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PREVALÊNCIA DE *WHITE STRIPING* E *WOODEN BREAST* EM FRANGOS DE CORTE SUPLEMENTADOS COM NÍVEIS CRESCENTES DE LISINA NA FASE DE CRESCIMENTO OU FINAL

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“Quanto mais aumenta nosso conhecimento,
mais evidente fica nossa ignorância.”

(John F. Kennedy)

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PREVALÊNCIA DE WHITE STRIPING E WOODEN BREAST EM FRANGOS DE CORTE SUPLEMENTADOS COM NÍVEIS CRESCENTES DE LISINA NA FASE DE CRESCIMENTO OU FINAL[1]

Autor: Rafael Fontana Abs da Cruz
Orientador: Sergio Luiz Vieira

RESUMO – Foram conduzidos dois experimentos para avaliar a prevalência e severidade das lesões de *white striping* (WS) e *wooden breast* (WB) em peitos de frangos alimentados com níveis crescentes de lisina digestível (Lis dig.) de 12 a 28 dias (Exp. 1) e de 28 a 42 dias (Exp. 2).Os testes foram conduzidos utilizando machos Cobb x Cobb 500 de empenamento lento com 1 dia de idade, ambos com 6 tratamentos e 8 repetições cada. O aumento da Lis dig. foi igualmente espaçado de 0,77 a 1,17% no Exp. 1 e de 0,68 a 1,07% no Exp. 2. A dieta com nível mais baixo de Lis dig. não foi suplementada com L-Lisina no experimentos e todos os outros aminoácidos (AA) essenciais estão de acordo ou excedem em até 5% as recomendações comerciais, a fim de não limitar o crescimento das aves. Foram selecionadas aleatóriamente quatro aves por repetição e processadas aos 35 e 42 dias nos Exp. 1 e 2, respectivamente. Os peitos desossados foram submetidos a avaliação de 3 pessoas para detectar a presença de WS e WB assim como fornecer os escores de WS (0-normal, 1-moderao, 2-severo) e WB (0-normal, 1-moderado leve, 2-moderado, 3-severo). O aumento da Lis dig. apresentou efeito positivo no peso vivo, peso da carcaça e peito bem como no rendimento de peito. A prevalência de WS e WB foi 32,3 e 85,9% no Exp. 1 e 87,1 e 89,1% no Exp. 2. Aves submetidas a dieta sem suplementação de Lis apresentaram os menores escores médios de WS e WB (0,22 e 0,78 no Exp. 1 e 0,61 e 068 no Exp. 2). Respostas lineares foram obtidas através das variáveis de desempenho para WS e WB no Exp. 1, enquanto que a resposta para as variáveis no Exp. 2 foram quadráticas. O aumento dos níveis de Lis melhora o desempenho zootécnico e as caracteristicas das carcaças, além de induzir a ocorrência e severidade das lesões de WS e WB, devido, provavelmente, aos níveis de Lis que maximizam o potencial genético para crescimento e rendimento de peito.

Palavras-chave: Miopatias, Lisina, Wooden breast, White striping, Frangos de corte.

[1]Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (73 p.) março, 2016.

PREVALENCE OF WHITE STRIPING AND WOODEN BREAST IN BROILERS FED DIETS WITH INCREASING LYSINE LEVELS IN GROWER OR FINISHER PHASE[2]

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ABSTRACT – Two experiments were conducted to evaluate the prevalence and severity of white striping (WS) and wooden breast (WB) in breast fillets from broilers fed diets with increasing digestible lysine (dig. Lys) from 12 to 28 d (Exp. 1) and from 28 to 42 d (Exp. 2). Trials were sequentially conducted using 1-d-old slow feathering Cobb × Cobb 500 male broilers, both with 6 treatments and 8 replicates each. Increasing dig. Lys levels were equally spaced from 0.77 to 1.17% in Exp. 1 and from 0.68 to 1.07% in Exp. 2. The lowest dig. Lys diet was not supplemented with L-Lys in either one of the studies and all other essential amino acids (AA) met or exceeded current commercial recommendations such that their dietary concentrations did not limit broiler growth. Four birds per pen were randomly selected from each replication and processed at 35 and 42 d in Exp. 1 and Exp. 2, respectively. Deboned breast fillets were submitted to a 3 subject panel evaluation to detect the presence of WS and WB as well as to provide scores of WS (0-normal, 1-moderate, 2-severe) and WB (0-normal, 1-moderate light, 2-moderate, 3-severe). Increased dig. Lys had a positive effect on body weight, carcass and breast weight as well as breast yield. White striping and WB prevalences were 32.3 and 85.9% in Exp 1 and 87.1 and 89.2% in Exp 2. Birds fed diets not supplemented with Lys had the lowest average WS and WB scores (0.22 and 0.78 in Exp. 1 and 0.61 and 0.68 in Exp. 2). White striping and WB presented linear responses to performance variables in Exp 1, whereas quadratic responses were observed for all variables in Exp 2. In conclusion, increasing Lys levels improved growth performance and carcass traits and induced the occurrence and severity of WS and WB lesions probably due to dig. Lys dietary levels that maximized the genetic potential for growth and breast meat yields.

Key words: Myophaties, Lysine, Wooden breast, White striping, Broilers.

[2]Master of Science dissertation in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (73 p.) march, 2016.

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RELAÇÃO DE ABREVIATURAS

AA	Aminoácidos
ALT	Alanina aminotransferase
AST	Aspartato aminotransferase
CA	Conversão alimentar
CK	Creatina quinase
Dig	Digestibilidade
GP	Ganho de peso
LDH	Lactato desidrôgenase
Lis	Lisina
LKR	Lisina-cetoglutarato redutase
MPP	Miopatia peitoral profunda
PSE-like	Pálida, macia e exsudativa
SDH	sacaropina desidrogenase
WS	White striping
WB	Wooden breast
α-KG	α-cetoglutarato

CAPÍTULO I

INTRODUÇÃO

O aumento do desempenho dos frangos de corte, ocasionados pela seleção genética, pela sanidade e pela nutrição, influenciou no aparecimento de alterações musculares. As miopatias que surgiram nos últimos anos foram denominadas *white striping* (ws) e *wooden breast* (WS), devido às características apresentadas pelo músculo do peito. *White striping* caracteriza-se por estriações brancas paralelas à fibra muscular, afetando principalmente a região cranial do músculo *pectoralis major* (Kuttappan et al., 2013). A miopatia WB é carcacterizada por áreas pálidas e com rigidez aumentada, sendo esta desordem restrita ao músculo do peito (Sihvo *et al.*, 2014).

Essas emergentes miopatias não apresentam etiologia conhecida. Frangos selecionados para maior rendimento de peito apresentam maior incidência de ws (Lorenzi *et al.*, 2014), demonstrando que fatores genéticos também são importantes no aparecimento das miopatias. Existe forte componente não genético que influencia o aparecimento das miopatias peitorais (Bailey *et al.*, 2015), sendo o ganho de peso e as dietas com maior densidades energéticas (Kuttappan *et al.*, 2012) fatores que desencadeiam o processo de ruptura das fibras musculares.

A lisina (Lis) é o segundo aminoácido (AA) limitante, quando utilizam-se dietas à base de milho e de farelo de soja, e apresenta função exclusiva de deposição proteica (Baker, 1997). A Lis é reconhecida pelos efeitos na composição da carcaça e sua exigência altera conforme a variável resposta estabelecida. Observam-se maiores exigências para características vinculadas à conformação da carcaça, como por exemplo, deposição de músculo peitoral (Kerr *et al.*, 1999).

Após a eclosão, as fibras musculares aumentam por hipertrofia (Zheng *et al.*, 2009) e a deficiência de Lis age reduzindo esse tipo de crescimento, especialmente nas fases iniciais de desenvolvimento (Sklan & Noy, 2003). O crescimento e o desenvolvimento muscular exigem um suprimento de proteínas, ou aminoácidos, presentes na dieta. Esse AA apresenta influência direta na deposição proteica e no aumento do peso do peito, sendo um possível fator para desencadear o aparecimento das desordens musculares.

Apesar de inúmeras pesquisas a respeitos das miopatias emergentes no mercado avícola, não houve tentativas de correlacionar a utilização de um AA específico com o aparecimento destas desordens. Esta dissertação teve como objetivo avaliar a prevalência e severidade das lesões de WS e WB em peitos de frangos alimentados com níveis crescentes de Lis digestível na fase de crescimento ou final.

REVISÃO BIBLIOGRÁFICA

Miopatias na indústria avícola

Nas últimas décadas, a indústria avícola passou por mudanças significativas nas áreas de nutrição, genética e sanidade. De 1957 a 2005, houve aumento de 400% no crescimento de frangos de corte e redução de 50% na conversão alimentar (Zuidhof *et al.*, 2014). Neste mesmo espaço de tempo, houve o incremento de 79% e de 85% no músculo *pectoralis major* (músculo peitoral) de machos e de fêmeas, respectivamente (Zuidhof *et al.*, 2014). Esses dados vão ao encontro da produção brasileira de carne de frango que passou de 5,98 milhões de toneladas em 2000 para 12,69 milhões em 2014. Esse crescimento deve-se ao aumento de consumo pela população mundial, pois a carne de frango é vista como saudável,com boas qualidades sensoriais, de fácil preparo e de menor custo em comparação às demais proteínas animais (Petracci *et al.*, 2015).

