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DETERMINAÇÃO DA EXIGÊNCIA DE LISINA PARA FRANGOS DE CORTE UTILIZANDO DIFERENTES MODELOS ESTATÍSTICOS

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“A paciência é amarga, mas seu fruto é doce.”

(Jean-Jacques Rousseau)

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DETERMINAÇÃO DA EXIGÊNCIA DE LISINA PARA FRANGOS DE CORTE UTILIZANDO DIFERENTES MODELOS ESTATÍSTICOS[1]

Autor: Henrique Scher Cemin
Orientador: Sergio Luiz Vieira

RESUMO - O objetivo desta dissertação foi estimar a exigência de lisina (Lis) para frangos de corte machos Cobb x Cobb 500 de 1 a 12 dias de idade (experimento 1), 12 a 28 dias de idade (experimento 2) e 28 a 42 dias de idade (experimento 3). Dietas basais foram formuladas para atingir ou exceder as exigências nutricionais, com exceção da Lis. Cinco níveis de Lis foram suplementados às dietas basais a partir de L-Lis HCl ou sulfato de L-Lis de modo que os níveis variaram de 0,97% a 1,37% de Lis digestível no experimento 1, 0,77% a 1,17% de Lis digestível no experimento 2 e 0,68% a 1,08% de Lis digestível no experimento 3 em incrementos de 0,08%. Os tratamentos foram distribuídos em um delineamento inteiramente casualizado com 8 repetições de 25 aves. Em cada experimento, 2200 aves foram alojadas em 88 unidades experimentais. Nos dias 1 e 12 (experimento 1), 12 e 28 (experimento 2) e 28 e 42 (experimento 3) as aves e a ração foram pesadas para determinar o ganho de peso (GP) e conversão alimentar (CA). No experimento 3, quatro aves por unidade experimental foram abatidas para determinação do rendimento de carcaça e peito. A biodisponibilidade relativa (RBV) das fontes de Lis foi avaliada através de uma regressão multivariada e comparada pelo teste t. A exigência de Lis foi estimada por três modelos de regressão: polinomial quadrática, broken-line linear e broken-line quadrática. A exigência foi representada como 95% do ponto de máxima resposta. Não houve diferença entre a RBV da Lis no sulfato de L-Lis em relação ao L-Lis HCl, portanto ambas as fontes foram utilizadas para estimar as exigências. As exigências encontradas variaram de acordo com o modelo estatístico e a variável analisada. A regressão broken-line quadrática apresentou o melhor ajustamento aos dados de desempenho, enquanto a regressão broken-line linear se ajustou melhor aos dados de rendimento de carcaça e peito. As regressões polinomial quadrática, broken-line linear e broken-line quadrática estimaram, respectivamente, as exigências como 1,190, 1,032 e 1,101% para GP e 1,226, 1,038 e 1,124% para CA no experimento 1; 1,021, 0,900 e 0,961% para GP e 1,064, 0,966 e 1,043% para CA no experimento 2; 0,949, 0,833 e 0,925% para GP, 0,978, 0,851 e 0,960% para CA, 0,933, 0,842 e 0,931% para rendimento de carcaça e 0,952, 0,839 e 0,921% para rendimento de peito no experimento 3. Os resultados demonstraram que as exigências de Lis foram consideravelmente influenciadas pelas diferentes regressões. Portanto, a escolha do modelo estatístico é crítica para a obtenção de estimativas precisas e coerentes.

Palavras chave: exigência, frangos de corte, lisina, regressão

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LYSINE REQUIREMENT OF MALE BROILERS USING DIFFERENT STATISTICAL MODELS[2]

Author: Henrique Scher Cemin
Advisor: Sergio Luiz Vieira

ABSTRACT - The objective of this thesis was to estimate lysine (Lys) requirement of male Cobb x Cobb 500 broilers from 1 to 12 days of age (experiment 1), 12 to 28 days of age (experiment 2), and 28 to 42 days of age (experiment 3). Basal diets were formulated to meet or exceed recommendations, except for Lys. Five graded levels of Lys were supplemented from L-Lys HCl or L-Lys sulfate to the basal diets. Dietary treatments ranged from 0.97% to 1.37% digestible Lys in experiment 1, 0.77% to 1.17% digestible Lys in experiment 2, and 0.68% to 1.08% digestible Lys in experiment 3 in 0.08% increments. Treatments were distributed in a completely randomized design with 8 repetitions of 25 birds each. A total of 2,200 birds per experiment were placed in 88 experimental units. At 1 and 12 days (experiment 1), 12 and 28 days (experiment 2), and 28 and 42 days (experiment 3), birds and feed were weighed to determine body weight gain (BWG) and feed conversion ratio (FCR). In experiment 3, four birds per experimental unit were processed for carcass and breast meat yield evaluation. Relative bioavailability (RBV) of Lys sources was assessed by a multivariate regression and compared by a t-test. Lysine requirement was estimated using three regression models: quadratic polynomial, linear broken-line, and quadratic broken-line. Requirements were represented as 95% of the asymptote. No difference was observed in Lys RBV in L-Lys sulfate compared to L-Lys HCl, thus both sources were used to estimate requirements. Requirement estimates varied according to statistic model and analyzed variable. Quadratic broken-line model presented the best fit to performance data (BWG and FCR), whereas linear broken-line model fitted better to carcass and breast meat yield data. Quadratic polynomial, linear broken-line, and quadratic broken-line estimates were, respectively, 1.190, 1.032, and 1.101% for BW gain and 1.226, 1.038, and 1.124% for FCR in experiment 1; 1.021, 0.900, and 0.961% for BW gain and as 1.064, 0.966, and 1.043% for FCR in experiment 2; and 0.949, 0.833, 0.925% for BW gain, 0.978, 0.851, and 0.960% for FCR, 0.933, 0.842, and 0.931% for carcass yield, and 0.952, 0.839, and 0.921% for breast meat yield in experiment 3. Results demonstrate that Lys requirements were considerably influenced by different regression models. Therefore, the choice of statistical model is crucial to obtain precise, coherent estimates.

Key words: broilers, lysine, regression, requirement

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RELAÇÃO DE ABREVIATURAS

AA	Aminoácido
CA	Conversão alimentar
GP	Ganho de peso
Lis	Lisina
L-Lis HCl	Hidrocloreto de L-lisina

CAPÍTULO I

INTRODUÇÃO

A lisina (Lis) é o segundo aminoácido (AA) limitante em dietas baseadas em milho e farelo de soja para frangos de corte. Em função do conceito de proteína ideal, expressar as exigências de AA em relação à Lis é uma estratégia adotada por muitos nutricionistas. A Lis foi escolhida como o AA referência por ser limitante em dietas práticas, ter função exclusiva de deposição proteica e simples análise laboratorial (Baker, 1997). Pequenas variações na avaliação das exigências de Lis alteram a inclusão dos outros AA essenciais, portanto a precisão da exigência de Lis é fundamental (Baker et al., 2002).

A Lis é rotineiramente suplementada em dietas práticas de frangos de corte. O aminoácido é comercializado em duas formas, hidrocloreto de L-Lis (L-Lis HCl) e sulfato de L-Lis. Apesar de dividirem etapas iniciais de produção, como a fermentação bacteriana, o processamento posterior difere e o produto final apresenta características distintas (Schutte & Pack, 1994). A biomassa bacteriana é removida no processo de produção de L-Lis HCl e mantida no sulfato de L-Lis, o que pode representar um aporte adicional de nutrientes (Schutte & Pack, 1994).

Devido principalmente à seleção genética, a taxa de crescimento, conversão alimentar e deposição proteica do frango de corte moderno melhoraram significativamente nas últimas décadas (Havenstein et al., 2003), o que pode indicar crescente exigência de AA e outros nutrientes. A recomendação do NRC (1994) é de 1,10% de Lis total entre 0 e 3 semanas, 1,00% de Lis total entre 3 e 6 semanas e 0,85% de Lis total entre 6 e 8 semanas. Entretanto, pesquisas mais recentes demonstram que o frango de corte moderno tem maior exigência de Lis (Labadan et al., 2001; Garcia e Batal, 2005; Dozier et al., 2008; Dozier et al., 2009; Dozier et al., 2010; Dozier et al., 2012), embora fatores possam influenciar as exigências de AA.

Um dos fatores que influencia profundamente estudos de determinação de exigência é a análise estatística. Ao testar diferentes modelos estatísticos em um mesmo conjunto de dados, Pesti et al. (2009) observaram grande variação na determinação da exigência de Lis. A escolha de um modelo que represente a resposta animal a um componente da dieta é complexa por causa da variabilidade das respostas de diferentes indivíduos ou unidades experimentais. É evidente que as regressões são preferíveis aos testes de média, entretanto cada modelo de regressão tem vantagens e desvantagens. Desta forma, a decisão do pesquisador é crítica para a correta interpretação das exigências nutricionais e deve ser fundamentada no conhecimento das características dos modelos estatísticos.

Pesquisas para determinação de exigência de Lis são abundantes na literatura. Entretanto, a comparação de resultados é dificultada pela utilização de diferentes modelos estatísticos e pela apresentação, na maior parte dos casos, de apenas um modelo. Além disso, é necessário considerar a possibilidade que as fontes L-Lis HCl e sulfato de L-Lis, por suas características intrínsecas, produzam resultados divergentes no desempenho de frangos de corte. Portanto, objetivou-se com esta dissertação avaliar a bioeficácia relativa da Lis no sulfato de L-Lis em comparação ao L-Lis HCl e determinar a exigência de frangos de corte até 42 dias de idade utilizando diferentes modelos estatísticos.

REVISÃO BIBLIOGRÁFICA

Digestão e absorção de aminoácidos

Os AA estão envolvidos, direta ou indiretamente, em virtualmente todos os processos celulares. Existem 20 AA na natureza que compõe proteínas, além de outros muito menos comumente encontrados, e todos eles diferem em suas cadeias laterais em estrutura, tamanho e carga elétrica. Os AA tem como característica um carbono α onde se ligam um grupo carboxila, um grupo amino, um hidrogênio e a cadeia lateral (Figura 1).

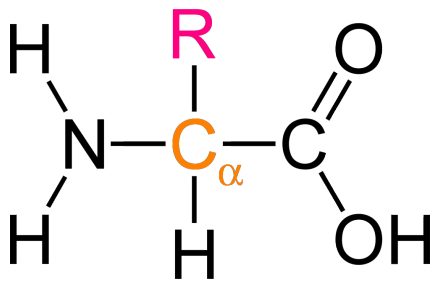

Figura 1. Estrutura química dos AA

Devido ao arranjo tetrahédrico das estruturas ao redor do carbono α, os grupos podem ocupar duas posições espaciais, portanto os AA tem dois estereoisômeros. Os isômeros são denominados de acordo com o sistema D ou L, de acordo com a localização dos grupos ao redor do carbono α. Apenas a forma L é utilizada na síntese proteica, embora o organismo seja capaz de usar a forma D através da desaminação ao cetoácido correspondente e reaminação ao AA na forma L por uma aminotransferase específica. Exceções são a Lis e a treonina, que não possuem aminotransferases (D'Mello, 2003).

De acordo com Macari et al. (2008), a digestão proteica em aves inicia no proventrículo, onde ocorre secreção de ácido clorídrico e pepsinogênio pelas células oxinticopépticas, estimulada pela visão, odor, expectativa do alimento e também pela presença física deste no trato gástrico (Burhol, 1982). O meio ácido tem importância na quebra da estrutura proteica, além de transformar o pepsinogênio em sua forma ativa, a pepsina. Esta, por sua vez, promove a hidrólise das ligações peptídicas.

A próxima etapa da digestão ocorre no intestino delgado. O pâncreas secreta diversas enzimas digestivas diretamente no intestino delgado através do duto pancreático. O tripsinogênio é ativado a tripsina através da ação de enteroquinases e reduz as proteínas a oligopeptídeos e AA livres (Smith & Hill, 1985). Peptidases de membrana completam a digestão proteica, agindo em oligopeptídeos com até seis AA resistentes à hidrólise. Di e tripeptídeos são absorvidos pelas células da mucosa intestinal por meio de transporte ativo envolvendo canais de Na^+, embora alguns AA como metionina e prolina possam ser transportados por um sistema sem dependência de Na^+ (Moretó, 1991). Os grupos de AA tem diferentes carreadores que podem ser divididos em: AA neutros; prolina, alanina e AA similares; AA básicos e AA ácidos (Bell & Freeman,

1984). Cerca de 20% dos AA disponíveis para absorção são utilizados para síntese da mucosa intestinal e entre 20 e 95% dos AA da dieta são catabolizados no intestino delgado (Wu et al., 2010). Em menor grau, os cecos também tem capacidade de absorção de AA. Prolina, metionina, leucina e fenilalanina podem ser absorvidos nesta região através de transportadores Na^+-dependentes (Obst & Diamond, 1989; Calonge et al., 1990; Moretó et al., 1991).

