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**ESTRATÉGIAS PARA REDUÇÃO PROTÉICA DE DIETAS PARA FRANGOS
DE CORTE**

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SUPLEMENTAÇÃO DE AMINOÁCIDOS NÃO TRADICIONAIS PARA MELHORAR A EFICIÊNCIA PRODUTIVA DE FRANGOS DE CORTE¹

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RESUMO

A presente tese foi realizada com o objetivo de estudar as relações entre valina (VAL) e isoleucina (ILE), determinar as exigências de valina e a razão pela qual dietas de baixo conteúdo protéico tendem a causar queda no desempenho de frangos de corte. Desta forma, três experimentos foram conduzidos utilizando dietas a base de milho e farelo de soja e linhagens de frangos de corte comercialmente disponíveis. No primeiro estudo foram avaliadas deficiências de VAL e ILE em dietas práticas para frangos de 14 a 35 dias de idade. Tanto VAL quanto ILE foram limitantes para o ganho de peso dos frangos, não sendo possível identificar com precisão a ordem de limitação destes dois aminoácidos, o que sugere uma co-limitação entre eles. No segundo experimento, foram determinadas as exigências de VAL para frangos entre 21 e 42 dias de idade. Os níveis de VAL digestível considerados ideais por meio de regressão linear foram de 0,82 e 0,81% para ganho de peso e conversão alimentar. Entretanto, quando utilizado o método de linha-quebrada o platô para estas mesmas respostas foi obtido com 0,81 e 0,76% de VAL digestível. No terceiro experimento, a suplementação de L-VAL, L-ILE, Glicina (GLY) e/ou L-Ácido glutâmico (L-GLU) (PB) em níveis idênticos aos apresentados por uma dieta controle tida como padrão da indústria indicou influência do nível de nitrogênio sobre o desempenho das aves. Glicina e ácido glutâmico melhoraram o ganho de peso e conversão alimentar das aves. Os benefícios no crescimento devidos ao fornecimento de GLY foram majoritariamente observados em fases iniciais do crescimento das aves enquanto a suplementação de GLU mostrou-se vantajosa ao longo de todo o período experimental. Adições de VAL, ILE, GLU e GLY resultaram em maior deposição de carne de peito em relação as dietas suplementadas somente com valina. Conclui-se que o fornecimento de dietas de reduzido conteúdo protéico fica restrito a utilização de relações precisas entre VAL, ILE e LYS, além do fornecimento adequado de glicina em fases iniciais. O fornecimento de L-GLU como fonte de nitrogênio em dietas de reduzido conteúdo protéico é uma alternativa para a obtenção de desempenho similar ao apresentado por frangos alimentados com dietas de maior conteúdo protéico.

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NON-TRADITIONAL AMINO ACID SUPPLEMENTATION TO IMPROVE THE BROILERS PRODUCTION EFFICIENCY²

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ABSTRACT

This thesis was carried out to understand the relationships between valine (VAL) and isoleucine (ILE), to determine the requirements of valine and why low protein diets tend to cause drop in broilers performance. Thus, three experiments were conducted using corn and soybean meal diets and broilers strain crosses commercially available. In first study were assessed deficiencies of VAL and ILE in practical diets for broilers from 14 to 35 days of age. VAL and ILE showed limitations on the body weight gain of broilers and it was impossible identify precisely the order of limitation of these two amino acids, suggesting a co-limitation between them. In the second experiment VAL requirements were determined for broilers between 21 and 42 days of age. Ideal digestible levels of VAL considering linear adjustments were 0.82 and 0.81% for body weight gain and feed conversion ratio. However, when used the method of broken-line linear model the plateau for these same responses were obtained with 0.81 and 0.76% of digestible VAL level. In the third experiment, the supplementation of VAL, ILE, Glycine (GLY) and/or Glutamic acid (GLU) as crude protein source at levels similar to those presented by a standard industry diet indicated a strong influence of nitrogen level on the performance of birds. GLY and GLU improved body weight gain and feed conversion of birds. The benefits in growth due to the supply of GLY were mostly observed in early stages of birds growth while the supplementation of GLU showed advantage throughout the experimental period. Additions of VAL, ILE, GLU and GLY showed a higher deposition of breast meat for diets supplemented only with valine. It is concluded that the provision of low protein content diets is restricted to the use of precise relationships between VAL, ILE and LYS, and providing adequate glycine in the early stages. The supply of L-GLU as a source of nitrogen in diets of low protein content is an alternative for obtaining similar performance presented by broilers fed diets of higher protein content.

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RELAÇÃO DE ABREVIATURAS E SÍMBOLOS

Aminoácido(s)	AA
Aminoácidos de cadeia ramificada	AACR
Aminoácidos essenciais	AAE
Aminoácidos não-essenciais	AANE
Arginina	ARG
Conversão alimentar	CA
Cistina	CYS
Gordura abdominal	GA
Ácido glutâmico	GLU
Glicina	GLY
Glicina+Serina	GLY+SER
Ganho de peso	GP
Isoleucina	ILE
Leucina	LEU
Lisina	LYS
Metionina	MET
Metionina+Cistina	MET+CYS
Pontos percentuais	p.p
Prolina	PRO
Proteína bruta	PB
Serina	SER
Treonina	THR
Triptofano	TRP
Valina	VAL

CAPÍTULO I

Introdução

Alterações no mercado mundial como a proibição do uso de ingredientes de origem animal e normas relacionadas a poluição ambiental, bem-estar animal e qualidade do produto final elevam os custos de produção em comparação à sistemas de produção sem estas exigências. Considerando a alta participação da ração nestes custos, é natural que esta seja foco de pesquisas.

Através da manipulação nutricional das rações não só é possível reduzir custos como modular respostas relacionadas a uma menor excreção de elementos potencialmente poluidores, como o nitrogênio. A obrigatoriedade do uso de dietas exclusivamente vegetais para frangos exportados para países da União Européia e Arábia Saudita passou a determinar a impossibilidade da reciclagem de ingredientes de origem animal dentro da própria indústria, anteriormente tida como uma alternativa de redução de custos. Dentre as fontes vegetais de proteína disponíveis no mercado brasileiro o farelo de soja destaca-se por ser o de maior inclusão nas dietas. Entretanto, este ingrediente apresenta como desvantagens o custo relativamente alto, a alta concentração de polissacarídeos não-amiláceos e de potássio, os quais aumentam a viscosidade da dieta e elevam o consumo e eliminação de água pelas aves. Estes fatores, em última análise, contribuem para o desenvolvimento de

condições adversas ao crescimento dos frangos através do aumento da proliferação de patógenos e fermentação da cama.

Desta forma, a redução do nível de proteína bruta da dieta por meio da diminuição da inclusão de farelo de soja nas dietas e menor excreção de nitrogênio pelas aves, sem perdas em desempenho zootécnico são pontos essenciais na busca por alternativas viáveis. Grandes avanços foram alcançados até a suplementação de treonina, o terceiro aminoácido limitante em dietas para frangos de corte. Contudo, a identificação do quarto aminoácido limitante e suas exigências carece de maiores estudos e aprofundamento. Da mesma forma, a redução acentuada do nível de proteína bruta das dietas geralmente leva à queda de desempenho. A busca do entendimento das causas desta perda de desempenho é atual e fundamental para que os custos de produção possam ser reduzidos.

Ante o exposto, para a realização da presente tese foram conduzidos três experimentos buscando determinar as relações entre valina e isoleucina, as exigências de valina e o efeito da suplementação de aminoácidos valina, isoleucina, glicina e ácido glutâmico em dietas a base de milho e farelo de soja sobre o desempenho de frangos de corte.

Revisão bibliográfica

Necessidades protéicas das aves

É geralmente aceito que o nível protéico ótimo de uma dieta é aquele suficiente para atender às exigências de aminoácidos (AA) das aves sendo o resultado da mistura heterogênea de diferentes matérias-primas. O crescimento dos animais depende da síntese protéica, a qual tem sua eficiência de utilização relacionada a quantidade de AA a serem catabolizados. As exigências de AA em uma determinada idade variam diretamente com a taxa de crescimento da ave. Portanto, as exigências de AA, como proporção do total de nutrientes consumidos por dia, diminuem em paralelo à redução do crescimento com a idade (Han & Baker, 1991).

A utilização dos AA presentes nas matérias-primas para síntese de proteína no organismo animal depende de fatores além da sua concentração na dieta. As proteínas dos alimentos são digeridas em taxas diversas e, assim, os AA são disponibilizados também em diferentes concentrações na mucosa intestinal para absorção. Vários fatores podem afetar a velocidade de digestão das proteínas, entre eles os inibidores das proteases, tais como: inibidores de tripsinas, lectinas, compostos polifenólicos e saponinas (Bryden, 1996; Hughes & Choct, 1999). Os vários polissacarídeos não amiláceos presentes em ingredientes protéicos vegetais, como os grãos e farelos de leguminosas

alteram a viscosidade intestinal e, portanto, a digestibilidade da dieta (Vieira & Lima, 2005).

A fixação de um nível mínimo de proteína bruta na formulação, como critério para assegurar níveis mínimos de AA e ótimo desempenho dos animais, continua sendo adotada por muitos nutricionistas. Isto eleva os custos de produção das rações e desconsidera o balanço aminoacídico e eventuais excessos destes nutrientes nas rações.

Além da grande influência sobre parâmetros de desempenho zootécnico e de rendimentos de cortes, o consumo de proteína e AA influencia o crescimento e desenvolvimento das penas de aves em crescimento. A maior parte da estrutura da pena é composta por queratina que é sintetizada na pena formando células da epiderme. Embora a estrutura da queratina seja muito rica em cistina e níveis marginais de metionina+cistina prejudiquem o desenvolvimento normal das penas, a deficiência de AA como a valina (VAL) também podem afetar o desenvolvimento destas (Farran e Thomaz, 1992ab).

Excessos de proteína das dietas são degradados e incorporados ao ácido úrico sintetizado por reações comuns às purinas, componentes do DNA. A glicina é parte integral do ácido úrico e, portanto, a eliminação desta é paralela à excreção urinária (Scott *et al.*, 1982). Portanto, supõe-se que a exigência de glicina seja maior em aves de rápido crescimento e em dietas com excesso ou desequilíbrio protéico. A treonina, sendo precursora da glicina, pode também ter suas exigências alteradas nestas condições. Da mesma forma, a excreção do ácido úrico demanda energia e o excesso de nitrogênio na dieta constitui desperdício de nitrogênio e de energia (McLeod, 1997).

As exigências de proteína das aves são, na verdade, exigências de

aminoácidos. Todavia, esta afirmação está baseada na necessidade de degradação das proteínas até os compostos típicos de absorção para posterior utilização nos processos de síntese no metabolismo animal. Independentemente da proteína original ingerida, AA, di- e tripeptídeos, são as unidades realmente absorvidas pelo animal. Da mesma forma, assume-se que do “pool” de AA em trânsito para os processos de síntese, preocupações com a demanda de aminoácidos não-essenciais (AANE) seriam irrelevantes, pois em dietas usuais estes apresentam-se em excesso. Assim, apenas a riqueza em AA essenciais é levada em consideração na formulação de rações comerciais. A menos que o conteúdo protéico das dietas seja reduzido de forma a restringir o suprimento de nitrogênio total da dieta considera-se desnecessária a quantificação dos AA não-essenciais.

A manipulação do conteúdo protéico das dietas também foi estudada em relação a temperatura ambiente. Altas temperaturas ambientes reduzem o consumo de alimento e a taxa de crescimento (Cemigua et al., 1983; Reece and Lott, 1983). Cheng et al. (1996) reportaram que o fornecimento de dietas de alta PB para frangos sob estresse por calor tem um efeito prejudicial no ganho de peso e composição da carcaça, bem como na eficiência de utilização do alimento, proteína e energia. Faria Filho et al. (2006) relataram ainda que dietas de baixa proteína bruta para frangos sob estresse por calor além de reduzir a proteína também aumentou a deposição de gordura em cortes. Segundo os autores a utilização destas dietas não é recomendada para frangos entre 43 e 49 dias de idade criados sob temperatura de 32 °C. Todavia, para temperaturas entre 20 e 25 °C sua utilização é benéfica.

Dietas de alto conteúdo protéico foram descritas como responsáveis

pelo aumento da resistência ao rompimento da pele de frangos, muito embora a razão esteja pouco elucidada. Uma quantidade maior de proteína bruta provê maior quantidade do AANE glicina, o qual representa cerca de 30% dos AA constituintes do colágeno, o que poderia explicar este fato (Leeson & Summers, 2001).

As concentrações relativas dos AA entre si também exercem efeito sobre a absorção e o metabolismo de síntese e degradação protéica do organismo animal, sendo comum a existência de antagonismos entre estes no momento de sua absorção. A disponibilidade comercial dos AA metionina, lisina (LYS), e treonina (THR) têm propiciado redução nos custos das dietas. Estes são, na prática, os três primeiros AA limitantes em dietas para frangos de corte. Contudo, a formulação de dietas com redução acentuada no conteúdo de proteína bruta das rações para frangos de corte permanece motivo de indagações e conjecturas.

Redução protéica

A adequação das dietas com os mais diversos ingredientes às exigências dos animais é muito difícil de ser realizada sem que ocorra a administração excessiva de alguns nutrientes. Comum é o caso dos AA, em que aqueles não suplementados individualmente nas dietas, geralmente excedem os níveis considerados suficientes para maximizar respostas de desempenho zootécnico, imunidade e rendimentos pós-abate. Neste contexto, a redução protéica das dietas ganha ênfase e relações mais precisas entre os AA passam a ser requeridas. Segundo Kidd & Kerr (1996), reduzindo-se a proteína bruta da dieta, é possível melhorar a eficiência de utilização do nitrogênio diminuindo sua excreção, aumentar a tolerância dos frangos a altas

temperaturas ambiente e reduzir o nível de amônia na cama. Ishibashi & Yonemochi (2002) afirmaram que a eficiência de utilização da proteína pode ser melhorada em níveis superiores a 50% pelo correto balanço aminoacídico da dieta e que para melhorar a conversão alimentar e reduzir a excreção de nitrogênio é necessário elucidar o conteúdo em aminoácidos das matérias-primas utilizadas na alimentação animal e sua disponibilidade além, é claro, de determinar as exigências dos animais. Todavia, a redução do conteúdo protéico em dietas para frangos de corte merece maior atenção que no caso de suínos, por exemplo. Reduções de 3 a 4 pontos percentuais (p.p.) no conteúdo protéico de rações para suínos são perfeitamente possíveis quando utilizada a suplementação de AA sintéticos, sem qualquer perda em desempenho zootécnico ou qualidade de carcaça (Hansen *et al.*, 1993; Ferreira *et al.*, 2006). Entretanto, quando reduções desta magnitude são realizadas em dietas para frangos estes resultados nem sempre são alcançados suplementando-se AA na forma sintética (Fancher and Jensen, 1989ab; Pinchasov *et al.*, 1990; Thornton *et al.*, 2005; Aftab *et al.*, 2006; Berres *et al.*, 2007).

Quando reduções desta magnitude são realizadas para o nível de proteína bruta, aminoácidos além da metionina, lisina e treonina passam a apresentar-se limitantes e a prejudicar o aproveitamento destes três primeiros AA para conversão em proteína corporal. É o caso da valina, isoleucina (ILE), arginina (ARG), triptofano (TRP) e para fases iniciais, mesmo da glicina (GLY). O balanço entre os aminoácidos essenciais é de grande importância na otimização do crescimento animal e o uso de aminoácidos sintéticos permite uma nutrição mais precisa, sem excessos de proteína bruta. De acordo com Kidd (2001), a disponibilidade econômica dos aminoácidos industriais, assim

como a melhor avaliação dos ingredientes e das exigências nutricionais permitem aos nutricionistas formularem rações com menores níveis protéicos.

Por outro lado, a adição de AA industriais em rações pode resultar em redução no consumo de alimento (Han and Baker, 1993; Carew et al., 1998; Si et al., 2004). O metabolismo dos AA envolve síntese e degradação de proteínas, incorporação do nitrogênio dos AA no ácido úrico, conversão dos esqueletos de carbono dos AA em glicose, gordura, energia ou CO_2 e H_2O e ainda formação de derivados não protéicos (Kidd & Kerr, 1996). Os esqueletos de carbono resultantes do catabolismo de AA podem gerar piruvato para produção de glicose ou energia e AANE para necessidades metabólicas tais como a síntese de proteína, creatina, serina, ácido úrico, sais biliares e glutatona, por exemplo. Alguns autores (Kumta and Harper, 1962; Peng and Harper, 1970) propuseram a hipótese aminostática, pela qual os níveis de aminoácidos presentes no plasma serviriam como um sinal para um mecanismo controlador do apetite. É possível que não somente níveis de aminoácidos mas também de seus metabólitos serviriam como sinal para regular o consumo de alimento. De acordo com Namroud et al. (2008) o retardo no crescimento e consumo de alimento em frangos alimentados com dietas de reduzida PB e suplementadas com grandes quantidades de AA é devido aos efeitos negativos do aumento da concentração sanguínea e excretória de amônia no metabolismo tecidual. A amônia é inevitavelmente liberada no metabolismo da proteína, mas é extremamente tóxica para células vivas, e os mecanismos sensíveis a mantem em níveis não tóxicos. Excessos ou desbalanços de AA originam esqueletos de carbono e amônia, a qual é posteriormente convertida em ácido úrico e excretada pelas aves.

Diminuir o consumo de ração para limitar a absorção e o catabolismo do excesso de aminoácidos pode ser um mecanismo para diminuir a concentração intracelular de amônia. Outra forma é a indução de atividades de enzimas envolvidas na desintoxicação de amônia. Segundo Noda (1975), o nível de amônia no sangue é um fator importante na regulação do apetite em ratos, mas não há nenhuma pesquisa confiável para investigar o mecanismo de controle em frangos de corte ou os fatores dietéticos eficazes sobre o nível de amônia no sangue.

O insucesso na obtenção de desempenho de frangos alimentados com dietas de reduzida PB semelhante ao apresentado por frangos que consomem dietas controle pode ser atribuído a insuficiência de nitrogênio para a síntese de AANE, especialmente GLY, SER, PRO, e GLU; a um decréscimo no nível de potássio ou alteração no balanço iônico; e a desbalanços entre aminoácidos. Contudo, a maioria destes fatores não explicam completamente essa queda no desempenho. Discrepâncias entre os efeitos da suplementação de AAE e AANE em dietas com reduzida PB podem ser atribuídas principalmente a severidade da redução do nível de PB, aos ingredientes utilizados nas dietas e a adequação da suplementação a ordem de limitação dos aminoácidos nestas dietas. Ainda assim, estas manipulações nas dietas podem não demonstrar sucesso na obtenção de crescimento similar ao apresentado por aves alimentadas com uma dieta controle positivo.

Aminoácidos essenciais

A determinação do conteúdo de PB de um alimento é bastante simplista e geralmente é obtida através da multiplicação do percentual de nitrogênio deste pelo fator de correção 6,25, o qual na realidade varia de uma

matéria-prima para outra. Portanto, parece mais apropriado considerar-se como nível protéico ótimo da ração aquele suficiente para atender às exigências das aves em aminoácidos. Quando o nível de PB da ração não apresenta restrições no momento da formulação este é determinado estritamente pelo nível do último aminoácido essencial fixo na fórmula, geralmente a treonina, e pela presença deste aminoácido nas fontes protéicas disponíveis.

A ordem de limitação dos AA das dietas é usualmente estudada por meio de experimentos com adição ou remoção dos mesmos das dietas (Edmonds *et al.*, 1985; Baker, 1989). Nos experimentos com adição, os AA são adicionados individualmente e em várias combinações a uma dieta com baixo conteúdo protéico ou AA-deficiente, enquanto em experimentos com remoção, os AA são removidos sequencialmente de uma dieta contendo os AA em níveis satisfatórios. Os experimentos com adição são mais diretos e apresentam menos excessos de AA, mas requerem muitos tratamentos. Os experimentos de remoção de AA são muito simples e mais eficientes do ponto de vista de desenho experimental, mas seus resultados podem ser confundidos pelos excessos de AA. Fernandez *et al.* (1994) demonstraram que o problema de excessos de AA no método de remoção pode ser minimizado pelo uso do conceito de proteína ideal na formulação das dietas.

Para a síntese protéica, os AA estão relacionados entre si de forma a sustentar as exigências de manutenção e crescimento. Portanto, inadequações na oferta do primeiro limitante levam ao catabolismo dos próximos em ordem de limitação. Na nutrição de AA, manutenção refere-se ao estado no qual a proteína não é depositada para crescimento, reprodução ou substituição de

penas (D'Mello, 1994). Normalmente, estas são devidas a perdas obrigatórias e são relativamente baixas comparadas àquelas necessárias para o crescimento. Por exemplo, frangos em crescimento têm 94% das exigências de valina utilizadas para suportar o crescimento e apenas 6% são exigidos para repor as perdas obrigatórias (Baker *et al.*, 1996).

Uma das primeiras definições de AAE foi a de que são aqueles que não podem ser sintetizados pelo organismo animal, a partir do material disponível às células, em velocidade suficiente para atender as exigências para um crescimento normal (Borman *et al.*, 1946). Cada AAE é único em seu catabolismo e a carência de um deles (limitante) geralmente acarreta o catabolismo parcial dos demais. A resposta das aves pode variar com os níveis de aminoácidos essenciais, com a magnitude da carência de algum deles, e com as relações entre os remanescentes (NRC, 1994). Exigências de AA são usualmente determinadas para ganho de peso e conversão alimentar. Entretanto, pela importância quantitativa na demanda a partir de AA da dieta, a composição corporal é relevante, permanecendo inalterada mesmo com alterações nos demais fatores que afetam as exigências protéicas das aves.

Muitos fatores podem influenciar as exigências de AA para frangos de corte durante o crescimento. Entre estes, fatores comuns são os níveis de proteína e energia e a presença de inibidores de proteases nas dietas, desafios microbiológicos, densidade de alojamento, disponibilidades de equipamentos, estresse por calor ou frio, bem como a composição corporal (Baker & Han, 1994) e sua taxa de crescimento. Concentrações inadequadas de um determinado AA levam ao catabolismo de outros. Antagonismos entre AA de cadeias laterais similares são reconhecidos, sendo que excessos de um afetam

as necessidades dos demais. São reconhecidos os antagonismos entre leucina-isoleucina-valina, arginina-lisina, e treonina-triptofano (NRC, 1994). Outros efeitos indiretos, entretanto, existem. Por exemplo, excesso de MET causa deficiência de THR devido ao aumento da oxidação da mesma pela elevação da atividade da enzima treonina desidratase. Da mesma forma, o excesso de LYS pode causar deficiência de treonina (Kidd & Kerr, 1996). Um problema na avaliação do efeito de diferentes AA fornecidos através da dieta sobre o desempenho é que muitos fatores mudam simultaneamente, incluindo consumo de alimento (Denbow, 1989; Kuenzel, 1994) e síntese protéica (Kang *et al.*, 1985; Klasing *et al.*, 1987). Num contexto experimental, a mudança de um único AA pode ser usada para determinar a exigência para aqueles AA, entretanto quando são alterados os níveis de mais de um AA, interações entre eles acontecem e as interpretações das respostas obtidas tornam-se mais difíceis. Dificuldades associadas com a análise de AA podem ser a maior fonte de variação de um experimento com AA e estas são freqüentemente negligenciadas (Ravindran & Bryden, 1999).

