \((P > 0.05)\) LOX as measured in vitro. In experiments 2 and 3, chicks 28 d of age did not decrease LKR activity \((P > 0.05)\) in response to a lysine-deficient diet. No changes in AASS protein abundance or mRNA were detected. Likewise, no differences in the mRNA abundances of AAOX or LYLOX were detected. The activity of AAOX did increase \((P < 0.05)\) in birds fed a lysine-adequate diets compared with those fed a lysine-deficient diet. Based on kinetic parameters and assumed concentrations, AAOX could account for about 20% of liver lysine oxidation in avians.

Key words: lysine, lysine α-ketoglutarate reductase, α-aminoadipate δ-semialdehyde synthase, lysyl oxidase, L-amino acid oxidase

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2705

Generally, lysine is second limiting in broiler diets and first limiting in turkey diets (Baker et al., 2003). Improving the efficiency of lysine use for protein synthesis can reduce feed costs and potentially the nitrogen and phosphorus input/output of poultry production systems. Essential for improving lysine nutriture is a thorough understanding of the pathways that degrade lysine and the regulatory mechanisms attendant to those pathways. For example, once mechanisms regulating lysine degradation are described, advanced biomolecular techniques could be used to enhance the efficiency of lysine utilization for protein synthesis by decreasing lysine degradation, an approach verified in cell culture (Cleveland et al., 2008). The importance of increasing the efficiency of lysine use for protein synthesis is now more important than ever as growers are increasing the quantity of lysine in pre-starter, starter, and grower diets (Agri Stats, 2008).

Given the plethora of reports investigating the effects of different dietary levels of lysine on poultry performance, it is surprising how little is known about lysine catabolism in poultry. Much of what we speculate to
be true about lysine metabolism in poultry is inferred from mammals. It is presumed that the primary route of lysine oxidation in poultry is via the intermediate saccharopine. Hutzler and Dancis (1968) reported the conversion of lysine to saccharopine by human tissues. Subsequently, the purification and physical properties of the enzyme responsible for this conversion in rat liver, lysine α-ketoglutarate reductase (LKR; E.C. 1.5.1.8), were reported (Noda and Ichihara, 1978). Later, a bifunctional enzyme α-aminoacidipate δ-semialdehyde synthase (AASS) that contained LKR and saccharopine dehydrogenase (SacD; E.C. 1.5.1.9) activities was described (Markovitz et al., 1984). Early reports suggested that the enzyme in both avian (Wang et al., 1973) and mammalian liver (Chu and Hegsted, 1976) responded to alterations in dietary lysine. It has been suggested (Cleveland et al., 2008) that decreases in LKR activity may be responsible for the conservation of lysine noted in rodents (Said and Hegsted, 1970). However, the adaptation of LKR to low levels of dietary lysine has not been extensively studied in poultry.

Arguably, from the standpoint of comparative nutrition, one aspect of lysine metabolism in poultry that has been acknowledged is the participation of l-amino acid oxidase (AAOX; E.C. 1.4.3.2), an enzymatic activity associated with microsomes (Shinwari and Falconer, 1967). Whereas l-lysine appears to be a poor substrate for AAOX in mammalian tissues (Holme and Goldberg, 1982; Mason et al., 2004), AAOX from avian liver seems to function well with l-lysine as a substrate (Struck and Sizer, 1960). However, reports describing the adaptive response of the enzyme to alterations in dietary lysine are lacking.

With respect to lysine metabolism, there may be a third pathway for lysine degradation that should be considered. Trackman and Kagan (1979) found that lysyl oxidase (LYLOX; E.C. 1.4.3.13), thought to act only on protein-bound lysine, oxidizes free diamines, including lysine. This finding certainly raises the question as to the contribution of LYLOX to lysine catabolism in vivo, in addition to its adaptive response. Given the paucity of knowledge about these 3 pathways and their adaptive responses, experiments were undertaken to increase our understanding of lysine catabolism and its regulation in chickens fed diets differing in lysine content.

