Extending daily feed access intervals does not influence lysine HCl utilization but enhances amino acid digestibilities in broiler chickens

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ABSTRACT Off-sex, male Ross 308 chickens were offered maize-soy diets without and with 3.5 g/kg lysine monohydrochloride (**HCl**), which contained 10.0 or 12.8 g/kg digestible lysine, from 7 to 28 D post-hatch. Birds were permitted access to diets at intervals of 12, 16, and 20 h/day. Lysine HCl improved weight gain (1,465 vs. 1,417 g/bird; $P < 0.025$) and feed conversion ratios (1.351 vs. 1.382; $P < 0.005$). Extending feed access intervals increased weight gain (1,542 vs. 1,303 g/bird; $P < 0.001$) and feed intake (2,142 vs. 1,748 g/bird; $P < 0.001$) but compromised feed conversion ratios (1.390 vs. 1.342; $P < 0.001$). Extending feed access intervals increased ($P < 0.001$) both relative crop and gizzard weights and amounts of digesta retained in these organs. Effective lysine HCl utilization in poultry irrespective of feeding frequency, as opposed to pigs, may stem from anticipatory feeding behavior, crop and gizzard functionality, and increased episodes of reverse peristalsis. Collectively, these properties appear to modulate the relative intestinal uptakes of unbound lysine and protein-bound amino acids including lysine. Instructively, extending daily feed access intervals from 12 to 20 h increased average ileal digestibility coefficients of 16 amino acids by 12.8% (0.830 vs. 0.736; $P < 0.001$), which was linearly related ($r = -0.834$; $P < 0.001$) to hourly feed intake rates. Birds given 12 h feed access consumed relatively more feed on an hourly basis and this may have contributed to lesser amino acid digestibilities. As treatment interactions ($P > 0.35$) between lysine HCl and feed access intervals for parameters of growth performance were not observed, it was concluded that feed access intervals do not influence lysine utilization. The implications of these findings are discussed.

Key words: amino acids, feed access, lysine HCl, poultry

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INTRODUCTION

Research completed by Ted Batterham in swine (Batterham, 1974; Batterham and O'Neill, 1978; Batterham and Murison, 1981; Batterham and Bayley, 1989) demonstrated that lysine monohydrochloride (**HCl**) utilization was compromised by restricted feeding regimes as opposed to ad libitum feeding. A significant interaction between feeding frequency and lysine supplementation for weight gain was reported in pigs as growth responses to 2 and 4 g/kg lysine (as lysine HCl) with once daily feeding were 57 and 31% inferior to those achieved from frequent-feeding (Batterham, 1974). Subsequently, Batterham and Bayley (1989) concluded that the more rapid absorption of lysine HCl by growing pigs fed once daily resulted in an imbalance of amino acids at the sites of metabolism but a better-balanced supply of amino acids was absorbed with frequent feeding. If applicable to poultry, these findings could have real implications for lysine HCl utilization and, in turn, the growth performance of broiler chickens and, also, starch and protein digestive dynamics (Liu and Selle, 2015, 2017).

Broiler chickens are fed on an unrestricted basis under practical conditions; however, this is subject to the lighting regimen adopted, which may consist of an "18 h on–6 h off" pattern of illumination. Assuming that Batterham's outcomes in pigs apply to poultry, it follows that longer periods of illumination and extended daily feeding periods would attenuate any negative impacts of the asynchronous arrival of synthetic or unbound amino acids vs. protein-bound amino acids at sites of protein synthesis. Thus, the objective of the present experiment was to determine if daily feed access intervals of 12,

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16, and 20 h influence lysine HCl utilization in broiler chickens as the implications of this possibility to the successful development of reduced-crude protein diets are readily apparent.

Lysine is usually the second limiting amino acid in diets for broiler chickens and is the foundation for the ideal protein ratio concept in the least-cost formulation of diets. The optimal amino acid ratios recommended by Texas A&M University are based on the true digestible lysine content of the diet where lysine equals 100 (Wu, 2014). Lysine HCl, a synthetic or unbound amino acid, has been almost invariably included in broiler diets to complement protein-bound lysine for decades, as have methionine and threonine (Kidd et al., 2013). Moreover, the digestive dynamics of unbound vs. protein-bound amino acids are inherently different. Unbound amino acids do not require digestion and are more rapidly absorbed from the small intestine than their protein-bound counterparts (Wu, 2009) and this has been demonstrated in pigs in respect of lysine and threonine (Yen et al., 2004). Consequently, unbound amino acids become available at sites of protein synthesis in advance of protein-bound amino acids. This asynchronous post-enteral availability of amino acids for skeletal protein deposition may compromise amino acid utilization and growth performance and hinder the development and acceptance of reduced crude-protein broiler diets.

There is now considerable interest in the successful development of reduced-crude protein diets for chicken-meat production. This is a highly desirable objective because the potential benefits include economic advantages, enhanced bird welfare and flock health, coupled with reduced nitrogen pollution and ammonia emissions (Selle et al., 2015; Liu and Selle, 2017; Garland, 2018; Lambert and Corrent, 2018; Moss et al., 2018). While the focus on this study is on lysine HCl utilization, reduced crude-protein diets will, axiomatically, contain substantially higher inclusions of numerous synthetic amino acids. Thus, the possibility that their utilization may be influenced by lighting regimen holds increasing relevance.

Male chickens were offered either lysine-inadequate diets (10.0 g/kg digestible lysine) or diets supplemented with 3.5 g/kg lysine HCl (12.8 g/kg digestible lysine). Feed trays were physically removed from bioassay cages at the appropriate times to guarantee the validity of the feed access intervals. The parameters assessed to evaluate lysine HCl utilization included growth performance or weight gain, feed intake, and feed conversion ratios (**FCR**) from 7 to 14, 7 to 21, and 7 to 28 D post-hatch and nutrient utilization. Parameters of nutrient utilization included apparent metabolizable energy (**AME**), metabolizable energy to gross energy ratios (**ME: GE**), nitrogen (**N**) retention, and N-corrected AME (**AMEn**). The crop and gizzard are two digestive organs that clearly differentiate the digestive tracts of pigs and poultry; therefore, relative weights of these organs and the weights and pH of their contents were monitored. The enrichment of broiler diets with lysine HCl has been shown to significantly increase the ileal digestibility of lysine per se but also the digestibility of 7 additional amino acids (Selle et al., 2007). Therefore, apparent distal ileal digestibility coefficients of protein (N) and amino acids and their disappearance rates were determined. Lysine has unique properties as it may be retained or persist in tissue pools rather than being deaminated (Benevenga and Blemings, 2007). Consequently, concentrations of free amino acids in plasma taken from the systemic circulation at 0, 3, and 6 h following feed withdrawal were determined in birds given 12 h daily feed access. Finally, mRNA expressions for amino acid (**CAT-1, PepT-1**) and glucose (**SGLT-1, GLUT-2**) transport systems were quantified.