Catabolismo da lisina

A Lis (Figura 2), assim como os demais AA, pode ser utilizada para produção de energia em três situações. Primeira, em dietas com alta PB, o excesso de AA é direcionado para a formação de energia através da gliconeogênese ou cetogênese. A segunda situação envolve o turnover proteico constante. Baseado na composição específica de AA, cada proteína requer um determinado pool de AA livres. Caso os AA disponíveis não sejam exatamente iguais aos necessários, aqueles não utilizados não podem ser estocados e devem ser utilizados por outro processo biológico ou catabolizados para formação de energia. O terceiro caso ocorre em eventos de privação de alimento. Quando carboidratos não estão disponíveis, o organismo utiliza proteínas corporais, especialmente do músculo esquelético, como fonte de energia.

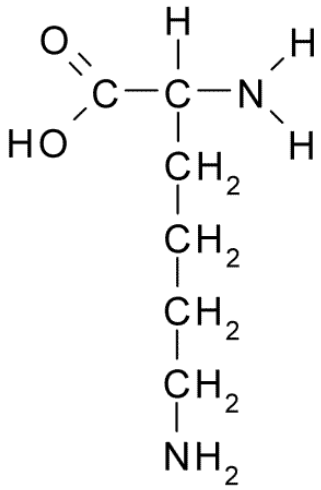

Figura 2. Estrutura química da Lis

O metabolismo da Lis inicia-se com a absorção intestinal. A Lis livre em excesso será catabolizada em diferentes células ou tecidos de maneira específica (Gatrell et al., 2013). Van Goudoever et al. (2000) demonstraram que a oxidação de Lis intestinal representa um terço da Lis total oxidada em suínos que receberam dietas com alta PB. A rota primária do catabolismo da Lis é chamada via da sacaropina, que ocorre no fígado. A nível celular, a reação ocorre na mitocôndria. Nesta rota, a Lis se combina com o α-cetoglutarato através da enzima lisina-cetoglutarato redutase para formar a sacaropina. Esta é então convertida em α-aminoadipato-6-semialdeído e glutamato pela

sacaropina desidrogenase. Por fim, o α-aminoadipato-6-semialdeído é convertido em acetil-CoA (Wu, 2013a). Pesquisas com ratos (Chu & Hegsted, 1976; Flodin, 1997) e suínos (Benevenga & Blemings, 2007) demonstraram que a atividade da enzima lisina-cetoglutarato redutase é diminuída em animais que receberam menos Lis, sugerindo um mecanismo de controle do catabolismo do aminoácido. A outra rota do catabolismo da Lis, menos comum no organismo, é denominada via do pipecolato. Esta rota ocorre no citosol das células, especialmente no cérebro. Nesta via, a Lis é convertida em ácido pipecólico e depois em ácido α-ceto-ε-aminocaproico, piperideina-2-carboxílico, piperideina-6-carboxilato para por fim ser transformada em acetil-CoA (Wu, 2013a).

A acetil-CoA produzida pelo catabolismo da Lis não pode ser convertida em glicose, portanto a Lis é considerada um aminoácido estritamente cetogênico. Os corpos cetônicos produzidos pelo seu catabolismo são utilizados no ciclo do ácido cítrico para produção de energia (Berg et al., 2002). A Lis, em quantidade menor que 1%, também é utilizada na biossíntese da carnitina através da metilação da Lis ligada a proteínas (Ball et al., 2007). Além disso, Ball et al. (2007) sugerem que todos os AA possuem uma taxa de oxidação obrigatória. A taxa de oxidação da Lis se diferencia dos demais AA por ser constante mesmo em casos de diminuição no seu consumo. Desta forma, decréscimos no consumo não são compensados por declínio na taxa de oxidação, implicando em maior sensibilidade da síntese proteica à deficiência de Lis do que de outros AA.

Fontes de suplementação de lisina

Em dietas de milho e farelo de soja para frangos de corte, a Lis é o segundo aminoácido limitante e sua suplementação é uma prática comum considerando os efeitos negativos da sua deficiência. Lisina cristalina na forma de hidrocloreto de L-Lis (L-Lis HCl) é a fonte mais comum de suplementação. Uma alternativa à esta é o sulfato de L-Lis. Ambas são produtos da fermentação bacteriana de carboidratos, porém diferem no processamento posterior. Na produção do sulfato de L-Lis, após a fermentação a biomassa bacteriana é removida e o íon cloreto é adicionado através de troca iônica. Em seguida ocorrem processos de evaporação, cristalização e secagem para formar o produto final. O sulfato de L-Lis passa pelo mesmo processo de fermentação bacteriana, porém a biomassa não é separada e o produto é mantido na forma de sulfato. Os processos de evaporação e granulação dão forma ao produto final. A biomassa bacteriana pode representar um aporte nutritivo adicional na forma de aminoácidos como metionina, treonina e arginina, além de fósforo (Schutte & Pack, 1994). A biomassa bacteriana apresentou alta digestibilidade em experimento realizado com suínos (D'Mello et al., 1976). Além disso, por ser um processo mais simples, sem necessidade de adição de ácido hidroclorídrico e menor geração de resíduos, a produção de sulfato de L-Lis apresenta vantagem do ponto de vista ambiental e financeiro (Kircher & Pfefferle, 2001).

De acordo com Ammermann et al. (1995), biodisponibilidade é o grau em que um nutriente é absorvido de maneira que possa ser utilizado no metabolismo animal. Izquierdo (1988) determinou que a L-Lis HCl é 100% biodisponível, portanto esta é considerada a fonte padrão nos experimentos de disponibilidade. O método mais utilizado para avaliar a biodisponibilidade é

através de ensaios de crescimento, pois as aves apresentam respostas rápidas tanto para a falta como para o excesso de determinados AA como a Lis (Yen et al., 1976).

Wang et al. (2007) não encontraram diferenças entre as fontes em frangos de corte entre 4 e 42 dias de idade. O consumo, entretanto, foi menor naqueles alimentados com dietas contendo sulfato de L-Lis. Os autores sugerem que os subprodutos da fermentação podem ter um efeito negativo no crescimento em inclusões muito altas. Efeitos das fontes no ganho de peso, conversão alimentar, consumo de ração e mortalidade não foram encontrados em experimento realizado por Ahmad et al. (2007) em frangos de corte fêmeas até 42 dias de idade.

Kircher & Pfefferle (2001) não encontraram diferença entre as fontes de Lis no desempenho de leitões entre 8 e 28 kg. Liu et al. (2007) encontraram resultados semelhantes para leitões entre 10 e 20 kg, bem como Ju et al. (2008) para leitões recém-desmamados. A análise de custo mostrou que o tratamento sem suplementação de Lis sintética apresentou o maior custo por kg de peso ganho. Entre as duas fontes, as dietas com sulfato de L-Lis custaram 6,7% menos por kg de peso ganho que as dietas com L-Lis HCl no cenário da época. Usualmente, o sulfato de L-Lis custa 40 a 65% do preço do L-Lis HCl, porém ambos são afetados pela flutuação do preço da soja (Ju et al., 2008).

Smirticky-Tjardes et al. (2004) compararam as duas fontes para leitões entre 5 e 10 kg e determinaram que o valor biológico relativo do sulfato de L-Lis é 99% do L-Lis HCl para ganho de peso (GP) e 97% para conversão alimentar (CA), porém nenhum dos dois valores diferiu significativamente de 100%. Portanto, a suplementação do aminoácido melhora o desempenho independentemente da fonte utilizada. Resultados semelhantes foram encontrados por Neme et al. (2001) em frangos de corte. A biodisponibilidade da Lis no sulfato de L-Lis em relação ao L-Lis HCl foi de 100,2%, porém sem diferença estatística entre elas. Os autores também não encontraram efeito no rendimento de carcaça e cortes comerciais. Liu et al. (2007) sugeriram biodisponibilidade de 101% para GP diário e 105% para CA em suínos entre 10 e 20 kg, porém sem diferença estatística para 100%. Valores de 99% GP e 107% para CA foram encontrados por Wang et al. (2007) para frangos de corte, estatisticamente iguais a 100%. Fontanillas et al. (2001) determinaram a bioeficácia entre 97 e 109% entre as fontes, sem diferença estatística.

Ao contrário das pesquisas anteriores, Bahadur et al. (2010) determinaram que aves suplementadas com sulfato de L-Lis apresentam melhor desempenho. Os autores sugerem que a melhoria é ocasionada pelos nutrientes como fósforo e aminoácidos presentes na biomassa bacteriana. Pesquisas com outras espécies são menos abundantes na literatura. Rodehutscord et al. (2000) determinaram que ambas as fontes são igualmente disponíveis para trutas arco-íris (*Oncorhynchus mykiss*).

De modo geral, os pesquisadores concluem que o sulfato de L-Lis apresenta biodisponibilidade semelhante ao L-Lis HCl. Portanto, ambas as formas podem ser utilizadas em dietas para aves e demais animais monogástricos.

Determinação da exigência de lisina

Numerosas pesquisas foram desenvolvidas para determinar a exigência de Lis para os frangos de corte modernos de rápido crescimento. A importância do AA pode ser explicada pelo fato de ser o segundo limitante em dietas baseadas em milho e farelo de soja e, por ter função única de deposição muscular, simples análise laboratorial e grande volume de estudos, foi escolhida como AA referência para a utilização do conceito de proteína ideal (Baker & Han, 1994). Este conceito determina que as dietas sejam formuladas com uma mistura ideal de AA que garanta a mantença e permita máximo crescimento, sem excesso ou deficiência (Mitchell, 1964). Como os AA são expressos em relação à Lis (AA/Lis), a precisão da exigência de Lis é crítica pois influencia a inclusão de todos os outros AA nas dietas.

Nos experimentos de determinação de exigência de AA, diversos fatores podem influenciar os resultados obtidos e devem ser levados em consideração. Ambiência, componentes da dieta, linhagem das aves, idade, sexo e modelo estatístico são alguns exemplos (Baker et al., 2002). Além disso, os pesquisadores optam por representar as exigências como 100, 99 ou 95% do ponto de máximo desempenho. Devido ao grande número de variáveis que influenciam a exigência de AA, deve-se confrontar resultados de diferentes pesquisas com cautela, levando em consideração que muitas vezes as comparações não são possíveis. Além disso, sabe-se que a maior parte dos experimentos é realizado em ambientes controlados e que em aviários comerciais as aves enfrentam adversidades como competição por comedouros, espaço e desafio sanitário que podem afetar a exigência de Lis.

Modelos estatísticos para determinação de exigências

Um ponto importante na experimentação com objetivo de estimar exigências é a análise estatística utilizada. Os pesquisadores muitas vezes optam por diferentes modelos, o que dificulta a comparação de resultados. Vedenov e Pesti (2008) testaram diversos modelos em diferentes bancos de dados e observaram que não há um modelo melhor para todos os tipos de resposta, portanto o objetivo do experimento deve guiar a escolha.

Ao testar diferentes modelos estatísticos para determinação de exigência, Pesti et al. (2009) observaram grande variabilidade entre eles. Uma maneira de comparar os modelos é através da avaliação do coeficiente de determinação (R^2), que indica o ajustamento dos dados ao modelo testado. Outro modo é através da observação da soma dos quadrados dos resíduos, porém este é menos utilizado uma vez que, por ser uma função linear do R^2, seus resultados são os mesmos.

Testes de média, como o teste de Tukey, não são ideais para determinação de exigências. O resultado não indica precisamente a exigência, mas sim um valor entre dois níveis testados. Não há dúvida que análises de regressão são mais apropriadas para este fim. Há diversas curvas que relacionam desempenho com componentes da dieta, como as regressões polinomial quadrática, broken-line linear e broken-line quadrática, entre outras (Figura 3).

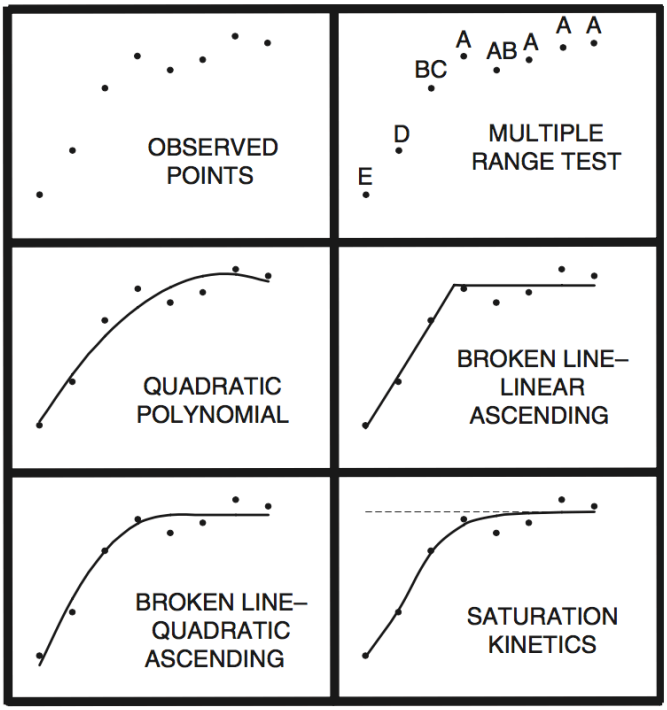

Figura 3. Modelos estatísticos para análise de exigências. Fonte: Pesti et al. (2009)

A regressão polinomial quadrática é de simples análise, tem um bom ajustamento aos dados e os pontos de máxima são facilmente obtidos. Entretanto, não é possível observar o platô que se espera de grande parte das respostas à nutrientes. Os modelos broken-line representam com fidelidade o conceito de exigência, definido como o nível nutricional que resulta em máxima resposta, porém são testes mais complexos e que exigem maior número de níveis testados para obter estimativas precisas (Pesti et al., 2009). A regressão broken-line linear tende a subestimar as exigências pois a resposta dos animais a níveis crescentes de nutrientes é claramente não linear, ela diminui à medida que se aproxima da exigência (Robbins et al., 2006). A regressão broken-line quadrática fornece valores de exigência mais precisos, porém a forma da curva não permite a determinação de limites de segurança a partir do qual o nutriente se torna tóxico ou prejudica o crescimento, o que é possível apenas com a regressão polinomial quadrática (Pesti et al., 2009).