Os AA apresentam uma larga variedade de funções, ainda que a sua participação no crescimento seja preponderante para os animais domésticos. As aves apresentam maior demanda dos AA sulfurados metionina (MET) e cistina (CYS) para deposição de penas e estes influenciam diretamente o desempenho zootécnico das aves além do rendimento de carcaça. A MET, além de participar da síntese protéica, é precursora da CYS e doadora de grupos metil (Warnick & Anderson, 1968). A LYS é utilizada quase que exclusivamente para síntese protéica (também atua na síntese de carnitina), enquanto a THR além de participar na formação de proteínas estruturais como

o músculo participa da formação da GLY, faz parte das enzimas digestivas e de outras secreções intestinais como o muco, as células da mucosa e as proteínas do sistema imunológico (Fernandez *et al.*, 1994; Kidd *et al.*, 1998). O conhecimento das exigências destes 3 primeiros aminoácidos limitantes para frangos é alicerçado em um grande volume de informações científicas. Reduções nos níveis de proteína bruta das dietas além das usuais são dependentes da correta identificação do quarto AA limitante bem como de sua relação com os demais AA presentes na dieta. Nesta perspectiva, uma vez determinado, espera-se que em futuro próximo seja corriqueiro o uso do quarto, e talvez do quinto AA limitantes a partir de fontes industriais em dietas para frangos.

Quarto aminoácido limitante

A ordem de limitação dos AAE tem sido fonte de preocupação e área prioritária de estudo há décadas. Um número considerável de pesquisas tem sido conduzido para definir a ordem de limitação dos AA em dietas com milho e farelo de soja (Schwartz & Bray, 1975; Edmonds *et al.*, 1985; Han *et al.*, 1992; Fernandez *et al.*, 1994, Corzo *et al.*, 2008), amplamente utilizadas nas rações de frangos de corte atualmente.

Sem dúvida, a suplementação de AA sintéticos é uma alternativa para a redução na inclusão de farelo de soja nas rações. No entanto, reduções protéicas com correspondentes suplementações de AAE, a partir de fontes industriais, nem sempre resultam em recuperação do desempenho animal. Diferentemente dos três primeiros, o quarto limitante é altamente dependente dos ingredientes usados nas dietas. Valina, isoleucina, arginina e triptofano são potenciais candidatos. Entre os AA referenciados na literatura como possível

quarto limitante, a valina vem demonstrando maior importância em dietas exclusivamente vegetais baseadas apenas em milho e farelo de soja (Schwartz & Bray, 1975; Edmonds *et al.*, 1985; Han *et al.*, 1992; Fernandez *et al.*, 1994; Kidd & Hackenhaar, 2006). Simulações de formulação deste tipo de dieta para frangos em crescimento apresentam relações entre valina e lisina abaixo de 77%, relação considerada ótima por alguns autores recentemente (Rostagno *et al.*, 2005). Por outro lado, teoricamente e baseando-se nas relações entre AA determinadas até o momento, quando as dietas são baseadas em milho ou trigo, farelo de soja e farinha de carne de frango ou quando trigo, farelo de soja e farinha de carnes são utilizados em uma mesma dieta, ILE passa a ser o quarto limitante. Em dietas baseadas em milho, farelo de soja e farinha de carnes, TRP é o aminoácido limitante após treonina. Quando o sorgo é utilizado no lugar do milho, ARG torna-se o quarto AA limitante para frangos de corte (Kidd and Hackenhaar, 2006).

Mack *et al.* (1999) usaram dietas a base de milho e farelo de soja com baixa proteína (17,2%) para avaliar as exigências para vários aminoácidos. As dietas experimentais foram fornecidas de 20 a 40 dias de idade. Entretanto, à exceção de TRP e ILE, o ganho de peso e conversão alimentar foram questionáveis, não permitindo uma boa base para o estabelecimento das exigências de TRP e ILE ou das relações TRP:LYS e ILE:LYS. Estes autores também obtiveram resultados sugerindo que a relação ideal THR:LYS foi de 59%, inferior a de 67% encontrada no estudo de Baker (1997). Estes estudos claramente demonstram a importância da realização de novos experimentos, a partir dos quais relações entre os AA possam ser calculadas.

As recomendações de VAL do NRC (1994) são majoritariamente baseadas em modelagem e ausência de desbalanços entre os AA de cadeia ramificada (AACR) VAL, ILE e leucina (LEU). A limitação de VAL é particularmente aparente a idades avançadas quando a proteína da dieta é reduzida e a participação dos grãos, especialmente milho, têm sua contribuição elevada. As proporções relativamente baixas de VAL e ILE na proteína do milho são acompanhadas por alta concentração de leucina. Dietas com alto conteúdo de leucina têm sido relatadas como responsáveis por um aumento das exigências de VAL e ILE em frangos e perus em crescimento (D'Mello & Lewis, 1970; Allen & Baker, 1972; Tuttle & Balloun, 1976). Dietas inadequadas no conteúdo de VAL não somente impactam negativamente sobre o ganho de peso e conversão alimentar, mas também causam anormalidades no desenvolvimento de penas e pernas (Anderson & Warnick, 1967; Robel, 1977; Farran & Thomas, 1992ab). Níveis extremamente elevados de leucina também ocasionam problemas de empenamento similares aos descritos para a carência de valina em dietas para frangos de corte (Penz *et al.*, 1984). Leclercq (1998) também observou redução no desempenho zootécnico de frangos consumindo dietas reconhecidamente deficientes em valina. Entretanto, não foram mencionadas anormalidades no desenvolvimento de penas e pernas. Desta forma, diferenças na expressão de uma deficiência de valina podem ser associadas à matéria-prima e ao nível de leucina empregados.

A deficiência dos AACR foi descrita por Konashi *et al.* (2000) como sendo responsável pela redução do peso de timo e bursa de Fabricius quando comparados com todos os outros grupos de AA essenciais. A degradação de VAL, ILE e LEU em animais inicia com uma transaminação seguida por

descarboxilação oxidativa dos respectivos cetoácidos. Esta reação é conduzida pelo complexo α -cetoácido desidrogenase de cadeia ramificada (Mathews & Van Holde, 1990). Embora o metabolismo da ILE seja majoritariamente conhecido, poucos estudos avaliando seus efeitos sobre o desempenho de frangos de corte foram conduzidos. Antagonismos entre estes três AA têm sido demonstrados em várias espécies animais como ratos, suínos, perus e frangos de corte (Sauberlich, 1961; Allen & Baker, 1972; Oestemer *et al.*, 1973; Tuttle & Balloun, 1976; Smith & Austic, 1978; Taylor *et al.*, 1984). Burnham *et al.* (1991) demonstraram que a elevação dos níveis de LEU em dietas de frangos alivia o efeito depressivo do fornecimento excessivo de isoleucina.

A mistura de ingredientes em uma ração que resultem em quantidades de AAs essenciais muito próximas das exigências para crescimento e manutenção dos frangos minimiza as inadequações entre os AACR e a excreção de nitrogênio (Mack *et al.*, 1999). Isoleucina também compete com o TRP para sua transferência no cérebro através da barreira sangue-cérebro (Wurtman, 1980; Tackman *et al.*, 1990). O triptofano tem um papel importante no cérebro como precursor do neurotransmissor serotonina que, entre suas muitas funções, destaca-se por seu maior efeito no comportamento do animal em relação à regulação do apetite e do ritmo circadiano (Blundell & Latham, 1978; Tackman *et al.*, 1990; Mullen & Martin, 1992). Como excessos de ILE na dieta são associados a uma redução marcada em consumo de alimento, talvez, então, este efeito também possa ser associado a uma deficiência secundária de triptofano no cérebro.

Existem evidências de participação de AA na modulação da resposta imunológica em aves e mamíferos. Deficiências de proteína na dieta ou de AA

alteram a resposta imunológica (Bhargava *et al.*, 1970, 1971; Glick *et al.*, 1981, 1983; Klasing, 1988; Payne *et al.* 1990; Umezawa *et al.*, 1990). Glick *et al.* (1981; 1983) demonstraram diferenças entre a resposta imunológica de frangos alimentados com uma dieta deficiente para todos os AA essenciais e outra controle.

As funções do sistema imunológico podem ser afetadas pelo ambiente, nutrição ou fatores estressantes. O fornecimento de dietas com desbalanços entre os AA pode prejudicar a habilidade da ave de montar uma resposta imunológica efetiva. Isto pode ocorrer em frangos alimentados com dietas limitantes em valina ou isoleucina e com excessos de leucina. Pesquisas com ratos demonstraram efeitos imunossupressivos de altas concentrações de leucina na dieta, sendo estes efeitos revertidos através do aumento dos níveis de VAL e ILE nestas dietas (Aschkenasy, 1979). Deficiências de ILE e VAL apresentaram efeitos imunossupressores em ratos (Petro & Bhattacharjee, 1981). Desta forma, pode-se afirmar que devido a similaridades estruturais, LEU pode antagonizar a absorção e retenção de VAL e ILE, ou ambos, e este pode ser o mecanismo envolvido na imunossupressão leucina-induzida (Benton *et al.*, 1956). Dependendo do perfil de ingredientes utilizado para compor uma dieta, a ordem de limitação de desempenho apresentada para os aminoácidos tende a ser diferente. Além disso, a medida que outros aminoácidos, menos limitantes, tem suas exigências atendidas com precisão, ou seja, sem excessos, o conceito de co-limitância ganha espaço, especificamente entre VAL e ILE (Corzo *et al.*, 2009).

Aminoácidos não-essenciais

Poucos trabalhos têm considerado a suplementação de VAL e ILE e

de AANE em dietas para frangos de corte, principalmente no Brasil. Dentre os AANE, os que apresentam potencial para serem utilizados sinteticamente em dietas para frangos de corte são a glicina, o ácido glutâmico e o ácido aspártico. Todavia, também existem estudos que avaliaram a suplementação de outros AANE como a prolina e a alanina. Embora a glicina possa ser sintetizada pelas aves, a taxa de síntese não é adequada para suportar crescimento máximo (Featherston, 1976). Há muitas funções no metabolismo intermediário nas quais a glicina está envolvida, mas esta é exigida primariamente para a formação de ácido úrico e excreção de nitrogênio (Coon et al., 1975; Corzo et al., 2004). O ácido glutâmico é essencial para a manutenção da mucosa intestinal por ser fonte de energia para o *turnover* da mucosa, por intermédio do ATP produzido a partir do ciclo de Krebs; fonte de nitrogênio para síntese de aminoácidos e outros compostos nitrogenados; precursor da glutathione, antioxidante intracelular, que auxilia na manutenção da integridade intestinal. Han *et al.* (1992) demonstraram que pintos com idade entre 1 e 21 dias, consumindo rações à base de milho e farelo de soja, com 19% de proteína bruta e suplementadas com metionina, lisina, treonina, arginina e valina, bem como o aminoácido não essencial ácido glutâmico, tiveram um desempenho equivalente àqueles alimentados com a dieta controle com 23% de proteína bruta. Já entre os 22 e os 42 dias de idade as aves que receberam 16% de proteína bruta e suplementação com estes aminoácidos, tiveram um desempenho semelhante àquelas que receberam uma dieta com 20% de proteína bruta. Não houve diferença no teor de gordura corporal entre as aves que receberam dietas de baixa proteína bruta suplementadas com aminoácidos e aquelas que receberam a dieta controle. Porém outros estudos (Pinchasov *et*

al., 1990; Colnago *et al.*, 1991) indicaram desempenhos inferiores em aves alimentadas com dietas à base de milho e farelo de soja em que se usou baixa proteína e suplementação com aminoácidos limitantes.

O grau de limitação de muitos AANE, quando a PB da dieta é reduzida, é desconhecido para frangos de corte. Trabalhos referentes à administração de AANE em dietas com baixo conteúdo protéico têm apresentado resultados promissores. É o caso do estudo de Corzo *et al.* (2005), em que frangos de corte machos dos 5 aos 21 dias de idade foram submetidos a dietas com baixo nível de PB e suplementação de AANE em comparação a uma dieta controle positivo (convencional), sendo o nível de PB da dieta controle 4 p.p. maior em relação a dieta suplementada com AANE. Neste estudo, verificou-se ganho de peso semelhante entre a dieta controle e a dieta com baixa PB suplementada com glicina, alanina, ácido aspártico, ácido glutâmico e prolina, sendo ambas superiores a dieta com baixa PB sem suplementação dos AANE. Estes resultados indicam a necessidade de suplementação de AANE em dietas iniciais com 4 p.p. a menos de PB.

Hussein *et al.* (2001) avaliando a suplementação não só de AANE como de treonina, triptofano e arginina em dois experimentos, encontraram resultados contraditórios. Em um primeiro experimento, a adição de arginina, triptofano e treonina à dietas com 17,3% de PB foram capazes de imprimir ganho de peso semelhante ao obtido por frangos consumindo uma dieta de alta PB (22,6%), o que não ocorreu com a suplementação de isoleucina ou ácido glutâmico. Contudo, em um segundo experimento, os pesquisadores obtiveram efeito positivo da suplementação de AANE ou energia extra em conjunto com arginina, triptofano e treonina sobre respostas de desempenho zootécnico. Os

autores destacaram a limitação de treonina das dietas com baixo conteúdo protéico.

Quando consideradas a retenção de nitrogênio e gordura bruta na carcaça de frangos consumindo dietas com baixa PB, ainda que suplementadas com glutamina e asparagina, comparativamente às daqueles que consumiram dietas convencionais, observou-se menor e maior retenção de nitrogênio e gordura, respectivamente, em carcaças de frangos que consumiram as dietas de baixo valor protéico. Da mesma forma, as aves que consumiram dietas com baixa PB, excretaram menos nitrogênio e apresentaram pior conversão alimentar (Bregendahl *et al.*, 2002).

De modo semelhante, Kerr & Kidd (1999) observaram que a adição única de ácido glutâmico (GLU) em dietas de baixo conteúdo protéico proporcionou ganho de peso e rendimento de carcaça inferior em relação à dieta controle. Contudo, quando estes pesquisadores adicionaram além de GLU, AAE, as respostas de rendimento de carcaça, percentual de gordura abdominal e rendimento de carne de peito foram similares às apresentadas por frangos consumindo a dieta controle. Em uma seqüência de estudos realizada por Dean *et al.* (2006), foi avaliada a suplementação de glicina em dietas com baixo conteúdo protéico. Os autores demonstraram ganho significativo sobre o desempenho das aves que consumiram dietas com baixa PB com níveis bastante superiores de GLY+SER aos comumente recomendados pelas tabelas de exigências de frangos de corte. Embora os resultados encontrados tenham sido semelhantes aos apresentados por frangos consumindo dietas controle com maior conteúdo protéico, a dieta de 16% de PB não teve aplicação prática em razão da alta quantidade de aminoácidos cristalinos ainda

não disponíveis comercialmente. Corzo et al. (2005) reportaram que frangos alimentados com dietas contendo 18% de PB suplementada com GLY ou L-LEU tiveram conversão alimentar igual a de frangos alimentados com uma dieta controle com 22% de PB.

Waldroup et al. (2005) reportaram que a suplementação de GLY em uma dieta de baixa PB melhoraram significativamente o ganho de peso, apesar de não restaurar completamente o desempenho obtido por frangos alimentados com dietas de 22 ou 24% de PB. Schutte et al. (1997) recomendaram 1.9% de GLY+SER total quando frangos foram alimentados com dietas de baixa PB fortificadas com aminoácidos essenciais. Por outro lado, alguns pesquisadores sugeriram que o grande fornecimento de aminoácidos essenciais em relação aos não essenciais em dietas de baixa PB tendem a melhorar o desempenho e prevenir o excesso de deposição de gordura, (Yamazaki et al., 1998); embora, outros achados rejeitem esta influência positiva (Yamazaki et al., 2006).

De acordo com a revisão de Pesti (2009), apesar do aumento dos níveis protéicos da maioria dos estudos resultar em melhora no ganho de peso e conversão alimentar, a decisão sobre os níveis adequados de PB ou da soma de AAE e AANE da dieta fica dependente de uma série de fatores relacionados ao retorno econômico.

Proteína ideal

Proposto por Mitchell (1964), o conceito de proteína ideal baseia-se em otimizar a utilização da proteína da dieta melhorando o desempenho das aves (Waldroup *et al.*, 1976; Baker, 1994; Mack *et al.*, 1999) e minimizando a excreção de nitrogênio (de Lange, 1993). Os nutricionistas de suínos foram os primeiros a focalizar esse problema, expressando as exigências de

aminoácidos como relativas à LYS para diferentes categorias de peso (Wang & Fuller, 1989; Chung & Baker, 1992). Embora as exigências para aminoácidos mudem por uma série de motivos, entre os quais a taxa de crescimento, as relações ideais permanecem similares e, uma vez determinadas, somente exigências precisas de LYS precisariam ser estabelecidas para os animais em diferentes condições ambientais (Baker, 1997).

A proteína ideal nada mais é que a mistura de aminoácidos ou proteínas capaz de atender as exigências dos animais para manutenção e crescimento com a máxima eficiência ou o menor desperdício possível. A LYS é o AA referência por ser usado quase que exclusivamente para deposição protéica, por ser um aminoácido essencial limitante em dietas para frangos e por sua determinação laboratorial ser bastante precisa (Baker, 1997). A utilização deste conceito facilita a inclusão de ingredientes alternativos na fórmula sem alteração no desempenho das aves e com redução nos custos de produção.

Quando utilizado o conceito de proteína ideal na formulação sem que níveis mínimos de proteína bruta sejam fixados é natural que ocorra uma redução do conteúdo de proteína bruta até que o nível mínimo do aminoácido limitante, geralmente um aminoácido não provido de forma sintética, seja obtido. Desta forma, a excreção de nitrogênio pelas aves é reduzida, e desde que a redução protéica não seja exacerbada o desempenho pode ser mantido similar a dietas de maior nível de proteína bruta.

Hipóteses e objetivos

Hipóteses

A alteração das relações VAL:LYS e ILE:LYS das dietas afetará o desempenho zootécnico e rendimentos de carcaça e cortes de frangos de corte e haverá uma ordem de limitação entre estes dois aminoácidos;

A alteração do nível de VAL das dietas afetará o desempenho zootécnico e rendimentos de carcaça e cortes de frangos de corte;

A alteração do nível protéico e do perfil aminoacídico das dietas afetará o desempenho zootécnico e rendimentos de carcaça e cortes de frangos de corte.

Objetivos

Geral

O objetivo da presente tese foi o de explorar possibilidades em virtude da necessidade de redução protéica das dietas.

Específicos

Entender a importância das relações entre VAL e ILE;

Determinar as exigências de VAL;

Determinar a razão pela qual o nível de proteína bruta das dietas, quando reduzido, determina respostas inferiores de desempenho e rendimentos de carcaça e cortes em frangos de corte.

CAPÍTULO II¹

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**Supplementing L-Valine and L-Isoleucine in low protein corn-soybean meal all
vegetable diets for broilers**

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Section: Metabolism and Nutrition

Running title: Valine and Isoleucine for broilers

Primary audience: Nutritionists

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SUMMARY

One study was conducted to evaluate performance and carcass yields of broilers fed diets having increased VAL and ILE to LYS ratios from 14 to 35 d. One thousand seven hundred and seventy-five Cobb x Cobb 500 male broilers were fed a corn-soybean meal all vegetable diets having 1.10% digestible LYS. This diet was formulated without crude protein restriction to attain the lowest digestible ILE to LYS ratio and without synthetic AA besides MET, LYS and THR. MET+CYS and THR to LYS ratios were kept at 75 and 65%. Crude protein was 18.7%, whereas VAL and ILE reached at 70 and 65% of LYS. Graded increases in ratios of VAL and ILE to LYS were created using the basal feed supplemented with L-VAL and L-ILE at the expense of kaolin as follow: 75 and 65, 80 and 65, 70 and 68, 70 and 71, 75 and 68, 80 and 71%. Treatments had 9 replications of 25 male broilers each. Performance differences were detected mainly from 14 to 21 d of age; feed intake was lower for broilers fed the basal diet, whereas body weight gain and feed conversion ratio were improved by increases in VAL and ILE to LYS ratios. From 14 to 35 d, low levels of VAL and ILE led to lower body weight gain but did not influence feed conversion ratio. No differences were found among treatments for mortality, carcass, cuts and abdominal fat yields. In general, digestible ratios of VAL and ILE to LYS between 70 and 75 and 65 and 68, respectively, should be sufficient for Cobb 500 birds from 14 to 35 d of age.

DESCRIPTION OF PROBLEM

The use of synthetic (MET sources) and crystalline (L-LYS sources and L-THR) amino acids (AA) in broiler feeds is well established as successfully attaining the needs of the first limiting amino acids. As amino acids follow in the limiting order of deficiency, the fourth limiting AA becomes more sensitive to the overall allowances of AA in macro ingredients. For instance, corn-soybean meal all vegetable diets, diets containing animal by-products, or even diets with other cereals such as sorghum have VAL, ILE, TRP or ARG as fourth limiting amino acids [1]. However, actual deficiencies of the fourth limiting amino acid in broiler diets are usually not seen in practical production even when frequent modifications in feed formulation potentially change the dietary AA profile. This is the consequence of CP safety margins adopted during linear formulation, which prevent the appearance of AA deficiencies after THR.

The use of ratios between essential amino acids and lysine has gained importance as a method to supply balanced protein in feeds for broilers. Diets formulated using this principle have been proposed by scientists to optimize animal utilization of dietary protein [2, 3, 4]. The literature has been presenting a progressive number of investigations directed to establish the ideal ratios between essential amino acids and lysine; however, information on this matter regarding the fourth limiting amino acid is still missing.

The use of synthetic amino acids has allowed a significant reduction in the dietary CP contents for broilers along the years. Further reductions are theoretically possible as long as identification of the fourth limiting amino acid is routinely performed as ingredients change during feed formulation.

Recent publications have indicated that ILE and VAL in broiler feeds are optimized at the ratio to LYS of 65 and 78%, when corn-soybean meal diets containing

blood cells or corn-peanut meal were respectively used [5, 6]. The use of all vegetable diets to feed animals destined for human consumption has been mandatory in some countries for a few years [7]. Presently, a considerable amount of broiler feeds produced in the world towards these markets is based on corn and soybeans as the only sources of protein besides synthetic amino acids. This type of diet has VAL and ILE as fourth and fifth limiting amino acids [1, 8, 9]. However, differences between supplemental needs of these amino acids are very small. Since VAL and ILE are branched chain amino acids, which present a known antagonism in broilers, excesses of one that can negatively affect the other can potentially occur if requirements of both are not assessed simultaneously. The objective of this investigation was to evaluate the performance and carcass yields of broilers fed all vegetable diets based on corn and soybean meal supplemented with VAL and ILE, individually or combined, simulating the practical feed formulation presently done for the international market requiring all vegetable feeds.