**MATERIALS AND METHODS**

**Birds and Diets**

Three separate experiments were conducted. In the first experiment, 32 one-day old straight-run Cobb × Cobb chicks (Southern Poultry Farms Inc., Harrisonburg, VA) were randomly divided into 4 groups of 8 birds each and allotted to individual cages. The required lysine content of a starter diet is 1.1% (NRC, 1994). A semipurified lysine-deficient diet was prepared (Biehl and Baker, 1997). Crude protein was determined in duplicate according to AOAC (1990) procedures using automated Kjeldahl equipment by Tecator (Tecator Inc., Herndon, VA). Using this semipurified diet (diet 1), 3 additional diets with graded levels of lysine were constructed (Table 1).

In all experiments, feed and water were provided ad libitum. In experiment 1, dietary treatments lasted from 1 d of age until euthanasia, which occurred 14 or 15 d later. Individual BW and feed intake were measured on d 14 for all 32 birds. Due to the length of time required for sample processing, the sample harvest was divided between 2 d, with 4 birds from each group killed on d 14 and the remaining birds killed on d 15. Individual liver weights were taken for chicks euthanized on d 14 and the relative liver size was calculated. Relative liver size was not calculated for the 16 birds killed on d 15. In experiment 1, liver LKR activity and in vitro lysine oxidation were measured on all 32 birds.

For experiments 2 and 3, a total of 25 chicks were used. Fourteen-day-old Ross × Ross chickens (Moyer’s Chicks Inc., Quakertown, PA) were housed in individual cages for a period of 13 d. Older birds were used so that a different portion of the growth cycle could be studied and to gain tissue for a more in-depth analysis. Chickens were fed diets that were either deficient or adequate in lysine (diets 1 and 2 of Table 1). On d 13 of the experiments, animals were euthanized and livers were removed and used in the following enzymatic assays. All experiments were approved by the West Virginia University Animal Care and Use Committee.

**Tissue Preparation**

Livers were harvested immediately after euthanasia and a portion (at least 0.5 g) was immediately frozen in liquid nitrogen for gene expression analysis. The remainder of the liver was placed in ice-cold H-medium [mannitol, 220 mM; sucrose, 70 mM; HEPES, 5 mM; ethylene glycol tetraacetic acid, 1 mM; β-mercaptoethanol, 5 mM; and BSA, 0.05% (wt/vol), pH = 7.4] for use in enzyme assays. Livers were minced with a razor blade and homogenized using a Potter-Elvehjem homogenizer. A 25% (wt/vol) homogenate was prepared, 5 mL was removed for further processing to determine AAOX activity, and the remainder of the 25% homogenate was used to determine LKR activity, lysine oxidation, and AASS protein abundance. For determining AAOX activity, the 25% homogenate was diluted to a 10% homogenate, centrifuged at 10,000 × g for 10 min at 4°C, and the supernatant was centrifuged for an additional hour at 100,000 × g at 4°C. The resulting microsomal pellet was resuspended in 1 mL of H medium and used to determine AAOX activity.

**LKR Activity Assay**

The LKR activity was measured spectrophotometrically as the lysine-dependent oxidation of NADPH at 340 nm as previously described (Blemings et al.,
A Beckman-Coulter DU-640 spectrophotometer was used. All liver samples were assayed for LKR activity in duplicate. Reaction mixtures contained 150 mM HEPES, 135 mM mannitol, 45 mM sucrose, 5 mM 2-mercaptoethanol, 0.05% (wt/vol) BSA, 0.25 mM NADPH, 15 mM α-ketoglutarate, 0.05% (vol/vol) polyoxyethylene octyl phenyl ether (Triton X-100), ±40 mM l-lysine HCl in a final volume of 2.09 mL. Reactions were started by the addition of lysine. All assays were conducted on the linear portion of the velocity curve with respect to both enzyme concentration and time.