MATERIALS AND METHODS

Experimental Design

The experimental design consisted of a 2 × 3 factorial array of dietary treatments. A maize-soy lysine-deficient diet (10.0 g/kg digestible lysine) was supplemented with 3.5 g/kg lysine HCl to generate a lysine-adequate diet (12.8 g/kg digestible lysine). These diets were offered to broiler chickens with 12, 16, and 20 h daily duration of feed access, which was guaranteed by the physical removal of feed trays from the bioassay cages. All feed trays were attached to the cages at 8.00 pm in the evening and appropriate trays were removed at 8.00 am, 12.00 noon, and 4.00 pm the following day.

Diet Preparation

A lysine-deficient, maize-soy diet was formulated to meet standard nutrient specifications, apart from lysine, as shown in Table 1 and a similar, but lysine-adequate, diet was supplemented with 3.5 g/kg lysine HCl. Both diets contained 1.87 g/kg d, l-methionine, but no other synthetic amino acids, and about 28 g/kg whey protein concentrate. The dietary electrolyte balance (**DEB**) was maintained at 220 mEq/kg in both diets by manipulating dietary inclusions of sodium chloride and sodium bicarbonate to avoid lysine HCl reducing DEB by virtue of its chloride content. Maize was coarsely ground (6.0 mm hammer-mill screen) prior to being blended into the complete diets, which were steam-pelleted at a temperature of 80°C with a conditioner residence time of 14 s. The two diets were analyzed for amino acid concentrations (Table 2) and lysine concentrations of either 10.5 or 12.6 g/kg were detected.

Bird Management

This study fully complied with the guidelines (2017/1252) specifically approved by the Research Integrity and Ethics Administration of The University of Sydney. A total of 288 off-sex, male Ross 308 chicks were procured from a commercial hatchery and were initially offered a standard starter diet. At 7 D post-hatch birds were individually identified (wing-tags) and

Table 1. Composition and nutrient specifications of experimental diets where amino acids are expressed on a digestible basis.

Composition (g/kg)	Nil lysine HCl	Plus lysine HCl	Nutrient specifications (g/kg)	Nil lysine HCl	Plus lysine HCl
Maize	591	587	AME (MJ/kg)	12.97	12.97
Soybean meal	275	275	Protein	190.3	193.9
Whey protein conc.	28.4	29.3	Calcium	8.25	8.25
Soy oil	47.2	46.9	Total phosphorus	6.75	6.74
Lysine HCl	0.0	3.50	Phytate phosphorus	1.97	1.97
d,l-Methionine	1.88	1.87	Non-phytate P	4.78	4.77
Sodium chloride	2.92	1.78	Fat	71.0	70.4
Sodium bicarbonate	1.77	3.44	Fiber	21.2	21.1
Limestone	5.75	5.75	Sodium	1.80	1.80
Dicalcium phosphate	22.8	22.8	Potassium	8.06	8.05
Choline Cl (60%)	0.80	0.80	Chloride	2.28	2.27
Vitamin-mineral premix[1]	2.00	2.00	DEB (mEq/kg)	220	220
Celite	20.0	20.0	Lysine	10.00	12.80
			Methionine	4.63	4.62
			Cysteine	2.77	2.78
			Threonine	6.67	6.70
			Tryptophan	2.14	2.15
			Isoleucine	7.50	7.53
			Leucine	14.80	14.84
			Arginine	10.97	10.97
			Valine	8.14	8.16

[1]The vitamin-mineral premix supplied per tonne of feed: [MIU] retinol 12, cholecalciferol 5, [g] tocopherol 50, menadione 3, thiamine 3, riboflavin 9, pyridoxine 5, cobalamin 0.025, niacin 50, pantothenate 18, folate 2, biotin 0.2, copper 20, iron 40, manganese 110, cobalt 0.25, iodine 1, molybdenum 2, zinc 90, selenium 0.3.

Table 2. Analyzed total amino acid concentrations in the two experimental diets.

Amino acid (g/kg)	Nil lysine HCl	Plus lysine HCl
Arginine	10.7	10.1
Histidine	5.0	4.8
Isoleucine	8.5	8.4
Leucine	16.9	16.5
Lysine	10.5	12.6
Methionine	2.8	3.0
Phenylalanine	9.1	8.7
Threonine	7.7	7.6
Valine	9.2	9.0
Alanine	9.2	8.9
Aspartic acid	18.9	18.2
Glutamic acid	34.3	32.9
Glycine	7.4	7.0
Proline	11.3	10.9
Serine	9.3	9.0
Tyrosine	3.9	3.7

allocated into bioassay cages on the basis of body-weights so that mean weights and variations within cages were nearly identical. Each of 6 dietary treatments was offered to 8 replicate cages (6 birds per cage) from 7 to 28 D post-hatch. Birds had unrestricted access to water and specified access to feed in an environmentally controlled facility, which remained illuminated at all times. An initial room temperature of 32 ± 1°C was maintained for the first week, which was gradually decreased to 22 ± 1°C by the end of the third week.

Data and Sample Collection, Chemical Analyses, Calculations

Growth performance (weight again, feed intake, FCR) was determined at 14, 21, and 28 D post-hatch in order to detect if the pattern of responses varied over the course of the feeding study. Weight gains and feed intakes were monitored over each experimental period, and the body-weights of any dead or culled birds were recorded on a daily basis to correct feed intakes and adjust FCR calculations. Hourly feed intake rates were also calculated.

Total excreta outputs and feed intakes were recorded from 25 to 27 D post-hatch to determine parameters of nutrient utilization by the classical approach. These parameters included apparent ME, metabolizable to GE ratios, nitrogen retention, and AMEn. Excreta were dried in a forced-air oven at 80°C for 24 h and the GE of excreta and diets were determined using an adiabatic bomb calorimeter. The AME values of the diets on a dry matter basis were calculated from the following equation:

$$\begin{aligned}\text{AME}_{\text{diet}}&(\text{MJ/kg DM})\\ &= [(\text{feed intake} \times \text{GE}_{\text{diet}})\\ &\quad -(\text{excreta output} \times \text{GE}_{\text{excreta}})]/\text{feed intake}\end{aligned}$$

ME: GE ratios were calculated by dividing AME by the GE of the appropriate diets. N contents of diets and excreta were determined using a nitrogen determinator (Leco Corporation, St Joseph, MI) and N retentions calculated from the following equation:

$$\begin{aligned}\text{N retention}(\%) = 100 \times [&(\text{feed intake} \times \text{N in diet})\\ &-(\text{excreta output} \times \text{N excreta})]/\\ &(\text{feed intake} \times \text{N in diet})\end{aligned}$$

AMEn (AMEn MJ/kg DM) values were calculated by correcting N retention to zero using the factor of

Table 3. Primers used for quantitative real-time PCR.

Enzyme	Accession number	Forward sequence (5′ to 3′)	Reverse sequence (5′ to 3′)
β-actin	NM_205,518	GAGAAATTGTGCGTGACATCA	CCTGAACCTCTCATTGCCA
GLUT-2	Z22932	CCGCAGAAGGTGATAGAAGC	ATTGTCCCTGGAGGTGTT
SGLT-1	XM_415,247	AGATTTGGAGGGCAGAGGAT	GCCCAAAGAGATTTGGATGA
PepT-1	AY029615	TACGCATACTGTCACCATCA	TCCTGAGAACGGACTGTAAT
CAT-1	XM_417,116	CACATGGATACGGTTTGCAG	GTCCATGCTTCTCTCCGTGT

36.54 kJ/g N retained in the body (Hill and Anderson, 1958).