MATERIALS AND METHODS

Broiler Husbandry

One thousand five hundred and seventy-five one-day-old Cobb X Cobb 500 male broiler chicks were placed in 63 pens, 1.70 x 1.65 m each. Chicks averaged 45 ± 0.42 g and were previously vaccinated for Infectious Bronchitis and Marek's disease at the hatchery. Temperature was 32 °C at placement, being reduced with age to provide comfort temperature throughout the study. Lighting was continuous until 10 d of age with natural day light to provide 12h of light thereafter. Water and feeds were provided *ad libitum*. All birds were fed the same commercial crumbled starter diet from 1 to 14 d (3,000 kcal/kg ME; 23.3% CP; and 1.27, 1.07, 0.82, 0.93, and 0.87% of dig LYS,

MET+CYS, THR, VAL, and ILE, respectively). Amino acid analyses were conducted for corn and soybean meal prior to feed formulation using High Performance Liquid Chromatography auto analyzer according to 914.12 AOAC Official Method [10]; and Near Infrared Spectroscopy System for CYS and TRP determination.

Experimental Diets

Treatments were applied from 14 to 35 d of age. A basal diet was formulated to attain the lowest digestible ILE to LYS ratio without a CP minimum value restriction and also without synthetic AA supplementation other than DL-MET, L-LYS and L-THR. Digestible LYS was fixed at 1.10% and ratios between essential AA and digestible LYS were as shown in Table 1. These ratios were based on Rostagno et al. [4], with exception of MET+CYS which was based on the Illinois University recommendation [2]. Through this feed formulation, ratios of VAL and ILE to LYS of 70 and 65% were obtained, whereas all other essential AA requirements were attended or slightly excessive. Six other treatments were then produced with graded increases of 5 and 3 percentage points in the ratios of VAL and ILE to LYS, respectively. These two AA were increased individually or simultaneously using L-VAL and L-ILE at the expense of kaolin [11] as formulated in the basal feed. Therefore, supplementations of VAL and ILE produced treatments with simultaneous ratios of VAL and ILE to LYS of 75 and 65, 80 and 65, 70 and 68, 70 and 71, 75 and 68, 80 and 71%, respectively.

Measurements

Feed intake, body weight gain and feed conversion corrected for the weight of dead birds were weekly evaluated. At 35 d of age, ten birds per pen were randomly taken from each pen following an 8h fasting and being then individually weighted.

Birds were then stunned by electric narcosis, bled for 3 minutes through a jugular vein cut, scalded at 60 °C for 45 seconds and de-feathered. Birds were manually eviscerated and then chilled in slush ice for 3 h and hung for 3 min to draw off water excess. Carcasses were then weighed and abdominal fat was collected and weighed. Commercial cuts were performed by skilled industry personnel producing deboned breast meat, thighs, drumsticks, wings, and cages, which were immediately weighed. Carcass yield was expressed relatively to live body weight whereas abdominal fat and cuts were expressed relatively to carcass weight. Birds were not submitted to any unnecessary discomfort.

Statistical Analyses

Seven treatments with nine replications were distributed in a randomized complete block design. The experimental unit was a pen with 25 birds and pen location was the blocking factor. Data were submitted to analysis of variance at a 5% of significance using the GLM procedure of SAS [12]. Significant differences were separated using Tukey test ($P < 0.05$). Mortality data were submitted to arcsine transformation prior statistic analysis.

RESULTS AND DISCUSSION

Resulting dietary treatments demonstrated slight deviations between analyzed and expected AA. These differences were considered acceptable since analyzed values followed expected trends for each treatment (Table 2).

Live performance results are shown in Table 3. From the body weight gain data, it was observed that the diets without VAL and ILE supplementation produced lower numerical means, which were statistically different ($P < 0.05$) from VAL and ILE supplementation at 75 and 68% ratios to LYS from 14 to 21 d but also to any level of supplementation of both AA from 21 to 28 d. There were no differences in the last week of observation, but the accumulated body weight gain from 14 to 35 d showed benefits of VAL or ILE ratios to LYS individually added to the feeds at 75 and 65, and 70 and 68%, respectively. Feed conversion from 14 to 21 d, was worse when not supplemented with VAL and supplemented at the ratio of 71% ILE. On the overall period, feed intake and feed conversion were not affected by the treatments, whereas body weight gain showed improvements when any supplementation of VAL or ILE was used.

Carcass evaluation performed at 35 d was not able to detect any differences due to the treatments. Feather abnormalities were not observed and leg problems were not related with the treatments ($P > 0.05$).

Data presented in this study showed supplementation effects of VAL and ILE in body weight gain and feed conversion; however, they failed to present any improvement in carcass evaluation. Requirements for maximum gain are generally lower than those for breast meat yield, which is lower than the requirement for feed conversion; and lastly, the requirement for minimum abdominal fat percentage is the highest [13].

However, this order of responses may be dependent of the studied AA, as shown by Schutte and Pack [14] for sulfur amino acids. Broiler performance and carcass

composition responses are expected to be less affected when AA supplementation becomes increasingly apart from MET and lysine. Muscle synthesis is not dependent of VAL and ILE compared to LYS and MET. Kidd et al. [15] related an increase in breast meat yield ($P < 0.08$) when branched chain amino acids were increased from deficient to adequate levels. In the present study, the impact of supplementing either VAL or ILE, were similar and without additive effects. Besides, responses were already maximized at the first level of supplementation.

Valine has been previously shown to be first limiting when compared to ILE in diets based on corn and soybean meal [6, 8, 9]. It is generally expected that increases in one essential amino acid leads to the need of further increasing the other essential AA to avoid limitation in animal protein synthesis and, therefore, limitation in growth. In the present study, VAL could have been limiting when ILE was increased, which then explains the worse feed conversion from 14 to 21 days when 70 and 71 VAL and ILE to LYS digestible ratios were used. In parallel, the lack of additive effects for VAL and ILE concomitantly supplemented, as well as discrepancies with higher levels of supplementation can also be attributed to interactions between branched-chain amino acids. Since VAL and ILE are branched chain amino acids, which present a known antagonism in broilers, individual excesses can negatively affect their counterpart [16, 17, 18]. In the case of VAL and ILE in corn-soybean meal all vegetable diets, this issue becomes relevant because these AA are almost co-limiting, i.e., they appear as fourth and fifth limiting at about the same dietary protein level.

Abnormalities on legs and feather have been related in the past when broilers were fed under the requirement [19, 20, 21, 22]. Severities of VAL deficiency in those early studies were likely to be more extensive than those in this work and, therefore, could explain the unnoticeable appearance of these characteristics in the present study.

Imbalances of AA in diets lead to abdominal fat increases in broilers and rats [23, 24]. However differences for this characteristic were not detected at levels studied in this work. The range of variation of VAL and ILE from the control diet to the highest supplementations was not capable of inducing such a change in the evaluated carcasses in this study.

Previous results or recommendations for VAL and ILE ratios to LYS for broilers have been based in diets formulated with a varied range of ingredients and studying individual VAL or ILE increases. In the present study, not only individual as well as associated supplementations of these two AA were evaluated. This fact associated to the natural interactions between them makes it more difficult to interpret the results. Early recommendations of VAL and ILE from NRC [25] allow present calculations of total ratios to LYS of 82% for VAL and 73% for ILE in diets from 21 to 42 d of age. Mack et al. [26] showed optimum responses with ideal VAL to LYS ratio of 81% and ILE to LYS of 71% for broilers from 20 to 40 d of age. Baker et al. [3] recommended ideal ratios of VAL to LYS and ILE to LYS of 77.5 and 61.4%, respectively. Rostagno et al. [4] currently suggest digestible ratios of VAL to LYS of 77 and ILE to LYS of 67% from 21 to 42 d and Corzo et al. [6] attributed optimum performance responses to VAL to LYS ratio of 78% from 21 to 42 days. Responses evaluated in the present study indicate that VAL and ILE ratios of 75 and 65%, respectively were able to optimize broiler performance but without affecting carcass characteristics. Reductions in protein towards deficiencies of VAL and ILE, lead to a parallel reduction in LEU, also a branched chain AA. Therefore, VAL and ILE increases from supplemented sources can potentially interact with LEU and affect protein synthesis.

CONCLUSIONS AND APPLICATIONS

1. Supplementation of VAL or ILE positively influenced broiler live performance responses.
2. VAL and ILE were both limiting in the test diets to support body weight gain.
3. In general, digestible ratios of VAL and ILE to LYS of 70 and 65% were sufficient for most parameters in Cobb 500 birds from 14 to 35 d of age, but to maximize body weight gain a VAL and ILE to LYS ratios of 75 and 65 or 70 and 68% must be provided, respectively.

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Table 1. Basal experimental diet composition (as-fed)

Ingredients	%
Corn, 7.5%	62.72
Soybean meal, 46%	28.70
Soybean oil	3.61
Dicalcium phosphate	1.90
Limestone	1.27
Sodium bicarbonate	0.41
Vitamin and mineral premix ^a	0.26
Common salt	0.20
Choline chloride, 75%	0.07
DL-Methionine, 99%	0.32
L-Lysine HCl, 78%	0.25
L-Threonine, 98%	0.10
Kaolin ^b	0.19
Calculated composition	
Apparent metabolizable energy, kcal/kg	3,100
Crude protein, %	18.70
Dig. LYS, %	1.10
Ca, %	0.98
Av. P, %	0.48
Na, %	0.21
Choline, ppm	1,700
Dig. AA to LYS, %	
MET+CYS	75
THR	65
VAL	70
ILE	65
ARG	106
TRP	18
His	42
LEU	142
Phe+Tyr	134

^a Supplied per kg of feed: Vitamin A: 8,000 IU; Vitamin D3: 2,000 IU; Vitamin E: 30 IU; Vitamin K: 2 mg; Thiamine: 2 mg; Riboflavin: 6 mg; Pyridoxine: 2.5 mg; Cyanocobalamin: 0.012 mg; Pantothenic acid: 15 mg; Niacin: 35 mg; Folic acid: 1 mg; Biotin: 0.08 mg; Fe: 40 mg; Zn: 80 mg; Mn: 80 mg; Cu: 10 mg; I: 0.7 mg; Se: 0.3 mg; Sodium monensin (CobanTM 40%, Elanco Animal Health, Division of Eli Lilly and Company): 0.275 g

^b Other treatments were composed by partial or total kaolin replacement with L-VAL 99% and/or L-ILE 99%

Table 2. Calculated and analyzed crude protein and total amino acids values of experimental diets, %

Calculated	Dig. AA to LYS, %						
	VAL 70 ILE 65	VAL 75 ILE 65	VAL 80 ILE 65	VAL 70 ILE 68	VAL 70 ILE 71	VAL 75 ILE 68	VAL 80 ILE 71
Crude protein	18.70	18.75	18.79	18.72	18.75	18.77	18.84
Methionine+Cystine				0.91			
Lysine				1.17			
Threonine				0.80			
Valine	0.86	0.92	0.98	0.86	0.86	0.92	0.98
Isoleucine	0.77	0.77	0.77	0.80	0.84	0.80	0.84
Arginine				1.21			
Tryptophan				0.22			
Leucine				1.59			
Analyzed							
Crude protein	18.50	18.00	18.60	19.10	18.90	19.00	18.60
Methionine+Cystine	0.91	0.92	0.90	0.92	0.89	0.90	0.94
Lysine	1.16	1.20	1.17	1.20	1.21	1.19	1.19
Threonine	0.78	0.81	0.79	0.82	0.78	0.80	0.80
Valine	0.86	0.90	0.99	0.87	0.87	1.00	0.94
Isoleucine	0.77	0.75	0.79	0.80	0.84	0.79	0.80
Arginine	1.22	1.21	1.25	1.27	1.27	1.27	1.19
Leucine	1.60	1.55	1.61	1.58	1.59	1.60	1.52

Table 3. Feed intake, body weight gain and feed conversion ratio of male broilers fed diets having different valine and isoleucine to lysine digestible ratios from 14 to 35 days of age

Digestible ratios to LYS, %		Feed intake, g				Body weight gain, g				Feed conversion			
VAL	ILE	14-21d	21-28d	28-35d	14-35d	14-21d	21-28d	28-35d	14-35d	14-21d	21-28d	28-35d	14-35d
70	65	719	1,096	1,511	3,323	484 ^b	712 ^b	889	2,084 ^b	1.486 ^{ab}	1.541	1.700	1.595
75	65	725	1,127	1,564	3,410	501 ^{ab}	751 ^a	891	2,142 ^a	1.449 ^a	1.502	1.756	1.592
80	65	737	1,132	1,539	3,403	502 ^{ab}	756 ^a	875	2,133 ^{ab}	1.466 ^{ab}	1.498	1.761	1.595
70	68	736	1,143	1,541	3,418	502 ^{ab}	754 ^a	888	2,144 ^a	1.467 ^{ab}	1.517	1.736	1.595
70	71	744	1,118	1,543	3,402	495 ^{ab}	752 ^a	889	2,137 ^{ab}	1.503 ^b	1.486	1.737	1.592
75	68	736	1,138	1,526	3,397	508 ^a	740 ^a	875	2,123 ^{ab}	1.449 ^a	1.539	1.745	1.600
80	71	724	1,111	1,527	3,353	489 ^{ab}	744 ^a	890	2,123 ^{ab}	1.481 ^{ab}	1.495	1.716	1.580
S.E.M.		3.05	4.78	6.40	10.82	1.99	3.49	3.42	5.44	0.005	0.006	0.008	0.003
P<		0.32	0.07	0.50	0.16	0.01	0.01	0.77	0.05	0.02	0.08	0.41	0.71

^{a, b} Means with different letters in the same column are different (Tukey, P≤0.05)

Table 4. Carcass, abdominal fat, and commercial cuts yields of male broilers with diets varying valine and isoleucine to lysine ratios at 35 days of age, %

Digestible ratios to LYS, %		Carcass	Abdominal fat	Breast meat ¹	Leg quarters ²	Wings
VAL	ILE					
70	65	74.7	1.88	29.8	32.3	11.1
75	65	74.1	1.89	29.2	32.8	11.0
80	65	74.4	1.82	29.4	32.5	11.1
70	68	74.8	1.80	29.3	32.7	11.0
70	71	74.3	1.88	29.1	32.3	11.0
75	68	74.2	1.87	29.4	32.5	11.1
80	71	74.1	1.91	29.2	32.5	11.1
S.E.M.		0.70	0.186	0.52	0.46	0.21
<i>P-value</i>		0.18	0.87	0.10	0.32	0.69

¹ *Pectoralis major* plus *P. minor*

² Thighs plus drumsticks

CAPÍTULO III¹

¹ Artigo submetido à revista Poultry Science em Fevereiro de 2010

Digestible Valine Requirements of Cobb × Cobb 500 Male Broilers From Twenty-One to Forty-Two Days of Age

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ABSTRACT

Valine is the fourth limiting amino acid (AA) for broilers fed diets having ingredients from vegetable origin. The present study evaluated live performance and processing yields of broilers fed corn-soybean meal diets with graded levels of L-VAL. One thousand five hundred and seventy-five male Cobb × Cobb 500 broilers were fed a corn-soybean meal diet with 23.2% CP; 1.25 % digestible (dig) LYS; 0.94% dig VAL; 0.92% Ca; and 0.46% non-pyrate P, from placement to 21 d of age. At 21 d of age, broilers were fed 7 experimental diets until 42 d of age with 9 replicate pens (25 birds per pen). The basal diet was formulated to contain 0.64% dig VAL and were all other essential AA at least 105% of their requirements. Valine was added to the basal diet at the expense of cornstarch to create 7 digestible VAL concentrations ranging from 0.64 to 0.98% in increments of 0.06%. The basal diet negatively affected body weight gain (BWG), and feed conversion ratio (FCR). No differences among dietary treatments were observed for parts yields. Digestible VAL requirements were 0.82 and 0.81, and 0.81 and 0.76 for BWG and FCR, respectively when using regression and broken-line models, respectively.

INTRODUCTION

Dietary amino acid (AA) supplements (MET, LYS, and THR) are used extensively in broiler production to lower diet cost without compromising the efficiency of growth. The inclusion of VAL to diets fed to broilers may further reduce production costs without altering performance objectives. Valine is considered the 4th limiting AA for broilers fed diets containing major ingredients only from vegetable origin (Kidd and Hackenhaar, 2005; Corzo et al., 2007). Valine deficiency results in decreased body weight gain (BWG), poor feed conversion, and reduced meat yields, as well as legs and feathers abnormalities (Anderson and Warnick, 1967; Robel, 1977; Farran and Thomas, 1992a,b; Corzo et al., 2004). Digestible (**dig**) VAL requirements estimates are limited for the modern broiler.

Corzo et al. (2008) evaluated dig VAL requirement of Ross × Ross 308 male broilers from 0 to 42 d of age. Determined dig VAL requirements were 0.91, 0.86, and 0.78% from 0 to 14, 14 to 28, and 28 to 42 d of age, respectively. From 28 to 42 d of age, dig VAL requirements ranged from 0.75 to 0.78% with growth responses having a higher need for VAL than meat yield estimates. Corzo et al. (2007) examined the dig VAL requirement of Ross × Ross 708 male broilers from 21 to 42 d of age and found that the values for BWG ranged from 0.69 to 0.74%. The VAL requirements for increased BWG were higher than those for increased breast meat yield.

Broiler strains have distinct growth curves, and it is expected that they have different nutrient requirements (Dozier et al., 2001, 2002). Studies estimating digestible VAL requirements of modern broilers have been mostly conducted with late-developing strains (Thornton et al., 2006; Corzo et al., 2007, 2008). Supposedly, requirements may be different with early-developing broilers due to differences in feed intake, growth rate,

and carcass yield and composition. The present study was designed to determine the dig VAL requirement of male Cobb × Cobb 500 broilers from 21 to 42 d of age based on growth performance and carcass yields.

MATERIALS AND METHODS

Broiler husbandry

One thousand five hundred and seventy-five one-day-old Cobb × Cobb 500 male broiler chicks were placed in 63 pens (1.70 × 1.65 m) in an open-sided house having built up litter. Pens were equipped with a tube feeder, and a drinker line with 3 nipples. Temperature at placement was 32 °C being reduced at 0.5 °C per day until 23 °C and lighting was continuous to 14 d with natural day light being used thereafter. Water and feed were provided *ad libitum*. Birds were managed according to the directives of Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul.

Treatments

Broilers were fed a common corn-soybean meal mash diet from 1 to 21 d of age (3,020 kcal AME/kg; 23.2% CP; 1.25% dig LYS; 0.94% dig VAL; 0.92% Ca; 0.46% non-phytate P). Amino acid analyses were conducted for corn and soybean meal previously to the feed formulation using High Performance Liquid Chromatography auto analyzer according to 914.12 and crude protein values according to 954.01 Official Methods (AOAC, 1998). A basal diet with 0.33% of cornstarch and a summit diet with 0.33% of L-VAL were manufactured to contain 0.64 and 0.97% of dig VAL, respectively. Synthetic MET, crystalline LYS, THR, ILE, ARG and TRP were added to ensure that performance would not be limited (Emmert and Baker, 1997; Rostagno et al., 2005) (Table 1). These two mash diets were mixed at different proportions to produce other 5 treatments having intermediate VAL concentrations ranging from 0.70

to 0.92%, by 0.06% increments. Composite samples of dietary treatments were submitted to these same analyzes carried out for corn and soybean meal to ensure that formulated diets were in agreement with the calculated values, except to TRP (Table 2).

Measurements

The overall body weight of birds from at 21 d was 1,055 g. Feed intake, BWG, feed conversion ratio (**FCR**) corrected for the weight of dead birds, and the mortality were determined from 21 to 42 d. Feed intake data did not take in consideration any possibly feed wastage. At 42 d, 10 birds were randomly selected from each pen, fasted for 8 h, individually weighed, for on line processing. Slaughter followed electrical stunning at 45 V for 3 s. Birds were then bled for 3 min after jugular vein cut, scalded at 60 °C for 45 s, and then had the feathers plucked. After manual evisceration, the carcasses were chilled in slush ice for 3 h. After chilling, carcasses with abdominal fat, lungs and kidneys, but without viscera and adjacent fats, feets, neck, and head were weighted. Abdominal fat were then removed from carcasses, and weighed. Carcasses were deboned to obtain weights of breast meat with skin (*Pectoralis major* plus *Pectoralis minor* muscles), thighs, drumsticks and wings with bone and skin, and cage.

Statistical analyses

Seven treatments with 9 replications each were distributed in a randomized complete block design. Pen was considered the experimental unit with pen location being the blocking factor. Mortality data were submitted to arcsine transformation prior to statistic analysis. Data were analyzed using the GLM procedure (SAS, 2001). Digestible VAL requirements were determined using: 1) Linear regression analysis when a significant ($P < 0.05$) response occurred 95% of the plateau, and 2) Broken-line methodology (Robbins et al., 2006).

RESULTS AND DISCUSSION

Analyzed Val in the experimental feeds followed a graded increasing sequence, however, Val analyzed values were higher than expected in the three first diets. Therefore, broilers may have received more dig Val than planned and this may explain the results obtained when birds were fed at 0.70% calculated dig Val.

Significant quadratic responses were observed for BWG and FCR (Figure 1 and 2). Estimations of digestible Val requirements following the quadratic equations for body weight gain and feed conversion were: 0.85% [BWG = $-1126.80 (\text{dig Val})^2 + 2021.2892 (\text{dig Val}) + 1246.4463$; $r^2 = 0.89$]; and 0.84% [FCR = $0.70474 (\text{dig Val})^2 - 1.24 (\text{dig Val}) + 2.23534$; $r^2 = 0.91$]. Estimations using the broken-line methodology for the same responses were: 0.81% [BWG = $2150.2 - 408.6 (0.8106 - \text{dig Val})$; $r^2 = 0.92$]; and 0.80% [FCR = $1.6921 + 0.2321 (0.7972 - \text{dig Val})$; $r^2 = 0.88$]. Feed intake, mortality, and carcass yields were not affected by the dietary treatments (Table 3). General means for thighs, drumsticks, wings and cage yields were 12.8, 19.4, 10.3, and 23.4%, respectively.

In the present study, dig Val requirements were observed at 0.85 and 0.84% for BWG and FCR, respectively, but a valid equation to estimate carcass and breast meat yields requirements was not found. Corzo et al. (2007) reported dig Val requirements ranging from 0.69 to 0.74% for Ross 708 broilers from 21 to 42 d of age. Requirement estimates were determined at 0.74, 0.73, and 0.70% for BWG, total breast weight, and total breast yield, respectively. Corzo et al. (2008) reported dig Val requirements at 0.77, 0.78, 0.77, 0.75, and 0.75%, for BWG, feed intake, FCR, carcass weight, and total breast weight, respectively.

