UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

SUPLEMENTAÇÃO DE AMINOÁCIDOS DE CADEIA RAMIFICADA EM DIETAS COM REDUÇÃO PROTEICA PARA FRANGOS DE CORTE

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Tese apresentada como um dos requisitos à obtenção do Grau de Doutor em Zootecnia
Área de Concentração Nutrição Animal

Porto Alegre (RS), Brasil
Março de 2015

CIP - Catalogação na Publicação

ANTONIOL MIRANDA, DANIEL JOSÉ
SUPLEMENTAÇÃO DE AMINOÁCIDOS DE CADEIA RAMIFICADA EM DIETAS COM REDUÇÃO PROTEICA PARA FRANGOS DE CORTE / DANIEL JOSÉ ANTONIOL MIRANDA. -- 2015.
88 f.

Orientador: SERGIO LUIZ VIEIRA.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, BR-RS, 2015.

1. FRANGOS DE CORTE. 2. PROTEÍNA. 3. AMINOÁCIDOS. 4. VALINA. 5. ISOLEUCINA. I. VIEIRA, SERGIO LUIZ, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Folha de homologação

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

(John F. Kennedy)

AGRADECIMENTOS

A Deus pelo dom da vida.

Aos meus familiares por serem a base da minha vida. Pelo apoio incontestável e incentivo, essenciais para a conquista de qualquer objetivo.

Ao Professor Sergio Luiz Vieira pela amizade, companheirismo e oportunidades durante o curso.

Aos amigos que diretamente ou indiretamente deram suporte no dia a dia durante estes 4 anos.

Aos colegas e amigos do grupo de pós graduação André Favero, André Ghiotti, Barbara, Catarina, Daniel, Diogo, Franciele, Jaime, Liliane, Rafael Barros, Vanessa; bolsistas e estagiários César, Fúlvio, Gabriela, Guilherme, Heitor, Henrique, Natacha, Natália, Patrícia, Silvana, Rafael Cruz e todos os que passaram pelo Aviário de Ensino e Pesquisa pela amizade e companheirismo.

À Universidade Federal do Rio Grande do Sul, aos professores e funcionários do Programa de Pós-Graduação em Zootecnia.

À Purdue University, prinpalmente ao professor Adeola pelo suporte durante o período de Doutorado Sanduíche.

À CAPES pela concessão da bolsa de estudos.

À empresa Ajinomoto Nutrição Animal pelo auxílio na realização desta tese.

A todos aqueles que de alguma forma contribuíram para a realização desta tese, meu muito obrigado!

SUPLEMENTAÇÃO DE AMINOÁCIDOS DE CADEIA RAMIFICADA EM DIETAS COM REDUÇÃO PROTÉICA PARA FRANGOS DE CORTE[1]

Autor: Daniel José Antoniol Miranda
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RESUMO - Esta tese foi realizada objetivando comparar 4 programas alimentares, em dietas à base de milho e farelo de soja, formuladas com ou sem restrição de proteína bruta (PB), suplementadas ou não com L-Val e L-Ile, e usando diferentes níveis de lisina digestível (dig.). Os programas alimentares (PRG) foram: PRG 1, PB limitada a um mínimo (22,4, 21,1, 19,8, 18,4% para as fases pré-inicial, inicial, crescimento e final, respectivamente, com relações de AA:Lis definidos apenas para Met+Cis (0,72) e Tre (0,65); PRG 2, a PB não foi restrita e as relações estendidas para Val (0,77) e Ile (0,67); PRG 3, mesmas restrições do PRG 2 e suplementadas com L-Val; PRG 4, mesmas restrições do PRG 3, e suplementadas com L-Ile. Dois experimentos foram realizados, um com 1.800 e outro com 4.800 pintos machos Cobb x Cobb 500 de 1 dia de idade. No primeiro experimento, as rações foram formuladas usando os 4 PRG e níveis de Lis dig. de 1,324%, 1,217%, 1,095% e 1,006% ou 5% maiores, para cada fase, totalizando 8 tratamentos e 9 repetições cada. Para o segundo experimento, os 4 PRG foram utilizados até 1-21 d, totalizando 4 tratamentos com 48 repetições. E de 22 a 42 dias, cada PRG foi subdividido nos 4 PRG, passando cada PRG da fase anterior a receber todos os 4 PRG para as fases de crescimento e final, totalizando 16 tratamentos com 12 repetições cada. Não houve interação entre os tratamentos em ambos os experimentos, com exceção para o ganho de peso (GP) e conversão alimentar (CA) de 36 a 43d, no primeiro experimento, onde aves alimentadas com PRG 2 demonstraram melhorias no GP e CA quando alimentadas com um aumento de 5% no nível de Lis. Também, o GP e a CA acumulados aos 35 e 43d foram melhores quando as aves foram alimentadas com o PRG 2, sem diferença estatística para o PRG 3 e 4. Um aumento em 5% no nível de Lis dig. resultou na melhoria da CA acumulativa aos 43d. A gordura abdominal, como porcentagem da carcaça eviscerada aos 43d, foi maior para as aves alimentadas com a PRG 1. No segundo experimento, as aves alimentadas com PRG 2 tiveram melhor CA de 22 a 42d e de 1 a 42d, mas sem diferença estatística para os PRG 3 e 4. As aves alimentadas com PGR 1 de 1 a 42d apresentou o menor GP e o pior CA sem diferenças entre os PGR 2 e 3, e os PGR 3 e 4, respectivamente. Os dados do presente estudo demonstram que a suplementação de dietas para frangos de corte com fontes sintéticas dos cinco primeiros AA limitantes permitiram resultados de desempenho semelhantes a uma dieta com PB restrita a um valor mínimo.

Palavras-chave: frango de corte, proteína, aminoácidos, valina, isoleucina

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[1] Tese de Doutorado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (189 p.) Março, 2015.

BRANCHED CHAIN AMINO ACIDS SUPPLEMENTATION IN DIETS WITH PROTEIN REDUCTION FOR BROILERS CHICKENS[2]

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Adviser: Sergio Luiz Vieira

ABSTRACT - This thesis was carried out to compare four corn-soy feeding programs formulated with or without crude protein (CP) restrictions supplemented with or without L-Val and L-Ile, and using different digestible (dig.) Lys levels. Feeding programs (PRG) were: PRG 1, CP was restricted to a minimum (22.4, 21.1, 19.8, 18.4% for pre starter, starter, grower and finisher phases, respectively) with AA to Lys ratios set only for TSAA (0.72) and Thr (0.65); PRG 2, CP was not restricted while minimum ratios were also extended to Val (0.77) and Ile (0.67); PRG 3, restrictions were as in PRG 2 but with L-Val added; PRG 4, restrictions were as in PRG 3, but with L-Ile added. For this two experiments were conducted, one using 1,800 and the other using 4,800 one-day-old Cobb 500 male broiler chicks. For the first experiment, feeds had formulated using the 4 PRG and dig. Lys as 1.324%, 1.217%, 1.095% and 1.006% or 5% higher, for each phase, totaling 8 treatments with 9 replicates each. For the second experiment, the 4 PRG were used from 1 to 21 d, totaling 4 treatments with 48 replicates. From 22 to 42d, each PRG was subdivided in 4 PRG having the same rational as it was done to 21d, where each of the 4 PRG began receiving all 4 PRG for grower and finisher phases, totaling 16 treatments with 12 replicates each. No interaction was found between treatments in both experiments, with one exception for body weight gain (BWG) and feed conversion ratio (FCR) from 36 to 43d, in the first experiment, with birds fed PRG 2 demonstrating improvements in BWG and FCR when fed the 5% increasing in dig. Lys. And also, cumulative BWG and FCR at 35 and 43d and in each individual feeding phases showed broilers from PRG 2 having the best BWG and FCR; however, mean separations using Tukey showed no difference from birds fed PRG 3 and 4. Feeding a dietary program with 5% increase in dig. Lys resulted in improvement in cumulative FCR at 43d. Abdominal fat, as a percentage of the eviscerated carcass at 43d, was highest for birds fed the PRG 1. For the second experiment, birds fed with PRG 2 led to the best FCR from 22 to 42d and from 1 to 42d, but without statistical difference from PRG 3 and 4. Birds fed PGR 1 from 1 to 42d showed the lowest BWG and the highest FCR without statistical differences from PGR 2 and 3 and PGR 3 and 4, respectively. Data from the current study demonstrate that the supplementation of broiler diets with crystalline sources of the first five limiting AA allowed performance results similar to those produced when CP is restricted to a minimum.