Segundo Branciari *et al.* (2009), as aves selecionadas nos programas de melhoramento genético apresentam um maior diâmetro da fibra muscular; no entanto, este aumento é associado à menor capilarização da estrutura muscular (Hoving-Bolink *et al.*, 2000). Essa diminuição pode ocasionar o acúmulo de resíduos metabólicos e, como consequência, danos ao tecido devido ao estresse oxidativo (MacRae *et al.*, 2006). Em perus, há indícios de danos musculares relacionados à esquemia, esta vinculada ao rápido ganho de peso (Sosnicki et al., 1991).

Em consequência do aumento da hipertrofia das células musculares, a incidência de anormalidades, como miopatia peitoral profunda (MPP) e pálida, macia e exsudativa (PSE-like), aumentou nos últimos 30 anos e, mais recentemente, WS e WB (Petracci *et al.*, 2015). A MPP foi umas das primeiras miopatias a serem descritas, porém continua sendo um problema de qualidade recorrente nas plantas frigoríficas. A miopatia denominada PSE-like reduz a habilidade da carne em reter a água durante o processamento e estocagem do produto (Petracci & Cavani, 2012).

Nos últimos anos, duas miopatias emergentes chamaram atenção da indústria avícola. *White striping* e WB acometem o músculo *pectoralis major* e são caracterizadas pela alta prevalência em grande parte das aves presentes nos lotes. Kuttappan *et al*. (2009) descreveram, pela primeira vez, a miopatia WS, suas características histológicas e a influência desta desordem na qualidade da carne. A miopatia denominada WS foi descrita por Sihvo *et al.* (2014) como um novo tipo de defeito do músculo peitoral ocorrido na Finlândia e em diversos outros países.

Estrutura e regeneração do tecido muscular esquelético

O músculo esquelético é formado por diversos feixes de fibras cilíndricas revestidos pelo epimísio (Figura 1). Os feixes musculares são separados entre si pelo perimísio, membrana de tecido conjuntivo que os mantém organizados. Dentro dos feixes, são encontradas as fibras musculares, separadas entre si pelo endomísio e formadas por miofibrilas compostas, principalmente, por duas proteínas: actina e miosina (Junqueira & Carneiro, 2004). Essas proteínas formam os sarcômeros, os quais se repetem diversas

vezes ao longo da miofibrila. Eles são responsáveis pela contração da fibra muscular através do deslizamento dos filamentos de actina sobre os de miosina (Galluzzo & Regenstein, 1978).

O tecido conjuntivo é formado por inúmeras céulas com funções de conexão de tecidos, de sustentação e de preenchimento (Junqueira & Carneiro, 2004). Os fibroblastos são as células mais abundantes do tecido conjuntivo e possuem a função de sintetizar as fibras colégenas, as fibras elásticas e a substância fundamental (Junqueira & Carneiro, 2004). Após a influência dos fatores de crescimento e de outros mediadores, produzidos principalmente pelos macrófagos, os fibroblastos são ativados e iniciam a produção de colágeno, processo denominado fibroplasia (Balbino *et al.*, 2005).

O músculo esqueletético, quando lesionado, apresenta capacidade de regeneração, porém, quando ocorre uma destruição celular de maior proporção, é possível observar proliferação de tecido conjuntivo (Gomes *et al.*, 2004). As células inflamatórias, principalmente os macrófagos, são fundamentais na regulação da homeostase do tecido. Eles são indispensáveis para o controle de danos e a remodelação do tecido sobre as lesões musculares (Mann *et al.*, 2011). Entretanto, quando a área muscular é substituída pelo tecido fibroso, ocorre uma inibição da regeneração completa (Kaariainen *et al.*, 2000). Após a degeneração da fibra, ocorre a revascularização e células inflamatórias são ativadas para a retirada do tecido necrótico e a ativação das células satélites (Philippou *et al.*, 2007). A formação de tecido conjuntivo de cicatrização é necessária para manter as extremidades das miofribrilas ligadas, prevenindo que a ruptura mantenham elas divididas em duas partes por um período longo de tempo (Kaariainen *et al.*, 2000).

Embora o fibroblasto seja necessário e fundamental para a homeostase dos tecidos e para a reparação de feridas, é um intermediário fundamental para doenças fibróticas crônicas, nas quais a inflamação persistente provoca atividade desregulada dos fribroblastos (Mann *et al.*, 2011). Além deles, quando ocorre falha na regeneração muscular, a cicatrização é infiltrada por adipócitos (Natajaran *et al.*, 2010).

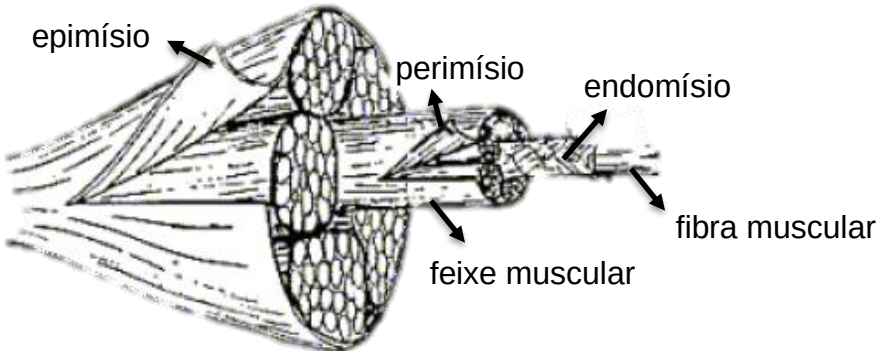

Figura 1. Organização do músculo estriado esquelético (Simões, 2009).

Características da miopatia *white striping*

White striping caracteriza-se por estriações brancas paralelas à fibra muscular. Acomete, principalmente, do músculo peitoral (Figura 2) e, em menor grau, das coxas e das sobrecoxas. Descrita inicialmente em 2009 (Kuttappan *et*

al., 2009), está miopatia afeta a qualidade da carne, pois aves acometidas pelo nível severo apresentam maiores níveis de gordura e menor conteúdo proteico comparados a peitos normais (Kuttappan *et al.*, 2012). Em consequência, há aumento da energia e aumento da relação colágeno/proteína total, diminuindo a digestibilidade da proteína (Petracci *et al.*, 2015).

De acordo com Kuttappan *et al.* (2012), a presença da WS (e o aumento da severidade) afeta negativamente a aceitação dos consumidores, quando baseados pela aparência. A principal razão para rejeitar os peitos acometidos pela miopatia foi a aparência gordurosa da carne. Além disso, a presença de estrias brancas e a coloração foram outros fatores decisivos para a rejeição do produto.

As lesões de WS afetam, de forma mais severa, a região cranial do músculo *pectoralis major*. Quando submetidos à análise histopatológica, as amostras apresentaram lesões miopáticas degenerativas com a substituição do músculo afetado por adipócitos e por fibrose (Kuttappan *et al.*, 2013). As lesões microscópicas incluem degeneração vacuolar/flocular, lise, mineralização, regeneração e inflamação intersticial com fibrose (Kuttappan *et al.*, 2013).

Mudanças sistêmicas ocorrem em graus severos de WS. Essas mudanças observadas estão relacionadas ao dano muscular causado pelo rompimento da fibra, resultando no aumento da circulação de creatina quinase (CK), alanina aminotransferase (ALT), aspartato aminotransferase (AST) e lactato desidrogênase (LDH). Entretanto, não são encontradas diferenças no perfil hematológico entre os graus de WS, sugerindo que não é causado por uma infecção (Kuttappan *et al.*, 2013).

Apesar do desconhecimento do agente causador, há fatores que desencadeiam este quadro. Aves de linhagem com alto rendimento de peito, machos, dietas com alta energia e aves mais pesadas ao abate são fatores que favorecem o aparecimento das lesões (Kuttappan *et al.*, 2012; Kuttappan *et al.*, 2013; Lorenzi *et al.*, 2014; Petracci *et al.*, 2015). Porém, todos esses fatores parecem estar vinculados à taxa de ganho de peso e peso ao abate.

Figura 2. Alterações características da miopatia *white striping* em peitos de frangos de corte.

Características da miopatia *wooden breast*

Nos últimos anos, foi descrito uma nova miopatia associado à qualidade da carne de peito, chamada *wooden breast*. Estas alterações estão restritas ao músculo *pectoralis major* e são catacterizadas por áreas pálidas

(Figura 3) e com rigidez aumentada (Sihvo *et al.*, 2014). A lesão é detectada manualmente, por meio da palpação, e acomete as aves a partir de 3 semanas de idade, podendo afetar mais de 50% de um lote (Mutryn *et al.*, 2015).