Digestible Val requirements for BWG and FCR were higher in the present study than in previous published research (Corzo et al., 2007, 2008). Rostagno et al. (2005)

reported that AA requirements were influenced by broiler growth rate. The BWG from 21 to 42 d of age was approximately 2.1 kg in the current research whereas it was 1.4 kg in the Corzo et al. (2007) study. Different strain crosses show distinct growth curves, and therefore, is expected that it have distinct nutrient requirements. The Cobb × Cobb 500 strain cross have an accelerated growth and respond better than Ross × Ross 308 to the higher dietary AA concentration with a higher breast meat and lower abdominal fat yields (Coneglian et al., 2010). As the plateau in the growth curve is reached is expected that muscle and bone accretion go lower and the fat tissue continues increasing. The increase in carcass fat content affects cuts yields negatively. In this way, for different body weights, differences between post-slaughter measures are expected. However, at a similar body weight, no differences between strain crosses are expected in carcass composition and quality. In this work, it became evident the higher development of birds in relation to this work. Thus, the highest recommendation of dig Val provided in the current study in relation to other researches carried out with Ross strain crosses makes sense. Amino density may also have contributed to differences with dig Val requirement estimates between studies. For example, the current research was designed to determine dig Val requirement, whereas other research was conducted to estimate a dig Val to dig Lys ratio (Corzo et al., 2007, 2008). Diet formulation to determine AA requirements utilizes a dig Lys concentration that will not limit growth performance, whereas designing a ratio study will utilize a dig Lys level that is sub-marginal to ensure that broilers do not over consume Lys so that an accurate ratio can be determined. Although some AA as Met, Lys, and yet Thr frequently exhibit effects on post-slaughter responses, less limiting AA as Val tend to not affect this responses, unless a very low level is used (Leclercq, 1998). This, possibly associated with the higher analyzed values than calculated of the three lower Val levels diets can explain the lack of effects in post-slaughter responses in the present study. In

order, a dig Val level of 0.81% is recommended to maximize male Cobb × Cobb 500 broilers performance from 21 to 42 days of age.

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Table 1. Ingredient and nutrient composition of the experimental diets, % “as-is”¹

Ingredients	Basal		Summit
	Without L-VAL	0.33% of L-VAL	
Corn		67.03	
Soybean meal 45.8% CP		23.91	
Soybean oil		2.97	
Dicalcium phosphate		2.05	
Limestone		0.97	
Sodium bicarbonate		0.73	
Premix ²		0.26	
Choline chloride 75%		0.07	
Salt		0.04	
DL-MET 99%		0.42	
L-LYS HCl		0.49	
L-THR		0.20	
L-ILE		0.18	
L-ARG		0.31	
L-TRP		0.04	
L-VAL	-		0.33
Cornstarch	0.33		-
Calculated composition			
AME, kcal/kg		3,150	
CP, %	17.57		17.83
Ca, %		0.95	
Non-phytate P, %		0.48	
Na, %		0.23	
Calculated true ileal digestible amino acids, %			
MET+CYS		0.85	
LYS		1.10	
THR		0.74	
VAL	0.64		0.97
ILE		0.76	
ARG		1.16	
TRP		0.21	
His		0.41	
LEU		1.28	
Phe+Tyr		1.26	
Total GLY+SER		1.42	

¹ Values standing done in the center of the table are common for all diets

² Supplied per kg of feed: Vitamin A: 8,000 IU; Vitamin D₃: 2,000 IU; Vitamin E: 30 IU; Vitamin K₃: 2 mg; Thiamine: 2 mg; Riboflavin: 6 mg; Pyridoxine: 2.5 mg; Vitamin B₁₂: 0.012 mg; Pantothenic acid: 15 mg; Niacin: 35 mg; Folic acid: 1 mg; Biotin: 0.08 mg; Fe: 40 mg; Zn: 80 mg; Mn: 80 mg; Cu: 10 mg; I: 0.7 mg; Se: 0.3 mg; Monensin sodium (CobanTM 40%, Elanco Animal Health, Division of Eli Lilly and Company): 0.275 g

Table 2. Calculated and analyzed composition for crude protein and total amino acids of the experiment diets, % “as-is”

CP or total amino acids	Digestible VAL, % ¹						
	0.64	0.70	0.75	0.81	0.86	0.92	0.97
	Calculated values ²						
CP	17.57	17.61	17.66	17.70	17.75	17.79	17.83
MET+CYS				0.90			
LYS				1.17			
THR				0.82			
VAL	0.72	0.78	0.83	0.89	0.94	1.00	1.05
ILE				0.82			
ARG				1.21			
TRP				0.23			
LEU				1.38			
	Analyzed values						
CP	17.43	17.56	17.73	17.78	17.87	17.90	17.97
MET+CYS	0.99	1.06	1.01	0.99	1.06	1.09	1.07
LYS	1.18	1.17	1.17	1.15	1.18	1.17	1.13
THR	0.83	0.81	0.78	0.79	0.78	0.79	0.78
VAL	0.80	0.83	0.87	0.89	0.96	1.00	1.05
ILE	0.84	0.88	0.86	0.82	0.81	0.82	0.82
ARG	1.43	1.37	1.38	1.37	1.32	1.34	1.34
LEU	1.45	1.46	1.43	1.39	1.39	1.38	1.37

¹Obtained values correspond to total AA analyzed in ingredients multiplied by the digestibility coefficients presented by Rostagno et al. (2005)

²Values standing alone in the center of the table are common for all diets

Table 3. Performance of Cobb × Cobb 500 broiler males fed graded increases on the VAL to LYS ratio from 21 to 42 d and post-slaughter yields at 42 days of age

Digestible VAL level, %	BWG ¹ , g/bird	FCR ² , g/g	Feed intake, g	Mortality, %	Carcass and parts yields, %		
					Carcass	Breast meat	Abdominal fat
0.64	2,081 ^b	1.733 ^b	3,607	0.67	78.59	31.64	2.10
0.70	2,111 ^{ab}	1.704 ^{ab}	3,610	1.00	78.62	31.46	1.94
0.75	2,112 ^{ab}	1.709 ^{ab}	3,610	1.11	78.74	31.92	1.83
0.81	2,156 ^a	1.691 ^a	3,646	1.22	79.14	31.67	1.86
0.86	2,159 ^a	1.691 ^a	3,650	0.56	78.78	31.30	1.88
0.92	2,143 ^{ab}	1.691 ^a	3,624	1.00	78.68	31.32	1.85
0.97	2,149 ^{ab}	1.695 ^a	3,642	0.67	79.02	31.54	1.80
Pooled SEM	7.37	0.003	11.20	0.12	0.11	0.09	0.03
ANOVA <i>P</i> -value	0.02	0.01	0.77	0.74	0.81	0.62	0.13
Regressions							
Linear <i>P</i> -value	0.02	0.03	-	-	-	-	-
Linear <i>r</i> ² -value	0.70	0.64	-	-	-	-	-
Quadratic <i>P</i> -value	0.01	0.01	-	-	-	-	-
Quadratic <i>r</i> ² -value	0.89	0.91	-	-	-	-	-
Broken-line <i>r</i> ² -value	0.01	0.01	-	-	-	-	-
	0.92	0.88	-	-	-	-	-
Requirements, %							
Quadratic regression ³							
Broken-line	0.85	0.84	-	-	-	-	-

¹ Body weight gain

² Feed conversion ratio

³ 95% of the maximum or minimum value

Figure 1. Body weight gain of male broilers fed diets varying in digestible VAL from 21 to 42 d of age. Digestible VAL requirement was determined at 0.81% by broken-line analysis [BWG = 2,146 – 305.8 (0.81 – dig VAL level); $r^2 = 0.55$]. Quadratic regression [BWG = –1,185.19497 (dig VAL level)² + 2,048.06293 (dig VAL level) + 1,264.79868; $r^2 = 0.56$] estimated the dig VAL requirement at of 0.86% to maximize body weight gain, with 95% of this value (upper value) being 0.82%

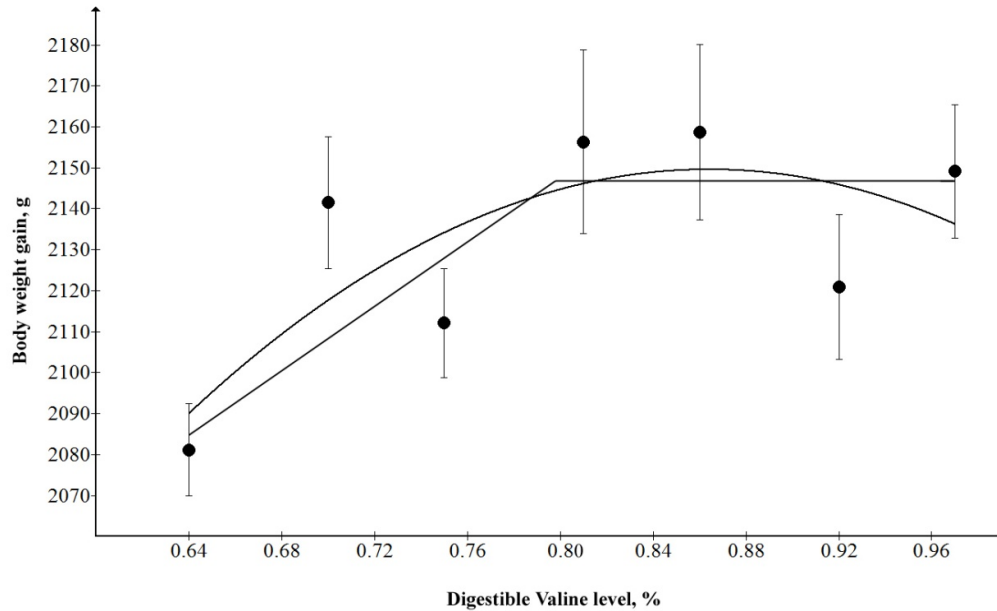
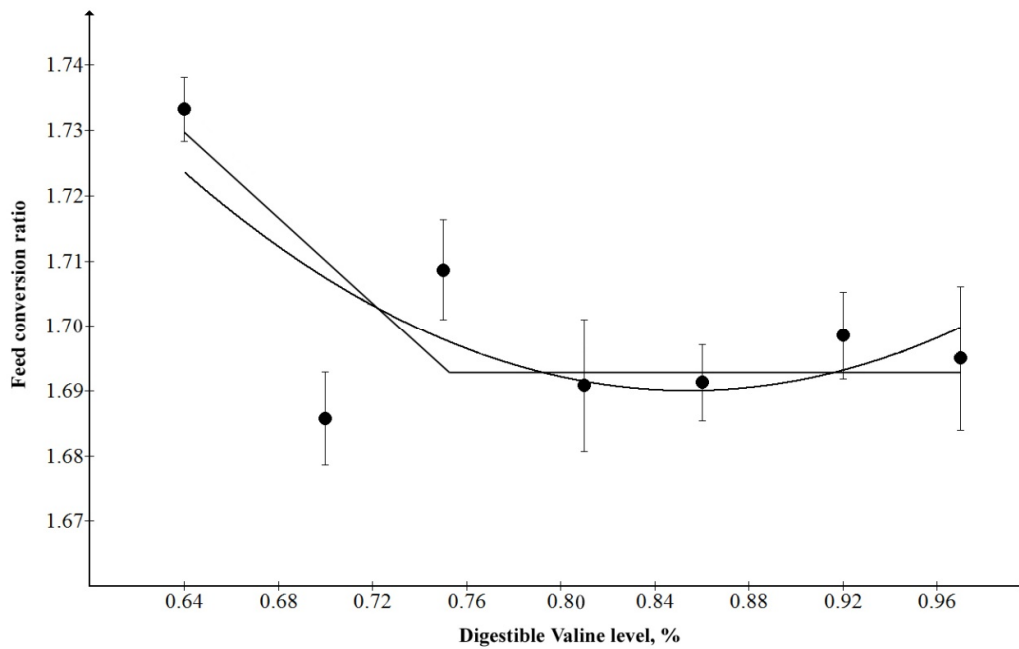


Figure 2. Feed conversion ratio of male broilers fed diets varying in digestible VAL from 21 to 42 d of age. Digestible VAL requirement determined at 0.76% by broken-line analysis [$FCR = 1.6939 + 0.2423 (0.7598 - \text{dig VAL level})$; $r^2 = 0.48$]. Quadratic regression [$FCR = 0.73365 (\text{dig VAL level})^2 - 1.25343 (\text{dig VAL level}) + 2.22534$; $r^2 = 0.53$] estimated the dig VAL requirement at 0.85% to maximize FCR, with 95% of this value (lower value) being 0.81%



CAPÍTULO IV¹

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**Broiler Responses to Reduced Protein Diets Supplemented with
Valine, Isoleucine, Glycine and Glutamic Acid**

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Running title: VAL, ILE, GLY, and GLU in broiler diets

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SUMMARY

Reduced performance is eventually observed when broilers are fed low protein diets even when industrial AA are provided to reach requirement levels. The reason for this detrimental effect is not precisely determined, but it is possible that non essential AA become limiting. The objective of this study was to evaluate the effects of individual or combined VAL, ILE, GLY and GLU supplementations at the same levels of these AA or CP content of a practical diet on live performance and meat yields of broilers. Two thousand and sixteen Ross × Ross 308 male broilers were fed 8 treatments with 9 replications (28 birds per pen) during a 42 d production period. Overall care of animals complied with animal welfare directives from Universidade Federal do Rio Grande do Sul. The control diet (Treatment 1) was formulated without CP restriction and maintaining a GLY + SER to LYS ratio of 155 or 158%; Other treatments, when not supplemented with L-GLU, had the CP content dependent of the AA to LYS fixed ratios in formulation, and this generated diets with lower crude protein contents than the control diet. Treatment 2 was formulated with L-VAL; Treatment 3 was formulated with L-VAL and GLY; Treatment 4 was formulated with L-VAL + L-GLU; Treatment 5 was formulated with L-VAL + GLY + L-GLU; Treatment 6 was formulated with L-VAL + L-ILE; Treatment 7 was formulated with L-VAL + L-ILE + GLY; Treatment 8 was formulated with L-VAL + L-ILE + GLY + L-GLU. Dietary CP varied with feeding phase and treatment, but reached 2.5 percentage points as the greatest obtained reduction. Glycine and GLU supplementation improved BW gain and feed conversion. Benefits in growth due to GLY supplementation were mainly observed in early phases of growth. Conversely, broilers fed diets supplemented with GLU exhibited advantages in growth throughout experimentation. Additions of ILE, GLU, and GLY increased breast meat yield

compared with broilers fed diets supplemented with only L-VAL. Isoleucine supplementation improved breast meat yield. Good broiler growth and meat yield can be obtained with GLY and GLU supplementation in reduced CP diets, which infers a need for nitrogen for the synthesis of non-essential amino acids.

Key words: amino acid, broiler growth, crude protein

DESCRIPTION OF PROBLEM

Investigations conducted with low CP diets fed to broilers have generally led to poor performance when comparison is made with broilers having adequate amino acids (AA) and non-essential nitrogen [1-3]. However, when formulation is based on an AA basis [4], a reasonable reduction in CP may produce acceptable growth performance [5]. Commercial availability of industrial AA has reduced dietary cost without compromising broiler performance. Threonine usage in broiler diets is a common practice and its inclusion is often dictated by corn, soybean meal, and fat prices. Diet costs can be eventually reduced with L-THR supplementation when large price spreads occur between soybean meal and fat.

L-Valine supplementation could potentially provide further reductions in diet cost without negatively influencing broiler performance. The order of limitation with VAL and ILE is dependent on ingredients used in diet formulation [6,7]. For example, VAL is the fourth limiting AA with corn and soybean meal being the primary ingredients [6, 8,9], whereas when animal protein meals are included in the diet VAL and ILE may be co-limiting [10]. L-Valine supplementation has not yielded strong evidence to provide it as a commercially available supplement [11]. Inadequacy of non-essential AA may limit performance when low CP diets containing L-VAL are fed.

Glycine has been shown to limit broiler performance in starter diets formulated without animal by-products [12] because its rate of endogenous synthesis cannot support maximum growth [13]. Glycine functions to support synthesis of uric acid and excretion of nitrogen [14,15]. The requirement for GLY seems to be higher in low CP diets than in high CP diets [16,17]. Glutamic acid has been included in the diet to provide a nitrogen source for synthesis of non- essential AA. However, most of

the studies with GLU addition with low CP diets have not been successful in reproducing similar performance compared with broilers fed diets having higher CP [1,17,18].

Research is warranted on the feasibility of L-VAL supplemented diets on broiler growth and meat yield. The addition of other than the three first limiting AA to corn-soybean meal diets containing L-VAL may improve performance of broilers. The objective of this study was to evaluate the effects of individual or combined VAL, ILE, GLY and GLU supplementations at the same levels of these AA or CP content of a practical diet on live performance and meat yields of broilers.

MATERIALS AND METHODS

Bird Husbandry

Two thousand and sixteen 1-d-old Ross × Ross 308 male broiler chicks were randomly distributed in 72 pens (1.70 × 1.65 m; 28 birds per pen). Each pen had reused pine shavings after one flock and was equipped with 1 tube feeder and 3 nipple drinkers. Chicks were vaccinated for Infectious Bronchitis and Marek's disease at the hatchery. Temperature was maintained at 32 °C at placement and gradually reduced to ensure comfort using a thermostatically controlled heater, fans and foggers. Birds were reared under a continuous lighting program from placement to 14 d and then it was gradually reduced to a 12:12 h L:D lighting schedule. Feed was provided in mash form and birds had free access to feed and water. Amino acid analyses were conducted for corn and soybean meal prior to feed formulation and on samples of experimental diets using accepted methodology [19]. Digestible AA values for corn and soybean meal were calculated using digestibility coefficients [20] and analyzed

total AA composition. Calculated digestible AA values for corn and soybean meal were used in diet formulation.

Treatments

Treatments were distributed in a 4 phase feeding program: 1 to 7, 8 to 21, 22 to 35 and 36 to 42 d of age. A corn-soybean meal all vegetable control diet (Treatment1) was formulated without CP restriction to meet or exceed Ideal AA recommendations [20]. Treatments 2 to 8 were formulated with individual or combined inclusions of industrial VAL, ILE, GLY and/or GLU to meet these same AA and/or CP levels of the control diet as showed in Table 1. These treatments, when not supplemented with L-GLU, had the CP content dependent of the AA to LYS fixed ratios in formulation, and this generated diets with lower crude protein contents than the control diet. Ingredient and nutrient composition of experimental diets are provided in Tables 2, 3, 4 and 5. These diets were individually mixed in 350 kg batches in a horizontal mixer, afterwards minor ingredients and corn pre-mix in 50 kg batches. Similar analyses were conducted on all experimental diets. Calculated and analyzed total AA and CP of diets were in good agreement (Table 6).

Measurements

Birds were not submitted to any unnecessary discomfort and followed directives of Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul.

Body weight gain, feed intake and feed conversion were determined from 1 to 7, 7 to 21, 21 to 35 and 35 to 42 d of age. Mortality was recorded daily and causes of death were classified as sudden death, ascites, leg abnormalities and other. At 42 d, 10 birds per pen were randomly selected and subjected to an 8 h pre-slaughter fasting but

had access to water. Subsequently, birds were individually weighed, hung on shackles, stunned by electric narcosis, bled for 3 min through a jugular vein cut until death, scalded at 60 °C for 45 s and then de-feathered. Processing followed with manual evisceration and chilling whole carcasses in slush ice for 3 h after which carcasses were hung for 3 min to remove water excess. Carcasses and abdominal fat were weighed. Then, carcasses were deboned by industry personnel to obtain breast meat (*Pectoralis major* plus *Pectoralis minor*), thighs, drumsticks, wings and cage weights. Yields of breast meat (*Pectoralis major* plus *Pectoralis minor* muscles), thighs, drumsticks, wings and cage were determined. Carcass weights were expressed as a proportion to live BW whereas meat parts were expressed relative to the whole carcass.

Statistical Analysis

Data were evaluated by the analysis of variance procedure using a randomized complete block design [21] with pen location within the experimental house being the blocking factor. Eight treatments with 9 replications were used and each pen was an experimental unit. Significant differences were separated using Tukey's multiple range tests. Mortality data were subjected to arcsine transformation for statistic analysis. Statistical significance was considered $P \leq 0.05$.

RESULTS

Live Performance

Live performance data is presented on Table 7. From 1 to 7 d of age, only broilers fed reduced CP diets supplemented with VAL and ILE had lower ($P \leq 0.05$) BW gain compared to broilers provided diets supplemented with VAL, VAL + GLY,

VAL + GLU, VAL + ILE + GLY and VAL + ILE + GLY + GLU, but had the same BW compared with broilers fed the control diet or the diets supplemented with VAL and VAL + GLY + GLU. Dietary treatments did not affect feed intake. Broilers fed diets supplemented with VAL + GLU, VAL + ILE + GLY, and VAL + ILE + GLY + GLU had better ($P \leq 0.05$) feed conversion compared with birds receiving diets containing VAL + ILE which had a similar feed conversion of birds fed the control diet and the diets supplemented with VAL + GLY and VAL + GLY + GLU.

From 1 to 21 d of age, dietary treatments did not alter BW gain or feed intake. Diets having added VAL + ILE + GLY exhibited similar feed conversion to the VAL + ILE treatment, which is in contrast to the previous period. Only birds fed diets supplemented with VAL and VAL + ILE + GLY had a similar feed conversion provided by the diet VAL + ILE. Other treatments had better feed conversions in relation to this diet. Broilers fed low CP diets supplemented with VAL, VAL + GLY, VAL + GLU, VAL + GLY + GLU, VAL + ILE + GLY and VAL + ILE + GLY + GLU had a similar feed conversion compared with the bird fed the Control diet supplemented with MET, LYS and THR.

From 1 to 35 and from 1 to 42 d of age dietary treatments did not alter BW gain or feed intake. Broilers fed diets supplemented with VAL + ILE and VAL + ILE + GLY had poorer feed conversion compared with the Control treatment or VAL + GLU fed birds from 1 to 35 d of age. Broilers fed low CP diets supplemented with VAL, VAL + GLY, VAL + GLU, VAL + GLY + GLU and VAL + ILE + GLY + GLU had a similar feed conversion compared with the bird fed the Control diet supplemented with MET, LYS and THR. Broilers fed the Control diet, VAL + GLU and VAL + ILE + GLY + GLU supplemented diets had improved feed conversion compared with diets supplemented with VAL + ILE in birds from 1 to 42 d of age

whatever did not differ from the broilers fed with diets containing VAL, VAL + GLY, VAL + GLY + GLU or VAL + ILE + GLY.

Dietary treatments did not influence the incidence of mortality (Table 8).

Carcass measurements

Dietary treatments did not affect carcass, wings or cage yields (Table 9). Only broilers fed the VAL + ILE + GLY + GLU supplemented diet had lower abdominal fat percentage than birds fed diets having added VAL + ILE. All other treatments resulted in similar abdominal fat percentage compared with VAL + ILE + GLY + GLU or VAL + ILE. Supplementing the diet with VAL + ILE + GLY + GLU increased breast meat yield compared with broilers fed diets having only added VAL, but the birds fed this diet had similar breast meat yield compared with all other treatments. All other treatments resulted in equal breast meat yield compared with birds fed diets supplemented with VAL + ILE + GLY + GLU. In contrast, birds fed diets supplemented with VAL and VAL + GLY had similar leg quarter yields to broilers given the control diet. Broilers receiving control diet had higher leg quarters yield than all treatments except those birds fed VAL or VAL + GLY supplemented diets.

DISCUSSION

Adding industrial MET, LYS and THR without fixing CP level on formulation lowers concentration of less limiting AA which can result in sub-marginal performance. This occurs due to the reduction of the intact protein source in diet. The next limiting AA should determine the level of CP. The reduction in CP and less limiting AA with the diet supplemented with VAL + ILE treatment led to poor cumulative feed conversion. One possible explanation is that the reduced CP diets did

not contain adequate amounts of non-essential AA or nitrogen. Glycine supplementation can be recommended for the initial stages, i.e. up to 21 d of age based on feed conversion responses. Glutamic acid supplementation produced similar responses with cumulative feed conversion compared with the control fed birds.