**In Vitro Lysine Oxidation**

Liver lysine oxidation (LOX) was determined using the procedure described by Blemings et al. (1998). Incubations were started by the addition of 1 mL of tissue homogenate to Erlenmeyer flasks (25 mL capacity) held in a 41°C oscillating water bath. Carbon dioxide was trapped in 1.5-mL microcentrifuge tubes containing 0.5 mL of base trap solution (ethanolamine:methylcellosolve, 1:2). Final concentrations of reagents in the incubation mixture were 10 mM l-lysine, 3 mM MgCl₂, 0.2 mM EDTA, 182 mM mannitol, and 61 mM sucrose in a final volume of 2 mL. The final specific activity of lysine was 4.2 bq/nmol. The radioactive lysine used was [U-14C] l-lysine. The recovery of 14CO₂ from lysine was linear over the 45 min incubation. Nonenzymatic production of 14CO₂ was corrected for by a sample containing a heat-killed homogenate preparation. Incubations were terminated by injecting 0.5 mL of 35% (wt/vol) perchloric acid into the mixture through the serum cap covering the flask. After the perchloric acid injection, flasks remained in the oscillating bath for at least 180 min, which was previously determined to be the minimal time for maximal recovery of 14CO₂. After at least 180 min, flasks were removed from the water bath, the base trap tubes were removed and placed into scintillation vials with 17 mL of Biosafe-II scintillation fluid, which were capped and vortexed. Radioactivity was determined using a Beckman LS 1800 (Beckman Coulter Inc., Somerset, NJ) liquid scintillation counter. The CV for this assay was less than 10%.

**AAOX Activity Assay**

The AAOX activity was measured as the formation of α-keto-ε-aminocaproate semicarbazone adduct at 248 nm (SPECTRAMax PLUS384 microplate spectrophotometer, Molecular Devices, Sunnyvale, CA) as previously described (Danson et al., 2002). Liver samples were assayed for AAOX activity in duplicate. Reaction mixtures contained 100 mM potassium phosphate, 250 mM semicarbazide, 25,000 units/L of catalase, 0.1% (vol/vol) triton-X 100, ±10 mM l-lysine HCl, and 25 µL of the microsomal suspension in a final volume of 1 mL. The reaction were started by the addition of lysine and allowed to incubate for 90 min with gentle shaking at 40°C. At the end of the 90 min of incubation, the reaction was stopped by the addition of 100

<table>
<thead>
<tr>
<th>Table 1. Dietary formulations</th>
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<tbody>
<tr>
<td>Ingredient (%)</td>
</tr>
<tr>
<td>Corn</td>
</tr>
<tr>
<td>Corn</td>
</tr>
<tr>
<td>Peanut meal</td>
</tr>
<tr>
<td>Soybean meal</td>
</tr>
<tr>
<td>Limestone, ground</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Vitamin-trace mineral mix</td>
</tr>
<tr>
<td>Choline chloride</td>
</tr>
<tr>
<td>l-Valine</td>
</tr>
<tr>
<td>l-Isoleucine</td>
</tr>
<tr>
<td>l-Tryptophan</td>
</tr>
<tr>
<td>Sodium monensin</td>
</tr>
<tr>
<td>Lysine-HCl (78.8%)</td>
</tr>
<tr>
<td>Measured lysine</td>
</tr>
<tr>
<td>Measured CP</td>
</tr>
</tbody>
</table>

1Supplied per kilogram of diet: manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B₆, 1.38 mg; niacin, 27.56 mg; pantothenic acid, 6.61 mg; thiamine, 2.20 mg; menadione, 0.83 mg; vitamin B₁₂, 0.01 mg; vitamin E, 16.53 IU; vitamin D₃, 2,133 IU; vitamin A, 7,716 IU.

2Teklad Test diets, Madison, WI.

3Coban-60 from Elanco Animal Health, Indianapolis, IN.

4Archer Daniels Midland Company, Decatur, IL.