Exigência de lisina na fase inicial

O NRC (1994) recomenda Lis total de 1,10% para frangos de corte de 1 a 21 dias de idade. Um compilado de 16 pesquisas sobre exigência de Lis na fase inicial foi realizado por Vazquez e Pesti (1997). Determinou-se que a exigência de Lis total é de 1,21% para GP e 1,32% para CA entre 1 e 21 dias de idade. Entretanto, trata-se de publicações antigas que podem não refletir a real exigência de frangos de corte modernos.

Utilizando a regressão linha quadrada linear, Labadan et al. (2001) determinaram a exigência de Lis total como 1,28% para GP e 1,21% para CA para frangos de corte Ross x Avian machos de 1 a 14 dias de idade. Garcia e

Batal (2005) estimaram a exigência de Lis digestível de frangos de corte Cobb 500 machos utilizando a mesma regressão como 1,00% para GP e 1,10% para CA, entretanto os autores consideraram 90% do ponto de máxima como a exigência. Além destes, Garcia et al. (2006) determinaram a exigência de Lis digestível como 0,97% para GP e 0,99% para CA para frangos de corte Cobb 500 machos de 7 a 21 dias de idade.

Através da regressão broken-line quadrática, Dozier et al. (2012) estimaram a exigência de frangos de corte machos Ross 708 como 1,27% para GP entre 1 e 14 dias de idade e como 1,18% para GP e 1,26% para CA na mesma idade na linhagem Hubbard x Cobb 500.

Bernal et al. (2014) observaram exigência de Lis digestível para frangos de corte Cobb 500 machos como 1,15% para GP e 1,22% para CA entre 10 e 21 dias de idade utilizando a regressão polinomial quadrática. Utilizando a mesma metodologia e linhagem, Siqueira et al. (2013) encontraram exigências de 8 a 22 dias como 1,17% para GP e 1,14% para CA.

Exigência de lisina nas fases crescimento e final

A recomendação do NRC (1994) para frangos de corte entre 22 e 42 dias de idade é de 1,00% de Lis total. Garcia et al. (2005) determinaram a exigência de Lis com a regressão broken-line linear como 0,97% para GP e 0,96% para CA em frangos de corte Cobb 500 de 21 a 38 dias de idade. Com a mesma análise estatística, Labadan et al. (2001) determinou a exigência de Lis total de machos Ross x Avian de 15 a 28 dias de idade como 1,13% para GP; entre 22 a 42 dias de idade, a exigência estimada foi de 0,99% para GP e 1,00% para CA.

Utilizando a regressão broken-line quadrática, Dozier et al. (2010) determinaram exigência de Lis digestível de machos Cobb 700 de 28 a 42 dias de idade como 0,965% para GP e 1,012% para CA. Dozier et al. (2009) determinaram a exigência de machos Ross TP16 de 14 a 28 dias de idade como 1,16% para GP e 1,20% para CA usando a broken-line quadrática e 1,18% para GP e 1,24% para CA utilizando a regressão polinomial quadrática.

Bernal et al. (2014) utilizaram a regressão polinomial quadrática e encontraram exigência de Lis digestível de 1,05% para GP e 1,07% para CA em machos Cobb 500 de 22 a 35 dias de idade.

De acordo com o NRC (1994), a exigência para frangos de corte de 43 a 56 dias de idade é de 0,85% de Lis total. Labadan et al. (2001) encontraram exigência de Lis total de machos Ross x Avian de 0,81% para GP e peso de peito utilizando a regressão broken-line linear. Dozier et al. (2008) utilizaram as regressões polinomial quadrática, broken-line linear e broken-line quadrática e encontraram, respectivamente, exigências de 0,86%, 0,79% e 0,91% para GP e 0,88%, 0,78% e 0,89% para CA para machos Ross 708 de 49 a 63 dias de idade.

HIPÓTESES E OBJETIVOS

Hipóteses

A Lis do sulfato de L-Lis apresenta bioeficácia relativa semelhante à Lis do L-Lis HCl.

As exigências de Lis nos períodos de 1 a 12, 12 a 28 e 28 a 42 dias de idade são influenciadas pelo modelo estatístico e pela variável resposta.

Objetivos

Determinar a bioeficácia relativa da Lis no sulfato de L-Lis em relação ao L-Lis HCl.

Determinar a exigência de Lis para frangos de corte machos Cobb x Cobb 500 de 1 a 12, 12 a 28 e 28 a 42 dias de idade.

Avaliar os diferentes modelos estatísticos para determinação da exigência de Lis

CAPÍTULO II[1]

[1]Artigo escrito conforme as normas do periódico Poultry Science

Estimation of digestible lysine requirements of male broilers from 1 to 42 days of age

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ABSTRACT Three experiments were conducted to estimate digestible Lys requirements of Cobb x Cobb 500 male broilers from 1 to 12, 12 to 28, and 28 to 42 d of age (experiment 1, 2 and 3, respectively) using different statistical models. For each experiment, 2,200 chicks were randomly distributed into 88 floor pens in a completely randomized design. Lysine deficient basal diets were formulated and five graded levels of Lys were supplemented from L-Lys HCl or L-Lys sulfate. Digestible Lys levels ranged from 0.97 to 1.37% in experiment 1, 0.77 to 1.17% in experiment 2, and 0.68 to 1.08% in experiment 3. Relative bioavailability (**RBV**) of Lys sources was evaluated by a multivariate regression analysis and compared by a *t*-test. Digestible Lys requirements were estimated by quadratic polynomial, linear broken-line, and quadratic broken-line models. No difference was observed in RBV of Lys, therefore both sources were used to estimate requirements. Overall, digestible Lys requirements varied among response variables and statistical models. The quadratic broken-line model provided the best fit among statistical models tested. Quadratic polynomial, linear broken-line, and quadratic broken-line estimates were predicted as 1.190, 1.032, and 1.101% for BW gain and 1.226, 1.038, and 1.124% for feed conversion ratio (**FCR**) in experiment 1; 1.021, 0.900, and 0.961% for BW gain and as 1.064, 0.966, and 1.043% for FCR in experiment 2; and 0.949, 0.833, 0.925% for BW gain, 0.978, 0.851, and 0.960% for FCR, 0.933, 0.842, and 0.931% for carcass yield, and 0.952, 0.839, and 0.921% for breast meat yield. The results suggest that Lys requirements vary greatly according to statistical analysis; therefore, the choice of an adequate model is critical to obtain accurate and reasonable estimates.

Key words: broiler, growth performance, lysine, regression model

INTRODUCTION

Lysine is the second limiting amino acid (**AA**) in practical corn-soybean meal diets. Based on the ideal protein concept, expressing AA requirements as ratios to Lys is a popular strategy. Lysine was chosen as the reference AA because of its limitation in standard corn-soybean meal diets, exclusive protein accretion function, and simple laboratorial analysis in feedstuffs (Baker, 1997). Thus, it is crucial to obtain precise Lys requirements since minor changes on its estimation alter the inclusion of all other indispensable AA (Baker et al., 2002).

Modern broiler chicken performance was considerably improved compared with commercial broilers of the past decades mainly due to genetic selection for growth rate, feed conversion ratio (**FCR**), and meat accretion (Havenstein et al., 2003). These improvements could indicate a greater AA requirement than determined with previous research. The NRC (1994) recommendations are 1.10% total Lys from 0 to 3 wk of age, 1.00% total Lys from 3 to 6 wk, and 0.85% total Lys from 6 to 8 wk of age. However, recent research (Labadan et al., 2001; Dozier et al., 2008, 2009a, 2010; Dozier and Payne, 2012) show that Lys requirements for modern broilers are higher than those previously recommended, although many factors such as strain, sex, age, type of diet, levels of other nutrients, environment, response criteria, and statistical model may influence AA requirements (Baker et al., 2002).

Pesti et al. (2009) tested different statistical models in the same data set and observed that Lys requirement estimates ranged from 0.90% to 1.28%. Thus, the choice of an appropriate statistical model is critical to interpreting nutritional requirements. It is clear that a regression model should be used in preference to multiple range tests (Lowry, 1992); however, each model has advantages and disadvantages. The objective of our

research was to estimate Lys requirements of Cobb x Cobb 500 male broilers in three feeding phases using different statistical models.

MATERIAL AND METHODS

Dietary treatments

A basal diet (Table 1) containing corn, soybean meal, and corn gluten meal was formulated to meet or exceed recommendations, except for Lys, from 1 to 12 d of age (experiment 1), 12 to 28 d of age (experiment 2), and 28 to 42 d of age (experiment 3). Digestible Lys levels in the basal diets were 0.97%, 0.77%, and 0.68% in experiments 1, 2, and 3, respectively. Five graded levels of Lys were added to the basal diet to generate a total of 6 digestible Lys levels in 0.08% of increment ranging from 0.97% to 1.37%, 0.77% to 1.17%, and 0.68% to 1.08% in experiments 1, 2, and 3, respectively. Two Lys sources were used, L-Lys HCl and L-Lys sulfate. Before diet formulation, near infrared reflectance spectroscopy was used to analyze the ingredients for CP and AA contents (Fontaine et al., 2001, 2002). Analyzed values were used to determine diet formulation. Digestible AA values for the ingredients were obtained by applying digestibility coefficients (Evonik, 2010) to analyzed total AA content. Despite the extensive variability existent among batches of the same ingredient, digestible coefficients were used rather than conducting digestible AA assays due to the variation associated with this measurement (Dozier et al., 2010). Diets were manufactured in mash form and representative samples of each complete feed were obtained to validate Lys concentrations.

Bird husbandry

All procedures utilized in this study were approved by the Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. Three experiments were conducted using Cobb x Cobb 500 male chicks allocated in a pen floor facility. For each experiment, 2,200 chicks were obtained from a commercial hatchery and vaccinated for Marek's disease and infectious bronchitis. Chicks were weighed before allotment to their respective treatments to ensure that each pen had similar BW range. Experiments 1, 2, and 3 were designed to estimate Lys requirement from 1 to 12, 12 to 28, and 28 to 42 d of age, respectively. Birds of experiment 1 received the experimental diets from placement to 12 d of age. Birds of experiments 2 and 3 received a standard commercial diet bases on corn-soybean meal until the start of the experimental period. At 12 and 28 d, respectively, birds of experiment 2 and 3 were equalized in 88 pens (25 birds per pen, 9.18 birds/m^2) to establish similar average weights at the start of the experimental period. Each pen was equipped with a hanging feeder, a nipple drinker line, and used litter. Birds were allowed *ad libitum* access to water and feed. Initial room temperature was set to 32ºC and was reduced by 1ºC every two d until 22ºC. Photoperiod was a continuous lighting schedule for experiment 1. For experiments 2 and 3, a continuous lighting schedule was used until 7 d of age and a 20L:4D h cycle thereafter. Mortality was recorded daily. Birds and feed were weighed on d 1 and 12 in experiment 1, d 12 and 28 in experiment 2, and d 28 and 42 in experiment 3 to determine BW gain, feed intake, and FCR. In experiment 3, 4 birds per pen were selected within the upper and lower 5% of each pen average at 43 d of age for processing. Feed was removed 8 h before slaughter. Birds were electrically stunned for 3 s and bled for 3 min through a jugular vein cut. Carcasses were scalded at 60ºC for 45 s, feathers were mechanically plucked, and evisceration was manually performed. Eviscerated carcasses (without feet and neck,

but with lungs) were statically chilled in slush ice for 3 h and hung for 3 min to allow the dropping of excess water before individual weighing. Carcass yield was expressed as a percentage of live weight, whereas breast meat yield was expressed as a percentage of the eviscerated carcass weight. Experiments 1, 2, and 3 were conducted in September 2014, November 2014, and July 2015, respectively.

Statistical Analysis

A gradient treatment structure was conducted as a completely randomized design in all experiments. The basal diet without supplemental Lys had 8 repetitions whereas the dose-response diets had 8 repetitions with L-Lys sulfate and 8 repetitions with L-Lys HCl supplementation for each experiment. Lysine sources relative bioavailability (RBV) was assessed using the slope-ratio model (Littel et al., 1997) by fitting data in a multivariate regression model with the following equation: $y = a + b_1x_1 + b_2x_2$, where a = common intercept, b_1 = slope of L-Lys HCl, b_2 = slope of L-Lys sulfate, x_1 = value for L-Lys HCl, and x_2 = value for L-Lys sulfate. The performance variables BW gain and FCR were regressed on supplemental Lys intake. Relative bioavailability was defined as b_2/b_1. An unpaired *t*-test was conducted to determine if RBV of Lys in L-Lys sulfate was different from in L-Lys HCl (Petrie and Watson, 1999). Growth performance data were fitted to linear and quadratic responses to explain potential Lys effects using PROC REG (SAS, 2009). Minimum digestible Lys requirements were estimated by linear and quadratic broken-line models (Robbins et al., 2006) and by submitting data to a quadratic polynomial regression analysis (Draper and Smith, 1981) and then determining the minimum Lys level required for maximal response (X at maximum Y value of quadratic response curve) when a significant ($P \leq 0.05$) response occurred. Because nutritionists

often decide to set subjective requirements at values other than 100% of the maximum response (Pesti et al., 2009), estimates for 99 and 95% of the calculated requirements were included.