Macroscopicamente, pode-se observar extensa área pálida, rígida e com ondulações. Material viscoso com petéquias ou pequenas hemorragias podem ser encontrado em graus mais severos de WS, concomitante com lesões de WS (Sihvo *et al.*, 2014). Observa-se degeneração multifocal e necrose caracterizadas pela perda das estriações e por ser infiltrado de células inflamatórias, principalmente macrófagos e heterófilos. As áreas afetadas apresentam espessamento difuso do interstício com quantidade variada de tecido conjuntivo, tecido de granulação ou fibrose separando as fibras musculares (Sihvo *et al.*, 2014).

A prevalência e etiologia da WS ainda é pouco conhecida. Há índícios de maior expressão gênica à hipóxia e ao estresse oxidativo em aves acometidas, porém não está claro se é primária ou secundária à doença (Mutryn *et al.*, 2015). Neste contexto, estudos demonstram a mudança no metabolismo glicolítico de aves selecionadas genéticamente para ganho de peso, através da diminuição da capilaridade em relação ao número de fibras (Sosnicki & Wilson, 1991). Segundo Mudalal *et al.* (2015), a seleção genética para ganho de peso e para rendimento de peito é a hipótese com maior suporte e os fatores que apresentam maior influência no aparecimento dessa anormalidade.

Além da aparência e da coloração da carne, a WS afeta a qualidade da carne refrigerada ou marinada. A carne de peito se apresenta endurecida, com diminuição na absorção de salmoura e maior perda por cocção que peitos afetados por WS ou normais (Mudadal *et al*., 2015). O fator principal na redução da qualidade é a diminuição da capacidade de reter água presente nessas amostras (Mudalal *et al.*, 2015).

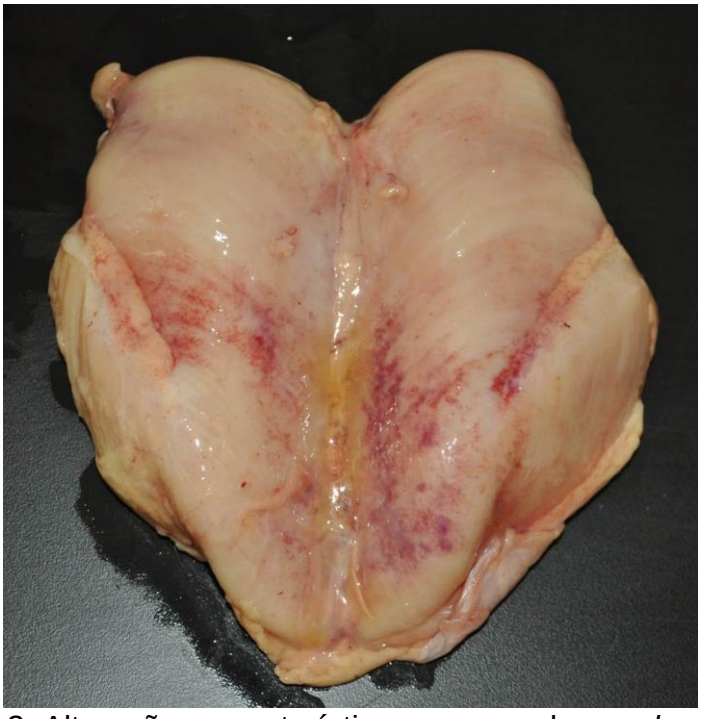

Figura 3. Alterações características severas de *wooden breast*

Digestão e absorção dos aminoácidos

Diversas interações entre estômago glandular e múscular e enzimas proteolíticas ocorrem para proporcionar a digestão das proteínas, finalizando com absorção de aminoácidos (AA) e de peptídeos pela membrana basolateral (D'Mello, 2003). Após a ação do ácido clorídrico e da pepsina, os polipeptídeos reagem com as enzimas secretadas na forma de zimogênio pelo pâncreas (tripsinogênio, elastase, quimiotripsinogênio e procarboxipeptidase A e B), sendo o produto destas reações oligopeptídeos de até seis AA (60%) e de AA livres (40%) (D'Mello, 2003). A última fase da digestão ocorre na membrana em forma de escova do intestino delgado por meio das enzimas citosólicas. Elas realizam a quebra dos oligopeptídeos da digestão pancreática em AA livre ou tri e em dipeptídeos (Freeman & Kim, 1978).

Os AA que irão participar da síntese de proteínas são transportados através da membrana basolateral à circulação hepática, por meio da veia porta. Os AA liberados pelo fígado formam um *pool* de AA na corrente sanguínea, que são absorvidos pelos tecidos e, aqueles destinados à síntese proteica, ligam-se a um RNA transportador específico no ribossomo (Rathmachier, 2000).

Metabolismo da lisina

A lisina (Lis) é reconhecida pelos efeitos na composição da carcaça e sua exigência altera conforme a variável estabelecida. Observam-se maiores exigências para características vinculadas à conformação da carcaça, como por exemplo, deposição de músculo peitoral (Schutte, J. B. & Pack, M., 1995), bem como a diminuição da deposição de gordura, como demonstrado por Moran e Bilgili (1990). O crescimento e o desenvolvimento muscular exigem um suprimento de proteínas, ou aminoácidos, presentes na dieta. A biossintese de proteínas nas aves é realizada por 20 AA, no entanto, nove destes não são sintetizados devido à ausência de enzimas específicas (D'mello, 2003).

Diversos artigos demonstram que a suplementação de lisina aumenta o peso do peito e a taxa de crescimento em frangos de corte (Garcia *et al.*, 2006; Dozier *et al.*, 2010; Carlos *et al.*, 2014). Em experimentos utilizando desempenho zootécnico, a suplementação de lisina aumenta a retenção de nitrogênio e a deposição muscular (Liu *et al.*, 2007), corroborando com Roy *et al.* (2000), os quais afirmam que esse aumento se deve à elevação da síntese e, consequentemente, à diminuição da degradação proteica.

Estruturalmente, as proteínas são polímeros de AA conectados por ligações peptídicas. Essas ligações são a junção entre um grupamento carboxila de um AA com o grupamento amina de outro. Para a síntese de proteínas e peptídeos, são necessários AA disponíveis simultaneamente no sítio de síntese, os quais são resultantes do catabolismo da dieta e das proteínas corporais. A reciclagem de AA corporais não apresenta alta eficiência e, por isso, grande parte deles devem ser supridos pela digestão de proteínas provenientes da dieta (Liao *et al.*, 2015).

A Lis é um aminoácido básico ou catiônico com uma longa cadeia lateral e seu metabolismo inicia com a absorção nos hepatócitos através de um sistema de transporte Na^+-independente. Após a absorção, a Lis que excede as necessidades para a síntese de proteínas e outras substâncias será catabolizada. A oxidação intestinal deste AA contribui com um terço de toda a

Lis oxidada em um suíno em crescimento (Liao *et al.*, 2015). Além da função primária de biosíntese de proteínas, a Lis apresenta-se como substrato para inúmeras moléculas não-proteicas, as quais incluem substâncias com baixo peso molecular (carnitina, poliaminas, amônia e uréia), outros AA ou derivados destes e moléculas não nitrogenadas (Wu, 2013).

Esse AA apresenta dois ciclos distintos para seu metabolismo, a via sacaropina e a via do ácido pipecólico, porém as duas convergem para um caminho comum de degradação (figura 4). A via sacaropina ocorre, predominantemente, no fígado. Inicialmente, a Lis junta-se com α-cetoglutarato (α-KG) formando a sacaropina através da lisina-cetoglutarato redutase (LKR). A sacaropina então é convertida em semialdeido-α-aminodiapínico e glutamato pela enzima sacaropina desidrogenase (SDH). O produto desta reação é a 6-semialdeído-α-aminodipato, sendo este convergido a Acetil-CoA para o ciclo de Krebs (Liao *et al.*, 2015). Uma pequena porção da Lis é catabolizada no cérebro pela via do ácido pipecólico. O grupamento α-amino é removido durante a formação de ácido pipecólico a partir da lisina nos peroxissomos celulares.