μL of 6 N HCl. Samples were centrifuged for 2 min at 2,000 × g at 4°C, and 200 μL of supernatant was loaded onto a 96-well UV transparent plate (UV-Star Plate, PGC Scientific, Fredrick, MD) and assayed using the SPECTRAmax PLUS384. All assays were conducted on the linear portion of the velocity curve with respect to both enzyme concentration and time.

### Real-Time PCR

Real-time, reverse-transcription (RT) PCR was performed on a Bio-Rad iCycler (Bio-Rad, Hercules, CA) as previously described (Higgins et al., 2005). Primers were designed to the chicken AASS, LYLOX, and AAOX mRNA sequences as described in Table 2. The primers for AASS are located in the LKR region of the gene. Acidic ribosomal protein Po (ARP) was used for the housekeeping gene. The PCR products were sequenced and primers were confirmed to be specific. Melt curve analysis showed a single product for AASS, LYLOX, AAOX, and ARP.

Approximately 200 mg of frozen liver from each chicken was used to isolate total RNA. The RNA was extracted using TRIzol (Invitrogen), back-extracted twice with a 5:1 mixture of acid phenol:chloroform, then back-extracted twice with a 25:24:1 mixture of phenol:chloroform:isoamyl alcohol. The RNA quality was assessed using OD\textsubscript{260}/OD\textsubscript{280} nm. An RNA pool was constructed by combining 10 μL from each RNA sample. This pool was used with each real-time RT-PCR reaction to serve as a reference for differences in reverse transcription efficiencies. For each liver sample and the pool, 2 μg of total RNA was treated with 2 units of RQ1 RNase-Free DNase (Promega, Madison, WI) and reverse transcribed using 1-μg random primers (Invitrogen) and 200 units of Maloney-Murine Leukemia Virus reverse transcriptase (Promega) with RNasin (Promega).

Each cDNA sample was analyzed in separate reaction wells of a 96-well PCR plate for AASS, LYLOX, AAOX, and ARP in duplicate PCR reactions using a step-down PCR procedure as follows. The reaction was heated at 95°C for 5 min, then 10 cycles of 95°C for 15 s, annealing for 30 s, and 72°C for 30 s. The annealing temperature was 70°C for the first cycle and was reduced by 1 degree per cycle in the following 9 cycles. Next, 30 PCR cycles were completed with an annealing temperature of 60°C. The cDNA generated from the reference pool was included on each plate as template for AASS, LYLOX, AAOX, and ARP primers to serve as a control. Real-time PCR reactions contained 7 μL of DNase-free water, 0.5 μL of forward AASS, LYLOX, or AAOX primer (25 μM) or ARP primer (50 μM), 0.5 μL of reverse AASS, LYLOX, or AAOX primer (25 μM) or ARP primer (50 μM), 10 μL of Bio-Rad, IQ SYBR Green Supermix, and 2 μL of cDNA diluted 1:4. Data were analyzed using an efficiency-corrected relative expression (Pfaffl, 2001). Gene expression data are presented as fold change, relative to the level of expression in the pooled sample, which was set at 1.0.

### Western Blotting

Electrophoresis (SDS-PAGE) was performed using polyacrylamide gels (4% stacking and 12% resolving) on a mini-gel setup. Ten lanes were used allowing for 9 samples and 1 molecular weight marker to be analyzed at a time. Samples (20 μL) containing 10 μg of protein were loaded into each lane and electrophoresed for 2.5 h at 80 V. Western blotting was used to determine AASS abundance. The primary antibody used was rabbit anti-chicken AASS. The specificity of primary antibody to the AASS protein has been previously determined (Kiess et al., 2008), and the antibody was determined to cross-react with chicken AASS (A. S. Kiess, unpublished data). The secondary antibody was goat anti-rabbit conjugated with horseradish peroxidase, and the detection used was Pierce SuperSignal West Pico Chemiluminescent substrate (Pierce Biotechnology Inc., Rockford, IL). Bands at approximately 52 kDa were detected by exposure to film for 5 min. The band intensity was quantified using densitometry (FluoroChem 8000, Alpha Innotech Corporation, San Leandro, CA), and data were expressed relative to a pooled sample that contained equal quantities of protein from all samples.