Key words: broiler, protein, amino acids, valine, isoleucine

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[2] Doctoral thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (189 p.) March, 2015.

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RELAÇÃO DE ABREVIATURAS

AA	Aminoácido
AACR	Aminoácidos de cadeia ramificada
CA	Conversão alimentar
Dig	Digestível
DL-Met	DL-Metionina
GP	Ganho de peso
Ile	Isoleucina
KIC	α-cetoisocaproato
KIV	α-cetoisovalerato
KMV	α-cetometilvalerato
L-Ile	L-Isoleucina
L-Lis	L-Lisina
L-Tre	L-Treonina
L-Val	L-Valina
Leu	Leucina
Lis	Lisina
Met	Metionina
PB	Proteína bruta
PRG	Programa alimentar
Tre	Treonina
Trp	Triptofano
Val	Valina

CAPÍTULO I

INTRODUÇÃO

Durante muitos anos, as dietas para aves e suínos foram formuladas com base na proteína bruta (PB), que era estimada por meio do conteúdo de nitrogênio total do alimento a ser avaliado, portanto, envolvendo um grande grupo de substâncias com estruturas semelhantes, porém com funções fisiológicas diferentes. Contudo, com o desenvolvimento da nutrição animal, a possibilidade de determinação de aminoácidos (AA) nos alimentos e o surgimento dos AA sintéticos no mercado, os nutricionistas sentiram a necessidade de considerar a formulação de dietas baseadas nas exigências dos animais por aminoácidos essenciais. Entretanto, as dietas práticas ainda continham considerável excesso de AA que representava uma fonte de energia de alto custo, maior gasto energético para excreção pelas aves, além de provocar danos para o ambiente devido à poluição causada pela excreção excessiva de nitrogênio pelas fezes e urina (Parsons & Baker, 1994), preocupação cada vez mais frequente, sendo esta responsável por poluição de água (nitritos e nitratos), eutrofização, volatilização de amônia e causador de chuva ácida.

Com isso, durante as formulações das dietas surgiu a necessidade de considerar os efeitos da digestibilidade dos AA para maximizar a absorção e síntese de proteínas nos tecidos e, por consequência, a eficiência de ganho, uma vez que as proteínas dos alimentos são diferentes e apresentam diferentes coeficientes de digestibilidade dos AA distintos, surgindo assim o conceito de proteína ideal.

O uso de relações de proteína ideal (relação AA com a Lis) tornou-se uma estratégica altamente utilizada pelos nutricionistas animais para a formulação de dietas para frangos de corte, permitindo a redução da quantidade de AA na dieta e melhor equilíbrio do balanço metabólico de AA (Lemme, 2003; Vieira & Angel, 2012). Contudo, usualmente o valor de PB não é restrito quando formuladas rações para frangos de corte usando o conceito de proteína ideal com a suplementação sintética dos três primeiros AA limitantes. Contudo, restringir em uma concentração mínima o valor da PB durante a formulação, torna-se uma estratégia conservadora utilizadas pelos nutricionistas para assegurar uma inclusão mínima de AA essenciais e não-essenciais.

A suplementação das dietas para frangos de corte com fontes de AA sintéticas tradicionais, comercialmente disponíveis (L-Lisina – L-Lis, DL-Metionina – DL-Met, e L-Treonina – L-Tre), tem permitido aos nutricionistas ótimos resultados, tanto referente ao desempenho dos frangos de corte no campo quanto em relação ao rendimento no frigorífico, tornando-se um padrão na indústria avícola atual (Hill & Kim, 2013; Kobayashi et al., 2013). A formulação de dietas para frangos de corte com uma adicional redução no valor de PB é possível com a entrada de outros AA sintéticos que estão sendo disponibilizados no mercado a preço competitivos. Como exemplo temos o uso da L-Valina (L-Val) que em dietas à base de milho e farelo de soja é o quarto AA limitante para frangos de corte e a L-Isoleucina (L-Ile) que segue como quinto AA limitante (Kidd et al., 2004a; Corzo et al., 2007; Berres et al., 2010b; Tavernari et al., 2013).

Dados publicados utilizando fontes de AA sintéticas como a L-Val e a L-Ile tem aumentado recentemente, contudo, a implementação comercial ainda é limitada devido as informações insuficientes dos efeitos da suplementação desses AA sintéticos utilizando diferentes programas de alimentação durante o crescimento dos frangos de corte. Contudo, dados sobre o desempenho e rendimentos de carcaça e cortes, utilizando dietas suplementadas ou não com L-Val e L-Ile se tornam necessários para que os nutricionistas possam utilizá-los de forma eficaz durante a formulação de rações. Relações ideais de valina (Val) e isoleucina (Ile) digestível com a lisina (Lis) que otimizam o crescimento de frangos de corte tem sido sugeridas entre 0,75 e 0,77, e 0,65 e 0,67, respectivamente (Kidd et al., 2004a; Corzo et al., 2009, Berres et al., 2010a,b; Corzo et al., 2011; Tavernari et al., 2013), porém os dados definidos destas relações para a otimização do rendimento de carcaça e cortes comerciais ainda são inconsistentes.

A redução proteica das dietas para frangos de corte por meio suplementação com AA sintéticos permite uma maior competitividade no mercado, principalmente devido a redução da inclusão de farelo de soja, pelo fato da proteína ser um nutriente caro em dietas avícolas comerciais. O aumento do custo dos ingredientes e da concorrência com a indústria de biocombustível para alimentos vegetais, como milho e soja, indicam um cenário de aumento de custos para ingredientes de alimentos nos próximos anos. A partir de 2007, os preços das principais culturas utilizadas na produção animal têm aumentado dramaticamente em termos reais, atingindo um pico em 2008 e depois em declínio em 2009 e 2010, seguido de fortes aumentos de novo em 2011 (Rosegrant et al., 2012). Portanto, reduzir a PB por meio do aumento do uso de AA sintético pode ajudar a manter a produção competitiva de carne e minimizar o impacto do aumento do custo e volatilidade de fontes de proteína. É importante notar que os lucros resultantes da melhoria dos rendimentos de carne ao utilizar dietas de alta densidade de AA dependem em grande parte do custo da ração e do preço de mercado da carne de frango (Kidd et al, 2004b;. Corzo et al., 2010).

A presente tese foi conduzida a fim de avaliar o efeito da suplementação com L-Val e L-Ile sintética em dietas com PB reduzida para frangos de corte submetidos a diferentes programas alimentares.

REVISÃO BIBLIOGRÁFICA

Digestão e absorção dos aminoácidos

As aves sintetizam proteínas que contém 20 L-AA e utilizam AA livres para realizar uma grande variedade de funções. No entanto, são incapazes de sintetizar nove desses AA devido à ausência de enzimas específicas. A digestão proteica nas aves inicia-se no proventrículo e termina no transporte de AA e peptídeos pela membrana basolateral do intestino delgado (D'Mello, 2003). A passagem do alimento pelo proventriculo estimula a secreção de ácido clorídrico e pepsinogênio através da células principais. O meio ácido além de promover a quebra parcial da molécula proteica, provoca a ativação do pepsinogênio que se converte em pepsina, uma endopeptidase, que hidrolisa ligações peptídicas entre os AA leucina-valina, tirosina-leucina e fenilalanina-tirosina (Macari et al., 2008).

No intestino ocorre secreção de diversas enzimas pelo pâncreas (aminopeptidases, carboxipeptidades e outras peptidases específicas) que atuarão sobre a proteína da ingesta. A tripsina é ativada pela enteroquinase que é liberada na presença de proteínas parcialmente desnaturadas provindas da moela a qual ativa os demais zimogênios, reduzindo então as proteínas em oligopeptídeos e AA livres (Smith & Hill, 1985).

Alguns oligopeptídeos com seis AA ou menos, sao resistentes à hidrolise luminal. Assim, é necessária a ação das peptidases de membrana para a completa digestão proteica. Grande parte dos oligopeptídeos são reduzidos, nas microvilosidades, em tri e dipeptídeos, os quais são absorvidos pelas células da mucosa intestinal por meio de transporte ativo envolvendo o íon Na^+, com diferentes sistemas carreadores para os vários grupos de AA (Leeson e Summers, 2001). Alguns estudos mostram que 20 a 97% dos AA da dieta são catabolizados no intestino delgado, com uma utilização inferior a 20% dos AA extraídos para a síntese proteica da mucosa intestinal (Burrin and Stoll, 2009; Wu et al., 2010; Bauchart-Thevret et al., 2011).