RESULTS

Experiment 1 – 1 to 12 d of age

Relative bioavailability of Lys in L-Lys sulfate was determined to be 97% of that in L-Lys HCl for BW gain and 95% for FCR (Table 2). However, neither values were different from 100% as determined by the unpaired *t*-test ($P > 0.05$). Therefore, both sources were used to estimate Lys requirements. Feeding Cobb x Cobb 500 birds increasing levels of Lys resulted in quadratic responses ($P \leq 0.01$) for BW gain, FCR, feed intake, and digestible Lys intake (Table 3). Quadratic polynomial regression equations estimated 95% of digestible Lys requirement at 1.190% and 1.226% for BW gain and FCR, respectively (Table 4). With the quadratic broken-line model, Lys requirements were predicted at 1.101% for BW gain and 1.124% for FCR, whereas linear broken-line model estimates were the lowest at 1.032% and 1.038% for BW gain and FCR, respectively. When averaged across variables, 95% of digestible Lys requirement was estimated as 1.208% using quadratic polynomial regression equation, 1.035% using linear broken-line model, and 1.113% using quadratic broken-line model. The best fit was provided by the quadratic broken-line model for both BW gain and FCR. Digestible Lys requirements estimates based on 100 and 99% of optimal responses were also calculated (Table 4).

Experiment 2 – 12 to 28 d of age

Slope-ratio analysis of BW gain and FCR showed that the RBV of Lys in L-Lys sulfate was 99 and 100%, respectively, of that in L-Lys HCl (Table 2). Comparison of slopes showed that sources were not different ($P > 0.05$). Therefore, both sources were used to estimate Lys requirements. Significant quadratic responses ($P \leq 0.01$) were observed for BW gain, FCR, feed intake, and digestible Lys intake (Table 5). Digestible Lys requirements presented were predicted based on 95% of the optimal response; estimates of 100 and 99% were also calculated (Table 6). Lys requirements were estimated at 1.021% for BW gain and 1.064% for FCR using quadratic polynomial regression. Linear broken-line model estimates were 0.900% for BW gain and 0.966% for FCR. As in Experiment 1, quadratic broken-line model provided a better fit and 95% of digestible Lys requirements were estimated at 0.961% and 1.043% for BW gain and FCR, respectively. When averaged across variables, digestible Lys requirement was predicted as 1.043% using quadratic polynomial regression, 0.933% using linear broken-line model, and 1.002% using quadratic broken-line model.

Experiment 3 – 28 to 42 d of age

Relative bioavailability of Lys in L-Lys sulfate for BW gain and FCR were determined to be 100 and 99%, respectively, of that in L-Lys HCl (Table 2). The unpaired *t*-test showed no difference between sources ($P > 0.05$). Thus, both sources were used to estimate Lys requirements. Providing broilers gradient concentrations of digestible Lys resulted in quadratic responses ($P \leq 0.01$) for BW gain, FCR, feed intake, digestible Lys intake, carcass yield, and breast meat yield (Table 7). Digestible Lys requirements presented were predicted based on 95% of the optimal response; estimates of 100 and 99% were also calculated (Table 8). Quadratic regression equations estimated digestible

Lys requirements at 0.949, 0.978, 0.933, and 0.952% for BW gain, FCR, carcass yield, and breast meat yield, respectively. Linear broken-line model estimates were the lowest at 0.833% for BW gain, 0.851% for FCR, 0.842 for carcass yield, and 0.839% for breast meat yield. Quadratic broken-line model estimated digestible Lys requirements at 0.925, 0.960, 0.931, and 0.921% for BW gain, FCR, carcass yield, and breast meat yield, respectively. When averaged across variables, digestible Lys requirements were calculated as 0.953% using quadratic regression analysis, 0.841% using linear broken-line model, and 0.934% using quadratic broken-line model. As in Experiments 1 and 2, quadratic broken-line model provided the best fit for BW gain and FCR. For the processing yield variables, linear broken-line model provided the best fit.

DISCUSSION

The basal diets utilized in the present research were based on corn, soybean meal, and corn gluten meal, and were formulated to contain CP and energy levels comparable to commercial diets. Amino acid requirement trials using diets with minimal protein content and high content of nitrogen from dispensable and indispensable AA often result in estimates that are not applicable to practical situations (Baker et al., 2002). It is crucial that requirement studies use a basal diet that is deficient in the test AA to produce accurate estimates. Amino acid analysis determined that the basal diets in experiments 1, 2, and 3 contained 1.12, 0.85, and 0.77% total Lys, respectively. Moreover, in experiments 1, 2, and 3, broilers fed the basal diets presented poor performance compared with broilers fed the dose-response diets with increasing Lys levels, demonstrating that Lys was deficient in the basal diets. Lysine levels tested in the present research seem adequate as indicated

by the significant quadratic broken-line and quadratic polynomial regression (Dozier et al., 2009b).

Two Lys sources were used to formulate the dose-response diets, L-Lys HCl and L-Lys sulfate. Both Lys forms are produced by fermentation of carbohydrates; however, post fermentation processing differs. The presence of biomass, which contains a small amount of other nutrients, is the main difference between sources: it is removed in L-Lys HCl processing and maintained in L-Lys sulfate (Schutte and Pack, 1994). Relative bioavailability assessed by the slope-ratio model indicated that both Lys sources produced equal responses on BW gain and FCR. These results were expected since no difference was observed in true digestibility of Lys in L-Lys sulfate compared to L-Lys HCl in cecectomized roosters (Neme et al., 2001). Ahamad et al. (2007) did not observe differences in BW gain, feed intake, and FCR when broilers were fed diets with L-Lys sulfate or L-Lys HCl. Moreover, similar results were observed in pigs (Smiricky-Tjardes et al., 2004) and rainbow trout (Rodehutscord et al., 2000).

Digestible Lys requirement varied depending upon response criterion. As observed in previous research (Labadan et al., 2001; Dozier et al., 2009a, 2009b, 2010; Dozier and Payne, 2012), digestible Lys requirement for FCR was higher than BW gain in all experiments. Apparently, as digestible Lys exceeds the concentration required for optimal BW gain, growth rate is maintained while feed intake decreases, thus resulting in a higher requirement for FCR than BW gain (Baker et al., 2002). Moreover, Lys increments produce more pronounced increases in BW gain than feed intake; because FCR is a ratio between these variables, as BW gain increases FCR is improved, thus increasing its requirement.

Comparable to our results, broilers fed diets with progressive additions of digestible Lys quadratically altered BW gain and FCR in all studied ages (Garcia and Batal, 2005; Dozier et al., 2009a, 2010; Dozier and Payne, 2012). Garcia and Batal (2005), using linear broken-line model, estimated digestible Lys requirement ranging from 1.01 to 1.10% from 1 to 7 d of age, which is in agreement with our results from experiment 1, although slightly different ages were tested. Conversely, Labadan et al. (2001) estimated total Lys requirements from 1 to 14 d of age as 1.28% for BW gain and 1.21% for FCR using linear broken-line model. Using the same model, Sklan and Noy (2003) estimated lower digestible Lys requirements (0.92 to 0.96%) from 1 to 7 d of age. Digestible Lys requirements from 1 to 14 d of age using the quadratic broken-line model were reported as 1.180% for BW gain, which is in close agreement to our findings, and 1.261% for FCR (Dozier and Payne, 2012).

Dozier et al. (2009b) estimated digestible Lys requirements from 14 to 28 d of age as 1.07% for BW gain and 1.10% for FCR, respectively, using quadratic regression analysis, and as 1.09% for BW gain and 1.15% for FCR, respectively, based on quadratic broken-line model. Although slightly lower, requirements from 12 to 28 d from experiment 2 are similar to those previously reported. Furthermore, quadratic regression analysis from Baker et al. (2002) estimated digestible Lys requirements from 8 to 21 d of age as 0.97% for BW gain and 1.03% for FCR using 90% of the maximal responses, which is in close agreement to our observations. Labadan et al. (2001) observed higher requirement estimate for BW gain as 1.13% total Lys from 14 to 28 d of age using linear broken-line model.

Dozier et al. (2010) estimated digestible Lys requirements from 28 to 42 d of age as 0.965% for BW gain, 1.012% for FCR, 0.963% for carcass yield, and 0.981% for breast

meat yield using quadratic broken-line model, which are comparable to our findings. Similarly, Labadan et al. (2001) using linear broken-line model determined total Lys requirements as 0.94% for BW gain and 0.95% for FCR. In contrast to our findings, Garcia et al. (2006) predicted unexpectedly high digestible Lys requirements as 0.97, 0.96, 0.94, and 0.98% for BW gain, FCR, and carcass yield, respectively, using the linear broken-line model.

The choice of an adequate statistical model is essential to the correct interpretation of requirement estimates. Multiple-range tests are not suitable to estimate requirements because the outcome is that the requirement is often between two tested concentrations of the AA, hence no precise requirement prediction is possible (Lowry, 1992; Pesti et al., 2009). There are many curves that relate performance variables with feed composition, such as quadratic polynomial, linear broken-line, quadratic broken-line, and saturation kinetics, which have been thoroughly reviewed by Pesti et al. (2009) and will be briefly discussed in the present research. Researchers often choose different models, and although not incorrect, it constitutes an additional complication to compare results along with distinct strains, ages, sex, and environmental conditions. The decision to use the quadratic regression analysis, linear broken-line model, and quadratic broken-line model was based on the abundance of Lys requirements research using these models (Labadan et al., 2001; Baker et al., 2002; Sklan and Noy, 2003; Garcia and Batal, 2005; Garcia et al., 2006; Dozier et al., 2008, 2009a, 2009b, 2010; Dozier and Payne, 2012).

According to Pesti et al. (2009), quadratic regression analysis is simple to fit to data and maximum responses are easily obtained; however, the analysis is not capable of characterizing the plateau that most nutritional responses are admitted to have. The broken-line models more approximately represent the concept of requirement, defined as

the minimum nutrient level that result in maximum response, but more levels of the tested nutrient are required to obtain accurate estimates. Vedenov and Pesti (2008) tested several nonlinear models, including linear and quadratic broken-line models, in different data sets and observed that no particular model is necessarily best for all nutritional response data, thus the objective of the experiments should indicate the choice of model.

Nevertheless, it is possible to compare models through R^2 and the sum of square residuals. Since the latter is a linear function of R^2 values, the order of fit is the same. Thus, data on sum of square residuals is not presented. In the present research, all models tested were statistically significant ($P \leq 0.01$). The model that provided the best fit was the quadratic broken-line model for BW gain and FCR and the linear broken-line model for carcass and breast meat yield. Conversely, Dozier et al. (2008, 2009b) observed better fit using quadratic regression than quadratic broken-line model. Pesti et al. (2009) compared six different models and observed that the quadratic regression provided the worst fit and both linear and quadratic broken-line models fitted to data very well. In addition to the excellent fit, broken-line models provide a clear definition of the requirement. The linear broken-line underestimates requirements because birds' response to increasing Lys levels is clearly nonlinear, it decreases as the AA approaches its requirement (Robbins et al., 2006). Thus, the quadratic broken-line model would provide the most accurate Lys requirement estimate. However, the shape of response curves of broken-line models does not allow researchers to determine what is the safe limit when the nutrient becomes toxic or impairs growth, which is possible with the quadratic regression curve (Pesti et al., 2009).

Although we attempted to contrast our results to previous research, several variables such as strain, sex, age, type of diet, statistical model, and method (factorial vs

empirical) must be observed before making direct comparisons. Overall, requirement estimates observed in the present research are notably higher than the recommended by the NRC (1994) and in agreement with recent research. The higher digestible Lys requirement observed in the present research may be due to genetic selection of the modern broiler, which allowed for less feed intake per unit of BW gain and greater meat accretion (Havenstein et al., 2003; Dozier et al., 2010). Estimates of Lys requirements vary among response variables and statistical models. Requirement estimates for FCR were higher than BW gain. The choice of an adequate statistical model is critical to obtain precise, coherent estimates. The quadratic broken-line model provided the best fit for growth performance variables, whereas the linear broken-line model fitted better to carcass and breast meat data.