### Protein Assay

Total protein was measured using Pierce Coomassie Plus Protein Assay Reagent (Thermo Scientific, Rockford, IL) with BSA as a standard.

### Table 2. Primer sequences used for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank accession no.</th>
<th>Primer sequence(^1)</th>
<th>Size of product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AASS</td>
<td>XM_416001</td>
<td>(F) GTGGGAGCTGACGACAGATGGGTTT3(^2)</td>
<td>2708 K</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(R) CTGGAAGGTCCTCTTCACACC3(^2)</td>
<td></td>
</tr>
<tr>
<td>LYLOX</td>
<td>NM_205481</td>
<td>(F) CCTTATCGGCGCGATTTAGA3(^2)</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(R) AGGGCTGCTGTGTAAGAAAT3(^2)</td>
<td></td>
</tr>
<tr>
<td>AAOX</td>
<td>XM_415327</td>
<td>(F) GTATCTCTGGGCTCTACACC3(^2)</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(R) GGTTGGATGTCCGACAAGTCT3(^2)</td>
<td></td>
</tr>
<tr>
<td>ARP</td>
<td>NW_001471461</td>
<td>(F) TGGCAGCTGATTAAGACCCG3(^2)</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(R) GAAGCTCAGCACTTCAAGGT3(^2)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)AASS = α-aminoadipate δ-semialdehyde synthase; LYLOX = lysyl oxidase; AAOX = l-amino acid oxidase; ARP = acidic ribosomal protein Po.

\(^2\)F = forward; R = reverse.
**RESULTS**

**Chick Growth and Performance and Liver Size.** Although the measured lysine content of the diets differed from the calculated content (Table 1), it is clear that diet 1 was lysine-deficient as the birds fed this diet gained less weight. Average BW of 14-d-old chicks fed the 4 diets are shown in Table 3. Dietary lysine level affected (\(P < 0.001\)) chick growth. The contrast for deficient (diet 1) versus adequate (diets 2–4) lysine treatments was significant. From the differences identified by these contrasts, it is clear that chicks fed diet 1 had significantly lower BW than chicks fed higher levels of lysine. The linear and quadratic effects were not significant.

The cumulative average feed intake for chicks fed diets 1 through 4 for 14 d is shown in Table 3. A diet effect (\(P < 0.0001\)) was detected. The contrast for deficient (diet 1) versus adequate (diets 2–4) lysine treatments was significant, indicating that chicks consuming diet 1 had significantly lower feed intake than chicks fed adequate or higher levels of lysine. The linear and quadratic effects were not significant.

The relative liver sizes of chicks in experiment 1 is shown in Table 3. The contrasts for deficient (diet 1) versus adequate dietary lysine treatments (diets 2–4) were significant. Additionally, there was a significant linear effect of dietary lysine on relative liver size.

**Lysine Metabolism.** The LKR activities in livers of chicks fed diets varying in lysine content in experiment 1 are shown in Table 3. An ANOVA shows a significant effect of diet on LKR activity. The contrast between deficient (diet 1) versus adequate (diets 2–4) diets was significant, indicating a lysine-deficient diet reduces LKR activity. There was no significant diet effect between the other diets. These results indicate that LKR enzyme activity responded to the low dietary level of lysine rather than the higher levels (diets 3 and 4), which were above the nutritional requirement (NRC, 1994) for chicks between 0 and 3 wk of age. To our knowledge, this report is the first to document an effect of dietary lysine on LKR activity measured spectrophotometrically in chickens. In vitro liver homogenate LOX data are shown in Table 3. A significant dietary effect was not detected. Contrasts for deficient versus adequate diets were not significant.