Após a absorção dos AA, estes são transportados para o fígado principalmente pela veia porta, sendo uma pequena quantidade pela via linfática. No fígado, parte dos AA é fixada pelas células hepáticas e o restante é liberado na corrente sanguínea formando um pool extracelular de AA livres. Nos tecidos após absorvidos pelas células, são convertidos em outros metabólitos ou ligam-se a um RNA tranpostador específico para ser utilizado na síntese protéica no ribossomo (Rathmacher, 2000).

A excreção de AA é condicionada primeiramente à sua desaminação, onde o esqueleto carbono originado é reaproveitado e o grupo amino usado na síntese do ácido úrico que nas aves, é retirado da corrente sanguínea e secretado via urina nos túbulos renais (Leeson e Summers, 2001).

Summers et al. (1992) observaram que o ganho de peso e a deposição de proteína na carcaça estão mais relacionados com o consumo de AA essenciais do que com o consumo de proteína ou nitrogênio. Estudos metabólicos indicam que a elevação do nível protéico da ração estimula o catabolismo protéico através da síntese de enzimas pancreáticas e intestinais e também das enzimas envolvidas na degradação dos AA essenciais. Enquanto o custo metabólico para incorporar um AA na cadeia protéica esta avaliado em

4 mol de ATP, enquanto o custo metabólico de excreção está estimado entre 6 e 18 mol de ATP, o que explica o alto custo energético a degradação dos AA (McLeod, 1997).

Metabolismo dos aminoácidos de cadeia ramificada

Os aminoácidos de cadeia ramificada (AACR) é uma classe de AA que possuem um grupamento R hidrofóbico e não polar. Esta família de AA são compostas pela Val, Ile e leucina (Leu) e suas volumosas cadeias, juntamente com a alanina, são importantes na estabilização da estrutura das proteínas pela promoção de interações hidrofóbicas em seu interior (Lehninger, et al. 2007). Portanto, são AA semelhantes em estrutura, além de compartilhar enzimas para sua transaminação e descarboxilação oxidativa (Harper *et al.* 1984).

Enquanto a maioria dos AA é metabolizada pelo fígado após a absorção intestinal, o primeiro passo da metabolização dos AACR ocorre no músculo esquelético (Matthews *et al.*, 1981). Esta etapa inicial é comum para Val, Ile e Leu e envolve uma reação reversível de transaminação, promovida pela enzima transaminase dos AACR. Esta reação envolve uma molécula de α-cetoglutarato e um dos AACR. O α-cetoglutarato desempenha um papel singular no metabolismo dos AA, pois aceita grupamentos amina destes e transforma-se em glutamato. O glutamato produzido pode ser utilizado na síntese de alanina e glutamina, e consequentemente auxiliar na síntese proteica. O AACR que doou o grupamento amina transforma-se em um α-cetoácido, sendo o α-cetoisovalerato (KIV), o α-cetoisocaproato (KIC) e o α-cetometilvalerato (KMV) respectivamente para Val, Leu e Ile (Lehninger *et al.*, 2007).

Os α-cetoácidos formados pela reação de transaminação nos músculos esqueléticos podem ser utilizados para a ressíntese de AACR no fígado (Holocek, 2002), ou passar por uma descarboxilação oxidativa catalisada pela enzima α-cetoácidos de cadeia ramificada desidrogenase. Esta reação é irreversível e ocorre principalmente no fígado, devido à mais alta atividade da enzima neste órgão (Wiltafsky *et al.*, 2010), e leva à formação de derivados da CoA: isobutiril-CoA a partir da Val, α-metil butiril-CoA a partir da Ile e isovaleril-CoA a partir da Leu. Estas acilas-COA são, então hidrogenadas e a partir da catálise com três enzimas relacionadas produzem finalmente propionil-COA até sucinil-COA (Val), acetil-COA e propionil-COA até sucinil-COA (Ile) e acetil-COA até acetato (Leu). Dessa forma, os produtos final do metabolismo da Val são glicogênicos, da Leu são cetogênicos e da Ile são glicogênicos e cetogênicos, ou seja, seus produtos podem ser destinados ao Ciclo de Krebs ou para a gliconeogênese hepática (Lehninger *et al.*, 2007).

Uma considerável interação tem sido relatada em resposta à ingestão desproporcional dos AACR, pois a adição de quantidades excessivas de Leu, em uma dieta com baixo teor de proteína, tem deprimido o crescimento, a ingestão de alimentos, e reduzido a associação de Val e Ile (Harper *et al.,*1984), tendo esses efeitos minimizados quando suplementadas pequenas quantidades de Val e Ile. Segundo Pelletier *et al.,* (1991), a alta ingestão de Leu aumenta a atividade da enzima cetoácido desidrogenase de cadeia ramificada em vários tecidos, diminuindo assim as concentrações de Val e Ile no sangue e tecidos.

A principal razão para o antagonismo entre os AACR é o aumento da atividade da enzima α-cetoácidos de cadeia ramificada desidrogenase estimulado por altos níveis de KIC que regula de maneira dose-dependente a atividade desta enzima, enquanto a Val e Ile e seus α-cetoácidos tem pouco ou nenhum efeito na regulação desta enzima. Como os três AACR compartilham as primeiras reações do seu metabolismo, um aumento na α-cetoácidos de cadeia ramificada desidrogenase gerado pelo excesso de KIC reflete-se em maior catabolismo de todos os AACR e, consequentemente, menor disponibilidade destes para a síntese proteica (Murakami *et al*., 2005).

Os efeitos negativos do excesso de Leu na dieta sobre o desempenho dos animais tem sido reportado por diversos autores. May *et al*., (1991) avaliaram o excesso de Leu dietética em ratos e verificaram que dietas com baixo nível protéico e excesso (suplementação de 10%) de Leu reduziram o desempenho dos animais, tendo a adição de 2,6% de Ile e 2,4% de Val na dieta insuficiente para recuperar a queda no desempenho desses animais. Wiltafsky *et al*., (2010) estudaram o impacto do excesso dietético de Leu para suínos em crescimento de 8 a 25 kg, sobre o desempenho, metabólitos sanguíneos, transcrição e cinética enzimática e verificaram que o aumento dietético de Leu reduziu o desempenho e aumentaram os níveis de Leu plasmática e KIC linearmente. Observaram também aumento linear da atividade da enzima α-cetoácidos de cadeia ramificada desidrogenase basal no fígado, enquanto no tecido hepático, os níveis de mRNA do receptor do hormônio de crescimento (GH), da subunidade do fator de crescimento similar a insulina I (IGF-I) reduziram significativamente com o aumento da Leu dietética.

Outras hipóteses para a diminuição do consumo levam em consideração que os animais percebem quando dietas apresentam o perfil aminoacídico desbalanceado (Ettle e Roth, 2004). O cérebro detecta a baixa concentração de um AA essencial e sinaliza para regular o consumo (Hao et al., 2005). Assim, um excesso de Leu pode exacerbar o requerimento de Val ou Ile, levando a uma deficiência. Além disso, alta concentração plasmática de Leu parece ser um sinal que proteína suficiente foi ingerida, resultando em regulação do consumo (Wiltafsky *et al*., 2009).

Seguindo esta linha, Calvert *et al*. (1982), ao realizarem experimento com alimentação forçada em frangos de corte estimou que 70% da diminuição do ganho de peso é devida ao menor consumo de ração. O mesmo foi confirmado por Fu *et al*. (2006) para suínos. A principal teoria para explicar este fato leva em consideração a importância do triptofano (Trp) na regulação do consumo. Este AA é um importante precursor da serotonina, neurotransmissor fortemente envolvido na regulação do apetite. A Leu compete com o Trp na passagem da barreira hematoencefálica, o que resulta em menor concentração de Trp no cérebro e consequentemente menor produção de serotonina, desencadeando a redução do consumo e do ganho de peso tanto em suínos (Henry *et al*., 1996), como em frangos de corte (Harrison e D'Mello, 1986).