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Table 1. Composition of basal diets

	Experiment 1	Experiment 2	Experiment 3
Ingredient, %			
Corn 7.8% CP	57.51	68.08	75.82
Soybean meal 45% CP	30.43	21.74	14.42
Corn gluten meal	5.00	5.50	5.80
Soybean oil	2.50	0.94	0.80
Dicalcium phosphate	1.45	0.96	0.54
Limestone	1.37	1.10	0.93
Salt	0.29	0.15	0.05
Sodium bicarbonate	0.36	0.42	0.59
DL-Met 99%	0.32	0.26	0.27
L-Thr 98.5%	0.14	0.14	0.09
L-Arg 98%	0.12	0.14	0.20
L-Ile 98.5%	0.08	0.08	0.06
L-Val 96.5%	0.13	0.11	0.07
L-Trp 98%	-	0.01	0.01
L-Leu 98.5%	-	0.03	-
Vitamin premix[1]	0.10	0.10	0.10
Mineral premix[2]	0.05	0.05	0.05
Choline chloride 60%	0.09	0.12	0.14
Coccidiostat[3]	0.05	0.05	0.05
Phytase[4]	0.01	0.01	0.01
Nutritional composition			
AME_n, kcal/kg	3,035	3,108	3,180
CP, %	22.82	19.48	18.85
Digestible TSAA[5], %	0.97 (1.02)	0.83 (0.87)	0.80 (0.81)
Digestible Lys, %	0.97 (1.12)	0.77 (0.85)	0.68 (0.77)
Digestible Thr, %	0.84 (0.97)	0.73 (0.81)	0.72 (0.79)
Digestible Val, %	1.05 (1.17)	0.89 (0.98)	0.87 (1.03)
Digestible Ile, %	0.92 (1.03)	0.78 (0.82)	0.74 (0.75)
Digestible Leu, %	1.97 (2.20)	1.79 (1.91)	1.77 (1.89)
Digestible Arg, %	1.38 (1.52)	1.16 (1.25)	1.12 (1.24)
Digestible Trp, %	0.22	0.18	0.17
Ca, %	1.05	0.84	0.68
Available P, %	0.52	0.42	0.33
Na, %	0.24	0.19	0.20
DEB[6], mEq/kg	220	190	180

[1]Composition per kg of feed: vit. A, 8,000 UI; vit. D3, 2,000 UI; vit. E, 30 UI; vit. K3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamine, 0.012 mg, panthothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin.
[2]Composition per kg of feed: iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.
[3]Poulcox® 40% Premix provided 200 g of monensin sodium per ton of feed (Huvepharma, Sofia, Bulgaria)
[4]HiPhos 1000 FTU
[5]Values in parenthesis represent analyzed total amino acid content.
[6]Dietary electrolyte balance represents dietary Na + K – Cl in mEq/kg of diet.

Table 2. Relative bioefficacy from L-Lys sulfate to L-Lys HCl[1]

Item	BW gain	FCR[2]
1 to 12 d of age[3]	$y = 193.70 + 33.13x_1 + 32.24x_2$	$y = 1.454 - 0.0500x_1 - 0.0475x_2$
12 to 28 d of age	$y = 644.95 + 32.89x_1 + 32.52x_2$	$y = 2.097 - 0.0289x_1 - 0.0291x_2$
28 to 42 d of age	$y = 818.07 + 34.07x_1 + 34.05x_2$	$y = 2.620 - 0.0316x_1 - 0.0313x_2$
R^2		
1 to 12 d of age	0.88	0.66
12 to 28 d of age	0.97	0.92
28 to 42 d of age	0.95	0.89
Relative bioefficacy[4]		
1 to 12 d of age	97.31	95.00
12 to 28 d of age	98.88	100.01
28 to 42 d of age	99.94	99.05
P value		
1 to 12 d of age	0.0001	0.0001
12 to 28 d of age	0.0001	0.0001
28 to 42 d of age	0.0001	0.0001

[1]Values are least square means of 8 replicates with 25 birds each for 0.97% digestible Lys and 16 replicates with 25 birds each for all other treatments.
[2]Feed conversion ratio corrected for mortality weight.
[3]Multivariate regression model is $Y = a + b_1x_1 + b_2x_2$, where Y is the dependent variable, a is the common intercept, b_1 is the slope of L-Lys HCl, x_1 is the dietary L-Lys HCl concentration, b_2 is the slope of L-Lys sulfate, and x_2 is the dietary L-Lys sulfate concentration.
[4]Relative bioefficacy calculated as b_2/b_1. Unpaired *t*-test conducted to evaluate if relative bioefficacy observed values were different than 100% were not significant ($P > 0.05$).

Table 3. Growth performance of broilers fed gradient levels of digestible lysine from 1 to 12 d of age[1] (Experiment 1)

Item	BW gain, g	FCR[2], g:g	Feed intake, g	Lys intake, mg/d
Digestible Lys, %				
0.97%	295.4	1.377	406.7	328.8
1.05%	338.9	1.259	426.7	373.3
1.13%	356.2	1.209	430.7	405.5
1.21%	359.7	1.182	425.2	428.7
1.29%	360.8	1.193	403.4	462.6
1.37%	357.8	1.200	429.2	490.2
SEM	0.002	0.007	0.002	5.50
Source of variation, P value				
Linear response	0.0001	0.0001	0.0035	0.0001
Quadratic response	0.0001	0.0001	0.0009	0.0001

[1]Values are least square means of 8 replicates with 25 birds each for 0.97% digestible Lys and 16 replicates with 25 birds each for all other treatments.
[2]Feed conversion ratio corrected for mortality weight.

Table 4. Digestible Lys requirements from 1 to 12 d of age (Experiment 1)

Model	Response variable	Equation	Estimated requirement (100, 99, 95%)	Probability	R^2
Quadratic polynomial[1]	BW gain	$y = -856.2 + 1949.5x - 777.8x^2$	1.253, 1.240, 1.190	0.0001	0.7099
	FCR	$y = 4.803 - 5.971x + 2.312x^2$	1.291, 1.278, 1.226	0.0001	0.6544
Linear broken-line[2]	BW gain	$y = 359.1 - 544.2 \times (1.086 - x)$	1.086, 1.075, 1.032	0.0001	0.7457
	FCR	$y = 1.196 + 1.473 \times (1.093 - x)$	1.093, 1.082, 1.038	0.0001	0.6601
Quadratic broken-line[3]	BW gain	$y = 359.1 - 1774.0 \times (1.159 - x)^2$	1.159, 1.147, 1.101	0.0001	0.7482
	FCR	$y = 1.193 + 4.018 \times (1.183 - x)^2$	1.183, 1.171, 1.124	0.0001	0.6673

[1]Quadratic polynomial regression model is $Y = \beta_0 + \beta_1 \times X + \beta_2 \times X^2$, where Y is the dependent variable, X is the dietary Lys concentration, and β_0 is the intercept, β_1 and β_2 are the linear and quadratic coefficients, respectively; maximum response concentration obtained by calculating $-\beta_1 \div (2 \times \beta_2)$.

[2]Linear broken-line model is $Y = \beta_0 + \beta_1 \times (\beta_2 - X)$, where $(\beta_2 - X) = 0$ for $X > \beta_2$, Y is the dependent variable, X is the dietary Lys concentration, β_0 is the value at the plateau, β_1 is the slope and β_2 is the Lys concentration at the break point.

[3]Quadratic broken-line model is $Y = \beta_0 + \beta_1 \times (\beta_2 - X)^2$, where $(\beta_2 - X) = 0$ for $X > \beta_2$, Y is the dependent variable, X is the dietary Lys concentration, β_0 is the value at the plateau, β_1 is the slope and β_2 is the Lys concentration at the break point.

Table 5. Growth performance of broilers fed gradient levels of digestible lysine from 12 to 28 d of age[1] (Experiment 2)

Item	BW gain, g	FCR[2], g/g	Feed intake, g	Lys intake, mg/d
Digestible Lys, %				
0.77%	1,040	1.734	1,803	1,157
0.85%	1,175	1.627	1,912	1,354
0.93%	1,250	1.567	1,958	1,517
1.01%	1,280	1.521	1,947	1,639
1.09%	1,275	1.508	1,922	1,746
1.17%	1,283	1.500	1,925	1,876
SEM	0.008	0.006	0.008	23.65
Source of variation, P value				
Linear response	0.0001	0.0001	0.0009	0.0001
Quadratic response	0.0001	0.0001	0.0001	0.0001

[1]Values are least square means of 8 replicates with 25 birds each for 0.77% digestible Lys and 16 replicates with 25 birds each for all other treatments.
[2]Feed conversion ratio corrected for mortality weight.

Table 6. Digestible Lys requirement from 12 to 28 d of age (Experiment 2)

Model	Response variable	Equation	Estimated requirement (100, 99, 95%)	Probability	R^2
12 to 28 d					
Quadratic polynomial[1]	BW gain	$Y = -1654.1 + 5483.8x - 2549.8x^2$	1.075, 1.064, 1.021	0.0001	0.8787
	FCR	$Y = 3.826 - 4.156x + 1.856x^2$	1.120, 1.109, 1.064	0.0001	0.9349
Linear broken-line[2]	BW gain	$Y = 1279.2 - 1255.1 \times (0.947 - x)$	0.947, 0.938, 0.900	0.0001	0.8867
	FCR	$Y = 1.504 + 0.827 \times (1.017 - x)$	1.017, 1.007, 0.966	0.0001	0.9130
Quadratic broken-line[3]	BW gain	$Y = 1279.2 - 4058.8 \times (1.012 - x)^2$	1.012, 1.002, 0.961	0.0001	0.9028
	FCR	$Y = 1.504 + 2.093 \times (1.098 - x)^2$	1.098, 1.087, 1.043	0.0001	0.9369

[1]Quadratic polynomial regression model is $Y = \beta_0 + \beta_1 \times X + \beta_2 \times X^2$, where Y is the dependent variable, X is the dietary Lys concentration, and β_0 is the intercept, β_1 and β_2 are the linear and quadratic coefficients, respectively; maximum response concentration obtained by calculating $-\beta_1 \div (2 \times \beta_2)$.

[2]Linear broken-line model is $Y = \beta_0 + \beta_1 \times (\beta_2 - X)$, where $(\beta_2 - X) = 0$ for $X > \beta_2$, Y is the dependent variable, X is the dietary Lys concentration, β_0 is the value at the plateau, β_1 is the slope and β_2 is the Lys concentration at the break point.

[3]Quadratic broken-line model is $Y = \beta_0 + \beta_1 \times (\beta_2 - X)^2$, where $(\beta_2 - X) = 0$ for $X > \beta_2$, Y is the dependent variable, X is the dietary Lys concentration, β_0 is the value at the plateau, β_1 is the slope and β_2 is the Lys concentration at the break point.

Table 7. Growth performance and processing yields of broilers fed gradient levels of digestible lysine from 28 to 42 d of age[1] (Experiment 3)

Item	BW gain, g	FCR[2], g/g	Feed intake, g	Lys intake, mg/d	Carcass yield[3], %	Breast meat yield[4], %
Digestible Lys, %						
0.68%	1,457	2.039	2,970	1,683	78.60	21.66
0.76%	1,604	1.899	3,046	1,929	78.98	23.11
0.84%	1,710	1.803	3,083	2,158	79.74	24.34
0.92%	1,768	1.728	3,054	2,341	80.11	25.24
1.00%	1,781	1.716	3,054	2,545	80.09	24.90
1.08%	1,771	1.706	3,021	2,719	79.87	25.14
SEM	0.012	0.012	0.008	35.16	0.12	0.15
Source of variation						
Linear response	0.0001	0.0001	0.6057	0.0001	0.0002	0.0001
Quadratic response	0.0001	0.0001	0.0022	0.0001	0.0001	0.0001

[1]Values are least square means of 8 replicates with 25 birds each for 0.68% digestible Lys and 16 replicates with 25 birds each for all other treatments.
[2]Feed conversion ratio corrected for mortality weight.
[3]Carcass yield expressed as a percentage of live weight.
[4]Breast meat yield expressed as a percentage of carcass weight.

Table 8. Digestible Lys requirement from 28 to 42 d of age (Experiment 3)

Model	Response variable	Equation	Estimated requirement (100, 99, 95%)	Probability	R^2
28 to 42 d					
Quadratic polynomial[1]	BW gain	$Y = -1414.2 + 6405.8x - 3203.9x^2$	0.999, 0.989, 0.949	0.0001	0.8515
	FCR	$Y = 4.601 - 5.635x + 2.739x^2$	1.029, 1.018, 0.978	0.0001	0.9259
	Carcass yield	$Y = 62.308 + 36.204x - 18.434x^2$	0.982, 0.972, 0.933	0.0001	0.2140
	Breast meat yield	$Y = -9.741 + 69.790x - 34.813x^2$	1.002, 0.992, 0.952	0.0001	0.6072
Linear broken-line[2]	BW gain	$Y = 1776.6 - 1544.2 \times (0.877 - x)$	0.877, 0.868, 0.833	0.0001	0.8475
	FCR	$Y = 1.717 + 1.434 \times (0.896 - x)$	0.896, 0.877, 0.851	0.0001	0.9210
	Carcass yield	$Y = 80.024 - 7.424 \times (0.886 - x)$	0.886, 0.877, 0.842	0.0001	0.2157
	Breast meat yield	$Y = 25.098 - 16.618 \times (0.883 - x)$	0.883, 0.874, 0.839	0.0001	0.6181
Quadratic broken-line[3]	BW gain	$Y = 1776.6 - 3704.9 \times (0.974 - x)^2$	0.974, 0.964, 0.925	0.0001	0.8520
	FCR	$Y = 1.710 + 3.007 \times (1.011 - x)^2$	1.011, 1.001, 0.960	0.0001	0.9271
	Carcass yield	$Y = 80.028 - 17.696 \times (0.980 - x)^2$	0.980, 0.970, 0.931	0.0001	0.2084
	Breast meat yield	$Y = 25.099 - 42.742 \times (0.969 - x)^2$	0.969, 0.959, 0.921	0.0001	0.6127

[1]Quadratic polynomial regression model is $Y = \beta_0 + \beta_1 \times X + \beta_2 \times X^2$, where Y is the dependent variable, X is the dietary Lys concentration, and β_0 is the intercept, β_1 and β_2 are the linear and quadratic coefficients, respectively; maximum response concentration obtained by calculating $-\beta_1 \div (2 \times \beta_2)$.