**Experiments 2 and 3**

**Chick Growth and Performance and Liver Size.** Consistent with experiment 1, in both experiments 2 and 3, birds fed the lysine-deficient diet (diet 1) gained less weight (\(P < 0.05\)) than birds fed the lysine-adequate diet (diet 2; Table 4). Birds receiving the lysine-deficient diet tended to consume less feed (\(P = 0.057\)) than those receiving the lysine-adequate diet (Table 4). In experiments 2 and 3, birds fed the lysine-deficient diet had a poorer feed conversion ratio (\(P < 0.05\)) than birds fed the lysine-adequate diet (Table 4). Moreover, birds consuming the lysine-deficient diet had smaller livers, but this was not the case when expressed relative to body size.

**Lysine Catabolism.** In contrast to the previous experiment, there was no effect of diet on LKR activity. However, chicks consuming the lysine-deficient diet had significantly lower LKR activity. There were no significant linear or quadratic effects of dietary lysine on LOX activity.

### Table 3. Effect of graded levels of dietary lysine on BW, feed consumption, relative liver size, lysine α-ketoglutarate reductase activity (LKR), and lysine oxidation (LOX) in 14-d-old chicks from experiment 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dietary lysine (%)</th>
<th>BW (g)</th>
<th>Cumulative feed intake (g)</th>
<th>Relative liver size (g/100 g)</th>
<th>LKR activity [nmol/(min·g)]</th>
<th>LOX activity [nmol/(min·g)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.07</td>
<td>282 ± 11*</td>
<td>315 ± 12*</td>
<td>2.98 ± 0.05*</td>
<td>620 ± 142*</td>
<td>1.17 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>1.25</td>
<td>450 ± 21</td>
<td>442 ± 26</td>
<td>2.64 ± 0.04**</td>
<td>1,425 ± 247</td>
<td>1.10 ± 0.12</td>
</tr>
<tr>
<td>3</td>
<td>1.73</td>
<td>435 ± 13</td>
<td>440 ± 17</td>
<td>2.81 ± 0.04</td>
<td>1,320 ± 173</td>
<td>0.95 ± 0.09</td>
</tr>
<tr>
<td>4</td>
<td>3.28</td>
<td>438 ± 20</td>
<td>433 ± 16</td>
<td>2.95 ± 0.07</td>
<td>1,410 ± 227</td>
<td>1.12 ± 0.09</td>
</tr>
</tbody>
</table>

*Values are means ± SEM (n = 8 for each treatment, except relative liver size, n = 4 for each treatment).

*Indicates that the contrast for deficient (1.07) versus all other treatments is significant.

**Indicates a significant linear effect.
had a significant reduction in liver AAOX activity. To more broadly define alterations in lysine catabolism as a function of dietary lysine, the relative abundance of AASS, AAOX, and LYLOX mRNA was determined. In no case was there a significant effect of diet on the expression of these 3 mRNA. Moreover, no differences in the abundance of the AASS protein between dietary treatments were detected. With respect to the Western blot, there was an interaction between diet and experiment, where numerically, the lysine-deficient diet had a significant reduction in liver AAOX activity. To more broadly define alterations in lysine catabolism as a function of dietary lysine, the relative abundance of AASS, AAOX, and LYLOX mRNA was determined. In no case was there a significant effect of diet on the expression of these 3 mRNA. Moreover, no differences in the abundance of the AASS protein between dietary treatments were detected. With respect to the Western blot, there was an interaction between diet and experiment, where numerically, the lysine-deficient diet had a significant reduction in liver AAOX activity.