Broilers fed the control diet exhibited better feed conversion compared with birds fed diets supplemented with VAL + ILE throughout experimentation but did not differ compared with the other amino acids supplemented diets. Optimum broiler performance is dependent on less limiting AA or non-essential nitrogen, particular for the young chick from 1 to 7 d of age. The amount of less limiting AA and/or non-essential nitrogen affected abdominal fat percentage. Nitrogen excretion is an energy dependent process [22], and broilers fed diets with excess nitrogen will result in less energy for fat deposition [23]. In the present research, the diet supplemented with VAL + ILE did not alter breast meat yield. ILE adequacy is known to influence breast meat yield [7].

Non-essential AA supplementation to low CP diets has been shown not to improve broiler performance [1,24-26]. However, Dean et al. [15] concluded that the addition of GLY to an AA-supplemented 16% CP diet supported growth and feed efficiency equal to broilers fed a 22% CP diet. Corzo et al. [27] also found positive effects with non-essential AA supplementation to diets with reduced CP content. Deschepper and Groote [28] reported that broilers fed a reduced CP diet supplemented with non-essential and essential AA grew as well as those fed a high-CP diet, but not when essential AA were included to the low CP diet. Moran and Stilborn [18] reported a response to GLU supplementation of reduced CP diets for broilers during the starter period. Broiler chicks need both essential AA and non-essential AA supplementation with low CP diets. Glycine may be utilized more

efficiently than GLU [28] when supplemented to low CP diets fed to young chicks. Essentially, differences in evaluated responses attributed to these two amino acids can reside in their metabolic route and in the age of broilers. Glutamic acid is known as a nitrogen source to form other amino acids, whereas its carbon skeleton is utilized to form carbohydrates. However, GLY is directly associated to nitrogen excretion because it is basically utilized in uric acid composition. Therefore, a higher protein diet typically required in the starter phases potentially leads to a higher proportion needs for nitrogen excretion and requires more GLY to form uric acid. This may explain a higher need for GLY in starter phases and the lower requirement of this amino acid in subsequent phases.

Han et al. [29] observed that low protein diets fortified with MET, LYS, THR, VAL, ARG and amino nitrogen from GLU, resulted in bird performance comparable to a high protein diet. In the present research, additions of L-VAL, L-ILE, GLY and L-GLU to the same levels supplied by a practical diet provided similar broiler growth rates. We suggest that GLY and GLU levels were sufficient to meet nitrogen needs for non-essential AA synthesis. Amino acid supplementation of low CP diets has not always resulted in bird performance comparable to those fed higher CP diets [30,31]. Discrepancies between the results may be attributed to the severity of dietary protein reduction, to the age of broilers, to the amino acid basis (as total or digestible) used in formulation and also to dietary designs.

CONCLUSIONS AND APPLICATIONS

1. Broilers fed diets supplemented with industrial amino acids had the same cumulative body weight gain, feed intake and feed conversion, and carcass, wings, cage and abdominal fat compared with the birds fed the Control diet, except the cumulative feed conversion of birds fed diets supplemented with VAL + ILE.
2. Higher cumulative feed conversion of broilers fed diets supplemented only with VAL + ILE was a consequence of poor performance occurring from 1 to 7 and 1

to 21 d of age, which provides evidence for adequate amino nitrogen being needed during the early growth phases.

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Table 1. Outline of dietary treatments (T1-T8) fed to male broilers throughout a 42 d production period¹

AA supplementation	T1	T2	T3	T4	T5	T6	T7	T8
L-VAL	-	+	+	+	+	+	+	+
L-ILE	-	-	-	-	-	+	+	+
GLY	-	-	+	-	+	-	+	+
L-GLU	-	-	-	+	+	-	-	+

¹DL-MET, L-LYS and L-THR were supplemented in all diets; other amino acids shown were supplemented to obtain the same ratio with LYS and/or crude protein of the control diet; - Not supplemented; + Supplemented.

Table 2. Ingredient and nutritional composition of the experimental diets (T1-T8) fed to male broilers from 1 to 7 d of age

Ingredients, %	T1	T2	T3	T4	T5	T6	T7	T8
Corn	44.32	48.53	48.33	45.52	45.67	52.60	52.21	47.11
Soybean meal 48% CP	47.29	43.51	43.53	43.83	43.82	39.81	39.85	40.38
Soybean oil	4.10	3.33	3.38	3.61	3.63	2.52	2.62	3.09
Monocalcium phosphate	1.65	1.66	1.66	1.67	1.67	1.68	1.68	1.69
Calcium carbonate	1.27	1.28	1.28	1.27	1.27	1.29	1.29	1.28
Sodium chloride	0.51	0.42	0.42	0.42	0.42	0.33	0.32	0.32
Sodium bicarbonate	0.01	0.14	0.14	0.15	0.15	0.28	0.28	0.29
Additives ¹	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
DL-MET 99%	0.33	0.36	0.36	0.36	0.36	0.39	0.39	0.40
L-LYS 78%	0.14	0.25	0.25	0.25	0.25	0.36	0.36	0.35
L-THR 98.5%	0.06	0.11	0.11	0.11	0.11	0.16	0.16	0.16
L-VAL 98.5%	-	0.06	0.06	0.06	0.06	0.12	0.12	0.12
L-ILE 98.5%	-	-	-	-	-	0.12	0.12	0.12
GLY 97%	-	-	0.13	-	0.13	-	0.25	0.26
L-GLU 99.4%	-	-	-	2.40	2.12	-	-	4.07
Premixes ²	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Choline 75%	0.02	0.03	0.03	0.03	0.03	0.04	0.04	0.04

Nutrients, energy and electrolytic balance, % or otherwise noted

CP	26.20	24.90	25.04	26.20	26.20	23.70	23.99	26.20
AME, kcal/kg				2,950				
Ca				0.94				
Non-phytate P				0.47				
Na+K-Cl, mEq/kg				250				
Choline, ppm				1,750				
Digestible LYS				1.33				

Digestible amino acid ratios to LYS, %

MET+CYS					71			
THR					65			
VAL					75			
ILE	69	65	65	65	65	69	69	69
TRP	22	21	21	21	21	19	19	19
ARG	120	113	113	113	113	105	105	105
MET	47	48	48	49	48	49	49	50
LEU	133	127	127	126	126	120	120	119
His	44	42	42	42	42	40	40	39
Phe	84	79	79	79	79	74	74	74
Phe+Tyr	144	136	136	135	135	127	127	127
GLY+SER	155	146	155	146	155	138	155	155

¹ Adsorbent (MT.x +, Olmix S.A.): 0.1%; Sodium monensin (CobanTM 40%, Elanco Animal Health, Division of Eli Lilly and Company): 0.0275%; Bacitracin methylene disalicylate (BMD 11%, Alpharma do Brasil Ltda.): 0.05%; Colistin sulphate 8% (Indukern do Brasil Química Ltda.): 0.0125%.

² Supplied per kg of diet: Vitamin A: 9,800 IU; Vitamin D₃: 2,520 IU; Vitamin E: 70 IU; Vitamin K₃: 3 mg; Thiamine: 2.5 mg; Riboflavin: 7 mg; Pyridoxine: 4 mg; Vitamin B₁₂: 0.025 mg; Pantothenic acid: 12 mg; Niacin: 50 mg; Folic acid: 1.5 mg; Biotin: 0.15 mg; Phytase: 0.14 g; Fe: 50 mg; Zn: 50 mg; Mn: 80 mg; Cu: 10 mg; I: 1 mg; Co: 1 mg; Se: 0.3 mg.

Table 3. Ingredient and nutritional composition of the experimental diets (T1-T8) fed to male broilers from 8 to 21 d of age

Ingredients, %	T1	T2	T3	T4	T5	T6	T7	T8
Corn	54.14	57.02	56.88	54.94	55.04	60.35	60.04	56.12
Soybean meal 48% CP	38.48	35.88	35.90	36.10	36.09	32.75	32.79	33.19
Soybean oil	3.18	2.66	2.69	2.85	2.86	2.03	2.10	2.46
Monocalcium phosphate	1.55	1.57	1.57	1.57	1.57	1.58	1.58	1.59
Calcium carbonate	1.22	1.23	1.23	1.22	1.22	1.24	1.24	1.23
Sodium chloride	0.27	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Sodium bicarbonate	0.32	0.41	0.41	0.42	0.42	0.52	0.53	0.53
Additives ¹	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
DL-MET 99%	0.27	0.29	0.29	0.29	0.29	0.31	0.31	0.32
L-LYS 78%	0.16	0.23	0.23	0.23	0.23	0.32	0.32	0.32
L-THR 98.5%	0.06	0.09	0.09	0.09	0.09	0.13	0.13	0.13
L-VAL 98.5%	-	0.04	0.04	0.04	0.04	0.09	0.09	0.09
L-ILE 98.5%	-	-	-	-	-	0.09	0.09	0.09
GLY 97%	-	-	0.09	-	0.09	-	0.19	0.20
L-GLU 99.4%	-	-	-	1.66	1.46	-	-	3.13
Premixes ²	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Choline 75%	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.06
Nutrients, energy and electrolytic balance, % or otherwise noted								
CP	22.66	21.77	21.87	22.66	22.66	20.74	20.96	22.66
AME, kcal/kg					3,000			
Ca					0.88			
Non-phytate P					0.44			
Na+K-Cl, mEq/kg					250			
Choline, ppm					1,700			
Digestible LYS					1.15			
Digestible amino acid ratios to LYS, %								
MET+CYS					71			
THR					65			
VAL					75			
ILE	69	65	65	65	65	69	69	69
TRP	22	21	21	21	21	19	19	19
ARG	119	113	113	113	113	105	105	105
MET	46	47	47	47	47	48	48	49
LEU	137	132	132	131	131	125	125	124
His	45	43	43	43	43	41	41	40
Phe	84	80	80	80	80	75	75	75
Phe+Tyr	144	138	138	137	137	129	129	129
GLY+SER	155	148	155	148	155	139	155	155

¹ Adsorbent (MT.x +, Olmix S.A.): 0.1%; Sodium monensin (Coban™ 40%, Elanco Animal Health, Division of Eli Lilly and Company): 0.0275%; Bacitracin methylene disalicylate (BMD 11%, Alpharma do Brasil Ltda.): 0.05%; Colistin sulphate 8% (Indukern do Brasil Química Ltda.): 0.0125%.

² Supplied per kg of diet: Vitamin A: 9,800 IU; Vitamin D₃: 2,520 IU; Vitamin E: 70 IU; Vitamin K₃: 3 mg; Thiamine: 2.5 mg; Riboflavin: 7 mg; Pyridoxine: 4 mg; Vitamin B₁₂: 0.025 mg; Pantothenic acid: 12 mg; Niacin: 50 mg; Folacin: 1.5 mg; Biotin: 0.15 mg; Phytase: 0.14 g; Fe: 50 mg; Zn: 50 mg; Mn: 80 mg; Cu: 10 mg; I: 1 mg; Co: 1 mg; Se: 0.3 mg.

Table 4. Ingredient and nutritional composition of experimental diets (T1-T8) fed to male broilers from 22 to 35 d of age

Ingredients, %	T1	T2	T3	T4	T5	T6	T7	T8
Corn	55.06	57.41	57.30	55.66	55.74	61.97	61.63	57.18
Soybean meal 48% CP	36.56	34.38	34.39	34.56	34.55	30.10	30.14	30.60
Soybean oil	4.50	4.08	4.11	4.25	4.26	3.26	3.31	3.72
Monocalcium phosphate	1.43	1.43	1.44	1.44	1.44	1.45	1.45	1.47
Calcium carbonate	1.14	1.15	1.15	1.14	1.14	1.16	1.16	1.15
Sodium chloride	0.21	0.21	0.21	0.21	0.21	0.20	0.20	0.21
Sodium bicarbonate	0.39	0.47	0.47	0.47	0.47	0.62	0.62	0.63
Additives ¹	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
DL-MET 99%	0.24	0.26	0.26	0.26	0.26	0.29	0.29	0.30
L-LYS 78%	0.12	0.18	0.18	0.18	0.18	0.31	0.31	0.30
L-THR 98.5%	0.04	0.07	0.07	0.07	0.07	0.12	0.12	0.12
L-VAL 98.5%	-	0.03	0.04	0.04	0.04	0.10	0.10	0.11
L-ILE 98.5%	-	-	-	-	-	0.10	0.10	0.10
GLY 97%	-	-	0.07	-	0.08	-	0.22	0.23
L-GLU 99.4%	-	-	-	1.40	1.24	-	-	3.54
Premixes ²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline 75%	0.03	0.04	0.04	0.04	0.04	0.05	0.05	0.05
Nutrients, energy and electrolytic balance, % or otherwise noted								
CP	21.75	20.99	21.08	21.75	21.75	19.57	19.83	21.75
AME, kcal/kg				3,100				
Ca				0.82				
Non-phytate P				0.41				
Na+K-Cl, mEq/kg				250				
Choline, ppm				1,600				
Digestible LYS				1.07				
Digestible amino acid ratios to LYS, %								
MET+CYS				72				
THR				65				
VAL				77				
ILE	70	67	67	67	67	70	70	70
TRP	22	21	21	21	21	19	19	19
ARG	122	116	116	116	116	105	105	105
MET	47	47	47	48	48	49	49	49
LEU	141	136	136	136	136	127	127	125
His	46	45	45	45	45	41	41	41
Phe	86	82	82	82	82	75	75	75
Phe+Tyr	148	142	142	142	142	130	130	129
Total GLY+SER:LYS	158	152	158	152	158	140	158	158

¹ Adsorbent (MT.x +, Olmix S.A.): 0.1%; Sodium monensin (Coban™ 40%, Elanco Animal Health, Division of Eli Lilly and Company): 0.0275%; Bacitracin methylene disalicylate (BMD 11%, Alpharma do Brasil Ltda.): 0.05%; Colistin sulphate 8% (Indukern do Brasil Química Ltda.): 0.0125%.

² Supplied per kg of diet: Vitamin A: 8,400 IU; Vitamin D₃: 2,160 IU; Vitamin E: 60 IU; Vitamin K₃: 2.57 mg; Thiamine: 2.14 mg; Riboflavin: 6 mg; Pyridoxine: 3.43 mg; Vitamin B₁₂: 0.02 mg; Pantothenic acid: 10.29 mg; Niacin: 42.86 mg; Folacin: 1.29 mg; Biotin: 0.13 mg; Phytase: 0.12 g; Fe: 40 mg; Zn: 40 mg; Mn: 64 mg; Cu: 8 mg; I: 0.8 mg; Co: 0.8 mg; Se: 0.26 mg.

Table 5. Ingredient and nutritional composition of experimental diets (T1-T8) fed to male broilers from 36 to 42 d of age

Ingredients, %	T1	T2	T3	T4	T5	T6	T7	T8
Corn	57.70	59.69	59.59	58.21	58.28	63.99	63.68	59.64
Soybean meal 48% CP	33.87	32.02	32.03	32.18	32.17	27.99	28.02	28.44
Soybean oil	4.75	4.40	4.42	4.54	4.55	3.59	3.67	4.04
Monocalcium phosphate	1.29	1.30	1.30	1.31	1.31	1.32	1.32	1.33
Calcium carbonate	1.06	1.07	1.07	1.07	1.07	1.08	1.08	1.08
Sodium chloride	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.19
Sodium bicarbonate	0.48	0.54	0.55	0.55	0.55	0.69	0.69	0.69
Additives ¹	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
DL-MET 99%	0.22	0.24	0.24	0.24	0.24	0.27	0.27	0.28
L-LYS 78%	0.13	0.18	0.18	0.18	0.18	0.30	0.30	0.29
L-THR 98.5%	0.04	0.06	0.06	0.06	0.06	0.11	0.11	0.12
L-VAL 98.5%	-	0.03	0.03	0.03	0.03	0.09	0.09	0.10
L-ILE 98.5%	-	-	-	-	-	0.09	0.09	0.09
GLY 97%	-	-	0.06	-	0.06	-	0.20	0.21
L-GLU 99.4%	-	-	-	1.18	1.04	-	-	3.22
Premixes ²	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Choline 75%	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04
Nutrients, energy and electrolytic balance, % or otherwise noted								
CP	20.64	20.00	20.08	20.64	20.64	18.66	18.89	20.64
AME, kcal/kg					3,150			
Ca					0.76			
Non-phytate P					0.38			
Na+K-Cl, mEq/kg					250			
Choline, ppm					1,500			
Digestible LYS					1.02			
Digestible amino acid ratios to LYS, %								
MET+CYS					72			
THR					65			
VAL					77			
ILE	70	67	67	67	67	70	70	70
TRP	22	21	21	21	21	19	19	19
ARG	121	116	116	116	116	105	105	105
MET	46	47	47	47	47	49	49	49
LEU	143	138	138	138	138	129	129	127
His	47	45	45	45	45	42	42	41
Phe	86	83	83	83	83	76	76	75
Phe+Tyr	148	143	143	143	143	131	131	130
GLY+SER	158	153	158	153	158	141	158	158

¹ Adsorbent (MT.x +, Olmix S.A.): 0.1%; Sodium monensin (Coban™ 40%, Elanco Animal Health, Division of Eli Lilly and Company): 0.0275%; Bacitracin methylene disalicilate (BMD 11%, Alpharma do Brasil Ltda.): 0.05%.

² Supplied per kg of diet: Vitamin A: 4,200 IU; Vitamin D₃: 1,080 IU; Vitamin E: 30 IU; Vitamin K₃: 1.29 mg; Thiamine: 1.07 mg; Riboflavin: 3 mg; Pyridoxine: 1.71 mg; Vitamin B₁₂: 0.01 mg; Pantothenic acid: 5.14 mg; Niacin: 21.43 mg; Folacin: 0.64 mg; Biotin: 0.06 mg; Phytase: 0.06 g; Fe: 30 mg; Zn: 30 mg; Mn: 48 mg; Cu: 6 mg; I: 0.6 mg; Co: 0.6 mg; Se: 0.13 mg.

Table 6. Calculated (C) and analyzed (A) values (%) of crude protein (CP) and total amino acids of experimental diets (T1-T8) fed to male broilers during a 42 d production period¹

	T1		T2		T3		T4		T5		T6		T7		T8	
	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A
1 to 7 d																
CP	26.2	25.8	24.9	24.8	25.0	24.5	26.2	26.4	26.2	26.0	23.7	23.1	24.0	24.4	26.2	25.7
LYS	1.43	1.60	1.43	1.53	1.43	1.32	1.43	1.46	1.43	1.29	1.42	1.29	1.42	1.51	1.42	1.51
VAL	1.09	1.13	1.09	1.16	1.09	1.01	1.09	1.12	1.09	0.99	1.08	1.01	1.08	1.15	1.08	1.12
ILE	1.00	1.08	0.94	1.00	0.94	0.87	0.94	0.95	0.94	0.84	0.99	0.91	0.99	1.10	0.99	1.01
GLY+SER	2.22	2.45	2.09	2.22	2.21	2.05	2.08	2.13	2.21	1.96	1.96	1.82	2.20	2.34	2.20	2.24
GLU	4.24	4.74	3.99	4.32	3.98	3.78	6.36	6.17	6.09	5.12	3.74	3.58	3.74	4.14	7.77	7.48
8 to 21 d																
CP	22.7	22.9	21.8	22.7	21.9	21.5	22.7	23.2	22.7	21.4	20.7	20.2	21.0	20.5	22.7	22.1
LYS	1.24	1.30	1.23	1.23	1.23	1.17	1.23	1.28	1.23	1.31	1.23	1.27	1.23	1.24	1.23	1.21
VAL	0.94	0.99	0.94	0.94	0.94	0.91	0.94	0.97	0.94	1.02	0.94	0.95	0.94	0.98	0.94	0.89
ILE	0.85	0.87	0.81	0.80	0.81	0.77	0.81	0.83	0.81	0.88	0.85	0.86	0.85	0.92	0.85	0.79
GLY+SER	1.91	1.99	1.82	1.79	1.90	1.80	1.82	1.87	1.90	2.03	1.71	1.74	1.90	1.99	1.90	1.79
GLU	3.65	3.85	3.48	3.51	3.48	3.37	5.12	4.92	4.93	5.00	3.27	3.31	3.27	3.36	6.37	6.06
22 to 35 d																
CP	21.7	21.9	21.0	20.6	21.1	20.8	21.7	21.2	21.7	19.9	19.6	18.1	19.8	19.6	21.7	21.3
LYS	1.16	1.19	1.16	1.20	1.16	1.16	1.16	1.20	1.16	1.16	1.15	1.15	1.15	1.13	1.15	1.15
VAL	0.91	0.93	0.90	0.93	0.90	0.92	0.90	0.95	0.90	0.90	0.90	0.90	0.90	0.86	0.90	0.88
ILE	0.82	0.85	0.78	0.81	0.78	0.82	0.78	0.83	0.78	0.76	0.81	0.82	0.81	0.79	0.81	0.81
GLY+SER	1.84	1.86	1.76	1.80	1.83	1.84	1.76	1.84	1.83	1.78	1.61	1.55	1.82	1.73	1.82	1.74
GLU	3.51	3.55	3.36	3.41	3.36	3.42	4.75	4.62	4.59	4.74	3.08	3.01	3.08	2.92	6.59	5.84
36 to 42 d																
CP	20.6	20.8	20.0	19.3	20.1	19.6	20.6	19.9	20.6	20.2	18.7	18.6	18.9	18.4	20.6	20.9
LYS	1.10	1.11	1.10	1.13	1.10	1.16	1.10	1.22	1.10	1.18	1.09	1.18	1.09	1.16	1.09	1.19
VAL	0.86	0.91	0.86	0.89	0.86	0.93	0.86	0.91	0.86	0.94	0.85	0.93	0.85	0.89	0.85	0.92
ILE	0.77	0.84	0.74	0.78	0.74	0.81	0.74	0.80	0.74	0.84	0.77	0.85	0.77	0.81	0.77	0.83
GLY+SER	1.74	1.77	1.67	1.73	1.73	1.86	1.67	1.83	1.73	1.85	1.53	1.67	1.72	1.76	1.72	1.85
GLU	3.33	3.47	3.20	3.34	3.20	3.45	4.37	4.35	4.24	4.30	2.93	3.20	2.93	2.94	6.13	6.87

¹Values were performed in duplicate by standard procedures [19].