[2]Linear broken-line model is $Y = \beta_0 + \beta_1 \times (\beta_2 - X)$, where $(\beta_2 - X) = 0$ for $X > \beta_2$, Y is the dependent variable, X is the dietary Lys concentration, β_0 is the value at the plateau, β_1 is the slope and β_2 is the Lys concentration at the break point.

[3]Quadratic broken-line model is $Y = \beta_0 + \beta_1 \times (\beta_2 - X)^2$, where $(\beta_2 - X) = 0$ for $X > \beta_2$, Y is the dependent variable, X is the dietary Lys concentration, β_0 is the value at the plateau, β_1 is the slope and β_2 is the Lys concentration at the break point.

CAPÍTULO III

CONSIDERAÇÕES FINAIS

As fontes sulfato de L-Lis e L-Lis HCl são igualmente eficientes e podem ser utilizadas sem distinção em dietas para frangos de corte de 1 a 42 dias de idade. A escolha do nutricionista pela fonte adotada deve ser baseada em outros fatores como preço e disponibilidade, uma vez que suas bioeficácias são similares.

As exigências de Lis observadas neste estudo são maiores que as recomendações anteriores do NRC (1994) e, de modo geral, estão em concordância com pesquisas recentes. A diferença na exigência de Lis do frango de corte moderno deve-se à intensa seleção genética para melhoria do desempenho zootécnico e incrementos na deposição de carne de peito.

A escolha do modelo estatístico adequado é fundamental para a obtenção de resultados precisos e coerentes. As regressões polinomial quadrática, broken-line linear e broken-line quadrática foram significativas para as variáveis analisadas. Através da comparação pelo coeficiente de determinação, o modelo que melhor se ajustou aos dados foi a regressão broken-line quadrática. Do ponto de vista estatístico, é incorreto afirmar que um modelo é superior ao outro: todos têm suas vantagens e desvantagens que devem ser analisadas para a seleção do modelo mais adequado à situação. É importante, portanto, fornecer informações do maior número de modelos possível, de modo que o nutricionista, através do seu conhecimento e experiência tome a decisão que julgar adequada.

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APÊNDICES

Apêndice 1. Normas para publicação no periódico Poultry Science

EDITORIAL POLICIES AND PROCEDURES

Poultry Science publishes the results of fundamental and applied research concerning poultry, poultry products, and avian species in general. Submitted manuscripts shall provide new facts or confirmatory data. Papers dealing with experimental design, teaching, extension endeavors, or those of historical or biographical interest may also be appropriate. A limited number of review papers will be considered for publication if they contribute significant additional knowledge, or synthesis of knowledge, to a subject area. Papers that have been, or are scheduled to be, published elsewhere will not be accepted. Publication of a preliminary report, such as an abstract, does not preclude consideration of a complete report for publication as long as it has not been published in full in a proceedings or similar scientific publication; appropriate identification of previously published preliminary reports should be provided in a title page footnote. Translation of an article into other languages for publication requires approval by the editor-in-chief. Opinions or views expressed in papers published by *Poultry Science* are those of the author(s) and do not necessarily represent the opinion of the Poultry Science Association or the editor-in-chief.

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Editor-in-Chief
Department of Animal and Avian Sciences, University of Maryland, College Park, Building 142, College Park, MD 20742
For information about the scientific content of the journal.
Nes Diaz
Managing Editor

CARE AND USE OF ANIMALS

Authors must make it clear that experiments were conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and laboratory techniques. Experiments shall be conducted in accordance with the principles and specific guidelines presented in *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 3rd edition, 2010 (Association Headquarters, Champaign, IL 61820); and, if applicable, *Guide for the Care and Use of Laboratory Animals* (United States Department of Human Health and Services, National Institutes of Health, Publication Number ISBN 0-309-05377-3, 1996); or *Guide to the Care and Use of Experimental Animals*, 2nd ed. Volume 1, 1993 (Canadian Council on Animal Care). Methods of killing experimental animals must be described in the text. In describing surgical procedures, the type and dosage of the anesthetic agent must be specified. Intra-abdominal and intrathoracic invasive surgery requires anesthesia. This includes caponization. The editor-in-chief of *Poultry Science* may refuse to publish manuscripts that are not compatible with these guides. If rejected solely on that basis, however, the paper may be resubmitted for reconsideration when accompanied by a written verification that a committee on animal care in research has approved the experimental design and procedures involved.

TYPES OF ARTICLES

Full-Length Articles

The majority of papers published in *Poultry Science* are full-length articles. The journal emphasizes the importance of good scientific writing and clarity in presentation of the concepts, apparatus, and sufficient background information that would be required for thorough understanding by scientists in other disciplines. One of the hallmarks for experimental evidence is repeatability. The results of experiments published in *Poultry Science* must be replicated, either by replicating treatments within experiments or by repeating experiments. Care should be taken to ensure that experiments are adequately replicated.

Research Notes

Research Notes are short notes giving the results of complete experiments but are less comprehensive than full-length articles. Preliminary or progress reports will not be accepted. The running head shall be "RESEARCH NOTE." Research Notes will be published as a subsection of the scientific section in which they were reviewed. Research Notes are limited to five printed pages including tables and figures. Manuscripts should be prepared according to the guidelines for full-length articles.

Symposium Papers

The symposium organizer or chair must present the proposal and tentative budget to the Board of Directors at the summer meeting one full year before the symposium is to be scheduled. The symposium chair must then develop detailed symposium plans, including a formal outline of the talks approved and full budgetary expectations, which must be brought to the Board of Directors at the January meeting prior to the meeting at which the symposium is scheduled. The symposium chair must decide whether or not the symposium is to be published and will inform the editor-in-chief of this decision at the January meeting. If the decision is not to publish the symposium, the individual authors retain the right to submit their papers for consideration for the journal as ordinary manuscripts. If publication is decided upon, all manuscript style and form guidelines of the journal shall be followed.

Manuscripts must be prepared electronically, including figures and tables, and then uploaded onto the *Poultry Science* Manuscript Central site within 2 weeks after the annual meeting. The symposium chair will review the papers and, if necessary, return them to the authors for revision. The symposium chair then forwards the revised manuscript to the editor-in-chief for final review. Final revisions by the author and recommendations for acceptance or rejection by the chair must be completed by December 31 of the year in which the symposium was presented. Manuscripts not meeting this deadline will not be included in the published symposium proceedings. Symposium papers must be prepared in accordance with the guidelines for full-length articles and are subject to review. Offprints and costs of pages are the responsibility of the author.

Invited Papers

Invited papers, such as the World's Poultry Science Association lecture, should be submitted online; the editorial office will then make these papers available to the editor-in-chief. These papers are subject to review, and all manuscript style and form guidelines of the journal shall be followed. Invited papers are exempt from page charges but not offprint charges.

Review Papers

Review papers are accepted only if they provide new knowledge or a high-caliber

synthesis of important knowledge. Reviews are not exempt from pages charges. All *Poultry Science* guidelines for style and form apply.

Invited Reviews

Invited Reviews will be approximately 10 published pages and in review format. The editor-in-chief will send invitations to the authors and then review these contributions when they are submitted. Nominations or suggestions for potential timely reviews are welcomed and should be sent directly to the editor-in-chief.

Contemporary Issues

Contemporary Issues in *Poultry Science* will address critical issues facing poultry scientists and the poultry industry. As such, submissions to this section should be of interest to any poultry scientist, to the industry, to instructors and faculty teaching contemporary issues classes, and to undergraduate and graduate students. The section will consist of short papers (approximately 2 published pages) written in essay format and will include an abstract, appropriate subheadings, and references.

Rapid Communications

We aim for receipt-to-decision times of a month or less, and accepted papers will have priority for publication in the next available issue of *Poultry Science*. These papers will present informative and significant new findings, such as tissue-specific gene expression profile data with full-length cDNA and genomic gene structure characterization. These papers will be short (2 to 4 published pages), adhere to journal format, and include references and an abstract. Rapid Communications should **not** be preliminary reports or incomplete studies. Authors will select Rapid Communications as the paper type when submitting the paper.

Book Reviews

Poultry Science publishes reviews of books considered to be of interest to the readers. The editor-in-chief ordinarily solicits reviews. Unsolicited reviews must be sent directly to the editor-in-chief for approval. Book reviews shall be prepared in accordance to the style and form requirements of the journal, and they are subject to editorial revision. No page charges will be assessed.

Letters to the Editor

The purpose of letters will be to discuss, critique, or expand on scientific points made in articles recently published in *Poultry Science*. Introduction of unpublished data will not be allowed, nor will material based on conjecture or speculation. Letters must be received within 6 months of an article's publication. Letters will be limited to 400 words and 5 references (approximately 3 double-spaced, typed pages including references). Letters shall have a title. Author name(s) and affiliation(s) shall be placed between the end of the text and list of references. Letters will be sent electronically directly to the editor-in-chief for consideration. The author(s) of the original paper(s) will be provided a copy of the letter and offered the opportunity to submit for consideration a reply within 30 days. Replies will have the same page restrictions and format as letters, and the titles shall end with "—Reply." Letters and replies will be published together. Acceptability of letters will be decided by the editor-in-chief. Letters and replies shall follow appropriate *Poultry Science* format and may be edited by the editor-in-chief and a technical editor. If multiple letters on the same topic are received, a representative letter concerning a specific article will be published. All letters may not be published. Letters and replies will be published as space permits.

SUBMISSION OF ELECTRONIC MANUSCRIPTS

Authors should submit their papers electronically (http://mc.manuscriptcentral.com/ps). Detailed instructions for submitting electronically are provided online at that site. Authors who are unable to submit electronically should contact Shauna Miller (shaunam@assochq.org) for assistance.

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REVIEW OF MANUSCRIPTS

After a manuscript is submitted electronically, the editorial office checks the manuscript. If a manuscript does not conform to the format for *Poultry Science*, it will be returned to the author (rejected) without review. Manuscripts that pass initial screening will be forwarded to the appropriate section editor, who pre-reviews the manuscript and may suggest rejection at this early stage for fatal design flaw, inappropriate replications, lack of novelty, deviation from the Instructions for Authors, or other major concerns.

The section editor assigns two reviewers, at least one of whom is an associate editor. Each reviewer has 3 weeks to review the manuscript, after which his or her comments are forwarded to the section editor. The section editor may recommend rejection or acceptance at this point, after which the manuscript and reviewer comments are made available to the editor-in-chief for a final decision. More commonly, the manuscript will

be sent back to the corresponding author for revision according to the guidelines of the reviewers. Authors have 6 weeks to complete the revision, which shall be returned to the section editor. Failure to return the manuscript within 6 weeks will cause the paper to be purged from the files. Purged manuscripts may be reconsidered, but they will have to be processed as new manuscripts. Section editors handle all initial correspondence with authors during the review process. The editor-in-chief will notify the author of the final decision to accept or reject. Rejected manuscripts can be resubmitted only with an invitation from the section editor or editor-in-chief. Revised versions of previously rejected manuscripts are treated as new submissions. Therefore, authors must complete a new Manuscript Submission and Copyright Transfer Form.

PRODUCTION OF PROOFS

Accepted manuscripts are forwarded by the editor-in-chief to the editorial office for technical editing and typesetting. At this point the technical editor may contact the authors for missing information or figure revisions. The manuscript is then typeset, figures reproduced, and author proofs prepared.

Proofs

Author proofs of all manuscripts will be provided to the corresponding author. Author proofs should be read carefully and checked against the typed manuscript, because the responsibility for proofreading is with the author(s). Corrections may be returned by fax (217-378-4083), mail, or e-mail. For faxed or mailed corrections, changes to the proof should be made neatly and clearly in the margins of the proof. If extensive editing is required, corrections should be provided on a separate sheet of paper with a symbol indicating location on the proof. Changes sent by e-mail to the technical editor must indicate page, column, and line numbers for each correction to be made on the proof. Corrections can also be marked using the note and highlight tools to indicate necessary changes. Author alterations to copy exceeding 10% of the cost of composition will be charged to the author. Editor queries should be answered on the galley proofs; failure to do so may delay publication. Proof corrections should be made and returned to the technical editor within 48 hours of receipt. The publication charge form should be returned with proof corrections so as not to delay publication of the article.

PUBLICATION CHARGES AND OFFPRINTS

Poultry Science has two options available for the publication of articles: conventional page charges and Open Access (OA).