**Correlation Analysis.** Using the data in experiments 2 and 3, correlations between indices of growth and lysine metabolism were performed to detect potential relationships between these variables. As expected, weight gain was positively correlated with feed consumed (r = 0.90, P < 0.0001) and liver weight (r = 0.85, P < 0.0001) and negatively correlated with feed conversion ratios (r = −0.67, P = 0.0002). In agreement with these findings was a positive correlation between feed consumption and liver weight (r = 0.77, P < 0.0001). Moreover, feed conversion ratios were negatively correlated with weight gain (r = −0.67, P = 0.0002) and liver size (r = 0.53, P = 0.0063). Liver weight and AAOX activity were positively correlated (r = 0.46, P = 0.04), as were relative liver size and LKR activity (r = 0.39, P = 0.06). The activity of AAOX was positively correlated with the AAOX mRNA abundance (r = 0.51, P = 0.02). However, there were no relationships between variables of AASS expression at the level of transcript or protein abundance or LKR activity.

### DISCUSSION

The BW data of this study are similar to that of Wang and Nesheim (1972) who fed 2 different strains of chicks, one with a high arginine requirement (HA) and the other with a low arginine requirement (LA), diets containing 0.9, 1.9, or 2.9% lysine. It was found that 0.9 and 2.9% lysine tended to depress growth by 3 wk. Compared with chicks fed 1.9% dietary lysine, chicks fed 0.9% lysine had about a 35% depression in growth rate in the HA strain and a 44% depression in growth rate in the LA strain (Wang and Nesheim, 1972). Results from the current study are similar; chicks consuming 1.07% lysine averaged 28% slower growth than chicks fed 1.25% dietary lysine. In a comparable study, an 11% growth depression was reported by Wang et al. (1973) for the LA strain when they were fed 0.96% lysine. However, Wang et al. (1973) did not find a significant difference in the BW at higher levels of lysine (1.46, 1.71, and 1.96% lysine). In contrast to the LA strain, the growth of the HA strain was depressed with additional dietary lysine supplementation (Wang et al., 1973). Collectively, these experiments clearly indicate that a lysine deficiency reduces growth, but effects of excess dietary lysine on growth may be dependent on additional factors such as genetics and age.

From the results of experiment 1, it is apparent that hepatic LKR responds to changes in dietary lysine. The effect is more pronounced when comparing low levels (deficient) to normal or higher levels (at or above the nutritional requirement levels) of lysine. The effect
of different levels of lysine in the present study is in close agreement with Wang et al. (1973) and Wang and Nesheim (1972) who found that increasing dietary lysine from deficient to adequate increased LKR activity. The measurements of LKR activities in Wang’s studies were based on saccharopine quantification. They saw an effect of genetic line on LKR activity, with lower enzymatic activity observed in the HA line. The LKR activity (expressed as μmol of saccharopine accumulated/h per g of liver) measured in the LA chicks fed 0.9, 1.9, and 2.9% lysine was 7.07, 10.24, and 10.16, respectively, whereas the LKR activity in HA chicks at the same levels of dietary lysine was 4.13, 7.01, and 8.41, respectively. These results indicate a quadratic response in the LA strain and a linear response in HA strain to dietary lysine. A similar response was observed in mammalian species with increased LKR activity due to increased dietary lysine (Chu and Hegsted, 1976; Muramatsu et al., 1984) and protein (Blemings et al., 1998). It also should be noted that the LKR activities measured spectrophotometrically in the current study are on the order of 3 to 9 times greater than those based on saccharopine quantification.

A previous study (Papes et al., 1999) examined changes in LKR activity and mRNA abundance in response to changes in lysine availability, and found a one-and-a-half-fold increase in LKR and SacD activities and about a 2-fold increase in LKR and SacD mRNA levels in both fasted mice and mice given excess lysine intraperitoneally. These studies in mammals are consistent with transcriptional regulation of LKR and presumably lysine degradation (Papes et al., 1999). However, in the current study, we did not detect increased LKR (AASS) mRNA as dietary lysine increased from deficient to adequate. The discrepancy between the studies may be due to difference in species, lysine level, or modes of mRNA detection (Northern blot versus RT-PCR). The majority of data from our laboratory support the conclusion that LKR activity is regulated posttranslationally (Kuess et al., 2008), as is the case in plants (Karchi et al., 1995).