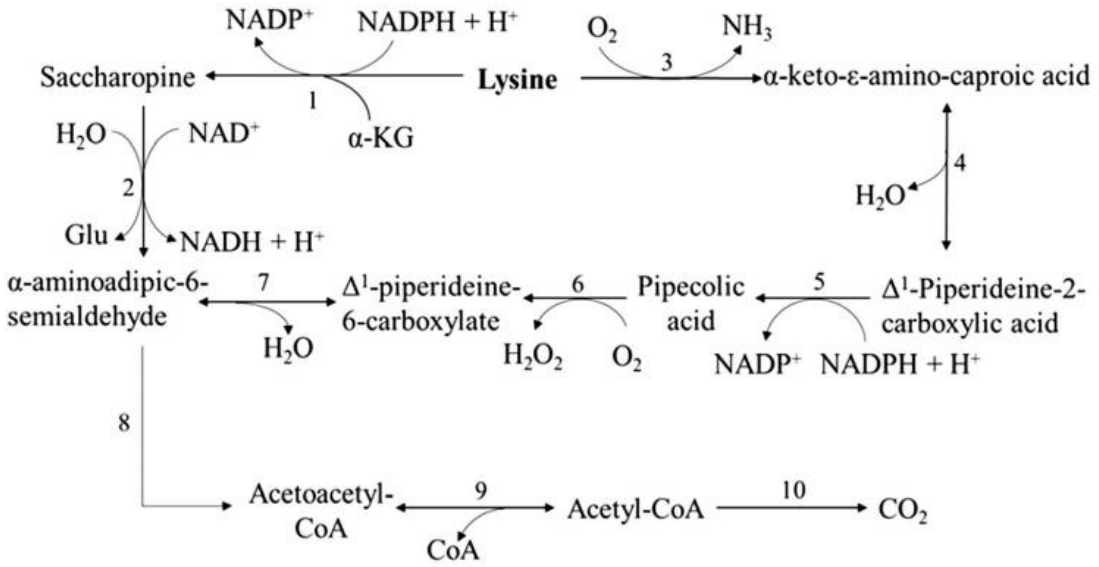

Figura 4. Vias de catabolismo da lisina (Liao *et al.*, 2015).

Exigência de lisina para frangos de corte na fase de crescimento e final

A Lis está extremamente associada à deposição muscular. Por ser o segundo AA limitante em dietas à base de milho e de farelo de soja e ser o AA referência no conceito de proteína ideal, diversos artigos foram publicados, nas últimas décadas, determinando a exigência de Lis para frangos de corte durante a fase de crescimento (Urdaneta-Rincon *et al.*, 2005; Rostagno *et al.*, 2007; Dozier *et al.*, 2009; Bernal *et al.*, 2013) e final (Garcia & Batal, 2005; Dozier *et al.*, 2010; Bernal *et al.*, 2013). Mudanças na exigência de Lis implicam em mudanças na concentração dos outros AA essenciais, sendo necessário obter estimativas mais precisas para otimizar a utilização dos demais AA. Apesar de diversos trabalhos determinarem a exigência de Lis, há muitos resultados

controversos na literatura. Isto se deve às diferentes metodologias, linhagens utilizadas e às variações ambientais (Conhalato, 1998). O método dose-resposta é tradicionalmente usado para estimar a exigência de Lis em frangos de corte (Sakomura & Rostagno, 2007).

Diversos estudos foram realizados durante a fase de crescimento para determinar a exigência de Lis. (Dozier *et al.*, 2009) utilizaram L-lisina HCl em frangos de corte machos Ross de 14 a 28 dias. Os autores obtiveram a exigência de 1,07% e 1,09% para ganho de peso (GP) e 1,10% e 1,15% para conversão alimentar (CA), aplicando a regressão quadrática e *broken-line*, respectivamente. Rostagno *et al.* (2007) avaliaram a exigência para machos Cobb 500 de 10 a 21 e 22 a 35 dias, elaborando os níveis com base nas Tabelas Brasileiras para Aves e Suínos (Rostagno *et al.*, 2011). Os autores sugerem que os níveis para melhor CA são de 1,16% e 1,04% de Lis dig. para os períodos analisados.

Bernal *et al.* (2013) determinaram a exigência de Lis em machos Cobb 500 durante o período de 10 a 21 dias e 22 e 35 dias. Obteve-se um efeito quadrático para GP e CA de 10 a 21 dias, sendo a exigência de Lis 1,15 e 1,22%, respectivamente. Os autores também observaram efeito quadrático na exigência de Lis para a fase de 22 a 35 dias, com o ponto de máxima de 1,05% para GP e 1,07% para CA. Não houve diferença estatística para rendimento de carcaça e gordura abdominal, porém a exigência de Lis para peso de peito foi de 1,16%, obtida através de regressão linear.

Utilizando a técnica de suplementação e de diluição, Siqueira *et al.* (2009) determinaram a exigência de Lis na fase de 8 a 22 dias para frangos machos Cobb 500. Os autores sugerem 1,17% para ganho de peso, independente do método utilizado e 1,14 ou 1,17% para CA, realizando o método de substituição e diluição, respectivamente.

Ao determinarem a exigência de Lis para frangos de corte Cobb 500 de 21 a 38 dias de idade, (Garcia *et al.*, 2006) encontraram 0,97 e 0,96% para GP e CA utilizando regressão linha quebrada. Diferente destes autores, (Dozier *et al.*, 2010) encontraram uma exigência de Lis maior para CA no período de 28 a 42 dias. Estes autores determinaram a exigência como 0,96% para GP e 1,01% para CA em machos Cobb 700.

HIPÓTESES E OBJETIVOS

Hipóteses

O desempenho zootécnico de frangos de corte suplementados com níveis crescentes de Lis apresenta comportamento quadrático.

As aves com maior peso corporal e maior peso do peito apresentam maiores escores de miopatias.

As aves suplementadas com baixos níveis de Lis não apresentam escores de miopatia.

Objetivos

Avaliar o efeito da suplementação de inclusões crescentes de lisina sintética no desempenho de frangos de corte na fase de crescimento e final.

Determinar a prevalência das miopatias em aves com maiores inclusões de lisina durante a fase de crescimento ou final.

Determinar a correlação entre a inclusão de lisina e o aparecimento das miopatias.

Determinar em qual fase apresenta maior influência no aparecimento das lesões.

CAPÍTULO II

PROCESSING AND PRODUCTS

DIETARY LYSINE AND BREAST MYOPATHIES

Occurrence of white striping and wooden breast in broilers fed grower and finisher diets with increasing lysine levels

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ABSTRACT Two experiments were conducted to evaluate the prevalence and severity of white striping (WS) and wooden breast (WB) in breast fillets from broilers fed diets with increasing digestible Lys from 12 to 28 d (Exp. 1) and from 28 to 42 d (Exp. 2). Trials were sequentially conducted using 1-d-old slow feathering Cobb × Cobb 500 male broilers, both with 6 treatments and 8 replicates. Increasing dig. Lys levels were equally spaced from 0.77 to 1.17% in Exp. 1 and from 0.68 to 1.07% in Exp. 2. The lowest dig. Lys diet was not supplemented with L-Lys in either one of the studies and all other essential AA met or exceeded current commercial recommendations such that their dietary concentrations did not limit broiler growth. Four birds per pen were randomly selected from each replication and processed at 35 and 42 d in Exp. 1 and Exp. 2, respectively. Deboned breast fillets were submitted to a 3 subject panel evaluation to detect the presence of WS and WB as well as to provide scores of WS (0-normal, 1-moderate, 2-severe) and WB (0-normal, 1-moderate light, 2-moderate, 3-severe). increased dig. Lys had a positive effect on BW, carcass and breast weight as well as breast yield. White striping and WB prevalences were 32.3 and 85.9% in Exp 1 and 87.1 and 89.2% in Exp 2. Birds fed diets not supplemented with Lys had the lowest average WS and WB scores (0.22 and 0.78 in Exp. 1 and 0.61 and 0.68 in Exp. 2). White striping and WB presented linear responses to performance variables in Exp 1, whereas quadratic responses were observed for all variables in Exp 2. In conclusion, increasing Lys levels improved growth performance and carcass traits and induced the occurrence and severity of WS and WB lesions probably due to dig. Lys dietary levels that maximized the genetic potential for growth and breast met yields.

Key words: broiler, breast myopathy, lysine, white striping, wooden breast

INTRODUCTION

Constant increases in the world demand for white meat have been spurring the broiler industry towards practices that increase its production. Breast meat as a proportion of total chicken meat has been significantly increasing mainly due to improvements in genetic selection, but also due to advances in health, farm management practices, and nutrition (Havenstein *et al.*, 2003; 2003; Zuidhof *et al.*, 2014).

As the most valuable cut from broiler chickens, breast meat must meet high quality market presentation standards. Therefore, it is of great importance to breeding companies as well as to broiler producers that breast muscle growth is sound such that the white meat quality delivered from it is not compromised. Recent reports of increased cases of breast muscle myopathies have brought concerns to the broiler meat industry because affected carcasses can be downgraded or less frequently condemned, leading to economic losses (Bailey *et al.*, 2015). This is the case of the white striping (**WS**) and wooden breast (**WB**) conditions, which appear to affect only the *Pectoralis major* as opposed to the deep breast myopathy which only affects the *Pectoralis minor*.

White striping is characterized by white striations appearing in parallel to the direction of muscle fibers in broiler breast fillets (Kuttappan *et al.*, 2013). Breast meat presenting WS has a slight increase in fat deposited, but so far there seems not to have any factor harmful to human health. Recent research shows that WS is not related to any specific commercial broiler strains (Kuttappan *et al.*, 2012; Kuttappan *et al.*, 2012; Kuttappan *et al.*, 2013; Kuttappan *et al.*, 2013; Petracci *et al.*, 2013; Ferreira *et al.*, 2014; Sihvo *et al.*, 2014; Mudalal *et al.*, 2015). Histologic reports of WS demonstrated alterations with loss of cross striations, variability in fiber size, floccular/vacuolar degeneration and lysis of fibers, mild mineralization, mononuclear cell infiltration,

lipidosis, interstitial inflammation, and fibrosis (Kuttappan *et al.*, 2013; Ferreira *et al.*, 2014). The etiology of WS is unknown, however broilers with higher growth rate and heavier breast weight have greater incidence of WS (Kuttappan *et al.*, 2012; Ferreira *et al.*, 2014).