Open Access

For authors who wish to publish their papers OA (available to everyone when the issue is posted online), authors will pay the OA fee when proofs are returned to the editorial office. Charges for OA are $1,500 if at least one author is a current professional member of PSA; the charge is $2,000 when no author is a professional member of PSA.

Conventional Page Charges

The current charge for publication is $100 per printed page (or fraction thereof) in the journal if at least one author is a professional member of PSA. If no author is a member of PSA, the publication charge is $170 per journal page.

Offprints

Offprints may be ordered at an additional charge. When the galley proof is sent, the author is asked to complete an offprint order requesting the number of offprints desired

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The cost to publish in color in the print journal is $600 per color image. A surcharge for offprints will also be assessed. At the time of submission on ScholarOne Manuscripts, authors will be asked to approve color charges for figures that they wish to have published in color in the print journal. Color versions of figures will be included in the online PDF and full-text article at no charge.

MANUSCRIPT PREPARATION: STYLE AND FORM

General

Papers must be written in English. The text and all supporting materials must use American spelling and usage as given in *The American Heritage Dictionary*, *Webster's Third New International Dictionary*, or the *Oxford American English Dictionary*. Authors should follow the style and form recommended in *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers*, 2006, 7th ed. Style Manual Committee, Council of Science Editors, Reston, VA.

Authors should prepare their manuscripts with Microsoft Word and upload them using the fewest files possible to facilitate the review and editing process.

Authors whose primary language is not English are strongly encouraged to use an English-language service to facilitate the preparation of their manuscript. A partial list of services can be found in the *Poultry Science* Manuscript checklist.

Preparing the Manuscript File

Manuscripts should be typed double-spaced, with lines and pages numbered consecutively, using Times New Roman font at 12 points. All special characters (e.g., Greek, math, symbols) should be inserted using the symbols palette available in this font. Complex math should be entered using MathType from Design Science (http://www.dessci.com). Tables and figures should be placed in separate sections at the end of the manuscript (not placed within the text). Failure to follow these instructions may result in an immediate rejection of the manuscript.

Headings

Major Headings. Major headings are centered (except ABSTRACT), all capitals, boldface, and consist of ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), ACKNOWLEDGMENTS (optional), APPENDIX (optional), and REFERENCES.

First Subheadings. First subheadings are placed on a separate line, begin at the left margin, the first letter of all important words is capitalized, and the headings are boldface and italic. Text that follows a first subheading should be in a new paragraph.

Second Subheadings. Second subheadings begin the first line of a paragraph. They are indented, boldface, italic, and followed by a period. The first letter of each important word should be capitalized. The text follows immediately after the final period of the subheading.

Title Page

The title page shall begin with a running head (short title) of not more than 45 characters. The running head is centered, is in all capital letters, and shall appear on the top of the title page. No abbreviations should be used.

The title of the paper must be in boldface; the first letter of the article title and proper names are capitalized, and the remainder of the title is lowercase. The title must not have abbreviations.

Under the title, names of authors should be typed (first name or initial, middle initial, last name). Affiliations will be footnoted using the following symbols: *, †, ‡, §, #, ‖, and be placed below the author names. Do not give authors' titles, positions, or degrees. Numbered footnotes may be used to provide supplementary information, such as present address, acknowledgment of grants, and experiment station or journal series number. The corresponding author should be indicated with a numbered footnote (e.g., 1Corresponding author: myname@university.edu). Note that there is no period after the corresponding author's e-mail address.

The title page shall include the name and full address of the corresponding author. Telephone and FAX numbers and e-mail address must also be provided. The title page must indicate the appropriate scientific section for the paper (i.e., Education and Production; Environment, Well-Being, and Behavior; Genetics; Immunology, Health, and Disease; Metabolism and Nutrition; Molecular, Cellular, and Developmental Biology; Physiology, Endocrinology, and Reproduction; or Processing, Products, and Food Safety).

Authors may create a full title page as a one-page document, in a file separate from the rest of the paper. This file can be uploaded and marked "not for review." Authors who choose to upload manuscripts with a full title page at the beginning will have their papers forwarded to reviewers as is.

Abbreviations

Author-derived abbreviations should be defined at first use in the abstract and again in the body of the manuscript. The abbreviation will be shown in bold type at first use in the body of the manuscript. Refer to the Miscellaneous Usage Notes for more information on abbreviations.

Abstract

The Abstract disseminates scientific information through abstracting journals and through convenience for the readers. The Abstract, consisting of not more than 325 words, appears at the beginning of the manuscript with the word ABSTRACT without a following period. It must summarize the major objectives, methods, results, conclusions, and practical applications of the research. The Abstract must consist of complete sentences and use of abbreviations should be limited. References to other work and footnotes are not permitted. The Abstract and Key Words must be on a separate sheet of paper.

Key Words

The Abstract shall be followed by a maximum of five key words or phrases to be used for subject indexing. These should include important words from the title and the running head and should be singular, not plural, terms (e.g., broiler, not broilers). Authors should consult a current "Subject Index" in Poultry Science for additional key words. Key words should be formatted as follows: Key words: . . .

Introduction

The Introduction, while brief, should provide the reader with information necessary for understanding research presented in the paper. Previous work on the topic should be summarized, and the objectives of the current research must be clearly stated.

Materials and Methods

All sources of products, equipment, and chemicals used in the experiments must be specified parenthetically at first mention in text, tables, and figures [i.e., (model 123, ABC Corp., Provo, UT)]. Model and catalog numbers should be included. Information shall include the full corporate name (including division, branch, or other subordinate part of the corporation, if applicable), city, and state (country if outside the United States), or Web address. Street addresses need not be given unless the reader would not be able to determine the full address for mailing purposes easily by consulting standard references.

Age, sex, breed, and strain or genetic stock of animals used in the experiments shall be specified. Animal care guidelines should be referenced if appropriate. Papers must contain analyzed values for those dietary ingredients that are crucial to the experiment. Papers dealing with the effects of feed additives or graded levels of a specific nutrient must give analyzed values for the relevant additive or nutrient in the diet(s). If products were used that contain different potentially active compounds, then analyzed values for these compounds must be given for the diet(s). Exceptions can only be made if appropriate methods are not available. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as appropriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phosphorus contents should also be specified.

When describing the composition of diets and vitamin premixes, the concentration of vitamins A and E should be expressed as IU/kg on the basis of the following equivalents:

Vitamin A

1 IU = 0.3 μg of all-trans retinol
1 IU = 0.344 μg of retinyl acetate
1 IU = 0.552 μg of retinyl palmitate
1 IU = 0.60 μg of β-carotene

Vitamin E

1 IU = 1 mg of DL-α-tocopheryl acetate
1 IU = 0.91 mg of DL-α-tocopherol
1 IU = 0.67 mg of D-α-tocopherol

In the instance of vitamin D3, cholecalciferol is the acceptable term on the basis that 1 IU of vitamin D3 = 0.025 μg of cholecalciferol.

The sources of vitamins A and E must be specified in parentheses immediately following the stated concentrations.

Statistical Analysis. Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the

interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately.
The experimental unit is the smallest unit to which an individual treatment is imposed. For group-fed animals, the group of animals in the pen is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use time-sequence analysis.
Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. For example, it is recommended that percentage data between 0 and 20 and between 80 and 100 be subjected to arc sin transformation prior to analysis. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals are stratified by ancestry or weight or if some other initial measurement should be accounted for, the model should include a blocking factor, or the initial measurement should be included as a covariate.

A parameter [mean (μ), variance (σ2)], which defines or describes a population, is estimated by a statistic (x, s2). The term **parameter** is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment. Standard designs are adequately described by name and size (e.g., "a randomized complete block design with 6 treatments in 5 blocks"). For a factorial set of treatments, an adequate description might be as follows: "Total sulfur amino acids at 0.70 or 0.80% of the diet and Lys at 1.10, 1.20, or 1.30% of the diet were used in a 2 × 3 factorial arrangement in 5 randomized complete blocks consisting of initial BW." Note that a factorial **arrangement is not a design**; the term "design" refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted).
Standard deviation refers to the variability in a sample or a population. The standard error (calculated from error variance) is the estimated sampling error of a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not "statistically significant" is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by "±" to a number implies that the second value is its standard error (not its standard deviation). Adequate reporting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of experimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of observations or the heterogeneity of the error variance is to be emphasized. Presenting individual standard errors clutters the presentation and can mislead readers.
For more complex experiments, tables of subclass means and tables of analyses of

variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator of each F statistic. Unbalanced factorial data can present special problems. Accordingly, it is well to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by Bonferroni t statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged. Fixed-range, pairwise, multiple-comparison tests should be used only to compare means of treatments that are unstructured or not related. Least squares means are the correct means to use for all data, but arithmetic means are identical to least squares means unless the design is unbalanced or contains missing values or an adjustment is being made for a covariate. In factorial treatment arrangements, means for main effects should be presented when important interactions are not present. However, means for individual treatment combinations also should be provided in table or text so that future researchers may combine data from several experiments to detect important interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

The terms significant and highly significant traditionally have been reserved for $P < 0.05$ and $P < 0.01$, respectively; however, reporting the P-value is preferred to the use of these terms. For example, use ". . . there was a difference ($P < 0.05$) between control and treated samples" rather than ". . . there was a significant ($P < 0.05$) difference between control and treated samples." When available, the observed significance level (e.g., $P = 0.027$) should be presented rather than merely $P < 0.05$ or $P < 0.01$, thereby allowing the reader to decide what to reject. Other probability (α) levels may be discussed if properly qualified so that the reader is not misled. Do not report P-values to more than 3 places after the decimal. Regardless of the probability level used, failure to reject a hypothesis should be based on the relative consequences of type I and II errors. A "nonsignificant" relationship should not be interpreted to suggest the absence of a relationship. An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of $P > 0.05$ to declare nonsignificance, such as indicating that a difference is not significant at $P > 0.05$ and subsequently declaring another difference significant (or a tendency) at $P < 0.09$. In addition, readers may incorrectly interpret the use of $P > 0.05$ as the probability of a β error, not an α error.

Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information contained in the data is sacrificed. Significant digits in data reported should be restricted to 3 beyond the decimal point, unless warranted by the use of specific methods.

Results and Discussion

Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Results section shall contain only the results and summary of

the author's experiments; there should be no literature comparisons. Those comparisons should appear in the Discussion section. Manuscripts reporting sequence data must have GenBank accession numbers prior to submitting. One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experiments are adequately replicated. The results of experiments must be replicated, either by replicating treatments within experiments or by repeating experiments.

Acknowledgments

An Acknowledgments section, if desired, shall follow the Discussion section. Acknowledgments of individuals should include affiliations but not titles, such as Dr., Mr., or Ms. Affiliations shall include institution, city, and state.

Appendix

A technical Appendix, if desired, shall follow the Discussion section or Acknowledgments, if present. The Appendix may contain supplementary material, explanations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel computer programs or mathematical computations would be appropriate. The Appendix will not be a repository for raw data.

References

Citations in Text. In the body of the manuscript, refer to authors as follows: Smith and Jones (1992) or Smith and Jones (1990, 1992). If the sentence structure requires that the authors' names be included in parentheses, the proper format is (Smith and Jones, 1982; Jones, 1988a,b; Jones et al., 1993). Where there are more than two authors of one article, the first author's name is followed by the abbreviation et al. More than one article listed in the same sentence of text must be in chronological order first, and alphabetical order for two publications in the same year. Work that has not been accepted for publication shall be listed in the text as: "J. E. Jones (institution, city, and state, personal communication)." The author's own unpublished work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and unpublished data must not be included in the References section.

References Section. To be listed in the References section, papers must be published or accepted for publication. Manuscripts submitted for publication can be cited as "personal communication" or "unpublished data" in the text.

Citation of abstracts, conference proceedings, and other works that have not been peer reviewed is strongly discouraged unless essential to the paper. Abstract and proceedings references are not apropriate citations in the Materials and Methods section of a paper. In the References section, references shall first be listed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the authors' names. As with text citations, two or more publications by the same author or set of authors in the same year shall be differentiated by adding lowercase letters after the date. The dates for papers with the same first author that would be abbreviated in the text as et al., even though the second and subsequent authors differ, shall also be differentiated by letters. All authors' names must appear in the Reference section. Journals shall be abbreviated according to the conventional ISO abbreviations given in journals database of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=journals). One-word titles must be spelled out. Inclusive page numbers must be provided. Sample references are given below. Consult recent issues of Poultry Science for examples not included below.

Article:

Bagley, L. G., and V. L. Christensen. 1991. Hatchability and physiology of turkey embryos incubated at sea level with increased eggshell permeability. Poult. Sci. 70:1412-1418.

Bagley, L. G., V. L. Christensen, and R. P. Gildersleeve. 1990. Hematological indices of turkey embryos incubated at high altitude as affected by oxygen and shell permeability. Poult. Sci. 69:2035-2039.

Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, with and without prior vaccination, to challenge with Marek's disease virus. Avian Dis. 50:354-365. doi:10.1637/7498-010306R.1

Book:

Metcalfe, J., M. K. Stock, and R. L. Ingermann. 1984. The effects of oxygen on growth and development of the chick embryo. Pages 205-219 in Respiration and Metabolism of Embryonic Vertebrates. R. S. Seymour, ed. Dr. W. Junk, Dordrecht, the Netherlands.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Federal Register:

Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Regist. 69:10137-10151.