At 27 D post-hatch, systemic blood samples (brachial vein) were taken at 8.00 am, 11.00 am, and 2.00 pm (or 0, 3, and 6 h post-prandial) from 3 birds in each replicate cage that were allowed 12 h duration of feed access. This was to determine concentrations of free amino acids in plasma over time. Blood samples were centrifuged and decanted plasma samples were then kept at −80°C prior to analysis. Concentrations of 20 proteinogenic amino acids in plasma taken from the brachial vein were determined using precolumn derivatization amino acid analysis with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC; Waters AccQTag Ultra; Waters Australia PL; www.waters.com) followed by separation of the derivatives and quantification by reversed phase ultra-performance liquid chromatography. All amino acids were detected by UV absorbance and this procedure is fully described in Selle et al. (2016).

At day 28, birds were euthanized by an intravenous injection of sodium pentobarbitone, the abdominal cavity opened, and pH of digesta within the gizzard and crop was determined in situ with a pH probe. The weights of full and emptied gizzards and crops were recorded and abdominal fat pad weights measured. The small intestine was removed and digesta was gently expressed in its entirety from the distal half of the ileum and pooled by cage, homogenized, freeze dried, and weighed to determine the apparent digestibility coefficients of protein (N) and amino acids. Acid insoluble ash (**AIA**) and protein (N) concentrations were determined by methods described in Siriwan et al. (1993). Amino acid concentrations of diets and digesta were determined via 24 h liquid hydrolysis at 110°C in 6 M HCl followed by analysis of 16 amino acids using the Walters AccQTag Ultra chemistry on a Waters Acquity ultra-performance liquid chromatography. Apparent digestibility coefficients were calculated by the following equation:

$$\text{Digestibility coefficient} = [(\text{Nutrient}/\text{AIA})_{\text{diet}} - (\text{Nutrient}/\text{AIA})_{\text{digesta}}]/(\text{Nutrient}/\text{AIA})_{\text{diet}}$$

Disappearance rates (g/bird/day) of amino acids and protein (N) were calculated from the product of dietary concentrations of nutrient (g/kg), daily feed intake (g/day) from 21 to 28 D post-hatch and the relevant apparent ileal digestibility coefficient.

For the analysis of gene expression, total RNA isolation was carried out using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. The concentration and purity of total RNA were monitored by measuring its optical density at 260 and 280 nm. One microgram of total RNA was reverse transcribed with a reverse transcription kit (Thermo Fisher Scientific (Waltham, MA)) according to the manufacturer's instructions. A quantitative real-time PCR assay was performed with the 7500 fluorescence detection system (Applied Biosystems, Foster City, CA) according to optimized PCR protocols using the SYBR-Green PCR kit (Thermo Fisher). Nested primers were designed (Table 3) within cloned chicken cDNA sequences with the Primer Express software, optimized for use with Applied Biosystems Real-Time PCR Systems. The PCR conditions were an initial denaturation step at 95°C for 2 min, 40 cycles at 95°C for 3 s and annealing and extension temperature at 60°C for 30 s. To confirm amplification specificity, we subjected the PCR products from each primer pair to a melting curve analysis and subsequent agarose gel electrophoresis. Gene expression was quantified using the comparative threshold cycle method, and the data were expressed as the value relative to the 12 h feed frequency and lysine deficiency group.

Statistical Analysis

The experimental data, as a 2 × 3 factorial array, was subject to analyses of variance using the IBM SPSS Statistics 24 program (IBM Corporation Somers, NY, USA). Experimental units were cage means (8 replicate cages of 6 birds per dietary treatment) and a probability level of less than 5% was considered statistically significant. Linear and quadratic regressions and Pearson correlations were examined and pair-wise comparisons drawn using Tukey's post-hoc analysis when appropriate.

RESULTS

The effects of lysine HCl and daily duration of feed access on growth performance from 7 to 14 and 7 to 21 D post-hatch are shown in Table 4. Dietary addition of lysine HCl significantly increased weight gain by 5.81 and 5.32% ($P < 0.005$) and improved FCR by 3.42 and 3.13% ($P < 0.01$) in both phases, respectively. Similarly,

Table 4. The effects of lysine HCl supplementation and 12, 16, and 20 h daily duration of feed access on body weight gain, feed intake, and feed conversion ratio from 7 to 14 and 7 to 21 D post-hatch.

Treatment		7 to 14 D post-hatch			7 to 21 D post-hatch		
Lysine HCl (g/kg)	Duration (hours)	BW Gain (g/bird)	Intake (g/bird)	FCR (g/g)	BW gain (g/bird)	Intake (g/bird)	FCR (g/g)
0	12	206	247	1.202	618	831	1.346
0	16	251	308	1.225	742	994	1.341
0	20	266	336	1.261	783	1054	1.346
3.5	12	221	261	1.186	657	850	1.295
3.5	16	259	305	1.180	767	1000	1.304
3.5	20	285	340	1.194	834	1089	1.308
SEM		5.037	4.767	0.0194	13.328	13.797	0.0124
Main effects: Lysine							
0		241[a]	297	1.229[a]	714[a]	961	1.344[a]
3.5		255[b]	302	1.187[b]	752[b]	979	1.302[b]
Duration							
12		213[a]	254[a]	1.194	637[a]	840[a]	1.320
16		255[b]	306[b]	1.203	754[b]	997[b]	1.322
20		276[b]	338[c]	1.228	808[c]	1071[c]	1.327
Significance (P =)							
Lysine (L)		0.002	0.185	0.008	0.001	0.088	< 0.001
Duration (D)		< 0.001	< 0.001	0.189	< 0.001	< 0.001	0.862
L × D interaction		0.503	0.214	0.378	0.647	0.593	0.809

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 5. The effects of lysine HCl supplementation and 12, 16, and 20 h daily duration of feed access on body weight gain, feed intake, and feed conversion ratio from 7 to 28 D post-hatch, mortality/cull rates, hourly feed intake rates and relative abdominal fat-pad weights.

Treatment		Growth performance					
Lysine HCl (g/kg)	Duration (hours)	BW Gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality (%)	Hourly feed intakes (g/bird)	Relative fat-pad weights (g/kg)
0	12	1,278	1734	1.359	2.1	107	12.2
0	16	1,464	2027	1.385	2.1	92	13.6
0	20	1,508	2114	1.403	0	76	13.2
3.5	12	1,329	1761	1.326	2.1	111	11.7
3.5	16	1,492	2013	1.350	0	93	12.1
3.5	20	1,576	2169	1.337	0	78	12.8
SEM		22.380	25.137	0.0112	1.476	1.583	0.3525
Main effects: Lysine							
0		1,417[a]	1958	1.382[b]	1.4	91[a]	13.0[b]
3.5		1,465[b]	1981	1.351[a]	0.7	94[b]	12.2[a]
Duration							
12		1,303[a]	1748[a]	1.342[a]	2.1	109[c]	12.0[a]
16		1,478[b]	2020[b]	1.367[b]	1.0	94[b]	12.9[b]
20		1,542[c]	2142[c]	1.390[c]	0	77[a]	13.0[b]
Significance (P =)							
Lysine		0.011	0.276	0.001	0.567	0.039	0.025
Duration		< 0.001	< 0.001	< 0.001	0.376	< 0.001	0.045
Lysine × Duration interaction		0.665	0.390	0.893	0.718	0.727	0.379

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

extending feed access intervals increased feed intake by up to 33.1 and 27.5% and weight gain by up to 29.6 and 26.8%, which were highly significant ($P < 0.001$) responses. However, significant treatment interactions ($P > 0.20$) were not observed.