Devido ao explanado, em aves e suínos, os requerimentos de Ile e Val aumentam em casos de excesso de Leu devido ao maior catabolismo dos primeiros, contudo, a ingestão excessiva de Val ou Ile causa uma depressão insignificante dos demais AACR (D'Mello & Lewis, 1970; Smith *et al.*, 1978; Burnham *et al.*, 1992). Além disso, o conteúdo proteico do milho e farelo de soja é proporcionalmente muito mais rico em Leu, apresentando valores de 0,37; 0,27 e 0,94% para o milho (7.88% PB) e 2,21; 2,12 e 3,50% para o farelo de soja (45% PB), para Val, Ile e Leu respectivamente (Rostagno *et al.*, 2011).

Valina

A valina (Figura 1) possui estrutura e função semelhante à Ile e Leu, é um AA alifático, hidrofóbico, que se encontra em quase totalidade no interior das proteínas. O farelo de soja, e a farinha de pescados e carnes, são fontes importantes de Val. Ela se incorpora às proteínas e às enzimas em um índice molar de 6,9% quando se compara com os outros AA (Duarte, 2009)

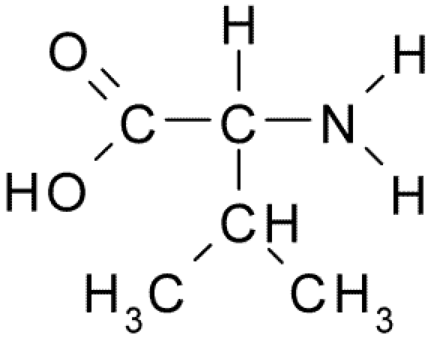

Figura 1. Estrutura química da valina

A Val é reconhecida atualmente como o quarto AA limitante em uma dieta à base de milho e farelo de soja para frangos de corte (Corzo et al., 2007; Berres et al., 2010b) e esta limitação é particularmente aparente com o avanço da idade dos animais, sendo importante conhecer sua exigência, especialmente para as dietas formuladas com baixos níveis de proteína onde são suplementados os AA sintéticos metionina (Met), Lis e treonina (Tre) (Corzo et al. 2004).As proporções relativamente baixas de Val e Ile na proteína do milho são acompanhadas por alta Leu, o que faz aumentar os requerimentos de Val e Ile para frangos de corte e perus (D’Mello & Lewis, 1970; Allen & Baker, 1972; Tuttle & Balloun, 1976).

Baker et al. (2002) avaliaram a exigência de Lis e diferentes relações Val/Lis digestível para frangos de corte e concluíram como melhor relação para a fase inicial (08 a 21 dias) era de 77,50%, com base no ganho de peso e da eficiência alimentar, valor similar aos apresentados por Baker (1997) e Mack et al. (1999).

Corzo et al. (2004) estimaram o requerimento de Val para frangos de corte no período de 42 a 56 dias de idade, consumindo dietas a base de milho, farelo de soja e glúten de milho contendo 0,60% de Val na dieta basal. Estes autores verificaram que o nível de 0,72% de Val na dieta maximizou o ganho de peso considerando 0,73% para conversão alimentar. A quantidade de gordura abdominal foi inalterada, e o peso da carcaça após o resfriamento foi maximizada utilizando 0,73% de Val na dieta, assim como o ganho de peso final.

Pesquisas realizadas por Rodehutscord & Fatufe (2005) com frangos de corte de 8 a 21 dias mostraram que a exigência de Val total foi 0,71% para uma melhor conversão alimentar, entretanto para máxima taxa de deposição protéica, o nível recomendado foi de 0,81%. Enquanto, segundo Rostagno et al. (2005) as recomendações de relação Val/Lis digestível pra frango de corte na fase inicial e (01-21 dias) e crescimento (22-42 dias) são 75 e 77%, respectivamente. Estes valores foram reavaliados pelo mesmo autor em 2011 e então, alterados para 77 e 78% para as fases inicial e crescimento, respectivamente.

Corzo et al (2007) utilizando dietas vegetais a base de milho e farelo de amendoim contendo diferentes níveis de Val digestível (0,59 a 0,84%), concluíram que a relação Val/Lis de 78% (0,74% na dieta) foi adequada para frangos de corte no período de 21 a 42 dias de idade. Experimentos executados por Corzo et al. (2008) com frangos machos de diferentes idades, concluíram que a exigência de Val digestível para frangos Ross de 0 a 14, 14 a 28 e 28 a 42 dias foi de 0.91, 0.86 e 0.78%, respectivamente. Levando em consideração o conteúdo de Lis das dietas experimentais, estes valores correspondem a uma relação Val/Lis entre 76 e 78%. Os mesmo autores não observaram efeitos no rendimento de carcaça e gordura abdominal, corroborando com os resultados apresentados por Leclercq (1998). Resultados semelhantes, foram encontrados por Tavernari et al. (2013) que relataram que a melhor relação Val/Lis digestível para a fase inicial (08 a 21 dias) de frangos de corte machos, foi de 76,5%, sendo este valor correspondente a 0,82% de valina na dieta.

Estudos de Helmbrecht *et al.* (2010) com frangos de corte Ross 308 dos 15 aos 29 dias de idade sugerem níveis de 0,86% de Val para GP, 0,80% para CA e 0,88% para peso de carcaça. Ganhos em peso de carcaça e peso de peito foram observados (Corzo *et al.*, 2004; 2007), mas em outros momentos o rendimento de carcaça como proporção do peso vivo ou o rendimento de peito como proporção da carcaça não foi afetado (Thornton *et al.*, 2006). Possivelmente, o aumento em peso da carcaça proporcionado pela suplementação crescente com Val elevou também o peso da musculatura peitoral e, portanto, não alterou as proporções dos mesmos.

Resultados semelhantes foram encontrados por Berres *et al.,* (2011) que realizaram trabalhos para estimar o nível de Val dietética capaz de otimizar o desempenho de frangos de corte de 21 a 42 dias de idade. Para compor os tratamentos foi utilizada uma dieta basal milho e farelo de soja, com incrementos de 0,6% de Val digestível, sendo a relação Val/Lis digestível encontrada pelos autores foi de 77 e 76% para ganho de peso e conversão alimentar respectivamente.

Isoleucina

A isoleucina (Figura 2) é um AA essencial e membro da família alifática de AA hidrofóbicos que se encontram principalmente no interior de proteínas e enzimas (Duarte , 2009). O núcleo da Ile é o mais hidrófobo entre todos os radicais dos AA. Essa hidrofobia permite a formação de ligações fracas com outros AA que contribuem na estrutura terciária e quartenária das proteínas. É glicogênica e cetogênica, formando o ácido acético (cetogênico) e o ácido propiônico (glicogênico) através do ácido metilbutírico (Leningher, 2007)

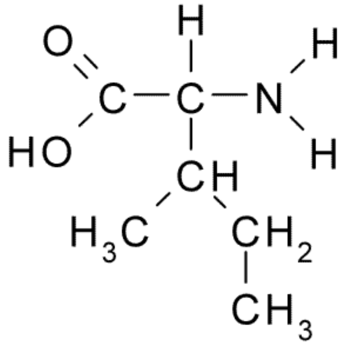

Figura 2. Estrutura química da isoleucina

A quantidade de isoleucina em grãos de cereais (milho, sorgo e trigo), farelo de soja e farinhas de origem animal indicam a possibilidade de este ser o próximo AA limitante depois da Val. Entretanto, a inclusão de ingredientes na dieta que tenham uma quantidade relativamente menor em Trp e arginina em relação à Ile pode mudar a ordem de limitação deste aminoácido. (Kidd et al., 2004b).

Hale et al., 2004 verificaram que a exigência de Ile total para frangos de corte Ross de 30 a 42 dias de idade foi de 0,58%, 0,62% e 0,66% para a conversão alimentar, rendimento de peito e ganho de peso, respectivamente, correspondendo a um relação Ile/Lis digestível entre 62 a 67%. Resultados semelhantes foram relatados por Kidd et al. (2004b) que concluíram que para ótimo desempenho de frangos de corte de 30 a 42 dias de idade o nível de Ile na dieta deve ser entre 0.64 a 0.66%.