Wooden breast is characterized by variable degrees of hardness in the *Pectoralis major* showing bulging and pale expansive areas (Sihvo *et al.*, 2014). Severe polyphasic myodegeneration with regeneration as well as a variable amount of interstitial connective tissue accumulation or fibrosis are observed microscopically (Sihvo *et al.*, 2014). Lesions are seen as early as at 3 wk of age and can affect a high proportion of birds in a flock (Mutryn *et al.*, 2015). Depending on the severity of the condition, WB may present surface hemorrhaging with a sterile exudate. So far, WS and WB are thought to be distinct myopathies since they are found independently of each other (Bailey *et al.*, 2015).

Increased growth rate as well as breast meat yields resulting from genetic selection have been suggested as leading causes of the increased presence of WS and WB in broiler chickens (Kuttappan *et al.*, 2012; Petracci & Cavani, 2012; Sihvo *et al.*, 2014); however, the analysis of data from two broiler lines that differed in terms of selection for breast yield showed that there is also a strong non-genetic component for all the breast muscle myopathy traits (Bailey *et al.*, 2015).

Post hatching muscle growth is mostly related to muscle cell hypertrophy instead of muscle hyperplasia (Sklan & Noy, 2003). Hypertrophy is attained by increasing cell diameter instead of length. Therefore, broilers with greater breast proportions have increased muscle cell diameters (Zheng *et al.*, 2009). However, the full expression of the genetic potential for growth and meat yields of the modern broiler can only be fulfilled by adequate nutrition. The implication of dietary Lys on broiler muscle cell hypertrophy

has been well established (Tesseraud *et al.*, 1996; Eits *et al.*, 2003; Sklan & Noy, 2003). Breast muscles are particularly sensitive to dietary concentration of Lys since it is its main essential AA representing approximately 7% of the total protein content (Munks *et al.*, 1945). Concentration of Lys in feed affects growth, but also carcass yield. Therefore, broilers fed diets with increased Lys have thicker myofibers regardless of genetics (Holsheimer & Veerkamp, 1992; Roy *et al.*, 2006; Sakomura *et al.*, 2015). By itself, Lys can modulate breast growth due to a higher synthesis to degradation ratio (Urdaneta-Rincon & Leeson, 2004; Mehri *et al.*, 2012; Carlos *et al.*, 2014)).

Usual determination of AA requirements target the optimization of growth rate, FCR, and breast meat yields; however, AA concentrations that optimize breast meat yields have shown to be higher than for the other responses (Moran & Bilgili, 1990; Holsheimer & Veerkamp, 1992; Huyghebaert *et al.*, 1994; Schutte, J. B. & Pack, M., 1995). Because dietary Lys is such an important factor for breast muscle growth, it is possible that its concentration in feed is capable of triggering, or at least modulating, the appearance of breast muscle myopathies. The objective of this study was to evaluate the prevalence of WB and WS in broilers fed grower or finisher diets with increasing digestible (**dig.**) Lys levels.

MATERIALS AND METHODS

Bird Husbandry

All procedures throughout the current study were approved by the Ethics and Research Committee of Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Two experiments (**Exp.**) were conducted using 1,200 one-d-old slow feathering Cobb x Cobb 500 male broileres each. Chicks were vaccinated for Marek's and infectious

bursal diseases at the hatchery and then randomly distributed into 48 pens of 1.65 x 1.65 m (9.2 birds/m^2, 25 birds per pen). Each pen had rice hulls bedding and was equipped with one 15 kg capacity tube feeder and 3 nipple drinkers. Mash feeds and water were available for *ad libitum* consumption. Mortality was recorded daily. Initial temperature was set to 32ºC being reduced by 1ºC every two days until 22ºC. A continuous lighting schedule was used until 7 d of age whereas a 20L:4D cycle was used thereafter.

Experimental Diets

Dietary treatments in Exp. 1 were provided from 12 to 28 d of age and in Exp. 2 from 28 to 42 d. Diets in both experiments were based on corn, soybean meal, and corn gluten meal (Table 1). Basal diets were formulated without supplemental Lys (0.77% dig. Lys in Exp. 1 and 0.68% of dig. Lys in Exp. 2, respectively), but had all other essential AA to meet or exceed commercial recommendations aiming to ensure dietary adequacy such that responses were only limited by Lys. Treatments were structured with the addition of increasing levels of dig. Lys in 0.08% increments from 0.77 to 1.17% in Exp. 1 and from 0.68 to 1.07% in Exp. 2 by adding L-Lysine HCl at the expense of sand. Common feeds were provided to all treatments in the periods before and after experimental phases of Exp. 1 (21.9 and 19.4% CP; 2,960 and 3,150 kcal/kg AME_n; 0.88 and 0.72% Ca; 0.42 and 0.35% Av. P from 1 to 12 d and 28 to 35 d, respectively) and before the experimental phase of Exp. 2 (21.9 and 20.5% CP; 2,960 and 3,050 kcal/kg AME_n; 0.88 and 0.78% Ca; 0.42 and 0.38% Av. P from 1 to 12 d and 12 to 28 d, respectively).

Broiler Performance Measurements

Birds and feeds were weighed at 12 and 28 d in Exp. 1 and at 28 and 42 d in Exp. 2. Four birds per pen were randomly selected from each pen at 35 and 42 d of age, respectively in Exp. 1 and 2. Birds were fasted for 6 h, individually weighed before electrical stunning (45 V for 3 s), bled for 3 min after carotid and jugular veins cut, scalded at 60ºC for 45 s, and mechanically defeathered. Evisceration was manually done and carcasses were statically chilled in slush ice for 3 h before processing. Breast fillets were manually removed from the carcasses. White striping and WB evaluations were immediately performed in boneless skinless breast. Carcass yield was expressed as a percentage of live weight and breast yield was expressed as a percentage of the eviscerated carcass weight.

White Striping and Wooden Breast Scores

Occurrence and severity of WS and WB were assessed by a 3 subject panel evaluation. First, deboned fillets were visually separated in groups by the presence or absence of WS and WB. Breast fillets presenting WS were classified in scores according to Kuttappan *et al.* (2013) as: normal (score 0) without any distinct white lines; moderate (score 1) presenting white lines in parallel to muscle fibers and that were < 1 mm thick; and severe (score 2) exhibiting white lines in parallel to muscle fibers and that were > 1 mm thick. Breast fillets presenting WB were classified as: normal (score 0) without any hardness or paleness areas; moderate light (score 1) mildly affected in cranial and/or caudal areas; moderate severe (score 2) moderately affected throughout the fillets; and severe (score 3) with surface hemorrhaging and the presence of a sterile exudate on the muscle surface.

Statistical Analysis

The study was conducted in a completely randomized design. Data were tested for normality previously to analysis and values that were not normal were square root transformed maintaining normal distribution of residuals. Live performance data were submitted to ANOVA using GLM procedures of SAS (Sas User's Guide, 2001) and, when significant, means were compared by Tukey test at 5%. Scores of WS and WB were analyzed using the non-parametric Kruskal-Wallis test (PROC NPAR1WAY). Linear and quadratic polynomial regressions were estimated (PROC REG) for WS and WB using dig. Lys, BW, carcass, and breast fillet weight as well as yield as independent variables.

RESULTS

Growth Performance and Processing Data

Growth performance and broiler processing data from Exp. 1 and 2 are presented in Table 2. Increasing dietary dig. Lys levels positively affected ($P < 0.01$) BW gain and carcass weight in both Exp. 1 and 2. Birds fed diets without Lys supplementation had the lowest BW and carcass weight. Body weight, carcass and weight, and breast yield increased quadratically ($P < 0.01$) when broilers were fed diets with increasing levels of dig. Lys. In Exp. 1, maximum responses at 35 d for BW, carcass weight, and breast weight were obtained using 1.08%, 1.07%, and 1.07% of dig. Lys, respectively. In Exp. 2, maximum responses at 42 d for BW gain, carcass weight, and breast weight of broilers were obtained using 0.99%, 0.98%, and 0.98% of dig. Lys, respectively. Quadratic increases ($P < 0.01$) were observed for breast meat yields at 35 and 42 d, with maximum responses obtained with 1.08% and 1.01% dig. Lys, respectively.

White Striping and Wooden Breast Occurrence and Severity

White striping and WB occurrences as percentages are shown in Figure 1. In Exp. 1, WS occurrence ranged from 18.8 to 56.3% among treatments, averaging 32.3%. Score 1 occurrence increased when broilers were fed diets with 1.01% dig. Lys and then moderately decreasead whereas score 2 tended to increase linearly using all levels of dig. Lys tested in Exp. 1 and 2. White striping occurence in Exp. 2 ranged from 58.1 to 100%, averaging 87.1% of the fillets having scores 1 or 2. Score 1 was consistent along dig. Lys levels, except at 0.92% dig. Lys, where the lowest value was observed. Furthermore, score 2 of WS increased when broilers were fed diets with until 0.92% dig. Lys and then tended to decrease.