The effects of dietary treatments on overall growth performance, mortality/cull rates, hourly feed intake rates, and relative fat-pad weights from 7 to 28 D post-hatch are shown in Table 5. Lysine HCl increased weight gain by 3.46% (1,465 vs. 1,416 g/bird; $P < 0.025$) and improved FCR by 2.24% (1.351 vs. 1.382; $P < 0.005$). Extending feed access intervals significantly ($P < 0.001$) increased weight gain and feed intake by up to 18.3 and 22.5%, respectively. However, increasing feed access intervals had negative impacts on FCR by up to 3.58% (1.390 vs. 1.342; $P < 0.001$). Again, significant treatment interactions ($P > 0.35$) for growth performance parameters were not observed. Increasing feed access intervals from 12 to 16 h depressed hourly feed intake rates by 13.8 and from 16 to 20 h by 18.1%

Table 6. The effects of lysine HCl supplementation and daily duration of feed access on relative weights and contents of gizzard and crop and pH of digesta in of gizzard and crop at 28 D post-hatch.

Treatment		Gizzard			Crop		
Lysine HCl (g/kg)	Duration (hours)	Weight (g/kg)	Contents (g/kg)	pH	Weight (g/kg)	Contents (g/kg)	pH
0	12	17.5	7.3	3.37	7.6	36.0	4.81
0	16	15.8	7.4	2.96	5.6	10.9	4.72
0	20	15.7	6.6	3.02	3.9	1.1	5.06
3.5	12	16.6	7.5	3.31	7.7	34.7	4.78
3.5	16	16.1	7.7	3.05	5.4	7.9	4.87
3.5	20	16.0	5.8	2.95	3.7	0.6	5.29
SEM		0.3422	0.3225	0.0798	0.2280	1.9093	0.0808
Main effects: Lysine							
0		16.3	7.1	3.12	5.7	16.0	4.86
3.5		16.3	7.0	3.10	5.6	14.4	4.98
Duration							
12		17.1[b]	7.4[b]	3.34[b]	7.7[c]	35.4[c]	4.79[a]
16		16.0[a]	7.6[b]	3.00[a]	5.5[b]	9.4[b]	4.79[a]
20		15.8[a]	6.2[a]	2.98[a]	3.8[a]	0.8[a]	5.18[b]
Significance (P =)							
Lysine		0.761	0.680	0.809	0.400	0.317	0.082
Duration		0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Lysine × Duration interaction		0.100	0.185	0.554	0.824	0.794	0.308

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 7. The effects of lysine HCl supplementation and daily duration of feed access on apparent metabolizable energy (AME), metabolizable: gross energy ratios (ME: GE), nitrogen (N) retention, and N-corrected AME (AMEn) at 25 to 27 D post-hatch.

Treatment					
Lysine HCl (g/kg)	Duration (hours)	AME (MJ/kg DM)	ME:GE ratio	N retention (%)	AMEn (MJ/kg DM)
0	12	13.42	0.802	66.66	12.36
0	16	13.23	0.791	67.39	12.10
0	20	13.27	0.793	67.77	12.17
3.5	12	13.49	0.801	69.14	12.44
3.5	16	13.34	0.792	68.17	12.22
3.5	20	13.33	0.791	66.88	12.24
SEM		0.0707	0.0042	0.6793	0.0559
Main effects: Lysine					
0		13.30	0.795	67.94	12.21
3.5		13.38	0.794	68.06	12.30
Duration					
12		13.46[b]	0.801[b]	68.90	12.40[b]
16		13.28[a]	0.791[b]	67.78	12.16[a]
20		13.30[a]	0.792[a]	67.32	12.21[a]
Significance (P =)					
Lysine (L)		0.171	0.789	0.828	0.069
Duration (D)		0.032	0.034	0.069	< 0.001
Interaction (L × D)		0.937	0.921	0.435	0.890

[a,b]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

culminating in a 29.4% (77 vs. 109 g/bird; $P < 0.001$) depression. Lysine HCl reduced relative abdominal fat-pad weights by 6.15% (12.2 vs. 13.0 g/kg; $P < 0.025$), whereas increasing feed access intervals generated heavier fat-pads. The negligible mortality rate of 1.04% was not related to treatment.

The effects of dietary treatments on relative gizzard and crop weights and pH of digesta in both organs are shown in Table 6 where duration of feed access had highly significant effects. An interval of 12 h duration increased gizzard weights by 8.23% (17.1 vs. 15.8 g/kg), gizzard contents by 19.4% (7.4 vs. 6.2 g/kg), and increased pH from 2.98 to 3.34, in comparison to 20 h feed access. In the same comparison, 12 h feed access increased crop weights by 103% (7.7 vs. 3.8 g/kg), remarkably increased crop contents from 0.8 to 35.4 g/kg and decreased crop pH from 5.18 to 4.79.

The effects of lysine HCl and duration of feed access on nutrient utilization parameters are shown in Table 7.

Table 8. The effects of lysine HCl supplementation and daily duration of feed access on apparent digestibility coefficient of protein (N) at 28 D post-hatch and protein (N) disappearance rates (g/bird/day) in the distal ileum from 2 to 28 D post-hatch.

Treatment		Protein (N) digestibility coefficient	Protein (N) disappearance rate
Lysine HCl (g/kg)	Duration (hours)		
0	12	0.705	22.74
0	16	0.746	28.02
0	20	0.793	31.00
3.5	12	0.695	23.24
3.5	16	0.757	28.43
3.5	20	0.797	32.29
SEM		0.0079	0.4743
Main effects: Lysine			
0		0.748	27.25
3.5		0.749	27.98
Duration			
12		0.700[a]	22.99
16		0.752[b]	28.22
20		0.795[c]	31.64
Significance (P =)			
Lysine (L)		0.830	0.065
Duration (D)		< 0.001	< 0.001
Interaction (L × D)		0.386	0.595

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 9. The effects of lysine HCl supplementation and daily duration of feed access on apparent ileal digestibility of amino acids at 28 days post-hatch.