Kidd et al. (2004a) avaliaram o efeito de diferentes níveis de iIle em dietas para frangos de corte machos. As aves que receberam dieta deficiente em Ile tiveram piora no ganho de peso, conversão alimentar e efeitos na carcaça quando comparadas as aves que receberam incremento de iIle nas dietas. A adição de Ile na dieta teste resultou em desempenho e rendimento de carcaça equivalentes às aves alimentadas com dieta controle a partir de fontes de proteínas intactas. Os autores concluíram como recomendação de Ile para frangos de corte é de 0,67 a 0,71% para a idade de 18 a 30 dias, 0,64 a 0,66% entre 30 a 42 dias e 0,55 a 0,66% para a idade de 42 a 56 dias.

Segundo Rostagno et al. (2005) as recomendações de relação Ile/Lis digestível pra frango de corte na fase inicial e (01-21 dias) e crescimento (22-42 dias) são 65 e 67%, respectivamente. Contudo, ao reavaliarem as exigências de Ile em 2011, verificaram que estes valores passaram para 67 e 68%, respectivamente. Resultados similares foram encontrados por Campos et al. (2009) que concluíram como melhor relação Ile/Lis digestível é de 67% para a fase inicial (07 a 21 dias), enquanto que para a fase final (28 a 40 dias) é de 70%.

Berres *et al*. (2010b) formularam dietas exclusivamente vegetais baseadas em milho e soja suplementadas com Met, Lis e Tre usando relações ideais destes AA com a Lis de 75% Met, 65% Thr, 70% Val, 18% Trp, 65% Ile e 106% Arginina. Esta dieta determinou perdas de crescimento que foram recuperadas quando L-Val ou L-Ile foram suplementadas para níveis de 75% e 68%, respectivamente, de 14 a 35 dias de idade. Segundo os autores, a observação geral dos resultados acima indicou relações ideais de Ile que maximizam as respostas zootécnicas de frangos de corte entre 65 e 75% da em relação a Lis.

Dozier III *et al.,* (2011) realizaram estudo para a determinação do quarto AA limitante para frangos de corte machos de 28 a 42 dias de idade recebendo dieta à base de milho, farelo de soja e subprodutos avícolas. Foram avaliados diferentes relações de Ile/Lis digestível (57, 62 e 67%) e Val/Lis digestível (66, 71 e 76%). Os autores verificaram que frangos alimentados com dietas com 62% Ile/Lis digestível e 76% Val/Lis digestível, apresentaram pior conversão alimentar e maior consumo de ração. Ao comparar o efeito da adição de Ile (62% para 67%) em dietas contendo 71% de Val/Lis digestível para ganho de peso em frangos de corte, não foi observada diferença significativa, entretanto, ao adicionar Val (de 71% para 76%) em dietas contendo 62% de Ile/Lis digestível foi observada melhora significativa de ganho de peso. Estes autores sugeriram a existência de uma co-limitação entre Ile e Val para crescimento em dietas à base de milho, farelo de soja e subprodutos avícolas.

Leucina

A leucina (Figura 3), assim como os demais AACR, é também um AA hidrofóbico e alifático. Mas ao contrário da Ile e Val, a Leu está presente em altas quantidades nos cereais e ingredientes comumente encontrados nas rações para frangos de corte. A soja, o principal alimento proteico utilizado nas rações possui baixos teores de Val e Ile (1,97 e 1,92%) quando comparados com Leu (3,19%) (Rostagno *et al*., 2011).

A Leu, mais do que Val e Ile, contribui significativamente para o aumento do catabolismo dos demais AACR. Todo processo de início de catabolismo de AA retirada do grupamento amino da molécula, no caso dos AACR a via de catabolismo é a mesma nos dois primeiros estágios. A primeira reação é catalisada pela AACR transferase, o que gera α-cetoácidos, que serão descarboxilados pela enzima α-cetoácidos de cadeia ramificada desidrogenase, sendo um processo irreversível, altamente regulado e limitado pela taxa de catabolismo de AACR, (May *et al*., 1991).

Pesquisas realizadas por Allen e Baker (1972), avaliaram o efeito do excesso dietético de Leu sobre a eficiência de utilização de Val e Ile para crescimento em frangos de corte de 8 a 14 dias de idade. Foram avaliados níveis crescentes de L-Val nas rações com ou sem suplementação de L-Leu (0 e 3%), sendo observado que o aumento de Leu na dieta provocou uma redução linear da eficiência de utilização da Val.

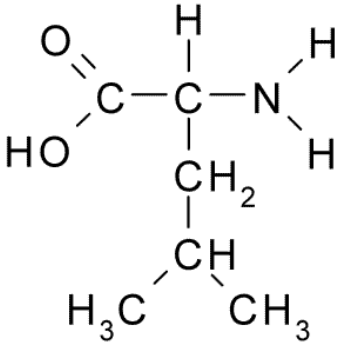

Figura 3. Estrutura da Leucina

A Leu não somente tem um papel como substrato para síntese proteica, mas também atua como um nutriente sinalizador que regula a síntese protéica em vários tecidos do corpo, incluindo músculo esquelético (Crozier *et al.,* 2005, Drummond & Rasmussen, 2008). Segundo Garlick (2005), em um estudo realizado com ratos, a capacidade da Leu em estimular a síntese proteica pode ser aumentada por um pequeno incremento de insulina, que por sua vez é induzido pela própria Leu. Entretanto, em concentrações fisiológicas de Leu e insulina de ratos em jejum e alimentados, a sensibilidade da síntese proteica à insulina é aumentada pela administração de Leu, de modo que a síntese proteica é estimulada por um nível moderadamente alto de insulina e Leu. Portanto, o papel da Leu é atuar em conjunto com a insulina para ativar a síntese proteica quando os AA e energia se tornam disponíveis.

Por outro lado, o papel da Leu como estimuladora da insulina implica a possibilidade de que a ingestão prolongada deste AA possa levar à resistência da insulina, similarmente ao que ocorre com casos de hiperglicemia prolongada. Isso pode levar à uma atenuação da estimulação da síntese proteica pela ingestão de alimentos. Além disso, como os mecanismos sinalização do estímulo para síntese proteica pela insulina são comuns àqueles envolvidos no metabolismo da glicose, sendo possível que uma superestimulação pela Leu possa gerar anormalidades no metabolismo da glicose (Layman & Baum, 2004).

Restrição proteica em dietas para frangos de corte

O nível proteico da ração passou a ser definido como o nível ótimo de AA necessários para que as aves possam apresentar seu máximo potencial produtivo, devendo ser considerado o custo dos ingredientes utilizados na formulação e o valor das carnes produzidas. Aminoácidos sintéticos tais como Lis, Met, Tre e Trp têm significativa participação na aplicabilidade do conceito de proteína ideal para aves e suínos. Também têm viabilizado pesquisas a fim de reduzir o nível de PB nas rações e atender às exigências nutricionais de AA com suplementação (Sabino *et al.,* 2004).

A manipulação da proteína da dieta tem sido proposta como um meio para melhorar o desempenho de frangos de corte em ambientes quentes pela redução da carga metabólica, melhorando o equilíbrio de AA da dieta, reduzindo desta forma a ingestão de proteína (Waldroup *et al.*, 1976). A redução do nível protéico da dieta implicaria em uma queda no catabolismo da proteína, resultando em um decréscimo na produção de calor e ajudando a ave a manter seu balanço energético em condições de elevadas temperaturas (Daghir, 1995).

De acordo com Noblet *et al.* (1987) e Roth *et al.* (1999), as dietas com baixos níveis de proteína, têm sido associadas com redução de perdas energéticas. Deve-se levar em consideração que o aumento do suprimento de proteína está diretamente associado com um maior “turnover” protéico (Roth *et al.* 1999; van Milgen *et al.* 2001) e ao aumento do peso das vísceras (Noblet *et al.,* 1987) com um subseqüente aumento da produção de calor. Com isso, a utilização de dietas de baixa proteína leva a diminuição da desaminação e produção de calor pelos animais devido aos excessos de proteína, além da menor produção e excreção de nitrogênio pela urina. Essas dietas, segundo esses autores, podem melhorar a disponibilidade energética para deposição de tecidos.