Wooden breast occurrence in Exp. 1 ranged from 65.6 to 100% among treatments, averaging 85.9% (Figure 1). Score 1 had consistent occurrence throughout all tested dig. Lys, whereas scores 2 and 3 tended to increase. Wooden breast occurrence in Exp. 2 ranged from 51.6 to 100% among treatments, averaging 89.2%. Score 1 ocurrence of WB was fairly constant along dig. Lys levels, except when 0.76 and 0.92% dig. Lys were tested, and these levels had the highest and lowest occurrences, respectively. Score 2 was prone to increase consistently with increasing dig. Lys levels, whereas score 3 increased remarkably until 0.92% dig. Lys and gradually decreased afterwards.

The average scores of WS and WB in broilers evaluated at 35 and 42 d are shown in Table 3. The severity of WS and WB was lower ($P < 0.01$) when broilers were fed diet without supplemental L-Lysine HCl and compared to broilers fed diets with 1.01% of dig. Lys in Exp. 1. In Exp. 2, means of WS and WB scores were higher ($P < 0.01$) in all dig. Lys levels compared to the basal diet with 0.68% of dig. Lys.

Regression Analysis of White Striping and Wooden Breast Scores

White striping and WB scores had a positive relationship with dig. Lys levels and performance variables in grower and finisher phases (Table 4). A linear response ($P < 0.05$) of WS and WB scores was observed in BW, breast weight, and breast yield of broilers at 35 d in Exp. 1. One exception was the relationship between WB and dig. Lys, which was quadratic and the score was estimated to be the highest at 1.10% dig. Lys. In Exp. 2, WS had quadratic responses ($P < 0.01$) for dig. Lys (0.96%), BW (3,400 g), breast weight (842 g), and breast yield (30.1%). A quadratic response ($P < 0.01$) of WB score was also observed for dig. Lys (0.98%), BW (2,598 g), breast weight (884 g), and breast yield (32.1%).

DISCUSSION

The best responses for broiler BW in the Exp 1 and 2 were estimated as 1.08% and 0.99% of dig. Lys, respectively. Estimations obtained in the present study are in agreement with those presented by Dozier et al. (2009; 2010) for BW gain, which were 1.07% dig. Lys from 14 to 28 d and 0.99% dig. Lys from 28 to 42 d. Values are higher than those observed with birds used in research from previous decades, which are likely related to less feed intake per unit of BW and higher rate of meat accretion of the modern broiler (Havenstein et al., 2003a; b). However, the objective of this study was not to reassess dig. Lys requirements, but to evaluate the effect of dietary increases in dig. Lys on WS and WB occurrence and severity.

Lysine is well known as an important AA for broiler growth performance and proper muscle development. It has been reported to increase carcass yield and alter its composition by increasing meat yield and reducing carcass fat (Leclrecq, 1998; Sterling, 2006). Dietary Lys plays an important role in breast muscle protein turnover by

modulating protein synthesis and breakdown rates (Tesseraud et al., 2001; Urdaneta-Rincon and Leeson, 2004). Furthermore, Lys deficiency results in reduced protein synthesis, especially on *Pectoralis major*, which is more sensitive to Lys than wings and thigh muscles (Tesseraud et al., 1996). Conversely to leg muscles, breast muscles are a direct product of genetic selection, have minor functional purpouse (McDonald and Swick, 1981), and represent a considerable protein store in deficiency states (Tesseraud et al., 1996).

In this study, WS occurrence was 31.3% in Exp. 1 and 89.0% in Exp. 2. Findings are in agreement with Russo et al. (2015), who observed 82.5% occurrence of WS in 55 d of age broilers with 3.6 kg mean BW and with Kuttapan et al. (2012a), who reported WS prevalence of 74.6% in birds with 3.0 kg average BW. Conversely, Petracci et al. (2013) reported WS occurrence as low as 12% in broiler chickens from 45 to 54 d of age reared under commercial conditions with average live weight of 2.75 kg. These differences may be influenced by BW (Petracci *et al.*, 2013), as well as growth rate (Kuttapan et al., 2012a) and strain (Kuttapan et al., 2013a). Kuttapan et al. (2013b) reported that WS is associated with increased occurrence of muscle damaged, which be a result of muscles outgrowing their supporting systems (Wilson et al., 1990). Reduced capillary density in heavier birds with higher percentage of breast meat could result in decreased supply of nutrients and oxygen and slower removal of lactic acid from breast muscle, which ultimately may lead to muscle damage (Hoving-Bolink et al., 2000).

Birds slaughtered with higher BW had higher severity of WS and WB lesions. The difference in myopathies severity between Exp. 1 and 2 could be explained by the different BW of broilers in both experiments (2.23 vs. 3.38 kg). In the present study, average scores of WS occurrence in broilers with 2.4 kg and 3.5 kg were 0.62 and 1.67,

respectively. These results are in agreemet with findings by Russo *et al.* (2015), who compared WS score in medium (2.59 kg) and heavy (3.64 kg) broilers and observed 0.84 and 1.09 average scores, respectively. Average WS severe score ocurrences were 9.4% in Exp 1. and 40.9% in Exp. 2, which is considerably higher than reported by Kuttapan et al. (2012a), Kuttapan et al. (2013a), Petracci et al. (2013), Ferreira et al. (2014), who observed severe score prevalence of 8.7%, 8.3%, 3.1%, and 2.5%, respectively. In both trials, increasing dig. Lys levels induced the occurrence and increased the severity of WS lesions, probably because these are Lys levels that can maximize the genetic potential of broilers.

Wooden breast occurrence was similar in both experiments (85.9 and 89.2% in Exp. 1 and 2, respectively). According to Mutryn *et al.* (2015) some degree of WB has been anecdotally reported to affect up to 50% of a flock. Conversely, Trocino et al. (2015) observed 12.2% average WB occurrence in broilers; however, 97% of breasts submitted to histological analysis presented damaged muscle fibers, which have been attributed to WB (Sihvo et al, 2014; Soglia et al., 2015).

Wooden breast severity was remarkably different between both experiments conducted. In Exp. 1, average occurrence of severe score was 8.9% and in Exp. 2, 34.4%. Moreover, a higher occurrence of low scores was observed in Exp. 1 than in Exp. 2 (52.1% vs. 30.1%). It is important to note that this score can easily be interpreted as normal breast in commercial slaughterhouses. Furthermore, WB mean score was 1.29 in Exp. 1 and 1.83 in Exp. 2. Based on these observations, the pronounced contrast in WB occurrence and severity between experiments seems to be related to BW and growth rate, similarly to WS. Trocino et al. (2015) observed that the occurrence of WB was doubled in males with 3.49 kg average BW compared with females with 2.85 kg average BW.

There is evidence of gene expression of intracellular calcium, possible fiber-type switching, hypoxia, and oxidative stress in lesions related to the WB disease (Mutryn *et al.*, 2015). Both myopathies have been reported to have low herdabilities and a marked non-genetic component (Bailey et al., 2015), which indicates the major role played by environmental, management, and nutritional factors in their incidence.

In conclusion, optimal dig. Lys levels resulted in improved broiler performance; however, birds with higher BW also presented higher proportions of myopathies occurrence and severity. These results are in agreement with other studies, demonstrating the influence of growth rate and slaughter weight in WS and WB (Kuttappan *et al.*, 2012; Kuttappan *et al.*, 2013; Ferreira *et al.*, 2014). Since BW and growth rate are direct results of increasing dig. Lys levels in broiler diets, myopathies do not seem to be associated with Lys itself but with gains in performance.

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Table 1. Ingredient and nutrient composition of the basal diet provided from 12 to 28 d and 28 to 42 d

	Experiment	
Item	1 (12 to 28 d)	2 (28 to 42 d)
Ingredients, %		
Corn	68.09	75.85
Soybean meal	21.74	14.42
Soybean oil	0.94	0.80
Corn gluten meal	5.50	5.80
Sodium bicarbonate	0.42	0.59
Dicalcium phosphate	0.96	0.54
Limestone	1.10	0.93
Salt	0.15	0.05
Vitamin and mineral mix[1]	0.15	0.15
DL-Methionine, 99%	0.26	0.27
L-Leucine, 98.5%	0.03	0.09
L-Threonine, 98.5%	0.14	0.20
L-Arginine, 98%	0.14	0.06
L-Isoleucine, 98.5%	0.08	0.07
L-Valine, 96.5%	0.11	0.01
L-Tryptophan, 98%	0.01	0.05
Choline chloride, 60%	0.12	0.14
Calculated nutrient composition, % unless noted		
AME_n, kcal/kg	3,108	3,180
CP	19.5	18.9
Ca	0.84	0.68
Av. P	0.42	0.33
Choline, mg/kg	1,550	1,500
Dig. Lys	0.77	0.68
Dig. Met	0.55	0.59
Dig. Met + Cys	0.83	0.80
Dig. Thr	0.73	0.72
Dig. Val	0.89	0.87
Dig. Ile	0.78	0.74
Dig. Leu	1.79	1.77
Dig. Arg	1.16	1.12

[1]Composition per kg of feed: vit. A, 8,000 UI; vit. D_3, 2,000 UI; vit. E, 30 UI; vit. K_3, 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, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; phytase, 100 mg, monensin sodium, 100 mg.