Other:

Choct, M., and R. J. Hughes. 1996. Long-chain hydrocarbons as a marker for digestibility studies in poultry. Proc. Aust. Poult. Sci. Symp. 8:186. (Abstr.)

Dyro, F. M. 2005. Arsenic. WebMD. Accessed Feb. 2006. http://www.emedicine.com/neuro/topic20.htm.

El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, assignee. US Pat. No. 6,766,767.

Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium, MD. Univ. Maryland, College Park.

Luzuriaga, D. A. 1999. Application of computer vision and electronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gainesville.

Peak, S. D., and J. Brake. 2000. The influence of feeding program on broiler breeder male mortality. Poult. Sci. 79(Suppl. 1):2. (Abstr.)

Tables

Tables must be created using the MS Word table feature and inserted in the manuscript after the references section. When possible, tables should be organized to fit across the page without running broadside. Be aware of the dimensions of the printed page when

planning tables (use of more than 15 columns will create layout problems). Place the table number and title on the same line above the table. The table title does not require a period. Do not use vertical lines and use few horizontal lines. Use of bold and italic typefaces in the table body should be done sparingly; such use must be defined in a footnote. Each table must be on a separate page. To facilitate placement of all tables into the manuscript file (just after the references) authors should use "section breaks" rather than "page breaks" at the end of the manuscript (before the tables) and between tables.
Units of measure for each variable must be indicated. Papers with several tables must use consistent format. All columns must have appropriate headings.

Abbreviations not found on the inside front cover of the journal must be defined in each table and must match those used in the text. Footnotes to tables should be marked by superscript numbers. Each footnote should begin a new line.

Superscript letters shall be used for the separation of means in the body of the table and explanatory footnotes must be provided [i.e., "Means within a row lacking a common superscript differ ($P < 0.05$)."]; other significant P-values may be specified. Comparison of means within rows and columns should be indicated by different series of superscripts (e.g., a,b, . . . in rows; x z . . . in columns) The first alphabetical letter in the series (e.g., a or A) shall be used to indicate the largest mean. Lowercase superscripts indicate $P \leq 0.05$. Uppercase letters indicate $P \leq 0.01$ or less.

Probability values may be indicated as follows: *$P \leq 0.05$, **$P \leq 0.01$, ***$v \leq 0.001$, and †$P \leq 0.10$. Consult a recent issue of Poultry Science for examples of tables.
Figures
To facilitate review, figures should be placed at the end of the manuscript (separated by section breaks). Each figure should be placed on a separate page, and identified by the manuscript number and the figure number. A figure with multiple panels or parts should appear on one page (e.g., if Figure 1 has parts a, b, and c, place all of these on the same page). Figure captions should be typed (double spaced) on a separate page.

Figure Size. Prepare figures at final size for publication. Figures should be prepared to fit one column (8.9 cm wide), 2 columns (14 cm wide), or full-page width (19 cm wide).

Font Size. Ensure that all type within the figure and axis labels are readable at final publication size. A minimum type size of 8 points (after reduction) should be used.

Fonts. Use Helvetica or Times New Roman. Symbols may be inserted using the Symbol palette in Times New Roman.

Line Weight. For line graphs, use a minimum stroke weight of 1 point for all lines. If multiple lines are to be distinguished, use solid, long-dash, short-dash, and dotted lines. Avoid the use of color, gray, or shaded lines, as these will not reproduce well. Lines with different symbols for the data points may also be used to distinguish curves.
Axis Labels. Each axis should have a description and a unit. Units may be separated

from the descriptor by a comma or parentheses, and should be consistent within a manuscript.

Shading and Fill Patterns. For bar charts, use different fill patterns if needed (e.g., black, white, gray, diagonal stripes). Avoid the use of multiple shades of gray, as they will not be easily distinguishable in print.

Symbols. Identify curves and data points using the following symbols only: □, ■, ○, ●, ▲, ▼, n, ,, e, r, +, or ×. Symbols should be defined in a key on the figure if possible.

File Formats. Figures can be submitted in Word, PDF, EPS, TIFF, and JPEG. Avoid PowerPoint files and other formats. For the best printed quality, line art should be prepared at 600 ppi. Grayscale and color images and photomicrographs should be at least 300 ppi.

Grayscale Figures. If figures are to be reproduced in grayscale (black and white), submit in grayscale. Often color will mask contrast problems that are apparent only when the figure is reproduced in grayscale.

Color Figures. If figures are to appear in color in the print journal, files must be submitted in CMYK color (not RGB).

Photomicrographs. Photomicrographs must have their unmagnified size designated, either in the caption or with a scale bar on the figure. Reduction for publication can make a magnification power designation (e.g., 100×) inappropriate.

Caption. The caption should provide sufficient information that the figure can be understood with excessive reference to the text. All author-derived abbreviations used in the figure should be defined in the caption.

General Tips. Avoid the use of three-dimensional bar charts, unless essential to the presentation of the data. Use the simplest shading scheme possible to present the data clearly. Ensure that data, symbols, axis labels, lines, and key are clear and easily readable at final publication size.

Color Figures. Submitted color images should be at least 300 ppi. The cost to publish each color figure is $600; a surcharge for color reprints ordered will be assessed. Authors must agree in writing to bear the costs of color production after acceptance and prior to publication of the paper.

MISCELLANEOUS USAGE NOTES

Abbreviations. Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD). A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the scientific field. Abbreviations may be used in heads within the paper, if they have been first defined within the text. The inside back cover of every issue of the journal lists abbreviations that can be used without

definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used less than three times must be spelled out in full rather than abbreviated. All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter, rather than the full term.
The abstract, text, each table, and each figure must be understood independently of each other. Therefore, abbreviations shall be defined within each of these units of the manuscript.

Plural abbreviations do not require "s." Chemical symbols and three-letter abbreviations for amino acids do not need definition. Units of measure, except those in the standard *Poultry Science* abbreviation *list, should be abbreviated as listed in the CRC Handbook for Chemistry and Physics* (CRC Press, 2000 Corporate Blvd., Boca Raton, FL 33431) and do not need to be defined.
The following abbreviations may be used without definition in *Poultry Science*.

A = adenine
ADG = average daily gain
ADFI = average daily feed intake
AME = apparent metabolizable energy
AMEn = nitrogen-corrected apparent metabolizable energy
ANOVA = analysis of variance

B cell = bursal-derived, bursal-equivalent derived cell
bp = base pairs
BSA = bovine serum albumin
BW = body weight

C = cytosine
cDNA = complementary DNA
cfu = colony-forming units
CI = confidence interval
CP = crude protein
cpm = counts per minute
CV = coefficient of variation

d = day
df = degrees of freedom
DM = dry matter
DNA = deoxyribonucleic acid

EDTA = ethylenediaminetetraacetate

ELISA = enzyme-linked immunosorbent antibody assay
EST = expressed sequence tag

g = gram
g = gravity
G = guanine
GAT = glutamic acid-alanine-tyrosine
G:F = gain-to-feed ratio
GLM = general linear model

h = hour
HEPES = N-2-hydroxyethyl piperazine-N□-ethane-sulfonic acid
HPLC = high-performance (high-pressure) liquid chromatography

ICU = international chick units
Ig = immunoglobulin
IU = international units

kb = kilobase pairs
kDa = kilodalton

L = liter*
L:D = hours light:hours darkness in a photoperiod

m = meter
μ = micro
M = molar
MAS = marker-assisted selection
ME = metabolizable energy
MEn = nitrogen-corrected metabolizable energy
MHC = major histocompatibility complex
mRNA = messenger ribonucleic acid
min = minute
mo = month
MS = mean square

n = number of observations
N = normal
NAD = nicotinamide adenine dinucleotide
NADH = reduced nicotinamide adenine dinucleotide
NRC = National Research Council
NS = not significant

PAGE = polyacrylamide gel electrophoresis
PBS = phosphate-buffered saline
PCR = polymerase chain reaction
pfu = plaque-forming units

QTL = quantitative trait loci

r = correlation coefficient
r2 = coefficient of determination, simple
R2 = coefficient of determination, multiple
RFLP = restriction fragment length polymorphism
RH = relative humidity
RIA = radioimmunoassay
RNA = ribonucleic acid
rpm = revolutions per minute

s = second
SD = standard deviation
SDS = sodium dodecyl sulfate
SE = standard error
SEM = standard error of the mean
SRBC = sheep red blood cells
SNP = single nucleotide polymorphism

T = thymine
TBA = thiobarbituric acid
T cell = thymic-derived cell
TME = true metabolizable energy
TMEn = nitrogen-corrected true metabolizable energy
Tris = tris(hydroxymethyl)aminomethane
TSAA = total sulfur amino acids

U = uridine
USDA = United States Department of Agriculture
UV = ultraviolet

vol/vol = volume to volume
vs. = versus
wt/vol = weight to volume
wt/wt = weight to weight
wk = week
yr = year
*Also capitalized with any combination, e.g., mL.

International Words and Phrases. Non-English words in common usage (defined in recent editions of standard dictionaries) will not appear in italics (e.g., in vitro, in vivo, in situ, a priori). However, genus and species of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. German nouns shall begin with capital letters.

Capitalization. Breed and variety names are to be capitalized (e.g., Single Comb White Leghorn).

Number Style. Numbers less than 1 shall be written with preceding zeros (e.g., 0.75).

All numbers shall be written as digits. Measures must be in the metric system; however, US equivalents may be given in parentheses. Poultry Science requires that measures of energy be given in calories rather than joules, but the equivalent in joules may be shown in parentheses or in a footnote to tables. Units of measure not preceded by numbers must be written out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract and in the body of the paper at first use. Units of measure for feed conversion or feed efficiency shall be provided (i.e., g:g).

Nucleotide Sequences. Nucleotide sequence data must relate to poultry or poultry pathogens and must complement biological data published in the same or a companion paper. If sequences are excessively long, it is suggested that the most relevant sections of the data be published in Poultry Science and the remaining sequences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submission of sequence data to one of the databases. The following statement should appear as a footnote to the title on the title page of the manuscript. "The nucleotide sequence data reported in this paper have been submitted to GenBank Submission (Mail Stop K710, Los Alamos National Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN."

Publication of the description of molecular clones is assumed by the editors to place them in the public sector. Therefore, they shall be made available to other scientists for research purposes.

Nucleotide sequences must be submitted as camera-ready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow Poultry Science guidelines.

Gene and Protein Nomenclature. Authors re required to use only approved gene and protein names and symbols. For poultry, full gene names should not be italicized. Gene symbols should be in uppercase letters and should be in italics. A protein symbol should be in the same format as its gene except the protein symbol should not be in italics.

General Usage. Note that "and/or" is not permitted; choose the more appropriate meaning or use "x or y or both."

Use the slant line only when it means "per" with numbered units of measure or "divided by" in equations. Use only one slant line in a given expression (e.g., g/d per chick). The slant line may not be used to indicate ratios or mixtures.

Use "to" instead of a hyphen to indicate a range.

Insert spaces around all signs (except slant lines) of operation (=,−, +, ×, >, or <_ when="""" these="""" signs="""" occur="""" between="""" two="""" items.="""" br="""" gt="gt" items="items" in="in" a="a" series="series" should="should" be="be" separated="separated" by="by" commas="commas" e.g.="e.g." b="b" and="and" c.br="c.br" _="_"> Restrict the use of "while" and "since" to meanings related to time. Appropriate substitutes include "and," "but," or "whereas" for "while" and "because" or "although" for "since."

Leading (initial) zeros should be used with numbers less than 1 (e.g., 0.01).

Commas should be used in numbers greater than 999.

Registered (®) and trademark (™) symbols should not be used, unless as part of an article title in the References section. Trademarked product names should be capitalized.

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The following information is available online and updated regularly. Please refer to these pages when preparing a manuscript for submission.

Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online:
http://www.oxfordjournals.org/our_journals/ps/for_authors/index.html.

SI Units. The following site (National Institute of Standards and Technology) provides a comprehensive guide to SI units and usage:
http://physics.nist.gov/Pubs/SP811/contents.html

Figure Preparation Guidelines. Current detailed information on figure and table preparation can be found at http://www.oxfordjournals.org/for_authors/figures.html

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Supporting material that is not essential for inclusion in the full text of the manuscript, but would nevertheless benefit the reader, can be made available by the publisher as online-only content, linked to the online manuscript. The material should not be essential to understanding the conclusions of the paper, but it should contain data that are additional or complementary and directly relevant to the article content. Such information might include more detailed methods, extended data sets/data analysis, or additional figures.
It is standard practice for appendices to be made available online-only as supplementary data. All text and figures must be provided in suitable electronic formats. All material to be considered as supplementary data must be submitted at the same time as the main manuscript for peer review. It cannot be altered or replaced after the manuscript has been accepted for publication, and it will not be edited. Please indicate clearly all material intended as supplementary data upon submission and name the files (e.g., "Supplementary Figure 1," "Supplementary Data," etc.). Also ensure that the supplementary data are referred to in the main manuscript where necessary, for example as "(see Supplementary data)" or "(see Supplementary Figure 1)."

Files submitted as supplementary material must be correct and complete, including figure legends, etc. These files will not be edited or reformatted, but are posted exactly as submitted.

VITA

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