Os avanços recentes na determinação das exigências de AA para aves e o aumento da disponibilidade dos AA sintéticos no mercado, permitiram que os níveis de PB das dietas sejam reduzidos, mantendo-se o suprimento de AA essenciais. Braga (1999) concluiu que essas dietas não têm efeito prejudicial sobre o desempenho e deposição protéica, mas, para níveis mais baixos de PB, existe um aumento na deposição de gordura abdominal (Cahaner et al., 1995; Alleman et al., 2000)

Segundo Zaviezo (2000) é possível trabalhar com níveis mínimos de PB em dietas comerciais para frangos de corte, de 21%, 18-19% e 16-17%, nas fases de 1 a 21 dias, 22 a 42 dias e 43 a 56 dias, respectivamente, desde que os AA Met, Lis e Tre sintéticos sejam suplementados de maneira correta. Resultados semelhantes foram encontrados por Burnham (2001) que utilizaram aves alimentadas com rações comerciais a base de milho e farelo de soja, com níveis de Lis digestível (%) e energia EM (Kcal/kg) de 1,10/3.100; 1,00/3.150 e 0,91/3.225 para as fases de 1-19 dias, 20-35 dias e 36-49 dias respectivamente, e demonstrou ser possível reduzir a proteína bruta para 20,5%; 19,6% e 18,2%, nas respectivas fases, sem detrimento ao desempenho do frango de corte. O mesmo autor em outro trabalho similar utilizando 308 pintos Ross, diferenciou somente as rações finais, onde formulou níveis de Lis digestíveis mais baixos, 0,87%, demonstrando que, para este nível de Lis mais baixo, é possível reduzir o nível de proteína destas dietas em até 16,5%, sem afetar desempenho e qualidade de carcaça das aves. Em ambos os trabalhos as rações foram suplementadas com os AA sintéticos Lis, Met e Tre, visando manter o perfil adequado dos AA essenciais.

Araújo *et al.* 2004 estudaram os efeitos da redução do nível proteico da dieta sobre o desempenho de frangos de corte na fase inicial e observaram que quando as aves foram alimentadas com a dieta referência, formulada para conter 22% PB e com base em AA digestíveis, obtiveram, estatisticamente mesmo ganho de peso e conversão alimentar que as aves alimentadas com as dietas contendo 22% PB e formulada com AA totais e com 20% PB formulada com aminoácidos digestíveis, já que o consumo de ração não foi alterado pelos tratamentos estudados. Estes resultados demonstraram ser possível diminuir o nível protéico da dieta na fase inicial desde que a formulação seja com base em aminoácidos digestíveis.

Segundo Aftab et al. (2006) o valor de PB da dieta de frangos de corte pode ser reduzido a um nível onde a relação energia e proteína (EM:PB) seja de 155, 180 e 195 respectivamente para o período de 0-21, 21-42 e 35-49 dias de idade. Transpondo essas relações para valores de PB dietética, utilizando uma dieta com 3200 kcal/kg EM, seriam 20,6, 17,8 e 16,4% respectivamente para cada fase, representando uma redução de cerca de 10% e 8% quando utilizado como referência o NRC (1994) e Rostagno et al. (2011), respectivamente.

Contudo, resultados experimentais são reportados na literatura demonstrando uma piora no desempenho zootécnico e rendimento de abate de frangos de corte consumindo dietas variando os valores PB (Pinchasov et al., 1990; Bregendahl et al., 2002; Si et al., 2004; Dean et al., 2006).

Muitas possibilidades são sugeridas para explicar o efeito negativo das dietas com redução proteica no desempenho de frangos de corte. Segundo Waldroup (2000) e Aftab et al. (2006) a não obtenção de um ótimo desempenho pelas aves que consomem dietas com níveis de PB reduzidos tem sido atribuída a fatores como: a variação do potássio dietético ou do balanço eletrolítico, em decorrência da redução na quantidade de farelo de soja nessas dietas, visto que esse alimento constitui a principal fonte de potássio; insuficiência de nitrogênio não específico para a síntese de AA não essenciais e alteração da relação entre AA essências e não essenciais, ocasionando um desbalanço entre determinados AA como a relação arginina/Lis ou AACR, além da relação inadequada de Trp e outros AA neutros (Val, Ile, Leu, fenilalanina e tirosina), que podem inibir a ingestão de alimentos pelos animais alimentados com dietas com nível reduzido de PB; tendência à redução do consumo de ração voluntário pelos frangos de corte devido a diferença na taxa de absorção e metabolismo de AA livres e AA intactos presentes na dieta, levando a um imbalanco de AA plasmático o que mostra deprimir o consumo de alimento (Austic et al., 2000).

Outras explicações para possíveis efeitos negativos das dietas com PB reduzida sobre o desempenho de frangos de corte poderiam ser explicadas pela síntese insuficiente de alguns AA não essenciais, com a glicina, para satisfazer a necessidade do rápido crescimento de tecidos durante uma determinada fase do crescimento de frangos de corte (Parr and Summers 1991; Corzo et al., 2004; Jiang et al., 2005; Dean et al., 2006), sugerindo-se, então, que a exigência deste aminoácido é superior ao proposto pelas tabelas de exigências para frangos de corte, principalmente quando utilizadas dietas com PB reduzida (Jiang et al., 2005; Dean et al., 2006

De acordo com Leeson *et al.* (2000) a piora no desempenho pode ser relacionada com a disponibilidade energética da dieta. A redução protéica aumenta o conteúdo de energia líquida da dieta. Sendo a ingestão de alimentos influenciada pelo suprimento energético o aumento da disponibilidade energética pode diminuir o consumo, diminuindo também a ingestão de AA. Dessa forma, supõe-se que manter constante o suprimento de energia líquida ao invés da energia metabolizável pode ser vantajoso nesse tipo de dieta.

Objetivando avaliar os efeitos do fornecimento de rações contendo níveis reduzidos de PB (15, 17 e 19%) e suplementadas com aminoácidos sintéticos sobre o desempenho de frangos de corte no período de 1 a 21 dias de idade, Silva *et al,* 2006, verificaram que a redução do teor de PB da ração para 15% afetou negativamente todas as características de desempenho, ao passo que a redução para 17% de PB influenciou negativamente o ganho de peso e a conversão alimentar.

Vasconcellos *et al.* (2010) avaliaram os efeitos da redução de proteína e suplementação de AA essenciais e L-Glicina, (nível glicina + serina total das dietas de baixa PB igual ao da dieta controle) sobre o desempenho de frangos de corte machos na fase de um a 21e de 22 a 42 dias de idade, ao reduzirem o nível protéico da dieta de 23% até 17% na fase de um a 21 dias e de 21% até 15% na fase de 22 a 42 dias idade observaram perda no desempenho. Segundo o autor, o desempenho inferior de aves alimentadas com dietas de baixa proteína pode estar relacionado ao fornecimento inadequado de nitrogênio para síntese dos AA não essenciais.

Em suma, conforme já mencionado anteriormente, a redução proteica em dietas pode ser benéfica ao desempenho de frangos de corte, com redução da carga metabólica (Waldroup et al., 1976), redução das perdas energéticas (Noblet et al., 1987; Roth et al., 1999), e redução de custos, principalmente pela redução da inclusão de farelo de soja nas rações cujo os custos tem oscilado bastante no mercado mundial nos últimos anos, elevando o valor em cerca de 163,33% (Index Mundi, 2013), contudo, deve-se manter um valor mínimo de nitrogênio não específico e as relações entre AA essências e não essenciais (Waldroup et al., 2000; Aftab et al., 2006).

HIPÓTESES E OBJETIVOS

Hipóteses

A suplementação de AACR na forma sintética em dietas com PB reduzida, mantém o desempenho zootécnico, rendimento de carcaça e peito em frangos de corte quando comparado a dietas com PB elevada e suplementadas apenas com os três primeiros AA limitantes.

Frangos de corte alimentados com dietas com PB restrita a um valor mínimo e suplementadas com apenas os três primeiros AA limitantes, tem seu desempenho prejudicado devido ao antagonismo entre os AACR presente, principalmente, em dietas à base de milho e farelo de soja.

O desempenho de frangos de corte é melhorado com aumento em 5% no nível de Lis digestível.

O uso de um programa alimentar deficiente em AACR no período de 1 a 21 dias de idade, pode ser melhorado quando utilizado programa alimentar com a suplementação de todos os AACR no período de 22 a 42 dias de idade, sem diferença no rendimento de carcaça.

Objetivos

Avaliar os efeitos da interação dos AACR em dietas formuladas sob condições práticas visando a redução proteica suplementadas com L-Val e L-Ile, além de DL-Met, L-Lis e L-Tre sobre o desempenho zootécnico e o rendimento de carcaça e corte comerciais de frangos de corte.