The observation that liver LKR activity was 1,000-fold greater than liver LOX is in agreement with the Blemings et al. (1998) study in rats. In chickens, although the liver represents the tissue with the greatest fractional rates of LKR and LOX metabolism, on an absolute basis the tissues with the greatest capacity to degrade lysine via LKR and LOX are the intestine and breast muscle, respectively (Manangi et al., 2005). This raises the possibility that lysine is converted to α-aminoacidic acid or α-ketoacidic acid in extrahepatic tissues via the saccharopine-dependent pathway or possibly by a saccharopine-independent pathway. These metabolites have lysine carbon, which presents the possibility that these metabolites subsequently get oxidized in the liver (Edmonds and Baker, 1987). There is evidence in pigs that there is a similar response in several tissues, both hepatic and extrahepatic, to up-regulate lysine oxidation, excretion, or both when dietary lysine is in excess. Young male pigs fed 4.6% (approximately 3 times the requirement) lysine increased α-aminoadipate (an intermediate of lysine metabolism) concentrations in plasma, liver, kidney, and muscle by 10-, 3-, 7-, and 5-fold respectively compared with the control group (1.15% lysine-fed animals). Free lysine concentrations in the plasma, liver, kidney, and muscle increased by 11-, 10-, 12-, and 33-fold, respectively. Of the lysine consumed (4.6% dietary lysine), 26% was excreted in the urine. A 3-fold increase in dietary lysine above the requirement results in increased plasma α-aminoadipate concentration and excretion of free lysine in the urine (Edmonds and Baker, 1987).

Transport of lysine into mitochondria via a carrier protein(s) may be a control point in lysine oxidation because, in rat liver, the initial enzymes of lysine degradation are housed exclusively in mitochondria (Blemings et al., 1994). In rats, LKR and SacD activities were 100 times the rate of LOX in vitro. Similarly, in the present study, LKR activity was ~1,000-fold LOX. However, in rats, the rates of mitochondrial lysine uptake and lysine oxidation were not significantly different (P > 0.05), consistent with a mitochondrial uptake limitation to lysine oxidation (Blemings et al., 1998). Presumably, the same is true in the avian, but data as to the subcellular/submitochondrial location of LKR/SacD as well as mitochondrial uptake rates of lysine in the avian are lacking.

The effects of low dietary lysine were much less noticeable in experiments 2 and 3, and this was presumably because the birds were already 2 wk old when the experiment began. The low lysine diet was still clearly deficient as evidenced by the improved animal performance with added lysine. However, the metabolic disturbances were less evident. For example, no differences in LKR activity were detected. However, this may be the first report of an effect of dietary lysine on AAOX activity. As noted above, the AAOX activity was positively correlated with AAOX mRNA. On the surface, the AAOX pathway seems minor relative to the saccharopine-dependent pathway given their relative activities. However, if one considers their respective activities in Table 4 were measured under maximal velocity conditions and that the Michaelis constant of LKR for lysine in chickens is about 7 mM (Manangi et al., 2005), whereas the Km of AAOX for lysine is about 1.6 mM (Mizon et al., 1970), if lysine averages 0.5 mM in liver, AAOX could account for about 20% of lysine degradation in liver. It would seem, at least in avian liver, that the AAOX pathway makes a considerable contribution to lysine oxidation.

In conclusion, improving the efficiency of lysine use for protein synthesis offers producers an opportunity to decrease feed costs. An improved understanding of the how lysine is degraded and how that degradation is regulated will suggest what interventions may be most fruitful in achieving improved efficiency. In the present study, we have shown that younger birds are more sensitive to low dietary lysine and that decreased LKR
activities do not necessarily translate into decreased lysine oxidation, at least in vitro. Additionally, AAOX may contribute about 20% to liver lysine oxidation and warrants further study. Future studies should address the potential mitochondrial transport limitation as well as discerning the relative importance of transcriptional and posttranslational regulation of LKR.

REFERENCES