Table 2. Body, carcass, and breast fillet (*Pectoralis major*) weights from broilers fed increased dig. Lys from 12 to 28 d and 28 to 42 d and processed at 35 and 42 d, respectively[1]

Dig. Lys, %[2]		Body weight, g		Carcass weight[3], g		Breast fillets[4]			
Exp. 1 (12 to 28 d)	Exp. 2 (28 to 42 d)	35 d	42 d	35 d	42 d	g		%	
						35 d	42 d	35 d	42 d
0.77	0.68	2,159^{d}	3,084^{c}	1,656^{d}	2,424^{c}	353^{c}	524^{c}	21.3^{c}	21.7^{d}
0.85	0.76	2,282^{c}	3,285^{b}	1,778^{c}	2,592^{b}	405^{b}	597^{b}	22.8^{bc}	23.0^{c}
0.93	0.84	2,323^{bc}	3,452^{a}	1,813^{bc}	2,764^{a}	429^{ab}	677^{a}	23.7^{ab}	24.5^{b}
1.01	0.92	2,415^{a}	3,517^{a}	1,896^{a}	2,837^{a}	463^{a}	728^{a}	24.4^{a}	25.7^{a}
1.09	1.00	2,389^{ab}	3,468^{a}	1,873^{ab}	2,793^{a}	454^{a}	698^{a}	24.2^{a}	25.0^{ab}
1.17	1.08	2,393^{ab}	3,513^{a}	1,866^{ab}	2,804^{a}	450^{a}	698^{a}	24.1^{a}	24.9^{ab}
SEM		14.9	27.0	13.3	24.4	4.1	11.8	0.21	0.23
P-value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Item		Regression equations[5]	*P*-value	r^2	Maximum response %
Body weight, g	35 d	$Y = -2.4787x^2 + 5.3709x - 0.5030$	< 0.001	0.720	1.08
	42 d	$Y = -4.5875x^2 + 9.0593x - 0.9471$	< 0.001	0.685	0.99
Breast weight, g	35 d	$Y = -1.1822x^2 + 2.5290x - 0.8925$	< 0.001	0.734	1.07
	42 d	$Y = -2.2425x^2 + 4.3833x - 1.4251$	< 0.001	0.746	0.98
Carcass weight, g	35 d	$Y = -2.4991x^2 + 5.3519x - 0.9789$	< 0.001	0.765	1.07
	42 d	$Y = -4.6018x^2 + 9.0202x - 1.5857$	< 0.001	0.760	0.98
Breast yield, %	35 d	$Y = -31.43x^2 + 67.8x - 12.29$	< 0.001	0.335	1.08
	42 d	$Y = -34.98x^2 + 70.5x - 10.20$	< 0.001	0.417	1.01

[a-d]Means followed for different letters in the same column differ by Tukey test ($P \leq 0.05$).
[2]Digestible Lys in Exp. 1 and 2, respectively.
[3]Eviscerated carcass without neck and feet.
[4]*Pectoralis major* weight or as a proportion of the eviscerated carcass.
[5]Quadratic polynomial model: $Y = \beta3 \times X^2 + \beta2 \times X + \beta1$; where Y is the dependent variable, X is the dietary level of dig. Lys, $\beta1$ is the intercept, $\beta2$ and $\beta3$ are the linear and quadratic coefficients, respectively; the maximum response levels were obtained by calculating: $-\beta2 \div (2 \times \beta3)$.

Table 3. White striping and wooden breast occurrence in broilers fed increasing dig. Lys from 12 to 28 d and from 28 to 42 d[1]

Dig. Lys, %[1]		White striping		Wooden breast	
Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
12 to 28 d	28 to 42 d	12 to 28 d	28 to 42 d	12 to 28 d	28 to 42 d
0.77	0.68	0.22[b]	0.61[c]	0.78[b]	0.68[c]
0.85	0.76	0.34[ab]	1.06[b]	1.09[ab]	1.35[b]
0.93	0.84	0.28[ab]	1.48[ab]	1.31[ab]	2.16[a]
1.01	0.92	0.72[a]	1.67[a]	1.44[a]	2.57[a]
1.09	1.00	0.44[ab]	1.35[ab]	1.56[a]	2.00[a]
1.17	1.08	0.50[ab]	1.50[ab]	1.53[a]	2.22[a]
SEM		0.057	0.064	0.070	0.109
P-value		0.019	<0.001	<0.001	<0.001

[a-c]Means followed for different letters in the same column differ by Bonferroni test ($P \leq 0.05$).

[1]White striping and wooden breast means of scores in broilers fed increasing dig. Lys from 12 to 28 d and processed at 35 d, and from 28 to 42 d and processed at 42 d.

Table 4. Regression analysis estimating white striping and wooden breast occurrence

Item	Regression equations[1]	*P*-value	r^2	Maximum score at
Dig. Lys from 12 to 28 d[2]				
White striping				
Dig. Lys, %	$Y = 0.615x - 0.235$	0.029	0.025	-
Body weight, g	$Y = 0.001x - 2.068$	<0.001	0.053	-
Breast weight, g	$Y = 0.0029x - 0.881$	< 0.001	0.081	-
Breast yield, %	$Y = 0.081x - 1.55$	< 0.001	0.080	-
Wooden breast				
Dig. Lys, %	$Y = - 4.37x^2 + 9.6500x - 4.14$	0.033	0.132	1.10
Body weight, g	$Y = 0.0015x - 2.534$	< 0.001	0.141	-
Breast weight, g	$Y = 0.0055x - 1.303$	< 0.001	0.364	-
Breast yield, %	$Y = 0.1474x - 2.409$	< 0.001	0.335	-
Dig. Lys from 28 to 42 d[3]				
White striping				
Dig. Lys, %	$Y = - 7.49x^2 + 14.44x - 5.74$	< 0.001	0.204	0.96
Body weight, g	$Y = - 0.000001x^2 + 0.0068x - 9.42$	< 0.001	0.197	3,400
Breast weight, g	$Y = - 0.000005x^2 + 0.0084x - 2.48$	< 0.001	0.284	842
Breast yield, %	$Y = - 0.0087x^2 + 0.524x - 6.5$	< 0.001	0.277	30.1
Wooden breast				
Dig. Lys, %	$Y = - 10.6100x^2 + 20.6900x - 8.57$	< 0.001	0.352	0.98
Body weight, g	$Y = - 0.000002x^2 + 0.0104x - 14.87$	< 0.001	0.370	2,598
Breast weight, g	$Y = - 0.000008x^2 + 0.0142x - 4.52$	< 0.001	0.467	884
Breast yield, %	$Y = - 0.0095x^2 + 0.6100x - 7.89$	< 0.001	0.379	32.1

[1]Linear equation: $Y = \beta 2 \times X + \beta 1$; where Y is the square root of lesion score, X is the independent variable, $\beta 1$ is the intercept, $\beta 2$ and is the linear coefficients; Quadratic polynomial equation: $Y = \beta 3 \times X^2 + \beta 2 \times X + \beta 1$; where Y is the square root of lesion score, X is the independent variable, $\beta 1$ is the intercept, $\beta 2$ and $\beta 3$ are the linear and quadratic coefficients, respectively; maximum response levels obtained by calculating $- \beta 2 \div (2 \times \beta 3)$.
[2]Birds processed at 35 d.
[3]Birds processed at 42 d.