Treatment		Amino acid digestibility coefficient							
Lysine HCl (g/kg)	Duration (hours)	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine
0	12	0.832	0.762	0.732	0.758	0.756	0.853	0.754	0.636
0	16	0.858	0.796	0.770	0.790	0.795	0.884	0.787	0.677
0	20	0.883	0.839	0.825	0.844	0.841	0.915	0.838	0.729
3.5	12	0.808	0.738	0.715	0.736	0.787	0.858	0.729	0.618
3.5	16	0.854	0.795	0.780	0.780	0.832	0.895	0.791	0.683
3.5	20	0.871	0.832	0.826	0.844	0.865	0.926	0.833	0.723
SEM		0.0055	0.0074	0.0079	0.0078	0.0066	0.0071	0.0079	0.0092
Main effects: Lysine									
0		0.858b	0.799	0.776	0.797	0.797a	0.884	0.793	0.681
3.5		0.844a	0.788	0.774	0.792	0.828b	0.893	0.785	0.675
Duration									
12		0.820[a]	0.750[a]	0.724[a]	0.747[a]	0.772[a]	0.885[a]	0.742[a]	0.627[a]
16		0.856b	0.795[b]	0.775[b]	0.794[b]	0.813[b]	0.889[b]	0.789[b]	0.680[b]
20		0.877[c]	0.835[c]	0.826[c]	0.844[c]	0.853[c]	0.921[c]	0.836[c]	0.726[c]
Significance (P =)									
Lysine (L)		0.006	0.078	0.762	0.428	< 0.001	0.120	0.193	0.424
Duration (D)		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
L x D interaction		0.240	0.270	0.240	0.115	0.587	0.909	0.178	0.429

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Extending the intervals depressed AME ($P < 0.05$), ME: GE ratios ($P < 0.05$), and AMEn ($P < 0.001$) to significant, but subtle, extents. Lysine HCl addition did not have any significant effects and N retention was not influenced by treatment.

The effects of dietary treatments on protein (N) ileal digestibility coefficients and disappearance rates are shown in Table 8. Increasing the duration of feed access from 12 to 20 h significantly ($P < 0.001$) increased both parameters by up to 13.6% (0.795 vs. 0.700) for protein (N) digestibility and 37.6% (31.64 vs. 22.99 g/bird/day) for protein (N) disappearance rates.

The effects of lysine HCl and feed access intervals on ileal amino acid digestibilities are shown in Tables 9 and 10. Addition of lysine HCl increased the digestibility of lysine per se by 3.89% (0.828 vs. 0.797; $P < 0.001$) but decreased digestibilities of arginine by 1.63% (0.844 vs. 0.858; $P < 0.01$) and glycine by 2.38% (0.697 vs. 0.714; $P < 0.05$). Increasing feed access intervals increased all amino acid digestibilities

Table 10. The effects of lysine HCl supplementation and daily duration of feed access on apparent ileal digestibility of amino acids at 28 D post-hatch.

Treatment		Amino acid digestibility coefficient								
Lysine HCl (g/kg)	Duration (hours)	Valine	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine	Tyrosine	Average
0	12	0.709	0.701	0.713	0.800	0.669	0.750	0.700	0.662	0.745
0	16	0.749	0.747	0.747	0.828	0.711	0.778	0.736	0.711	0.780
0	20	0.805	0.826	0.796	0.868	0.762	0.821	0.790	0.784	0.832
3.5	12	0.684	0.674	0.691	0.779	0.638	0.724	0.677	0.627	0.727
3.5	16	0.754	0.753	0.745	0.828	0.706	0.779	0.740	0.712	0.785
3.5	20	0.803	0.819	0.783	0.862	0.747	0.811	0.779	0.775	0.828
SEM		0.0082	0.0094	0.0094	0.0069	0.0089	0.0073	0.0088	0.0117	0.0078
Main effects: Lysine										
0		0.754	0.758	0.752	0.840	0.714b	0.783	0.742	0.719	0.786
3.5		0.747	0.749	0.739	0.831	0.697a	0.771	0.732	0.704	0.780
Duration										
12		0.696[a]	0.688[a]	0.702[a]	0.789[a]	0.654[a]	0.737[a]	0.688	0.645[a]	0.736[a]
16		0.752[b]	0.750[b]	0.746[b]	0.828b	0.708[b]	0.779[b]	0.738	0.711[b]	0.782[b]
20		0.804[c]	0.823[b]	0.790[c]	0.865c	0.754[c]	0.816[c]	0.785	0.779[c]	0.830[c]
Significance (P =)										
Lysine (L)		0.277	0.245	0.112	0.120	0.027	0.057	0.174	0.139	0.349
Duration (D)		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
L x D interaction		0.176	0.236	0.555	0.305	0.363	0.193	0.347	0.277	0.312

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 11. The effects of lysine HCl supplementation and daily duration of feed access on apparent disappearance rates (g/bird/day) of amino acids from 21 to 28 D post-hatch.

Treatment		Amino acid disappearance rate (g/bird/day)							
Lysine HCl (g/kg)	Duration (hours)	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine
0	12	1.463	0.627	1.023	2.106	1.305	0.392	1.128	0.806
0	16	1.759	0.762	1.252	2.556	1.597	0.474	1.371	0.999
0	20	1.883	0.836	1.399	2.845	1.761	0.511	1.521	1.118
3.5	12	1.368	0.594	1.007	2.035	1.662	0.432	1.064	0.787
3.5	16	1.629	0.718	1.233	2.478	1.973	0.506	1.296	0.978
3.5	20	1.786	0.811	1.409	2.825	2.212	0.564	1.471	1.115
SEM		0.0240	0.0120	0.0209	0.0410	0.0268	0.0074	0.0221	0.0210
Main effects: Lysine									
0		1.730b	0.742b	1.225	2.502	1.555a	0.459a	1.340b	0.974
3.5		1.620a	0.707a	1.216	2.446	1.949b	0.500b	1.277a	0.960
Duration									
12		1.416[a]	0.610[a]	1.015[a]	2.070[a]	1.484[a]	0.412[a]	1.096[a]	0.797[a]
16		1.691[b]	0.740[b]	1.242[b]	2.517[b]	1.785[b]	0.490[b]	1.333[b]	0.988[b]
20		1.835[c]	0.823[c]	1.404[c]	2.835[c]	1.987[c]	0.537[c]	1.496[c]	1.117[c]
Significance (P =)									
Lysine (L)		< 0.001	0.001	0.628	0.098	< 0.001	< 0.001	0.001	0.370
Duration (D)		< 0.001	< 0.001	< 0.001	< 0.001	<0.001	< 0.001	< 0.001	< 0.001
L × D interaction		0.635	0.732	0.748	0.743	0.192	0.357	0.851	0.887

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

to highly significant extents. The increase from 12 to 20 h access increased the average digestibility coefficient of 16 amino acids by 12.8% (0.830 vs. 0.736; $P < 0.001$).

The effects of dietary treatments on amino acid disappearance rates in the distal ileum are displayed in Tables 11 and 12. Lysine HCl addition increased the disappearance rate of lysine per se by 25.3% (1.949 vs. 1.555 g/bird/day; $P < 0.001$) and that of methionine by 8.93% (0.500 vs. 0.459 g/bird/day; $P < 0.001$). Alternatively, lysine HCl addition decreased the disappearance rates of arginine, histidine, phenylalanine, aspartic acid, glutamic acid, proline, serine, and tyrosine to significant extents ($P = 0.043$–<0.001). Increasing feed access intervals increased all amino acid disappearance rates to highly significant extents. The increase from 12 to 20 h access increased the total disappearance rate of 16 amino acids by 36.7% (28.89 vs. 21.13 g/bird/day; $P < 0.001$).