Avaliar os efeitos de programas alimentares formulados a um custo mínimo, com PB restrita ou não, suplementados ou não com L-Val e L-Ile, e, ainda, com níveis de Lis digestível comerciais ou acrescidos em 5% sobre o desempenho e rendimento de carcaça e cortes comercias de frangos de corte.

Avaliar os efeitos de programas alimentares formulados a um custo mínimo, com PB restrita ou não, suplementados ou não com L-Val e L-Ile e utilizados em diferentes fases de crescimento sobre o desempenho e rendimento de carcaça e cortes comercias de frangos de corte.

CAPÍTULO II[1]

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[1] Artigo submetido ao Journal of Applied Poultry Research em Maio de 2014 e publicado em Dezembro de 2014.

Running Title: L-Valine and L-Isoleucine for Broilers

Broiler responses to feeds formulated with or without minimum crude protein restrictions and using supplemental L-valine and L-isoleucine

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Section: Metabolism and Nutrition

Primary Audience: Nutritionists, feed mill personnel

Key words: broiler, protein, amino acids, valine, isoleucine

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SUMMARY

Comparisons of corn-soy feeding programs formulated using different protein constraints were used in the present study. A total of 1,800 one-day-old slow feathering Cobb x Cobb 500 male broiler chicks were placed in 72 floor pens of 25 birds each with 9 birds/m^2. Feeding programs (PRG) were composed of pre starter (PST,1-7 d), starter (ST, 8-21 d), grower (GR, 22-35 d) and finisher (FN, 36-43 d) diets formulated as: PRG 1 = CP restricted to a minimum (22.4, 21.1, 19.8, and 18.4% for PST, ST, GR, and FN, respectively) with amino acid-to-Lys ratios only set for TSAA (0.72) and Thr (0.65); PRG 2 = CP not restricted, whereas ratios of amino acids to Lys were also extended to Val (0.77) and Ile (0.67); PRG 3 and PRG 4 were the same as PRG 2, but with L-Val added in PRG 3 and L-Val and L-Ile in PRG 4. Feeds were formulated to have digestible Lys of 1.324, 1.217, 1.095, and 1.006% for PST, ST, GR, and FN, respectively, or 5% higher. A completely randomized design was used with a 4 x 2 factorial (4 PRG and 2 digestible Lys) design with 9 replications per treatment. Overall, no interactions between PRG and digestible Lys were observed, with 1 exception observed for BW gain and FCR from 36 to 43 d. Birds fed PRG 2 showed improvements in BW gain and FCR when fed the diet 5% higher in digestible Lys. Cumulative BW gain and FCR results at 35 and 43 d, as well as in each individual feeding phase, showed broilers from PRG 2 having the best results, but without mean separation significant differences when compared with PRG 3 and 4. Feeding a dietary program with 5% increase in digestible Lys resulted in improved cumulative FCR only when cumulativelt measured from placement at 43 d. Abdominal fat, as a percentage of the eviscerated carcass at 43 d, was lowest for birds fed PRG 2 diets. Formulation of having traditional recommendations of Lys with Val- and Ile-to-Lys rations of 0.77 and 0.67, without restricting CP, led to the best results overall in performance and fat pad deposition; therefore, if the goal is to reduce CP, similar results should be achieved by adding L-Ile with further reductions in protein if L-Val is also added. Data obtained in this study support the applicability of supplementation with L-Val and L-Ile when ideal AA ratios are followed.

DESCRIPTION OF PROBLEM

Formulating broiler feeds maintaining minimum restriction in CP is a common practice when linear least cost feed formulation is used. Preserving safety margins for minimum daily intakes of protein as well as essential and non essential amino acids (**AA**) is a main justification behind this practice. Providing dietary AA for broilers using crystalline sourcesas supplements has shown to produce adequate performance in the case of L-Lys, DL-Met, and L-Thr [1, 2]. However, differences exists on the next limiting AA after Thr in broiler diets due to the ingredients used to formulated the diets [3, 4]. Using ideal protein ratios (AA-to-Lys) has become a popular strategy to formulate dietary in for broiler diets. This formulating approach targets reducing the amount of absorbed AA that are in relative excess compared to the first-limiting AA, avoiding excess oxidation, and allowing the maintenance of a better AA balance [5, 6]. Therefore, CP is usually not restricted when formulating broiler feeds using the ideal protein concept with more than 3 crystalline AA. The effect of further reducing the total dietary protein content when adding more crystalline AA than that usually supplemented (L-Val and L-Ile, for instance) has gained importance recently.

Energy and nutrient recommendations for broilers should allow maximum meat production; however, maximizing economic profitability is more difficult because main production targets vary between broiler integrators. Allowances of AA in broiler feeds that maximize broiler economic productivity depend not only on performance and proportion of carcass components, but also the mix of products being marketed and their price. Dietary AA concentrations that maximize growth, FCR, and breast meat yield are different; for instance, optimum essential AA concentrations that maximize production decrease from breast meat yields to whole carcass and then BW gain [7-9]. Whereas breast meat is presently the main commercial broiler chicken product marketed in the United States, differences exist in other markets around the world. Therefore, the decision on the optimum AA density that should be used in feeds depends on the desired market weight, product mix, broiler live cost, and also on the genetic potential [5].

Protein, and therefore AA, is a costly nutrients; therefore, low-AA density diets minimize costs. However, they may limit broiler meat yield (as a proportion of the whole body) and frequently do not allow for maximizing profits [9, 10]. This is especially true when consideration is given to breast meat yield in relation to breast meat market prices [10]. Conversely, economic profits resulting from improved meat yields when using high-AA density diets are largely dependent of the feed cost and meat market price [11, 12].

Concerns with the constant increases in feed ingredient costs without corresponding increases in meat market price usually lead to feed formulations targeting cost reductions. These frequently do not allow for maintaining live performance within expected parameters. Reducing dietary CP with the use of crystalline sources of Lys, Met, and Thr is an important part of the strategy to reach this objective [6, 9, 10]. Opportunities exist for the commercial use of crystalline Val and Ile, which can further reduce minimum dietary CP, as these are fourth- and fifth-limiting AA in corn-soy diets [3, 4]. Data on the use of these 2 crystalline AA sources have increased recently; however, direct implementation is difficult because feed formulation for broilers differs widely around the world. Therefore, generating data on broiler live performance as well as the proportion of the diverse market meat cuts in diets with or without Val and Ile, is of importance for nutritionists to determine the value of their use in feed formulation. Data that optimize growth have been published on Val and Ile-to-Lys ratios, which indicate values of 0.75 to 0.77 and 0.65 to 0.67, respectively [4, 13-19].

The objective of the present study was to evaluate the effects of feeding programs formulated using least cost linear feed formulation, restricting or not CP to a minimum, with or without supplementation of L-Val and L-Ile. Evaluations were also performed using feeding programs with the same CP and AA restrictions as presented above, but with digestible Lys at 5% over commercial use concentrations maintaining the digestible Ly-to-AA ratio constant as defined for each diet strategy. Observations extended from live performance to the processing yields of the major cut-up.

MATERIAL AND METHODS

Bird husbandry

All animal procedures implemented throughout the current study avoided unnecessary animal discomfort and were approved by the Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. A total of 1,800 one-day-old slow feathering Cobb x Cobb 500 male broiler chicks [20], vaccinated for Marek's disease at the hatchery, were randomly distributed into 72 pens (1.65 x 1.65 m; 9.2 birds/m^2, 25 birds per pen). Each pen had rice hull bedding and was equipped with one 15 kg capacity tube feeder and three nipple drinkers. Average temperature was 32ºC at placement and was reduced by 1ºC every 2 days until 23ºC to provide comfort throughout the study with the use of thermostatically controlled heaters, as well as fans and foggers when needed. Lighting was continuous until 7 d of age, with a 14L:10 D cycle used afterwards. Birds had *ad libitum* access to water and were provide mash feeds.

Experimental Diets

Experimental feed ingredient and nutrient content are presented in tables 1, 2, 3, and 4. Least cost linear feed formulation was used to formulate corn-soybean meal diets based on duplicate AA analysis of the corn and soybean meal used. As with the ingredients, analysis of AA was also done in all experimental diets using the methodology outlined in method L257 of the Official Journal of The European Community [21]. Briefly, Cys and Met were oxidized to cysteic acid and methionine sulphone, prior to hydrolysis. Hydrolysis was done with 6 mol/L HCl during 23 hours, the pH of the hydrolizate adjusted to 2.2, and amino acids separated by ionic exchange chromatography and determined using colorimeter at 570 nm, or 440 nm in the case of Pro, after reaction with nihydrin. Feeds were formulated using digestible AA obtained by multiplying total AA from analyses by the coefficients of digestibility set forth on the 2011 Brazilian Tables [22].