Table 7. Body weight gain, feed intake and feed conversion of Ross 308 male broilers fed control diets or diets supplemented with different industrial AA to provide these same ratios to LYS or CP levels during a 42 d production period¹

Body weight gain, g/bird	1 to 7d	1 to 21d	1 to 35d	1 to 42d
Control	136 ^{ab}	984	2,520	3,120
VAL	135 ^{ab}	973	2,489	3,100
VAL + GLY	140 ^a	993	2,508	3,122
VAL + GLU	140 ^a	976	2,495	3,123
VAL + GLY + GLU	136 ^{ab}	981	2,500	3,108
VAL + ILE	131 ^b	962	2,472	3,096
VAL + ILE + GLY	142 ^a	979	2,476	3,072
VAL + ILE + GLY + GLU	140 ^a	993	2,506	3,110
SD	6.27	28.22	44.09	53.76
P=	0.01	0.37	0.34	0.53
Feed intake, g/bird				
Control	144	1,264	3,557	4,675
VAL	142	1,264	3,551	4,686
VAL + GLY	145	1,280	3,587	4,717
VAL + GLU	142	1,248	3,504	4,656
VAL + GLY + GLU	141	1,261	3,547	4,676
VAL + ILE	141	1,272	3,572	4,741
VAL + ILE + GLY	146	1,280	3,562	4,660
VAL + ILE + GLY + GLU	143	1,274	3,553	4,638
SD	5.66	38.89	72.53	94.57
P=	0.50	0.68	0.50	0.40
Feed conversion, g:g				
Control	1.060 ^{ab}	1.285 ^a	1.412 ^a	1.498 ^a
VAL	1.048 ^{ab}	1.299 ^{ab}	1.427 ^{ab}	1.511 ^{ab}
VAL + GLY	1.038 ^{ab}	1.289 ^a	1.430 ^{ab}	1.511 ^{ab}
VAL + GLU	1.018 ^a	1.278 ^a	1.404 ^a	1.490 ^a
VAL + GLY + GLU	1.040 ^{ab}	1.286 ^a	1.419 ^{ab}	1.504 ^{ab}
VAL + ILE	1.079 ^b	1.324 ^b	1.445 ^b	1.531 ^b
VAL + ILE + GLY	1.024 ^a	1.307 ^{ab}	1.439 ^b	1.517 ^{ab}
VAL + ILE + GLY + GLU	1.019 ^a	1.283 ^a	1.418 ^{ab}	1.491 ^a
SD	0.029	0.021	0.019	0.021
P=	0.01	0.01	0.01	0.01

¹Values are least-square means of 9 replicate pens with each pen having 28 birds at 1 d of age.

^{a, b} Means within an age and measured characteristic followed by differing letters are statistically different ($P \leq 0.05$).

Table 8. Mortality (%) of Ross 308 male broilers fed control diets or diets supplemented with different industrial AA to provide these same ratios to LYS or CP levels during a 42 d production period¹

Treatment	Ascites	Sudden death	Legs abnormalities	Others	All
Control	0.00	1.59	0.00	0.40	1.98
VAL	0.00	0.79	0.00	1.19	1.98
VAL + GLY	0.00	1.19	0.79	1.59	3.57
VAL + GLU	0.00	1.36	0.40	3.21	4.96
VAL + GLY + GLU	0.40	0.79	0.40	0.79	2.38
VAL + ILE	0.00	1.43	0.52	0.15	2.03
VAL + ILE + GLY	0.00	1.19	0.79	0.79	2.78
VAL + ILE + GLY + GLU	0.00	2.38	0.40	1.19	3.97
SD	0.43	2.20	1.52	2.26	3.38
<i>P</i> =	0.46	0.78	0.93	0.22	0.53

¹Values are least-square means of 9 replicate pens with each pen having 28 birds at 1 d of age.

Table 9. Carcass, abdominal fat, and parts yields of Ross 308 male broilers fed diets with different amino acid profiles during a 42 d production period¹, %

Treatment	Carcass	Abdominal fat	Breast meat ²	Leg quarters ³	Wings	Cage
Control	78.62	1.88 ^{ab}	29.04 ^{ab}	33.51 ^a	10.51	24.63
VAL	78.73	2.04 ^{ab}	28.99 ^b	33.29 ^{ab}	10.57	24.54
VAL + GLY	78.69	1.94 ^{ab}	29.22 ^{ab}	33.11 ^{ab}	10.55	24.69
VAL + GLU	78.53	1.83 ^{ab}	29.33 ^{ab}	32.91 ^b	10.66	24.62
VAL + GLY + GLU	78.45	1.93 ^{ab}	29.32 ^{ab}	32.96 ^b	10.49	24.73
VAL + ILE	78.60	2.06 ^b	29.27 ^{ab}	32.93 ^b	10.47	24.43
VAL + ILE + GLY	79.09	2.03 ^{ab}	29.52 ^{ab}	32.88 ^b	10.42	24.52
VAL + ILE + GLY + GLU	78.50	1.81 ^a	29.73 ^a	32.76 ^b	10.62	24.45
SD	0.72	0.17	0.50	0.36	0.17	0.41
<i>P</i> =	0.67	0.01	0.04	0.01	0.09	0.72

¹Values are least-square means of 9 replicate pens with 10 birds per pen.

Carcass yield expressed relative to live weight, whereas other data are relative to carcass weight.

² *Pectoralis major* plus *Pectoralis minor* muscles.

³ Thighs plus drumsticks.

^{a, b} Means followed by differing letters are statistically different ($P \leq 0.05$)

CAPÍTULO V

Considerações finais

Ainda que existam certas discrepâncias, resultados promissores têm sido demonstrados. A perspectiva de redução dos níveis protéicos das formulações, têm impulsionado novos estudos na área, abrangendo além dos AAE menos limitantes valina, isoleucina, triptofano e arginina, os AANE glicina e ácido glutâmico. Respostas positivas à utilização destes AA propiciarão, a médio prazo, sua produção sintética em escala industrial com conseqüente queda dos custos e maximização na utilização das novas formulações.

Não só as exigências de cada aminoácido devem ser atendidas como o equilíbrio entre eles, principalmente lisina, aminoácidos sulfurados, treonina e, num futuro próximo, valina, isoleucina e mesmo glicina. Desta forma, a excreção de nitrogênio pelas aves será minimizada, economizando energia para os processos de manutenção e crescimento.

A tendência é que os nutricionistas e formuladores passem a utilizar relações fixas entre os AA e lisina nas fórmulas, conforme a fase de desenvolvimento das aves, alterando somente o nível de lisina das dietas em função das respostas esperadas sob as mais diversas condições ambientais. Novos estudos avaliando as interações de VAL, ILE e LEU devem ser conduzidos.

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APÊNDICES

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

Journal of Applied Poultry Research Instructions to Authors Editorial Policies and Procedures

The mission of *Journal of Applied Poultry Research* (JAPR) is to provide practical, reliable, and timely information to those whose livelihoods are derived from the commercial production of poultry and those whose research benefits this sector; address topics of near-term application based on appropriately designed studies and critical observations; encourage scientific approaches to practical problem solving; and present information comprehensible to a broad readership.

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Headings

Major Headings. Major headings are centered, boldface, in all capital letters, and consist of SUMMARY, DESCRIPTION OF PROBLEM, MATERIALS AND METHODS, RESULTS AND DISCUSSION, CONCLUSIONS AND APPLICATIONS, and REFERENCES AND NOTES.

Major headings in review articles, field reports, and symposium articles may vary from those listed here, but should include SUMMARY, CONCLUSIONS AND APPLICATIONS, and REFERENCES AND NOTES.

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- The title should be indicative of the content. It should capture the interest of all who might benefit from information in the manuscript. However, the length of the title should be kept to a minimum.
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- The running title should be 30 characters or less, including spaces.
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The Summary (12 to 16 lines) is not an abstract. It is intended to give readers with diverse backgrounds a general appreciation of the manuscript contents. It should be written so that even those not directly interested in the topic will enjoy reading at least this section to keep abreast of areas other than their own. This section should not include details of materials and methods or a detailed review of the results. Keep the summary free-flowing, giving the reader a general, not specific, idea of what the study revealed. Do not include reference citations in the summary.

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This section will acquaint the reader with the problem, citing field experiences where appropriate. Readability is of utmost importance. Detailed literature reviews may not be appropriate for this section.

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This section begins with observed results and their interpretation. Descriptive subheadings may precede all major paragraphs and changes in subject emphasis. This section should discuss specifically how findings address the problem described in the Description of Problem section and how they are related to published works.

Statements regarding statistically significant differences between treatments in results should be included in the text, tables, and figures. Statements regarding differences should be avoided unless they are supported by statistical analyses and meet the stated level of probability (e.g., $P < 0.05$).

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

- **Symbols.** Identify curves and data points using the following symbols only: □, ■, ○, ●, ▲, ▼, ◻, ◼, ◇, ◆, +, 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 Power-Point files and other formats. For the best printed quality, line art should be prepared at 600 ppi.

Grayscale and color images and photomicrographs should be at least 300 ppi.

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

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

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

- **Caption.** The caption should provide sufficient information so that the figure can be understood without 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 3-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 the key are clear and easily readable at the final publication size.

Color Images. The cost to publish in color is \$995 per figure; there is also a surcharge for color offprints. The corresponding author should complete a Color Charge Agreement form, available on the journal Web site (<http://japr.fass.org/misc/ifora.dtl>) and should fax (217-378-4083) that form to the JAPR editorial office when a manuscript with color figures is accepted for publication.

Sample References

NOTE: The headings that appear above the following sample references and notes are for clarification in these instructions, but they are not used in an actual paper, except for *Acknowledgments*.

Journal Article

Dansky, L. M., and F. W. Hill. 1952. Application of the chromic oxide indicator

method to balance studies with growing chicks. *J. Nutr.* 47:449–459.

Snow, J. L., M. W. Douglas, and C. M. Parsons. 2003. Phytase effects on amino acid digestibility in molted laying hens. *Poult. Sci.* 82:474–477.

Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, with and without prior vaccination, to challenge with Marek's disease virus. *Avian Dis.* doi:10.1637/7498-010306.R1

Monograph

NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Dissertation

Heskett, E. A. 2003. Efficacy of a recombinant herpes virus of turkeys vector vaccine, expressing genes to Newcastle disease virus and Marek's disease virus, in chickens and turkeys against exotic Newcastle disease virus challenge. PhD Diss. Univ. Florida, Gainesville.

Trade Publication

Wilgus, H. S. 1973. Temperature-programmed feeding schedules and other means of conserving protein in market turkey production. *Feedstuffs* 45(27):27–31.

Book or Chapter in Book

AOAC International. 2007. *Official Methods of Analysis of AOAC International*. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.

Whittow, G. C. 1976. Regulation of body temperature. Pages 146–173 in *Avian Physiology*. P. D. Sturkie, ed. Springer-Verlag, New York, NY.

Proceedings

Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Pages 3–5 in *Proc. 2nd Mid-Atlantic Nutr. Conf.*, Timonium, MD. Univ. Maryland, College Park.

Federal Register

USDA, Plant and Animal Health Inspection Service. 2004. Blood and tissue collections at slaughtering and rendering establishments, final rule. 9CFR part 71. *Fed. Regist.* 69:10137–10151.

Laboratory Procedure

The extract was added to 30 mL of hexane, made to 100 mL with 10% aqueous Na₂SO₄.

Personal Communication

Wilson, H. R. 2005. Univ. Florida, Gainesville. Personal communication.

Proprietary Product

Incubator, Petersime, Zulte, Belgium. Avizyme TX, Finnfeed International, Marlborough, Wiltshire, UK. Thymol, 99% purity, Acros Organics, Geel, Belgium.

Statistical Procedure

If a note has an embedded reference, the reference is cited by number (as in the text) or parenthetically within the note:

Data were analyzed by ANOVA with flock as the independent variable. When differences among flocks were significant, means were separated using Duncan's multiple range test (SAS User's Guide, 2001, Version 8 ed., SAS Institute Inc., Cary, NC). Pearson product-moment correlation coefficients were calculated between average percentage cracks from each flock recorded every

week and average values for egg-specific gravity, breaking strength, percentage shell, shell thickness, and shell weight per unit of surface area. Significance implies $P < 0.05$.

Statistical Software

SAS User's Guide. 2001. Version 8 ed. SAS Inst. Inc., Cary, NC.

US Patent

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

Web Site

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

Acknowledgments

The advice and technical assistance of Thomas Jones (affiliation, location) are acknowledged.

Abbreviations

The following abbreviations may be used without definition in the *Journal of Applied Poultry Research*. Plurals do not require "s". Chemical symbols and 3-letter abbreviations for amino acids do not need definition. Other abbreviations should be defined at first use in the summary and the main text, as well as in each table or figure in which they appear. Abbreviations are boldface at first use in the main text. Abbreviations should not be used in the manuscript title, running title, or to begin a paragraph or sentence. They can be used in section headings if previously defined. This list appears inside the back cover of each issue of the journal.

ADF	acid detergent fiber
ADFI	average daily feed intake
ADG	average daily gain
AME	apparent metabolizable energy
AMEn	nitrogen-corrected apparent metabolizable energy
ANOVA	analysis of variance
BSA	bovine serum albumin
BW	body weight
°C	Celsius
cDNA	complementary DNA
CF	crude fiber
cfu	colony-forming units (following a numeral)
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
EE	ether extract
°F	Fahrenheit
FCR	feed conversion ratio
FE	feed efficiency

ft	foot
g	gram
gal	gallon
G:F	gain-to-feed ratio
GLM	general linear model
h	hour
HEPES	<i>N</i> -(2-hydroxyethyl)piperazine- <i>N'</i> -2-ethanesulfonic acid
HPLC	high-performance (high-pressure) liquid chromatography
ICU	international chick units
Ig	immunoglobulin
IL	interleukin
i.m.	intramuscular
in.	inch
i.p.	intraperitoneal
IU	international units
i.v.	intravenous
kcal	kilocalorie
L	liter (also capitalized with any combination, e.g., mL)
lb	pound
L:D	hours of light:hours of darkness in a photoperiod
LSD	least significant difference
m	meter
μ	micro
<i>M</i>	molar
ME	metabolizable energy
ME _n	nitrogen-corrected metabolizable energy
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
min	minute
mo	month
MS	mean squares
<i>n</i>	number of observations
<i>N</i>	normal
NAD	nicotinamide adenine dinucleotide
NADH	reduced form of NAD
NDF	neutral detergent fiber
NRC	National Research Council
NS	not significant
PBS	phosphate-buffered saline
ppm	parts per million
<i>r</i>	correlation coefficient
<i>r</i> ²	coefficient of determination, simple
<i>R</i> ²	coefficient of determination, multiple
RH	relative humidity
RIA	radioimmunoassay
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
s.c.	subcutaneous

SD	standard deviation
SE	standard error
SEM	standard error of the mean
SNP	single nucleotide polymorphism
SRBC	sheep red blood cells
TBA	thiobarbituric acid
T	cell thymic-derived cell
TME	true metabolizable energy
TME _n	nitrogen-corrected true metabolizable energy
TSAA	total sulfur amino acids
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

Supplemental Information (Online)

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

Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online (<http://japr.fass.org/misc/ifora.dtl>).

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>

Manuscript Central Instructions. Manuscripts are submitted online (<http://mc.manuscriptcentral.com/psa>). Full user instructions for using the Manuscript Central system are available online; click the “Get Help Now” link on the top right of the main page (<http://mc.manuscriptcentral.com/psa>).

Apêndice 2. Normas para publicação de artigos no periódico Poultry Science

POULTRY SCIENCE INSTRUCTIONS TO AUTHORS

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

For information on the scientific content of the journal, contact the editor-in-chief, Dr. Colin G. Scanes, 335 Chapman Hall, 2310 East Hartford Ave., University of Wisconsin, Milwaukee, WI 53201; e-mail: scanes@uwm.edu (with cc to cscanes@wi.rr.com). For assistance with Manuscript Central, manuscript submission and copyright forms, or page charge and offprint orders, contact Jeremy Holzner, editorial assistant, Headquarters Office, 2441 Village Green Place, Champaign, IL 61822 (FAX: 217-378-4083; jeremyh@assoqh.org). For other information or to submit a paper, contact Susan Pollock, managing editor, Headquarters Office, Poultry Science Association, Inc., 2441 Village Green Place, Champaign, IL 61822 (telephone: 217-356-7641; FAX: 217-378-4083; journals@assoqh.org).

Care and Use of Animals

Authors must make it clear that experiments were conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and laboratory techniques. Experiments shall be conducted in accordance with the principles and specific guidelines presented in *Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, 1st revised edition, 1999 (Association Headquarters, 2441 Village Green Place, Champaign, IL 61822); and, if applicable, *Guide for the Care and Use of Laboratory Animals* (United States Department of Human Health and Services, National Institutes of Health, Publication Number ISBN 0-309-05377-3, 1996); or *Guide to the Care and Use of Experimental Animals*, 2nd ed. Volume 1, 1993 (Canadian Council on Animal Care). Methods of killing experimental animals must be described in the text. In

describing surgical procedures, the type and dosage of the anesthetic agent must be specified. Intra-abdominal and intrathoracic invasive surgery requires anesthesia. This includes caponization. The editor-in-chief of *Poultry Science* may refuse to publish manuscripts that are not compatible with these guides. If rejected solely on that basis, however, the paper may be resubmitted for reconsideration when accompanied by a written verification that a committee on animal care in research has approved the experimental design and procedures involved.

Types of Articles

Full-Length Articles. The majority of papers published in *Poultry Science* are full-length articles. The journal emphasizes the importance of good scientific writing and clarity in presentation of the concepts, apparatus, and sufficient background information that would be required for thorough understanding by scientists in other disciplines. The results of experiments published in *Poultry Science* must be replicated, either by replicating treatments within experiments or by repeating experiments.

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

Symposium Papers. The symposium organizer or chair must present the proposal and tentative budget to the Board of Directors at the summer meeting one full year before the symposium is to be scheduled. The symposium chair must then develop detailed symposium plans, including a formal outline of the talks approved and full budgetary expectations, which must be brought to the Board of Directors at the January meeting prior to the meeting at which the symposium is scheduled.

The symposium chair must decide whether or not the symposium is to be published and will inform the editor-in-chief of this decision at the January meeting. If the decision is not to publish the symposium, the individual authors retain the right to submit their papers for consideration for the journal as ordinary manuscripts. If publication is decided upon, all manuscript style and form guidelines of the journal shall be followed. Manuscripts must be prepared electronically, including figures and tables, and then uploaded onto the *Poultry Science* Manuscript Central site within 2 weeks after the annual meeting. The symposium chair will review the papers and, if necessary, return them to the authors for revision. The symposium chair then forwards the revised manuscript to the editor-in-chief for final review. Final revisions by the author and recommendations for acceptance or rejection by the chair must be completed by December 31 of the year in which the symposium was presented. Manuscripts not meeting this deadline will not be included in the published symposium proceedings. Symposium papers must be prepared in accordance

with the guidelines for full-length articles and are subject to review. Offprints and costs of pages are the responsibility of the author.

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

Review Papers. Review papers are accepted only if they provide new knowledge or a high-caliber synthesis of important knowledge. Reviews are not exempt from page charges. All *Poultry Science* guidelines for style and form apply.

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

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

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

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

Letters to the Editor. The purpose of letters will be to discuss, critique, or expand on scientific points made in articles recently published in *Poultry Science*. Introduction of unpublished data will not be allowed, nor will material based on conjecture or speculation. Letters must be received within 6 months of an article's publication. Letters will be limited to 400 words and 5 references (approximately 3 double-spaced, typed pages including references). Letters shall have a title. Author name(s) and affiliation(s) shall be placed between the end of the text and list of references. Letters will be sent electronically directly to the editor-in-chief for consideration. The author(s) of the original paper(s) will be provided a copy of the letter and offered the opportunity to submit for consideration a reply within 30 days. Replies will have the same page

restrictions and format as letters, and the titles shall end with “—Reply.” Letters and replies will be published together. Acceptability of letters will be decided by the editor-in-chief. Letters and replies shall follow appropriate *Poultry Science* format and may be edited by the editor-in-chief and a technical editor. If multiple letters on the same topic are received, a representative letter concerning a specific article will be published. All letters may not be published. Letters and replies will be published as space permits.

SUBMISSION OF ELECTRONIC MANUSCRIPTS

Authors should submit their papers electronically (<http://mc.manuscriptcentral.com/ps>). Detailed instructions for submitting electronically are provided online at that site. Authors who are unable to submit electronically should contact the editorial office (jeremyh@assochoq.org) for assistance.

Copyright Agreement

Authors shall complete the Manuscript Submission and Copyright Release form for each new manuscript submission; faxed copies are acceptable. The form is published in *Poultry Science* as space permits and is available online (<http://ps.fass.org>). The copyright agreement is included in the Manuscript Submission and Copyright Release Form and must be completed by all authors before publication can proceed. The corresponding author is responsible for obtaining the signatures of coauthors. Persons unable to sign copyright agreements, such as federal employees, must indicate the reason for exemption on the form.

The Poultry Science Association grants to the author the right of republication in any book of which he or she is the author or editor, subject only to giving proper credit to the original journal publication of the article by the Association. The Poultry Science Association, Inc. retains the copyright to all materials accepted for publication in the journal. Please address requests for permission to reproduce published material to the editor-in-chief. All tables must be original material. If an author wishes to present data previously published in tabular form, copyright permission to reproduce the table must be obtained by the author and forwarded to the PSA editorial office, even when the format of the table submitted with the manuscript is different than the table already published.

If an author desires to reprint a figure published elsewhere, copyright permission to use the figure must be obtained by the author and forwarded to the PSA editorial office.

REVIEW OF MANUSCRIPTS

After a manuscript is submitted electronically, the editorial office informs the appropriate section editor, who assigns two reviewers, at least one of whom is an associate editor. Each reviewer has 3 weeks to review the manuscript, after which his or her comments are forwarded to the section editor. The section editor may recommend rejection or acceptance at this point, after which the manuscript and reviewer comments are made available to the editor-in-chief for a final decision. More commonly, the manuscript will be sent back to the corresponding author for revision according to the guidelines of the reviewers.

Authors have 6 weeks to complete the revision, which shall be returned to the section editor. Failure to return the manuscript within 6 weeks will cause the paper to be purged from the files. Purged manuscripts may be reconsidered, but they will have to be processed as new manuscripts.

Section editors handle all initial correspondence with authors during the review process. The editor-in-chief will notify the author of the final decision to accept or reject. Rejected manuscripts can be resubmitted only with an invitation from the section editor or editor-in-chief. Revised versions of previously rejected manuscripts are treated as new submissions. Therefore, authors must complete a new Manuscript Submission and Copyright Release Form.

PRODUCTION OF PROOFS

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

Proofs

Author proofs of all manuscripts will be provided to the corresponding author. Author proofs should be read carefully and checked against the typed manuscript, because the responsibility for proofreading is with the author(s). Corrections may be returned by fax, mail, or e-mail. For faxed or mailed corrections, changes to the proof should be made neatly and clearly in the margins of the proof. If extensive editing is required, corrections should be provided on a separate sheet of paper with a symbol indicating location on the proof. Changes sent by e-mail to the technical editor must indicate page, column, and line numbers for each correction to be made on the proof. Corrections can also be marked using the note and highlight tools to indicate necessary changes. Author alterations to copy exceeding 10% of the cost of composition will be charged to the author. Editor queries should be answered on the galley proofs; failure to do so may delay publication. Proof corrections should be made and returned to the technical editor within 48 hours of receipt.

Publication Charges and Offprints

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

OA. For authors who wish to publish their papers OA (freely available to everyone when the issue is posted online), authors will pay the OA fee when proofs are returned to the editorial office. Charges for OA are \$2,400 if at least one author is a current professional member of PSA; the charge is \$3,100 when no author is a professional member of PSA.

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

Offprints and Color Charges. Offprints may be ordered at an additional charge. Authors who submit articles containing color illustrations are responsible for paying the additional charge for color printing, including the printing of any reprints they order, and must agree in writing prior to publication

to pay the additional charges. When the galley proof is sent, the author is asked to complete an offprint order requesting the number of offprints desired and the name of the institution, agency, or individual responsible for publication charges.