The effects of lysine HCl and timing of post-prandial blood collection on concentrations of free amino acids in plasma taken from the systemic circulation are

Table 12. The effects of lysine HCl supplementation and daily duration of feed access on apparent disappearance rates (g/bird/day) of amino acids from 21 to 28 D post-hatch.

Treatment		Amino acid disappearance rate (g/bird/day)								
Lysine HCl (g/kg)	Duration (hours)	Valine	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine	Tyrosine	Total
0	12	1.072	1.060	2.215	4.509	0.814	1.393	1.070	0.425	21.41
0	16	1.320	1.316	2.704	5.439	1.007	1.685	1.310	0.531	26.08
0	20	1.477	1.553	3.091	5.937	1.124	1.850	1.466	0.609	28.99
3.5	12	1.032	1.006	2.106	4.296	0.749	1.323	1.022	0.389	20.87
3.5	16	1.278	1.261	2.552	5.129	0.930	1.599	1.253	0.496	25.30
3.5	20	1.466	1.521	2.994	5.754	1.062	1.794	1.423	0.582	28.79
SEM		0.0227	0.0028	0.0698	0.0812	0.0184	0.0267	0.0235	0.0347	0.4197
Main effects: Lysine										
0		1.290	1.310	2.670b	5.295b	0.982b	1.642b	1.282b	0.522b	25.49
3.5		1.259	1.263	2.551a	5.060a	0.914a	1.572a	1.232a	0.489a	24.99
Duration										
12		1.052[a]	1.033[a]	2.160[a]	4.403[a]	0.782[a]	1.358[a]	1.046[a]	0.407[a]	21.13[a]
16		1.299[b]	1.289[b]	2.628[b]	5.284[b]	0.968[b]	1.642[b]	1.281[b]	0.513[b]	25.69[b]
20		1.472[c]	1.537[c]	3.043[c]	5.845[c]	1.093[c]	1.822[c]	1.444[c]	0.596[c]	28.89[c]
Significance (P =)										
Lysine (L)		0.103	0.072	0.043	0.001	< 0.001	0.002	0.013	0.001	0.147
Duration (D)		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	<0.001
L × D interaction		0.740	0.915	0.917	0.714	0.906	0.852	0.949	0.912	0.785

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 13. The effects of lysine HCl supplementation and timing of post-prandial blood collection on concentrations of free essential and non-essential amino acids in the systemic circulation at 27 D post-hatch.

Treatment		Amino acid (μmol/L)							
Lysine HCl (g/kg)	Timing (hours)	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine
0	0	443e	97	139	287	194b	92d	165	1113
0	3	332d	74	118	265	143ab	60b	158	939
0	6	226c	61	117	256	113a	55b	151	997
3.5	0	221c	72	109	236	703d	68c	140	781
3.5	3	158b	58	93	219	543c	47a	143	700
3.5	6	98a	52	95	222	510c	44a	135	668
SEM		13.47	3.75	3.39	6.63	18.80	2.27	2.82	49.12
Main effects: Lysine									
0		334	78[b]	125[b]	269[b]	150	69	158[b]	1016[b]
3.5		159	60[a]	99[a]	226[a]	586	53	139[a]	716[a]
Timing									
0		332	84[c]	124[b]	262[b]	449	80	152[b]	947[b]
3		245	66[b]	105[a]	242[a]	343	54	150[b]	819[a]
6		162	57[a]	106[a]	239[a]	311	49	143[a]	833[a]
Significance (P =)									
Lysine (L)		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Timing (T)		< 0.001	< 0.001	< 0.001	0.002	< 0.001	< 0.001	0.006	0.024
L × T interaction		0.005	0.114	0.504	0.455	0.006	0.020	0.177	0.568

[a-d]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

shown in Tables 13 and 14. Significant treatment interactions were observed for arginine, lysine, methionine and glutamic acid (P = 0.02–0.005). Otherwise, lysine HCl addition depressed plasma concentrations of histidine, isoleucine, phenylalanine, threonine, valine, aspartate/asparagine, serine, and tyrosine to significant extents (P = 0.017–<0.001) but did not influence tryptophan and alanine (P > 0.70). The lapsed time of 6 h after feed withdrawal significantly depressed (P 0.024–<0.001) plasma concentrations of histidine (32.1%), aspartate/asparagine (16.6%), isoleucine (14.5%), valine (13.4%), threonine (12.5%), glycine (11.7%), alanine (9.3%), leucine (8.8%), and phenylalanine (5.9%). However, concentrations were significantly increased in the case of serine (7.2%) and tyrosine (50.9%).

The linear effects of time elapsed following feed withdrawal on concentrations of free amino acids in systemic plasma offered diets without and with 3.5 g/kg lysine HCl are shown in Table 15. In birds offered diets without lysine HCl there were no significant linear effects for threonine, alanine, and serine. Concentrations of glutamic acid (r = 0.553; P = 0.005) and tyrosine (r = 0.741; P < 0.001) linearly increased from

Table 14. The effects of lysine HCl supplementation and timing of post-prandial blood collection on concentrations of free essential and non-essential amino acids in the systemic circulation at 27 D post-hatch.

Treatment		Amino acid (μmol/L)							
Lysine HCl (g/kg)	Timing (hours)	Tryptophan	Valine	Alanine	Aspartic + Asparagine	Glutamic acid	Glycine	Serine	Tyrosine
0	0	86	250	1483	397	258a	695	733	179
0	3	74	208	1308	349	255a	573	727	209
0	6	76	213	1313	333	324b	580	783	253
3.5	0	87	183	1431	361	294b	567	692	147
3.5	3	71	151	1395	310	252a	467	685	173
3.5	6	78	164	1331	300	392c	535	744	238
SEM		2.01	5.58	66.61	10.45	12.23	19.24	20.00	9.12
Main effects: Lysine									
0		79	223[b]	1368	360[b]	279	616[b]	747[b]	214[b]
3.5		79	166[a]	1386	323[a]	313	523[a]	707[a]	186[a]
Timing									
0		86[c]	217[b]	1457	379[b]	276	631[b]	712[a]	163[a]
3		72[a]	180[a]	1351	330[a]	253	520[a]	706[a]	191[b]
6		77[b]	188[a]	1322	316[a]	358	557[a]	763[b]	246[c]
Significance (P =)									
Lysine (L)		0.900	< 0.001	0.746	< 0.001	0.002	< 0.001	0.017	0.001
Timing (T)		< 0.001	< 0.001	0.005	< 0.001	< 0.001	< 0.001	0.012	< 0.001
L × T interaction		0.312	0.297	0.584	0.949	0.020	0.093	0.997	0.497

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

Table 15. The linear effects of time elapsed following feed withdrawal on concentrations of free amino acids in systemic plasma (μmol/L) of broiler chickens offered diets without and with 3.5 g/kg lysine HCl at 27 D post-hatch.