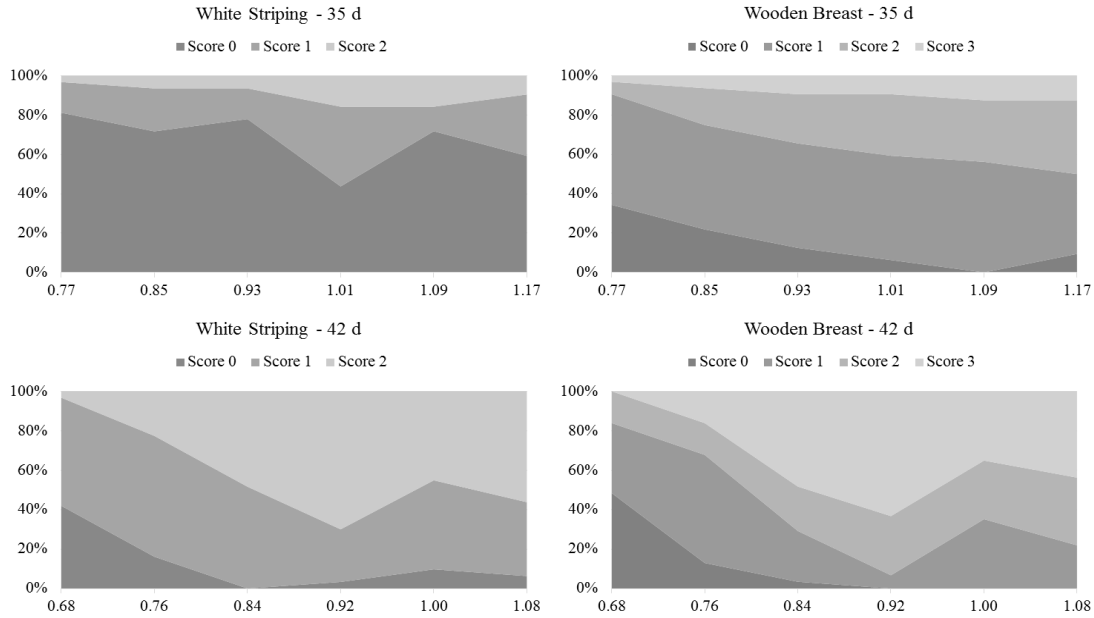

Figure 1. Occurrence (%) of breast fillets presenting white striping[1] and wooden breast[2] scores in broilers fed increasing dig. Lys levels from 12 to 28 d and processed at 35 d (Exp. 1); and from 28 to 42 d and processed at 42 d (Exp. 2).

[1]White striping scores were evaluated according to Kuttappan *et al.* (2013): as score 0 (normal, without white lines in parallel to muscle fibers), score 1 (moderate, with white lines < 1 mm thick, and score 2 (severe, with white lines > 1 mm thick).

[2]Wooden breast scores were: score 0 (normal, without any hardness or paleness areas), score 1 (moderate light, mildly affected at the cranial and/or caudal areas), score 2 (moderate severe, affected throughout the fillet), and score 3 (severe, with surface hemorrhaging and exudate on the surface).

CAPÍTULO III

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APÊNDICES

Apêndice 1. Normas para publicação de artigos no periódico Journal of Applied Poultry Research

POULTRY SCIENCE INSTRUCTIONS TO AUTHORS [1]

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.

Contact Information for Journal Staff

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Care and Use of Animals

Authors must make it clear that experiments were con- ducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and lab- oratory techniques. Experiments shall be conducted in accordance with the principles and specific guidelines pre- sented 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, Na- tional Institutes of Health, Publication Number ISBN 0-309-05377-3, 1996); or *Guide to the Care and Use of Experi- mental Animals,* 2nd ed. Volume 1, 1993 (Canadian Coun- cil on Animal Care). Methods of killing experimental ani- mals 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 in- vasive surgery requires anesthesia. This includes capon- ization. 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 pub- lished in *Poultry Science* are full-length articles. The jour- nal 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 ex- periments are adequately replicated.

Research Notes. Research Notes are short notes giv- ing the results of complete experiments but are less com- prehensive than full-length articles. Preliminary or prog- ress reports will not be accepted. The running head shall be “RESEARCH NOTE.” Research Notes will be pub- lished 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.

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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 approxi- mately 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.

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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*. Intro- duction 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 publica- tion. 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 electroni- cally 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 let- ters 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 pub- lished as space permits.

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After a manuscript is submitted electronically, the edi- torial 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. Manu- scripts that pass initial screening will be forwarded to the appropriate section editor, who pre- reviews the manu- script and may suggest rejection at this early stage for fatal design flaw, inappropriate replications, lack of nov- elty, 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 sec- tion editor may recommend rejection or acceptance at this point, after which the manuscript and reviewer com- ments 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 manu- scripts 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 sub- missions. Therefore, authors must complete a new Manu- script 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 type- setting. At this point the technical editor may contact the authors for missing information or figure revisions. The manuscript is then typeset, figures reproduced, and au- thor proofs prepared.

Proofs

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

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

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MANUSCRIPT PREPARATION: STYLE AND FORM

General

Papers must be written in English. The text and all sup- porting materials must use American spelling and usage as given in *The American Heritage Dictionary, Webster's Third New International Dictionary,* or the *Oxford Ameri- can 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 Microboldface 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). Affili- ations will be footnoted using the following symbols:

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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

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Major Headings. Major headings are centered (ex- cept ABSTRACT), all capitals, boldface, and consist of ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), ACKNOWLEDGMENTS (optional), AP- PENDIX (optional), and REFERENCES.

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Abbreviations

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Abstract

The Abstract disseminates scientific information through abstracting journals and through conveniencefor 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). Key words should be formatted as follows: **Key words:** . . .

Introduction

The Introduction, while brief, should provide the read- er 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 num- bers 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 refer- ences.

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 deal- ing with the effects of feed additives or graded levels of a specific nutrient must give analyzed values for the rel- evant additive or nutrient in the diet(s). If products were used that contain different potentially active compounds, then analyzed values for these coupounds must be given for the diet(s). Exceptions can only be made if appropri- ate methods are not available. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as ap- propriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phos- phorus 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 equiv- alents:

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 ac- ceptable 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. Statisti- cal 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. Refer- ence to a statistical package without reporting the sourc- es 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 anal- ysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar ex- periments 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 chemi- cal analyses of the same sample usually do not consti- tute independent experimental units. Measurements on the same experimental unit over time also are not inde- pendent and must not be considered as independent ex- perimental 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 ex- ample, 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 pro- cedures are based on the assumption that experimental units have been assigned to treatments at random. If ani- mals 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 measure- ment 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 vari- able, 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 ar- rangement in 5 randomized complete blocks consisting of initial BW.” Note that **a factorial arrangement is not a de- sign**; 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 er- ror 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 ex- perimental 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 ob- servations 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* sta- tistic. Unbalanced factorial data can present special prob- lems. Accordingly, it is well to state how the computing was done and how the parameters were estimated. Ap- proximations should be accompanied by cautions con- cerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was de- signed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evalu- ated by Bonferroni *t* statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally ar- ranged. 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 impor- tant 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 tradition- ally have been reserved for $P < 0.05$ and $P < 0.01$, re- spectively; 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 con- sequences of type I and II errors. A "nonsignificant" rela- tionship 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 con- tained 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 Re- sults 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 submit- ting. One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experi- ments are adequately replicated. The results of experi- ments 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 Dis- cussion section or Acknowledgments, if present. The Appendix may contain supplementary material, expla- nations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel com- puter 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, re- fer 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 un- published work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and un- published data must not be included in the References section.

References Section. To be listed in the References sec- tion, papers must be published or accepted for publica- tion. Manuscripts submitted for publication can be cited as “personal communication” or “unpublished data” in the text.

Citation of abstracts, conference proceedings, and oth- er 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 list- ed alphabetically by author(s)’ last name(s), and then chronologically. The year of publication follows the au- thors’ names. As with text citations, two or more publi- cations 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 abbrevia- tions given in journals database of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/que- ry.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 in- creased 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 chal- lenge 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. Reg- ist. 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, as- signee. 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 elec- tronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gaines- ville.
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 fea- ture 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 prob- lems). 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 place- ment 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 super- scripts indicate $P \leq 0.05$. Uppercase letters indicate $P \leq 0.01$ or less.

Probability values may be indicated as follows: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, and $\dagger P \leq 0.10$. 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 identi- fied 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 publi- cation. 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. Sym- bols 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 de- scriptor by a comma or parentheses, and should be consistent within a manuscript.
• ***Shading and Fill Patterns.*** For bar charts, use dif- ferent 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 ap- parent only when the figure is reproduced in gray- scale.
• ***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 cap- tion or with a scale bar on the figure. Reduction for publication can make a magnification power desig- nation (e.g., 100×) inappropriate.
• ***Caption.*** The caption should provide sufficient in- formation 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 read- able 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 scien- tific 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 ab- breviations 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 edi- tors 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 abbrevia- tion following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbre- viations 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, ab- breviations shall be defined within each of these units of the manuscript.

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
IL interleukin
IU international units
kb kilobase pairs
kDa kilodalton
L liter*
L:D hours light:hours darkness in a photoperiod (e.g., 23L:1D)
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 2
R coefficient of determination, multiple

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 ab- breviated 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
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., invitro, in vivo, in situ, a priori). However, genus and spe- cies of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. Ger- man 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 writ- ten 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 se- quences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submis- sion of sequence data to one of the databases. The fol- lowing statement should appear as a footnote to the title on the title page of the manuscript. “The nucleotide se- quence data reported in this paper have been submitted to GenBank Submission (Mail Stop K710, Los Alamos Na- tional Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN.”
Publication of the description of molecular clones is as- sumed 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 are re- quired to use only approved gene and protein names and symbols. For poultry, full gene names should not be itali- cized. Gene symbols should be in uppercase letters and should be in italics. A protein symbol should be in the same format as its gee 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 num- bered 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 <, etc.) when these signs occur between two items.
Items in a series should be separated by commas (e.g., a, b, and c).
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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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 preparation can be found at http:// www.oxfordjournals.org/for_authors/figures.html

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Apêndice 2. Peso vivo das aves aos 12, 28 e 35 dias do experimento 1, kg