MANUSCRIPT PREPARATION:

STYLE AND FORM

General

Papers must be written in English. The text and all supporting materials must use American spelling and usage as given in *The American Heritage Dictionary*, *Webster's Third International Dictionary*, or the *Oxford American English Dictionary*. Authors should follow the style and form recommended in *Scientific Style and Format. The CBE Manual for Authors, Editors, and Publishers*. 6th ed. Council of Biology Editors Style Manual Committee. Cambridge Univ. Press, Cambridge, UK. Authors should prepare the main text, tables, and figure captions in MS Word. Details on figure preparation and file formats are provided in the Figures section of these instructions.

Preparing the Manuscript File

Manuscripts should be typed double-spaced, with lines and pages numbered consecutively, using Times New Roman font at 12 points. All special characters (e.g., Greek, math, symbols) should be inserted using the symbols palette available in this font. Complex math should be entered using MathType from Design Science (<http://www.dessci.com>). Equations created using the new Equation Builder feature in Microsoft Word 2007 may not be compatible with earlier versions of Word or other software used in our journal composition system. Tables and figures should be placed in separate sections at the end of the manuscript (not placed in the text). Failure to follow these instructions may result in an immediate rejection of the manuscript.

Headings

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

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

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

Title Page. The title page shall begin with a running head (short title) of not more than 45 characters. The running head is centered, is in all capital letters, and shall appear on the top of the title page. No abbreviations should be used. The title of the paper must be in boldface; the first letter of the article title and proper names are capitalized, and the remainder of the title is lowercase. The title must have no abbreviations, and numbers must be given in words rather than in numerals (e.g., One-Day-Old Broilers).

Under the title, names of authors should be typed with initial capital letters and a

space between initials (e.g., T. E. Smith). Affiliations will be footnoted using the following symbols: *, †, ‡, §, #, □, and be placed below the author names. Do not give authors' titles, positions, or degrees. Numbered footnotes may be used to provide supplementary information, such as present address, acknowledgment of grants, and experiment station or journal series number. The corresponding author should be indicated with a numbered footnote (e.g.,¹Corresponding author: myname@university.edu). Note that there is no period after the corresponding author's e-mail address. The title page shall include the name and full address of the corresponding author. Telephone and FAX numbers and e-mail address must also be provided. The title page must indicate the appropriate scientific section for the paper (i.e., Education and Production; Environment, Well-Being, and Behavior; Genetics; Immunology, Health, and Disease; Metabolism and Nutrition; Molecular, Cellular, and Developmental Biology; Physiology, Endocrinology, and Reproduction; or Processing, Products, and Food Safety). Authors may create a full title page as a one-page document, in a file separate from the rest of the paper. This file can be uploaded and marked "not for review." Authors who choose to upload manuscripts with a full title page at the beginning will have their papers forwarded to reviewers as is.

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

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

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

Key words: . . .

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

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

able to determine the full address for mailing purposes easily by consulting standard references. Age, sex, breed, and strain or genetic stock of animals used in the experiments shall be specified. Animal care guidelines should be referenced if appropriate.

Papers must contain analyzed values for those dietary ingredients that are crucial to the experiment. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as appropriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phosphorus contents should also be specified.

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

Vitamin A

1 IU = 0.3 µg of all-*trans* retinol

1 IU = 0.344 µg of retinyl acetate

1 IU = 0.552 µg of retinyl palmitate

1 IU = 0.60 µg of β-carotene

Vitamin E

1 IU = 1 mg of dl-α-tocopheryl acetate

1 IU = 0.91 mg of dl-α-tocopherol

1 IU = 0.67 mg of dl-α-tocopherol

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

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

Statistical Analysis. Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately. The experimental unit is the smallest unit to which an individual treatment is imposed. For group-fed animals, the group of animals in the pen is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use timesequence analysis. Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data

transformations or other techniques are helpful. For example, it is recommended that percentage data between 0 and 20 and between 80 and 100 be subjected to arc sin transformation prior to analysis. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals are stratified by ancestry or weight or if some other initial measurement should be accounted for, the model should include a blocking factor, or the initial measurement should be included as a covariate. A parameter [mean (μ), variance (σ^2)], which defines or describes a population, is estimated by a statistic (\bar{x} , s^2). The term **parameter** is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment.

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

For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator of each F statistic. Unbalanced factorial data can present special problems.

Accordingly, it is well to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by

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

The terms significant and highly significant traditionally have been reserved for $P < 0.05$ and $P < 0.01$, respectively; however, reporting the P -value is preferred to the use of these terms. For example, use “. . . there was a difference ($P < 0.05$) between control and treated samples” rather than “. . . there was a significant ($P < 0.05$) difference between control and treated samples.” When available, the observed significance level (e.g., $P = 0.027$) should be presented rather than merely $P < 0.05$ or $P < 0.01$, thereby allowing the reader to decide what to reject. Other probability (α) levels may be discussed if properly qualified so that the reader is not misled. Do not report P values to more than 3 places after the decimal. Regardless of the probability level used, failure to reject a hypothesis should be based on the relative consequences of type I and II errors. A “nonsignificant” relationship should not be interpreted to suggest the absence of a relationship.

An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of $P > 0.05$ to declare nonsignificance, such as indicating that a difference is not significant at $P > 0.05$ and subsequently declaring another difference significant (or a tendency) at $P < 0.09$. In addition, readers may incorrectly interpret the use of $P > 0.05$ as the probability of a β error, not an α error. Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information contained in the data is sacrificed. In most cases, 2 or 3 significant digits (not decimal places) are sufficient.

Results and Discussion

Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Results section shall contain only the results and summary of the author’s experiments; there should be no literature comparisons. Those comparisons should appear in the Discussion section.

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. Review copies shall have authors’ institutions omitted.

Appendix

A technical Appendix, if desired, shall follow the Discussion section or

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

References

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

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

Article:

Bagley, L. G., and V. L. Christensen. 1991. Hatchability and physiology of turkey embryos incubated at sea level with increased eggshell permeability. *Poult. Sci.* 70:1412–1418. Bagley, L. G., V. L. Christensen, and R. P. Gildersleeve. 1990.

Hematological indices of turkey embryos incubated at high altitude as affected by oxygen and shell permeability. *Poult. Sci.* 69:2035–2039.

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

Book:

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

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

Federal Register:

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

Other:

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

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

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

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

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

Tables

Tables must be created using the MS Word table feature and inserted in the manuscript after the references section. When possible, tables should be organized to fit across the page without running broadside. Be aware of the dimensions of the printed page when planning tables (use of more than 15 columns will create layout problems). Place the table number and title on the same line above the table. The table title does not require a period. Do not use vertical lines and use few horizontal lines. Use of bold and italic typefaces in the table body should be done sparingly; such use must be defined in a footnote. Each table must be on a separate page. To facilitate placement of all tables into the manuscript file (just after the references) authors should use “section breaks” rather than “page breaks” at the end of the manuscript (before the tables) and between tables. Units of measure for each variable must be indicated. Papers with several tables must use consistent format. All columns must have appropriate headings. Abbreviations not found on the inside front cover of the journal must be defined in each table and must match those used in the text. Footnotes to tables should be marked by superscript numbers. Each footnote should begin a new line. Superscript letters shall be used for the separation of means in the body of the table and explanatory footnotes must be provided [i.e., “Means within a row lacking a common superscript differ ($P < 0.05$).”]; other significant P -values may be specified. Comparison of means within rows and columns should be indicated by different series of superscripts (e.g., a,b, . . . in rows; x–z . . . in columns) The first alphabetical letter in the series (e.g., a or A) shall be used to indicate the largest mean. Lowercase

superscripts indicate $P \leq 0.05$. Uppercase letters indicate $P \leq 0.01$ or less.

Probability values may be indicated as follows: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and † $P \leq 0.10$. Consult a recent issue of *Poultry Science* for examples of tables.

Figures

To facilitate review, figures should be placed at the end of the manuscript (separated by section breaks). Each figure should be placed on a separate page, and identified by the manuscript number and the figure number. A figure with multiple panels or parts should appear on one page (e.g., if Figure 1 has parts a, b, and c, place all of these on the same page). Figure captions should be typed (double spaced) on a separate page.

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

- **Font Size.** Ensure that all type within the figure and axis labels are readable at final publication size.

A minimum type size of 8 points (after reduction) should be used.

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

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

- **Axis Labels.** Each axis should have a description and a unit. Units may be separated from the descriptor by a comma or parentheses, and should be consistent within a manuscript.

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

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

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

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

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

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

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

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

Color Figures. Submitted color images should be at least 300 ppi. The cost to publish each color figure is \$995; 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. The form “Color Charge Agreement” is available on the journal web site (<http://ps.fass.org>) and should be completed and returned to PSA Headquarters upon submission.

Miscellaneous Usage Notes

Abbreviations. Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD).

A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the scientific field. Abbreviations may be used in heads within the paper, if they have been first defined within the text. The inside back cover of every issue of the journal lists abbreviations that can be used without definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal (or the updated list at <http://ps.fass.org/>) for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used less than three times must be spelled out in full rather than abbreviated. All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter, rather than the full term. The abstract, text, each table, and each figure must be understood independently of each other. Therefore, abbreviations shall be defined within each of these units of the manuscript.

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

A	adenine
ADG	average daily gain
ADFI	average daily feed intake
AME	apparent metabolizable energy
AMEn	nitrogen-corrected apparent metabolizable energy
ANOVA	analysis of variance
B	cell bursal-derived, bursal-equivalent derived cell
bp	base pairs
BSA	bovine serum albumin
BW	body weight

C	cytosine
cDNA	complementary DNA
cfu	colony-forming units
CI	confidence interval
CP	crude protein
cpm	counts per minute
CV	coefficient of variation
d	day
df	degrees of freedom
DM	dry matter
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetate
ELISA	enzyme-linked immunosorbent antibody assay
EST	expressed sequence tag
g	gram
<i>g</i>	gravity
G	guanine
GAT	glutamic acid-alanine-tyrosine
G:F	gain-to-feed ratio
GLM	general linear model
h	hour
HEPES	<i>N</i> -2-hydroxyethyl piperazine- <i>N'</i> -ethane-sulfonic acid
HPLC	high-performance (high-pressure) liquid chromatography
ICU	international chick units
Ig	immunoglobulin
i.m.	intramuscular
i.p.	intraperitoneal
IU	international units
i.v.	intravenous
kb	kilobase pairs
kDa	kilodalton
L	liter*
L:D	hours light:hours darkness in a photoperiod
m	meter
μ	micro
<i>M</i>	molar
MAS	marker-assisted selection
ME	metabolizable energy
ME _n	nitrogen-corrected metabolizable energy
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
min	minute
mo	month
MS	mean square
n	number of observations
<i>N</i>	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
r ²	coefficient of determination, simple
R ²	coefficient of determination, multiple
RFLP	restriction fragment length polymorphism
RH	relative humidity
RIA	radioimmunoassay
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
s.c.	subcutaneous
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
TME _n	nitrogen-corrected true metabolizable energy
Tris	tris(hydroxymethyl)aminomethane
TSAA	total sulfur amino acids
U	uridine
USDA	United States Department of Agriculture
UV	ultraviolet
vol/vol	volume to volume
vs.	versus
wt/vol	weight to volume
wt/wt	weight to weight
wk	week
yr	year

*Also capitalized with any combination, e.g., mL.

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

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

Number Style. Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits. Measures must be in the metric

system; however, US equivalents may be given in parentheses.

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

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

Therefore, they shall be made available to other scientists for research purposes. Nucleotide sequences must be submitted as camera-ready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow *Poultry Science* guidelines.

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

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

Use "to" instead of a hyphen to indicate a range. Insert spaces around all signs (except slant lines) of operation (=, −, +, ×, >, or <, 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.

Supplemental Information (Online)

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

Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online (<http://ps.fass.org/misc/ifora.dtl>).

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 and Table Preparation Guidelines. Current detailed information on figure and table preparation can be found at <http://ps.fass.org/misc/ifora.dtl>

Manuscript Central Instructions. Manuscripts are submitted online (<http://mc.manuscriptcentral.com/psa>). Full user instructions for using the Manuscript Central system are available on the Manuscript Central home page.

Apêndice 3. Ganho de peso (GP, g), conversão alimentar (CA), consumo de ração (Cons, g) e mortalidade de 14 a 35 dias de idade (referente ao artigo do Capítulo II).

VAL:lys	ILE:lys	Bloco	GP				CA				Cons				Mort
			14-21 d	21-28 d	28-35 d	14-35 d	14-21 d	21-28 d	28-35 d	14-35 d	14-21 d	21-28 d	28-35 d	14-35 d	
70	65	I	465	690	868	2022	1,511	1,532	1,680	1,591	702	1057	1458	3218	0
70	65	II	471	661	886	2018	1,490	1,598	1,656	1,597	701	1056	1467	3222	8
70	65	III	477	720	914	2111	1,456	1,514	1,695	1,573	694	1090	1549	3320	0
70	65	IV	482	728	909	2119	1,519	1,511	1,690	1,588	733	1100	1537	3366	4
70	65	V	485	712	884	2081	1,480	1,489	1,712	1,581	718	1060	1514	3290	4
70	65	VI	482	729	863	2074	1,467	1,509	1,729	1,591	706	1100	1493	3299	0
70	65	VII	482	747	886	2114	1,488	1,502	1,723	1,592	717	1122	1527	3365	0
70	65	VIII	504	728	892	2124	1,473	1,550	1,755	1,618	743	1129	1565	3437	0
70	65	IX	506	690	898	2094	1,494	1,660	1,662	1,621	755	1146	1493	3394	0
75	65	I	503	740	887	2130	1,498	1,503	1,795	1,618	754	1111	1593	3447	8
75	65	II	498	730	893	2121	1,331	1,480	1,763	1,564	662	1081	1574	3318	0
75	65	III	493	761	884	2138	1,486	1,425	1,791	1,589	733	1084	1583	3399	0
75	65	IV	498	736	893	2128	1,470	1,520	1,737	1,591	733	1118	1552	3384	0
75	65	V	499	761	897	2157	1,492	1,563	1,781	1,633	745	1190	1597	3523	8
75	65	VI	501	806	867	2173	1,522	1,468	1,826	1,621	762	1183	1583	3522	8
75	65	VII	531	737	897	2165	1,360	1,589	1,618	1,539	723	1170	1452	3333	0
75	65	VIII	499	727	871	2097	1,407	1,522	1,734	1,583	703	1106	1510	3318	0
75	65	IX	485	758	928	2171	1,474	1,447	1,759	1,587	715	1098	1632	3444	4
80	65	I	501	724	798	2023	1,451	1,497	1,754	1,574	727	1084	1400	3185	0
80	65	II	505	792	828	2125	1,491	1,458	1,870	1,626	753	1154	1548	3455	0
80	65	III	488	743	924	2155	1,470	1,492	1,721	1,578	717	1109	1591	3401	0
80	65	IV	505	772	898	2174	1,466	1,460	1,764	1,587	740	1127	1583	3450	0
80	65	V	514	745	861	2120	1,481	1,502	1,834	1,631	762	1118	1578	3458	0
80	65	VI	483	731	911	2126	1,424	1,488	1,664	1,549	688	1088	1516	3292	0
80	65	VII	522	775	873	2170	1,465	1,517	1,778	1,609	764	1176	1553	3493	0
80	65	VIII	503	768	917	2189	1,467	1,529	1,681	1,577	738	1175	1542	3452	4
80	65	IX	502	750	866	2118	1,482	1,537	1,780	1,623	743	1154	1542	3439	0
70	68	I	477	778	902	2156	1,459	1,501	1,736	1,589	696	1167	1565	3426	4
70	68	II	478	714	878	2069	1,471	1,552	1,719	1,604	702	1107	1509	3318	0
70	68	III	505	742	934	2180	1,482	1,536	1,663	1,578	748	1139	1553	3440	0
70	68	IV	505	756	864	2125	1,487	1,561	1,650	1,578	750	1181	1426	3353	8
70	68	V	523	726	874	2123	1,445	1,566	1,723	1,601	756	1136	1506	3398	0
70	68	VI	490	766	871	2127	1,508	1,432	1,750	1,580	738	1098	1525	3361	0
70	68	VII	505	772	912	2188	1,426	1,529	1,747	1,596	720	1180	1593	3493	0
70	68	VIII	523	784	883	2189	1,419	1,472	1,814	1,597	742	1154	1601	3497	0
70	68	IX	513	749	872	2134	1,507	1,503	1,823	1,630	773	1125	1590	3478	9
70	71	I	492	741	918	2151	1,517	1,429	1,701	1,565	746	1058	1562	3367	0
70	71	II	539	721	898	2158	1,500	1,576	1,703	1,604	809	1136	1530	3463	0
70	71	III	485	746	918	2149	1,512	1,550	1,637	1,578	733	1156	1503	3391	4
70	71	IV	479	752	846	2077	1,474	1,415	1,798	1,585	706	1064	1521	3291	0
70	71	V	497	763	886	2146	1,506	1,423	1,737	1,569	748	1086	1539	3367	0

70	71	VI	498	775	874	2147	1,486	1,419	1,799	1,589	741	1100	1571	3412	0
70	71	VII	498	763	871	2133	1,475	1,491	1,772	1,602	735	1138	1543	3417	0
70	71	VIII	483	754	849	2085	1,549	1,532	1,833	1,658	748	1154	1556	3456	4
70	71	IX	482	757	945	2184	1,510	1,539	1,654	1,582	728	1164	1563	3455	4
75	68	I	522	771	900	2194	1,470	1,510	1,766	1,606	768	1165	1590	3522	0
75	68	II	522	689	857	2067	1,387	1,531	1,687	1,559	723	1054	1446	3223	0
75	68	III	521	733	882	2135	1,384	1,573	1,801	1,615	721	1153	1588	3447	8
75	68	IV	513	744	878	2135	1,476	1,544	1,765	1,615	757	1149	1550	3449	8
75	68	V	506	730	846	2082	1,452	1,542	1,678	1,575	735	1125	1419	3279	0
75	68	VI	481	709	898	2088	1,458	1,540	1,656	1,569	701	1091	1488	3275	4
75	68	VII	513	759	901	2173	1,467	1,536	1,705	1,590	752	1166	1536	3453	0
75	68	VIII	501	750	864	2116	1,485	1,548	1,802	1,637	744	1162	1558	3462	8
75	68	IX	495	774	847	2116	1,462	1,526	1,841	1,637	724	1181	1560	3465	0
80	71	I	483	754	882	2118	1,478	1,477	1,709	1,566	713	1114	1507	3317	0
80	71	II	481	712	917	2110	1,516	1,546	1,623	1,573	729	1101	1488	3318	0
80	71	III	477	724	823	2024	1,467	1,440	1,751	1,557	699	1043	1442	3152	4
80	71	IV	488	785	882	2155	1,491	1,412	1,849	1,598	728	1108	1630	3444	0
80	71	V	510	727	904	2142	1,481	1,565	1,708	1,606	756	1138	1544	3438	0
80	71	VI	490	798	915	2204	1,460	1,435	1,690	1,546	716	1146	1546	3408	0
80	71	VII	493	713	905	2112	1,475	1,557	1,733	1,611	728	1111	1569	3403	4
80	71	VIII	477	734	879	2090	1,510	1,538	1,669	1,587	720	1130	1467	3317	0
80	71	IX	500	746	903	2149	1,453	1,482	1,715	1,573	726	1105	1549	3380	0

Apêndice 4. Dados referentes ao abate de frangos de corte (artigo do Capítulo II).

VAL:lys	ILE:lys	Rendimentos, %					
		Bloco	Carcaça	Gordura	Peito	Pernas	Asas
70	65	I	74,43	1,70	29,47	32,34	11,34
70	65	II	74,48	2,10	29,26	32,23	11,07
70	65	III	74,60	2,01	29,67	33,00	11,08
70	65	IV	74,98	1,74	30,59	31,73	11,37
70	65	V	74,53	1,90	29,68	32,54	11,05
70	65	VI	74,97	1,83	29,95	32,07	11,18
70	65	VII	74,97	1,82	29,66	33,00	10,99
70	65	VIII	74,38	1,72	29,84	32,62	11,22
70	65	IX	75,26	2,07	29,94	31,50	10,97
75	65	I	73,86	2,35	28,58	31,87	11,18
75	65	II	73,32	1,59	29,12	32,68	11,18
75	65	III	75,03	1,79	30,59	32,19	10,84
75	65	IV	72,80	2,14	28,84	33,44	11,15
75	65	V	73,67	2,07	28,78	33,26	11,18
75	65	VI	74,28	1,88	28,47	33,07	11,06
75	65	VII	74,54	1,72	29,62	32,34	10,40
75	65	VIII	74,38	1,95	28,78	33,60	11,37
75	65	IX	75,08	1,53	29,55	32,47	10,97
80	65	I	73,86	2,07	29,27	31,73	11,47
80	65	II	73,98	2,03	30,18	32,21	11,23
80	65	III	74,31	1,79	28,98	32,85	11,06
80	65	IV	73,14	1,87	29,17	33,18	10,84
80	65	V	73,42	2,07	29,56	32,71	11,28
80	65	VI	75,30	1,68	29,13	32,48	11,23
80	65	VII	74,61	1,71	29,26	31,95	10,97
80	65	VIII	75,58	1,51	29,68	32,36	11,30
80	65	IX	75,15	1,62	29,66	32,69	10,90
70	68	I	74,36	1,87	28,21	32,85	10,80
70	68	II	74,05	1,99	29,07	32,65	10,97
70	68	III	74,77	1,75	29,47	32,85	10,85
70	68	IV	76,63	1,94	29,88	32,14	10,91
70	68	V	75,06	1,82	29,12	32,66	11,25
70	68	VI	74,29	1,59	28,16	33,28	11,01
70	68	VII	73,69	1,74	30,04	32,81	11,32
70	68	VIII	74,82	1,71	29,97	32,53	10,70
70	68	IX	75,32	1,81	29,37	32,65	11,38
70	71	I	73,84	1,79	28,78	31,83	10,90
70	71	II	74,65	2,08	29,55	31,64	10,85
70	71	III	73,91	1,74	29,23	32,40	10,70
70	71	IV	74,58	1,96	29,09	32,73	11,28
70	71	V	74,56	1,64	28,55	32,34	10,92
70	71	VI	74,68	1,91	28,92	33,37	11,31
70	71	VII	74,50	2,06	29,79	32,03	11,28
70	71	VIII	73,97	1,87	28,36	32,14	11,15
70	71	IX	74,30	1,88	29,42	32,60	10,99
75	68	I	73,99	2,05	29,84	31,52	11,15
75	68	II	73,40	1,48	29,61	31,77	11,44
75	68	III	74,30	1,93	29,67	32,19	11,08
75	68	IV	74,96	2,22	28,46	33,39	11,11
75	68	V	72,69	1,78	28,89	33,21	11,41
75	68	VI	74,10	1,71	29,07	32,90	11,04
75	68	VII	74,76	1,69	30,13	32,98	11,04
75	68	VIII	74,66	1,92	29,30	32,83	10,94
75	68	IX	74,48	2,04	29,35	32,11	11,09
80	71	I	73,61	2,11	29,36	32,16	11,23
80	71	II	74,55	1,73	29,85	32,90	11,06
80	71	III	74,73	1,74	29,64	31,95	11,06
80	71	IV	74,48	2,21	28,80	32,46	11,17
80	71	V	74,03	1,96	28,10	32,84	11,19
80	71	VI	72,69	2,06	29,00	32,97	11,24
80	71	VII	74,59	1,79	28,66	32,43	10,65
80	71	VIII	75,08	1,65	30,13	32,31	11,36
80	71	IX	72,78	1,92	29,25	32,35	11,27

Apêndice 5. Ganho de peso (GP, g), conversão alimentar (CA), consumo de ração (Cons, g) e mortalidade (Mort, %) de frangos de corte no período de 21 a 42 dias de idade e rendimentos de carcaça, gordura e cortes aos 42 dias de idade (referentes ao artigo do Capítulo III).