Amino acid	Without lysine HCl		With lysine HCl	
	Correlation coefficient	Significance	Correlation coefficient	Significance
Arginine	r = −0.884	$P < 0.001$	r = −0.939	$P < 0.001$
Histidine	r = −0.792	$P < 0.001$	r = −0.670	$P < 0.001$
Isoleucine	r = −0.663	$P < 0.001$	r = −0.495	$P = 0.014$
Leucine	r = −0.602	$P = 0.002$	r = −0.304	$P = 0.149$
Lysine	r = −0.795	$P < 0.001$	r = −0.737	$P < 0.001$
Methionine	r = −0.862	$P < 0.001$	r = −0.826	$P < 0.001$
Phenylalanine	r = −0.576	$P = 0.003$	r = −0.250	$P = 0.239$
Threonine	r = −0.278	$P = 0.188$	r = −0.413	$P = 0.045$
Tryptophan	r = −0.509	$P = 0.011$	r = −0.401	$P = 0.052$
Valine	r = −0.659	$P < 0.001$	r = −0.380	$P = 0.067$
Alanine	r = −0.338	$P = 0.061$	r = −0.209	$P = 0.328$
Aspartate/Asparagine	r = −0.645	$P = 0.001$	r = −0.687	$P < 0.001$
Glutamic acid	r = +0.553	$P = 0.005$	r = +0.621	$P = 0.001$
Glycine	r = −0.571	$P = 0.004$	r = −0.230	$P = 0.279$
Serine	r = +0.316	$P = 0.133$	r = +0.401	$P = 0.052$
Tyrosine	r = +0.741	$P < 0.001$	r = +0.860	$P < 0.001$

0 to 6 h. There were significant negative linear relationships for the balance of eleven amino acids including lysine (r = −0.795; $P < 0.001$). In birds offered diets with lysine HCl there were no significant linear effects for leucine, phenylalanine, tryptophan, valine, alanine, glycine, and serine. Again, concentrations of glutamic acid (r = 0.621; $P = 0.005$) and tyrosine (r = 0.860; $P < 0.001$) linearly increased. And again, there were significant negative linear relationships for the balance of 7 amino acids including lysine (r = −0.737; $P < 0.001$).

The effects of lysine HCl and daily feed access intervals on mRNA expression for SGLT-1, GLUT-2, PepT-1, and CAT-1 are shown in Table 16. Feed access intervals significantly increased ($P < 0.05$) mRNA expression for SGLT-1 and PepT-1 but decreased CAT-1. The effect of lysine HCl on GLUT-2 mRNA expression closely approached significance ($P = 0.054$) but did not influence the other transporters.

DISCUSSION

The Ross 308 performance objective for male chickens from 7 to 28 D post-hatch calls for a weight gain of 1,387 g/bird with an FCR of 1.479. Birds receiving lysine-supplemented diets in this study comfortably exceeded this objective with a mean weight gain of 1,465 g/bird with an FCR of 1.351. This discussion focuses on the 2 major outcomes of the present study, which are the influence of feed access intervals on lysine

Table 16. The effects of lysine HCl supplementation and daily duration of feed access on mRNA expression of SGLT-1, GLUT-2, PepT-1, and CAT-1 at 28 days post-hatch.

Treatment		mRNA expression			
Lysine HCl (g/kg)	Duration (hours)	SGLT-1	GLUT-2	PepT-1	CAT-1
0	12	1.13	1.43	1.01	1.13
0	16	1.87	2.42	1.48	1.03
0	20	1.31	1.42	3.54	0.71
3.5	12	0.87	0.57	0.16	0.99
3.5	16	1.21	0.87	4.92	0.88
3.5	20	1.80	1.51	4.44	0.67
SEM		0.2444	0.4784	1.3634	0.1346
Main effects: Lysine					
0		1.44	1.75	2.01	0.96
3.5		1.29	0.98	3.18	0.85
Duration					
12		1.00[a]	1.00	0.59[a]	1.06[b]
16		1.54[b]	1.64	3.20[a,b]	0.95[a,b]
20		1.56[b]	1.46	3.33[b]	0.69[a]
Significance (P =)					
Lysine (L)		0.478	0.054	0.302	0.322
Duration (D)		0.045	0.389	0.043	0.027
L × D interaction		0.066	0.240	0.296	0.902

[a-c]means within columns not sharing a common suffix are significantly different at the 5% level of probability.

HCl utilization and amino acid digestibilities in broiler chickens.

The present study established that daily feed access intervals of 12, 16, and 20 h did not influence the utilization of lysine HCl in broiler chickens because treatment interactions were not observed for all parameters assessed including growth performance. Moreover, the feed access intervals investigated are relevant to practical lighting regimen. Data generated by Nonis and Gous (2006) on the utilization of synthetic amino acids in broiler breeders indicates that Batterham's observations in pigs may apply to poultry. However, Baker and Izquierdo (1985) had investigated the effect of meal frequency and spaced lysine HCl ingestion in broiler chickens and concluded that total dietary lysine utilization was not compromised by spaced ingestion of diets containing lysine HCl. Baker and Izquierdo (1985) suggested that this was because lysine is conserved in tissue pools rather than being catabolized; however, this suggestion was largely based on data generated in rats (Yamashita and Ashida, 1969; Chu and Hegsted, 1976). Nevertheless, it follows that if lysine is not deaminated but conserved in tissue pools then the asynchronous availability of unbound lysine and protein-bound amino acids at sites of protein synthesis lysine would not be a constraint. However, in the present study free lysine concentrations in systemic plasma linearly declined in birds offered diets without (r = −0.795; $P < 0.001$) and with (r = −0.795; $P < 0.001$) lysine HCl over a post-prandial period of 6 h. While not conclusive, this finding does not support the suggestion that lysine is retained in tissue pools in poultry.

There may be a different explanation for the dichotomy between pigs and poultry given the function of the crop and gizzard in poultry and the occurrence of reverse peristalsis (Svihus, 2014). There is transient storage of large quantities of feed in the crop and Boa-Amponsem et al. (1991) noted that birds accustomed to intermittent feeding exhibit significantly increased crop contents; this was unequivocally demonstrated in the present study. The overall average daily feed intake from 21 to 28 D post-hatch was 150.6 g/bird; however, birds given 12 h feed access retained 49.3 g of digesta in their crops in absolute terms, which represented 33% of daily feed intake. In contrast, birds given 16 and 20 h feed access retained 8.3 g of digesta in their crops and there was a linear decline (r = 0.925; $P < 0.001$) in absolute crop contents as duration of feed access increased. This outcome is reflected in the relative weight of crop contents (Table 6). Moreover, there was a linear decline (r = 0.906; $P < 0.001$) in relative crop weights with increasing hours of feed access (Figure 1).

Another aspect is anticipatory feeding where birds increase consumption prior to a "lights-off" period of darkness or scotoperiod (Classen et al., 2016). Duve et al. (2011) suggested that a scotoperiod of longer than 4 h is required to induce anticipatory feeding behavior. It is noteworthy that 12 h birds consumed 16.0% more feed than 16 h birds and 41.6% more than 20 h birds on an hourly basis when feed was available (Table 5), which is a clear indication of anticipatory feeding behavior in the present study. The effects of daily feed access intervals of 6, 15, and 24 h have been shown to induce a similar pattern of feed intake rates and anticipatory feeding (Liu et al., 2016).

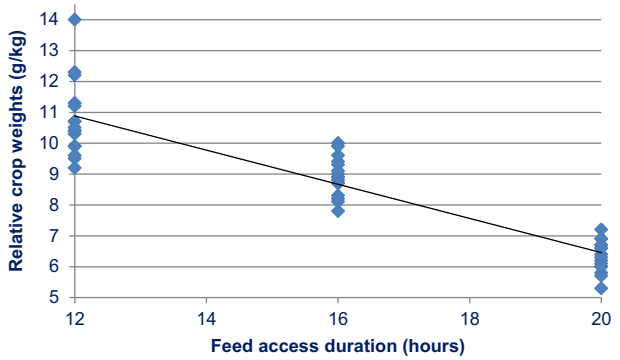

Figure 1. Linear relationship (r = 0.931; $P < 0.0001$) between feed access duration and relative crop weights.

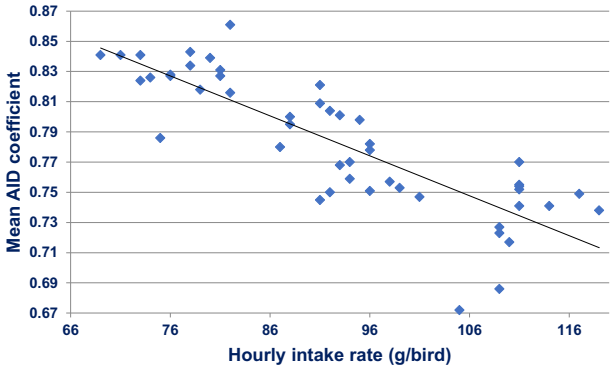

Figure 2. Linear relationship (r = −0.834; $P < 0.0001$) between hourly feed intake rates and average ileal digestibility coefficients of 16 amino acids.

Reverse peristalsis in poultry may be in response to fasting and appears to play little role in fed birds (Clench and Mathias, 1992; Godwin and Russell, 1997). Reverse peristalsis includes the gastric reflux, the small intestinal reflux, and the cloaca–caecal reflux. The gizzard has been described as the "pacemaker" of gut motility and promotes reverse peristalsis (Ferket, 2000). That 12 h birds had heavier relative gizzard weights (8.23%) and contents (19.4%) than 20 h birds (Table 6) is suggestive of enhanced gizzard functionality and increased episodes of reverse peristalsis in response to 12 h daily fasting periods. Thus, the dichotomy between pigs and poultry in respect of lysine HCl utilization does appear related to reverse peristalsis, crop, and gizzard functionality modulating the relative intestinal uptakes of unbound and protein-bound lysine.

Interestingly, longer feed access intervals significantly increased amino acid digestibility coefficients. Increases ranged from 4.1% for methionine (0.921 vs. 0.885; $P < 0.001$) to 15.8% for threonine (0.726 vs. 0.627; $P < 0.001$) across the essential amino acids as feed access intervals were extended from 12 to 20 h (Table 9). Moreover, average amino acid digestibility significantly increased by 12.8% from 0.736 to 0.830 as shown in Table 10. This outcome was not anticipated; however, it appears to be associated with reductions in hourly feed intake rates from 109 to 94 and 77 g/bird observed as intervals were extended from 12 to 16 and 20 h (Table 5). Instructively, there is a highly significant negative linear relationship between hourly feed intake rates and average ileal digestibility coefficients of 16 amino acids, as shown in Figure 2 and highly significant ($P < 0.001$) negative linear relationships were observed for all amino acids on an individual basis. Thus, extending feed access intervals reduced hourly feed intake rates and presumably reduced gut passage rates that appear to have resulted in increased amino acid digestibility coefficients. This may be a manifestation of relative "over-consumption" by birds given 12 h feed access compromising protein digestion and/or absorption of amino acids. "Over-consumption" has been shown to compromise efficiency of feed conversion in modern genotype broiler chickens (Svihus, 2011). Feed intake rates have been shown to influence amino acid digestibilities in a quadratic manner in cannulated weaner pigs (Goerke et al., 2012) and effects of diet type on rates of passage and amino acid digestibilities have been reported in broilers (Rochell et al., 2012). However, the likelihood is that the impact of feed access intervals per se on amino acid digestibility has not been previously investigated in poultry. Given that the differences in amino acid digestibilities induced by feed access intervals in the present study are tangible, this finding has obvious implications for lighting regimen adopted under practical conditions.

The significant impacts of lysine HCl addition on amino acid digestibilities were confined to increases in lysine but decreases in arginine and glycine digestibilities. The positive impact on lysine per se is to be expected and the negative impact on arginine appears to be a manifestation of the established antagonism between lysine and arginine (Austic and Scott, 1975). Lysine HCl addition significantly increased disappearance rates of lysine per se and methionine in the distal ileum but significantly decreased disappearance rates of eight amino acids. These outcome are not consistent with data generated by Selle et al. (2007) who found that the addition of 1.8 g/kg lysine, as lysine HCl, to broiler diets significantly increased the ileal digestibility of isoleucine, methionine, phenylalanine, valine, aspartic acid, glutamic acid, and tyrosine. This was considered to be attributable to the up-regulation of lysine transport systems in the jejunum with overlapping amino acid specificities (Torras-Llort et al., 1998).

In conclusion, daily duration of feed access intervals did not influence lysine HCl utilization as treatment interactions between feed access intervals and lysine HCl addition for weight gain, feed intake, and FCR and other parameters assessed were not observed. This outcome is in complete contrast to outcomes reported in pigs by Batterham and colleagues. However, extending

feed access intervals significantly increased relative crop and gizzard weights and their contents. It is proposed that the different outcomes in pigs and poultry in respect of lysine HCl utilization stems from anticipatory feeding behavior, crop and gizzard functionality, and increased episodes of reverse peristalsis, which modulate the relative intestinal uptakes of unbound and protein-bound lysine. Extending feed access intervals from 12 to 16 and 20 h daily substantially increased average ileal digestibility coefficients of 16 amino acids from 0.736 to 0.782 and 0.830, which was not anticipated. Instructively, as hourly feed intake rates were linearly related ($r = -0.834$; $P < 0.001$) to average ileal digestibility coefficients, it appears that higher feed intake rates in birds with 12 h feed access may have been compromising digestibility of amino acids. Thus, the findings of the present study hold implications for the development of reduced crude-protein diets and lighting regimen for chicken-meat production. First, it appears that lighting regimen adopted in practice will not influence the utilization of lysine HCl and, by extension, the balance of synthetic amino acids. Second, the inference is that feeding intervals, or lighting regimen, influence the digestibility of amino acids. This may not have been previously reported in poultry and, if confirmed, has obvious implications for the formulation of broiler diets based on digestible amino acids.

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