VAL	GP	CA	Cons	Mort	Rendimentos, %				
					Carcaça	Gordura	Asas	Peito	Pernas
0,64	2118	1,725	3695	1	79,09	1,87	10,29	32,51	32,09
0,64	2094	1,745	3652	1	79,70	2,68	10,09	30,64	30,53
0,64	2044	1,744	3576	0	78,13	1,96	10,44	30,84	33,19
0,64	2144	1,750	3736	1	76,79	2,00	10,35	30,41	33,20
0,64	2059	1,743	3562	0	78,18	2,17	9,81	31,98	32,22
0,64	2051	1,730	3543	1	78,98	1,93	10,39	32,66	31,01
0,64	2052	1,728	3557	0	78,20	1,80	10,63	31,78	32,62
0,64	2081	1,733	3542	1	79,56	2,40	10,38	32,15	31,35
0,70	2107	1,702	3535	0	80,78	1,81	10,17	31,85	31,85
0,70	2170	1,678	3637	1	78,07	2,00	10,39	31,66	31,64
0,70	2145	1,676	3683	1	78,42	1,85	10,31	32,33	32,40
0,70	2069	1,717	3564	2	78,69	2,24	9,97	30,53	32,84
0,70	2104	1,722	3552	1	79,13	1,68	10,45	32,12	32,45
0,70	2203	1,689	3683	1	77,04	2,16	10,37	30,39	33,08
0,70	2130	1,672	3550	0	77,42	1,98	10,34	31,07	32,13
0,70	2219	1,667	3687	0	79,88	1,80	10,42	31,58	32,17
0,70	2126	1,662	3594	0	78,12	1,92	10,13	31,65	32,60
0,75	2116	1,690	3608	1	78,99	1,76	10,44	30,58	32,64
0,75	2130	1,705	3615	0	79,96	1,50	9,75	33,80	31,00
0,75	2064	1,697	3599	1	78,95	2,04	10,45	31,93	32,77
0,75	2074	1,743	3502	2	78,55	1,69	10,37	31,84	32,37
0,75	2102	1,689	3651	1	78,86	1,75	10,39	31,05	32,96
0,75	2152	1,737	3733	4	77,63	1,72	10,26	32,59	31,96
0,75	2120	1,735	3576	0	77,38	1,90	10,37	32,04	31,97
0,75	2182	1,687	3700	1	79,06	1,90	10,42	31,37	31,78
0,75	2069	1,696	3496	0	79,23	2,20	10,05	32,09	31,68
0,81	2090	1,690	3558	0	78,56	2,01	10,44	30,33	32,76
0,81	2181	1,703	3666	0	79,82	2,11	10,00	31,82	32,38
0,81	2082	1,681	3419	0	79,53	1,54	10,21	32,59	32,70
0,81	2156	1,643	3665	1	78,88	1,52	10,50	32,39	32,19
0,81	2181	1,700	3801	2	78,65	2,00	10,03	31,63	32,65
0,81	2288	1,743	3776	2	78,31	1,90	10,41	31,68	32,02
0,81	2113	1,651	3618	2	79,66	1,90	10,22	31,53	31,76
0,81	2212	1,712	3738	1	78,65	1,70	10,31	31,91	31,98
0,81	2105	1,690	3571	3	80,24	2,07	10,10	31,11	31,92
0,86	2188	1,696	3723	2	79,59	1,96	10,61	30,60	32,13
0,86	2221	1,701	3765	1	80,04	1,86	10,06	31,38	32,04
0,86	2237	1,695	3776	1	77,65	1,96	10,19	30,71	32,85
0,86	2123	1,688	3616	0	77,61	2,04	10,14	31,09	32,46
0,86	2134	1,703	3609	0	77,54	1,66	10,04	31,78	32,76
0,86	2109	1,691	3557	0	78,09	1,91	10,57	32,25	32,99
0,86	2047	1,686	3506	1	79,28	2,23	10,42	30,39	31,58
0,86	2139	1,713	3623	0	80,52	1,63	10,47	31,52	31,17
0,86	2229	1,694	3678	0	78,74	1,71	10,23	31,95	32,49
0,92	2152	1,650	3616	0	77,99	2,30	10,22	30,84	31,83

0,92	2105	1,680	3609	1	79,22	1,90	10,38	31,72	31,52
0,92	2097	1,714	3655	3	79,36	2,12	10,15	31,18	31,80
0,92	2129	1,743	3578	0	77,49	1,87	10,41	31,07	34,13
0,92	2037	1,681	3474	0	77,56	1,76	9,97	30,60	33,86
0,92	2097	1,705	3546	0	78,54	1,51	10,16	31,98	32,47
0,92	2106	1,691	3548	1	79,39	1,59	10,87	31,71	31,48
0,92	2129	1,684	3619	1	79,35	1,86	10,39	32,49	32,19
0,92	2235	1,700	3775	3	79,19	1,73	10,18	30,31	31,86
0,97	2151	1,689	3672	0	79,11	1,92	10,33	31,30	32,06
0,97	2110	1,707	3605	2	78,36	2,39	10,24	29,90	31,94
0,97	2208	1,708	3720	0	78,82	1,94	10,26	31,95	31,62
0,97	2141	1,685	3696	2	78,72	1,81	10,46	30,67	32,93
0,97	2094	1,727	3507	0	78,44	1,59	10,54	31,97	32,31
0,97	2170	1,674	3652	1	80,18	1,50	9,93	32,42	32,22
0,97	2084	1,683	3557	0	79,33	1,66	10,42	31,81	32,60
0,97	2155	1,707	3745	1	79,19	1,83	10,95	31,60	31,74
0,97	2229	1,738	3625	0	78,99	1,56	10,12	32,26	31,76

Apêndice 6. Ganho de peso (GP, g), conversão alimentar (CA), consumo de ração (Cons, g) e causas de mortalidade de frangos de corte (referentes ao artigo do Capítulo IV).

U.E.	BLOCO	GP				CA				Cons				Causas mortalidade (1 – 42 d), %				
		1-7 d	1-21 d	1-35 d	1-42 d	1-7 d	1-21 d	1-35 d	1-42 d	1-7 d	1-21 d	1-35 d	1-42 d	Ascite	Morte Súbita	Pernas	Outros	Total
T1	I	141	982	2548	3162	1,076	1,308	1,409	1,491	152	1284	3591	4714	0	0	0	0	0
T1	II	127	976	2509	3096	1,101	1,283	1,409	1,496	140	1251	3534	4633	0	0	0	0	0
T1	III	144	988	2504	3031	1,035	1,294	1,412	1,519	149	1278	3536	4605	0	0	0	0	0
T1	IV	143	1008	2551	3153	1,065	1,267	1,394	1,495	152	1277	3555	4714	0	0	0	0	0
T1	V	131	964	2428	2998	1,033	1,252	1,403	1,489	135	1206	3406	4464	0	0	0	0	0
T1	VI	133	961	2451	3042	1,091	1,314	1,425	1,510	145	1262	3493	4593	0	4	0	0	4
T1	VII	135	955	2494	3137	1,016	1,280	1,398	1,473	137	1223	3488	4621	0	4	0	4	7
T1	VIII	137	1013	2611	3235	1,063	1,262	1,402	1,486	146	1278	3661	4808	0	4	0	0	4
T1	IX	136	1011	2586	3226	1,058	1,305	1,452	1,525	144	1319	3753	4920	0	4	0	0	4
T2	I	139	971	2496	3089	1,005	1,291	1,411	1,502	140	1253	3521	4639	0	0	0	0	0
T2	II	117	934	2461	3101	1,122	1,295	1,411	1,493	131	1210	3474	4629	0	0	0	0	0
T2	III	131	976	2473	3082	1,083	1,308	1,444	1,515	142	1276	3570	4669	0	0	0	4	4
T2	IV	147	989	2517	3096	1,034	1,309	1,426	1,533	152	1295	3589	4746	0	0	0	0	0
T2	V	137	978	2514	3120	1,036	1,316	1,435	1,522	142	1287	3607	4748	0	0	0	0	0
T2	VI	148	987	2525	3116	1,043	1,299	1,417	1,519	154	1282	3578	4733	0	0	0	0	0
T2	VII	136	984	2490	3074	1,037	1,298	1,432	1,515	141	1277	3565	4658	0	4	0	0	4
T2	VIII	129	961	2471	3179	1,028	1,284	1,442	1,510	132	1234	3563	4801	0	0	0	0	0
T2	IX	134	976	2453	3043	1,048	1,290	1,423	1,496	141	1259	3491	4553	0	4	0	7	11
T3	I	136	977	2521	3137	1,032	1,317	1,449	1,529	140	1287	3653	4798	0	0	0	4	4
T3	II	140	998	2534	3133	1,036	1,274	1,427	1,506	145	1272	3615	4717	0	4	0	4	7
T3	III	145	980	2479	3070	1,020	1,305	1,421	1,507	148	1279	3523	4626	0	4	0	0	4
T3	IV	141	1000	2477	3053	1,040	1,293	1,436	1,509	147	1293	3557	4607	0	0	7	0	7
T3	V	144	1015	2503	3133	1,045	1,286	1,444	1,521	151	1306	3615	4766	0	0	0	0	0
T3	VI	144	998	2506	3116	1,045	1,278	1,413	1,493	150	1276	3541	4653	0	0	0	0	0
T3	VII	145	1021	2506	3144	1,049	1,298	1,457	1,543	152	1325	3651	4850	0	0	0	0	0
T3	VIII	134	977	2541	3161	1,031	1,273	1,398	1,476	139	1243	3554	4665	0	4	0	4	7
T3	IX	132	969	2509	3152	1,043	1,279	1,425	1,515	138	1240	3576	4774	0	0	0	4	4
T4	I	144	1008	2567	3162	0,965	1,266	1,403	1,490	139	1277	3602	4713	0	0	0	4	4
T4	II	81	850	2389	2961	1,168	1,264	1,375	1,485	94	1074	3284	4398	0	0	0	0	0

T4	III	134	976	2512	3146	1,048	1,285	1,389	1,465	140	1254	3489	4610	0	0	0	14	14
T4	IV	141	976	2434	2994	1,000	1,275	1,402	1,487	141	1244	3414	4451	0	0	0	0	0
T4	V	143	1013	2523	3161	1,005	1,274	1,409	1,474	144	1291	3555	4658	0	0	4	4	7
T4	VI	139	976	2491	3085	1,036	1,295	1,416	1,502	144	1264	3529	4632	0	0	0	0	0
T4	VII	135	1002	2486	3111	1,063	1,300	1,434	1,512	144	1303	3565	4704	0	0	0	4	4
T4	VIII	145	1008	2556	3137	0,988	1,263	1,407	1,495	144	1274	3595	4691	0	7	0	0	7
T4	IX	145	995	2548	3192	1,020	1,301	1,441	1,514	148	1294	3673	4834	0	4	0	0	4
T5	I	139	998	2473	3060	1,010	1,268	1,413	1,491	140	1265	3494	4563	0	0	0	4	4
T5	II	139	964	2491	3111	1,046	1,290	1,406	1,485	145	1244	3503	4619	0	0	0	4	4
T5	III	124	922	2362	2963	1,098	1,323	1,437	1,508	136	1220	3395	4468	0	0	0	0	0
T5	IV	140	999	2483	3085	1,051	1,300	1,453	1,533	147	1299	3607	4730	0	0	0	0	0
T5	V	136	976	2526	3130	1,042	1,280	1,405	1,487	141	1249	3550	4656	0	0	0	0	0
T5	VI	144	1002	2523	3135	1,010	1,255	1,391	1,473	145	1257	3510	4618	0	7	0	0	7
T5	VII	146	1009	2585	3238	1,059	1,277	1,417	1,499	154	1288	3665	4853	4	0	0	0	4
T5	VIII	122	964	2505	3115	1,076	1,279	1,408	1,555	131	1234	3528	4843	0	0	0	0	0
T5	IX	137	998	2508	3130	0,964	1,258	1,424	1,511	132	1256	3570	4730	0	0	4	0	4
T6	I	126	946	2469	3156	1,073	1,326	1,452	1,511	136	1254	3586	4768	0	0	0	0	0
T6	II	134	970	2468	3100	1,096	1,328	1,431	1,496	146	1288	3531	4638	0	0	0	0	0
T6	III	131	960	2488	3082	1,098	1,368	1,464	1,549	144	1313	3644	4774	0	4	0	0	4
T6	IV	134	940	2421	3043	1,048	1,332	1,441	1,507	141	1252	3488	4586	0	0	0	0	0
T6	V	127	979	2442	3003	1,118	1,338	1,463	1,595	142	1309	3574	4790	0	0	0	0	0
T6	VI	135	967	2505	3124	1,063	1,320	1,434	1,512	144	1277	3592	4725	0	7	0	0	7
T6	VII	131	934	2471	3118	1,049	1,319	1,453	1,520	137	1231	3590	4737	0	0	4	0	4
T6	VIII	127	935	2436	3110	1,096	1,360	1,482	1,551	139	1271	3611	4825	0	0	0	0	0
T6	IX	142	1026	2531	3098	1,040	1,318	1,449	1,546	148	1352	3668	4791	0	4	0	4	7
T7	I	135	968	2464	3070	1,035	1,293	1,416	1,487	140	1251	3488	4566	0	7	0	0	7
T7	II	144	986	2461	3025	0,975	1,294	1,431	1,511	141	1276	3523	4570	0	4	0	0	4
T7	III	140	981	2519	3121	1,077	1,312	1,434	1,507	151	1286	3611	4703	0	0	0	4	4
T7	IV	145	993	2514	3117	1,044	1,314	1,439	1,514	151	1305	3617	4718	0	0	0	0	0
T7	V	131	955	2401	3012	1,027	1,278	1,424	1,489	135	1221	3420	4484	0	0	0	4	4
T7	VI	152	993	2487	3080	1,014	1,286	1,439	1,520	154	1276	3579	4681	0	0	7	0	7
T7	VII	144	978	2468	3031	1,040	1,307	1,434	1,525	149	1278	3539	4624	0	0	0	0	0
T7	VIII	151	1009	2521	3121	0,986	1,310	1,470	1,562	149	1322	3704	4875	0	0	0	0	0

T7	IX	138	961	2463	3077	1,016	1,309	1,452	1,534	140	1257	3576	4719	0	0	0	0	0
T8	I	141	988	2563	3153	0,975	1,268	1,395	1,482	137	1253	3576	4674	0	4	0	0	4
T8	II	125	966	2491	3155	1,078	1,287	1,408	1,476	134	1243	3506	4656	0	4	0	4	7
T8	III	134	974	2530	3135	1,059	1,281	1,400	1,493	141	1247	3541	4681	0	0	0	0	0
T8	IV	147	989	2478	3002	0,995	1,272	1,401	1,501	146	1258	3472	4507	0	4	0	4	7
T8	V	133	1003	2487	3070	0,986	1,248	1,391	1,468	131	1252	3458	4507	0	4	0	0	4
T8	VI	149	1026	2501	3100	1,048	1,290	1,427	1,510	156	1323	3570	4682	0	0	0	0	0
T8	VII	151	1022	2517	3101	1,019	1,284	1,419	1,497	154	1312	3571	4643	0	0	0	0	0
T8	VIII	144	998	2486	3088	1,005	1,288	1,422	1,498	144	1286	3534	4626	0	4	0	0	4
T8	IX	140	999	2562	3190	1,005	1,273	1,418	1,493	141	1271	3631	4763	0	4	4	4	11

Apêndice 7. Rendimentos de carcaça, gordura e cortes comerciais de frangos de corte aos 42 dias de idade (referentes ao artigo do Capítulo IV), %.

U.E.	Bloco	Carcaça	Gordura	Peito	Pernas	Asas	Dorso
T1	I	77,05	2,16	28,92	33,33	10,29	25,16
T1	II	80,71	1,76	28,81	33,83	10,35	24,51
T1	III	77,89	1,81	29,20	33,43	10,35	24,63
T1	IV	78,37	1,96	29,18	33,51	10,87	24,37
T1	V	78,25	1,97	28,41	33,82	10,84	24,40
T1	VI	80,23	1,70	29,58	33,35	10,46	24,16
T1	VII	78,43	2,05	28,71	33,42	10,38	24,74
T1	VIII	78,79	1,58	29,10	33,17	10,59	24,81
T1	IX	77,87	1,90	29,48	33,73	10,44	24,89
T2	I	77,54	1,91	28,59	33,70	10,59	25,21
T2	II	79,71	2,14	28,93	33,03	10,47	24,46
T2	III	78,60	2,36	28,69	32,73	10,48	25,29
T2	IV	79,40	1,95	29,88	33,06	10,48	23,88
T2	V	79,54	2,12	28,25	33,25	10,85	24,46
T2	VI	78,69	2,17	28,63	33,62	10,73	24,73
T2	VII	79,81	2,13	29,18	33,23	10,30	24,59
T2	VIII	77,64	1,85	29,22	32,92	10,68	24,44
T2	IX	77,65	1,77	29,53	34,07	10,53	23,78
T3	I	77,79	2,02	29,36	32,80	10,34	25,04
T3	II	79,70	1,90	28,45	33,00	10,37	25,57
T3	III	79,13	2,01	28,81	33,69	10,79	24,32
T3	IV	79,55	1,69	29,96	33,02	10,38	24,60
T3	V	77,61	2,07	29,19	33,03	10,77	24,64
T3	VI	79,63	1,96	29,26	33,27	10,39	24,39
T3	VII	79,50	2,05	28,68	32,86	10,79	24,85
T3	VIII	77,86	1,74	30,10	32,98	10,67	24,16
T3	IX	77,47	2,02	29,19	33,33	10,46	24,63
T4	I	78,47	1,75	28,35	32,82	10,57	25,46
T4	II	79,65	1,67	30,01	33,18	10,77	23,66
T4	III	78,58	1,89	30,11	32,68	10,32	24,38
T4	IV	78,81	1,82	29,67	32,52	10,76	24,05
T4	V	78,57	1,89	29,40	32,78	10,87	24,69
T4	VI	78,48	2,00	28,15	33,22	10,73	25,36
T4	VII	78,17	1,96	29,13	32,40	10,63	25,17
T4	VIII	78,61	1,84	29,47	33,31	10,47	24,33
T4	IX	77,45	1,64	29,69	33,29	10,83	24,48
T5	I	76,98	1,70	29,04	32,84	10,57	25,64
T5	II	80,44	2,21	28,29	33,05	9,99	25,28
T5	III	78,02	2,12	29,48	32,79	10,39	24,43
T5	IV	78,37	1,90	29,90	33,09	10,51	24,33
T5	V	79,06	1,74	29,26	32,87	10,57	24,59
T5	VI	77,71	1,87	29,40	32,83	10,75	24,73
T5	VII	78,48	2,10	28,49	33,80	10,68	24,68
T5	VIII	78,40	1,99	30,35	32,68	10,45	24,38
T5	IX	78,62	1,79	29,70	32,72	10,49	24,48
T6	I	77,37	1,90	29,14	32,69	10,39	25,61
T6	II	79,76	1,99	28,57	33,08	10,56	25,13
T6	III	78,51	2,18	30,06	33,37	10,43	23,50
T6	IV	77,64	2,02	29,03	33,35	10,71	24,43

T6	V	79,41	1,82	28,52	33,04	10,62	24,66
T6	VI	79,18	2,03	28,86	33,11	10,49	24,52
T6	VII	77,68	2,18	29,30	33,19	10,60	24,27
T6	VIII	78,91	2,30	29,47	32,18	10,40	23,69
T6	IX	78,93	2,11	30,48	32,34	10,04	24,08
T7	I	78,53	1,83	29,83	32,20	10,41	24,90
T7	II	78,99	1,89	30,07	32,20	10,46	25,05
T7	III	80,90	1,81	29,35	33,46	10,29	24,06
T7	IV	78,66	2,12	28,86	33,41	10,58	24,51
T7	V	78,39	2,01	29,25	32,70	10,74	24,74
T7	VI	79,08	1,91	29,23	33,22	10,34	24,39
T7	VII	78,68	2,10	28,90	33,15	10,62	24,90
T7	VIII	80,06	2,38	29,59	32,88	10,15	24,03
T7	IX	78,55	2,27	30,56	32,71	10,21	24,07
T8	I	78,26	1,62	30,54	32,75	10,30	24,24
T8	II	79,00	1,97	29,81	33,02	10,55	24,12
T8	III	79,39	1,69	29,80	32,31	10,37	24,46
T8	IV	77,69	1,81	29,70	32,96	10,90	24,57
T8	V	78,13	1,92	29,01	33,07	10,61	24,74
T8	VI	78,87	1,85	28,91	32,82	10,72	24,99
T8	VII	78,87	2,01	29,41	32,95	10,74	24,42
T8	VIII	78,28	1,92	30,08	32,27	10,79	24,24
T8	IX	78,02	1,50	30,31	32,66	10,61	24,26

Apêndice 8. Modelo geral do programa utilizado no pacote estatístico SAS (2001) para análise de linha quebrada (referente ao artigo do Capítulo III).

```
data one;
input x y;
cards;
0.64 1.733
0.70 1.704
0.75 1.709
0.81 1.691
0.86 1.691
0.91 1.695
;
data fill;
do x=0.64 to 0.91 by 0.001; y=.; output; end;
run;
data one; set one fill; run;
proc sort data=one; by x;

proc nlin;
parameters l=1.6921 u=0.2321 r=0.7972;
z1=(x<r)*(r-x);
model y=l+u*(z1);
output out=ppp p=predy;
run;

proc gplot;
title 'VAL Requirements';
goptions hpos=45 vpos=45 ftext=swiss;
symbol1 v=dot c=black;
symbol2 i=join v=none c=black;
plot y*x predy*x/overlay;
run;
quit;
```

VITA

Josemar Berres, primogênito de Pedro Celso Berres e Arsicia Maria Berres, nasceu em Cândido Godói, RS, aos 12 de junho de 1981. Coursou o ensino fundamental nos colégios Medianeira (La Salle) em Cerro Largo, RS e Pio X em Palmeira das Missões, RS. Concluiu o ensino médio na Escola de 1º e 2º Graus da Universidade Regional Integrada de Frederico Westphalen, RS. Em 1999 ingressou no Curso de Zootecnia da Universidade Federal de Santa Maria, RS, obtendo o Grau de Zootecnista em janeiro de 2004. Iniciou, em março de 2004, o curso de mestrado na área de Produção Animal, subárea Nutrição de Não-ruminantes, no Programa de Pós-graduação em Zootecnia da Universidade Federal do Rio Grande do Sul, Porto Alegre, RS. No ano de 2006 prosseguiu em suas pesquisas na área de nutrição de frangos de corte quando iniciou seu doutorado nesta mesma instituição, permanecendo nesta até o presente momento.