The feeding phases used were: pre starter (**PST**, 1-7 d), starter (**ST**, 8-21 d), grower (**GR**, 22-35 d) and finisher (**FN**, 36-43 d). Diets were formulated 2,960, 3,050, 3,100 and 3,200 kcal/kg

AME_n; 0.92, 0.84, 0.76 and 0.66% Ca; 0.46, 0.42, 0.38 and 0.33% available P, for PST, ST, GR, and FN, respectively. Nutrient and AME_n used as well as ideal protein ratios, were based on the 2011 Brazilian Tables [22]. Supplementation with crystallinec AA was done using DL-Met (99%), L-Lys HCl (78.0%), L-Thr (98.5%), L-Val (96.5%), or L-Ile (98.5%).

Eight dietary treatments were used based on a factorial arrangement of 4 protein restriction strategies (**PRG**) and 2 digestible Lys concentrations (a standard [19] or with 5% greater digestible Lys, but maintaining digestible Lys to digestible AA ratios). In PRG 1, diets were formulated using a CP minimum of 22.4, 21.1, 19.8, and 18.4%, for PST, ST, GR, and FN, respectively, but minimum digestible AA to digestible Lys ratios were only set for digestible TSAA (0.72) and digestible Thr (0.67). In PRG 2, no minimum was set on CP and minimum ratios were extended to include digestible Val (0.77) and digestible Ile (0.67) with no use of crystalline Val or Ile. In PRG 3, restrictions were the same as in PRG 2, but crystalline L-Val was included as ingredients; and in PRG 4, restrictions were the same as in PRG 2, but both crystalline L-Val and L-Ile were used as ingredients. Levels of digestible Lys were 1.324, 1.217, 1.095, and 1.006% (Control) or 1.390, 1.278, 1.150, and 1.056% in the PRG diets having 5% higher digestible Lys for PST, ST, GR, and FN, respectively.

Broiler Performance Measurements

Mortality was checked twice daily and mortality weights recorded. Field performance was evaluated at 7, 21, 35, and 43 d of age and FCR was corrected for the weight of dead birds. At the end of the study birds were processed for evaluation of carcass yields and commercial cuts using 6 birds randomly selected from each pen. Processed birds were fasted for 8 h and individually weighed before being electrically stunned with 45V for 3s, then bled for 3 min after a jugular vein cut, followed by scalding at 60ºC for 45 s with feathers being mechanically plucked. Evisceration was manually done and carcasses were statically chilled in slush ice for approximately 3 h. Eviscerated carcasses (without feet and neck but with lungs) were hung for 3 min to remove excess water before individual weighing. Abdominal fat was removed and weighed separately. Carcass yield was expressed as a percentage of live weight, whereas commercial cuts and abdominal fat were expressed as a percentage of the eviscerated carcass weight.

Statistical Analysis

The current study was conducted as a completely randomized design using a factorial arrangement of 4 PRG and 2 digestible Lys levels. The 8 resulting treatments had 9 replicates of 25 birds each, totaling 1,800 birds. Mortality, carcass, and cut yield data were analyzed after arcsine transformation $[(\% \text{ data}/100) + 0.05]^{0.5}$. Normality and homoscedasticity of the data were verified by the Shapiro-Wilk test [23]. The normal and homogeneous data were submitted to a 2-way ANOVA using the GLM procedure of SAS [24]. Significance was accepted at the $P \leq 0.05$ and mean differences were separated using Tukey's HSD test [25].

RESULTS AND DISCUSSION

Slight deviations were observed between analyzed and formulated CP and AA in the feeds (Table 1, 2, 3 and 4). These were considered acceptable because analyzed values followed the expected trends in the formulated values. Of note is that when the CP minimum was removed versus the PRG 1 treatment and the diets formulated to a minimum digestible Lys, digestible TSAA, digestible Thr, digestible Ile, an digestible Val without including crystalline Val or Ile (PRG-2) the CP in the resulting diets increased from 22.4 to 24.8% in the PST, 21.1 to 22.9% in the ST, 19.8 to 20.6% in the GR, and 18.4 to 19.0% in the FN. When crystalline Val was used (PRG 3), the CP was the same or slightly lower in the GR and FN, respectively, as that seen in the diets formulated with a minimum CP (PRG 1) and digestible AA to digestible Lys ratio minimums set only for TSAA and Thr. If both crystalline Val and Ile (PRG 4) were used, the CP in ST, GR and FN diet reached the CP concentrations (ST) or below (GR, FN) the PRG 1 diets.

Growth performance and carcass yields results are shown in Tables 5 and 6, respectively. No differences were observed between any treatment for mortality or feed intake ($P > 0.05$); therefore, these data is not shown in tables. Overall means for cumulative mortality and feed intake were 2.44 % and 5,288 g, respectively.

Results from each feeding phase demonstrated that PRG 1 resulted in the lowest BW gain ($P < 0.05$) throughout all the feeding phases as compared to PRG 2, 3 and 4, except in the FN phase. In the GR phase, birds fed with PRG 3 showed the best FCR ($P<0.05$); however, no difference when means were separate as compared to PRG 4 ($P > 0.05$). No difference in BW gain or FCR ($P > 0.05$) was seen between any PRG treatment in the FN phase. Cumulative BW gain and FCR results from 1 to 35 and 1 to 43 d are similar to that of data obtained in each individual phase, with broilers fed PRG 2 having higher BW gain and lowest FCR compared with PRG 1 ($P < 0.05$), but no difference ($P>0.05$) from those fed PRG 3 and 4.

Feeding any PRG treatment with 5% greater digestible Lys throughout all feeding phases did not result in any improvement on BW gain or FCR in any individual period or cumulative to 35 d ($P > 0.05$). However, cumulative FCR measured at 43 d was improved in birds fed the increased digestible Lys treatments ($P < 0.014$).

No differences were observed for carcass or for the yields of commercial cuts (Table 7) between PRG and increased digestible Lys treatments ($P > 0.05$). One exception was abdominal fat weight as a percent of eviscerated carcass weight, that was lower for birds fed PRG 2 ($P < 0.011$) but no different to PRG 3 and 4 fed birds ($P > 0.05$).

Interactions between PRG and digestible Lys were not observed for any evaluated response; an exception was BW gain and FCR from 36 to 43 d. In this period, increasing 5% of digestible Lys led to improvements in BW gain and FCR ($P < 0.05$) when birds were fed with PRG 2 Trt diets (Table 6).

Crystalline AA supplementation of Met, Lys, and Thr has allowed the poultry industry to reduce feed formulation costs without impairment in broiler field performance [5]. Reduced feed costs are obtained when these crystalline AA are added to broiler diets due to their effect in reducing the need of protein ingredients, such as soybean meal [6, 7]. As broiler production continues to increase around the world, the demand for soybean meal will continue to increase. Starting in 2007, the prices of major grains, including soy, increased dramatically in real terms and reach their peak in 2008, declined in 2009 and 2010, but did not go back to their previous levels, and moved sharply upward again in 2011 [26]. The commercial use of crystalline L-Val and L-Ile, respectively the fourth and fifth limiting AA in corn-soy diets, seems to allow for improvements in economic returns [3, 4]. Studies conducted recently have demonstrated that diets supplemented with L-Val and L-Ile allow broiler growth and processing performances that are comparable to industry standard feeds formulated only with DL-Met, L-Lys, and L-Thr [3, 4, 19]. In the present study, the highest BW gain was associated with the lowest FCR obtained with PRG 2, but without significant differences ($P<0.05$) by mean separations from PRG 3 and 4. This outcome cannot be solely credited to the setting of minimums for digestible Val and Ile to digestible Lys ratios. These minimum ratios were the same in PRG2, 3 and 4 diets, but how these were achieved varied depending on use or not of crystalline Val or Ile. The PRG 2 diets required greater CP to achieve the minimums set for digestible Val (0.77) and digestible Ile (0.67) to digestible Lys ratios. Even though the differences seen between PRG 2 and PRG 3 and 4 are not significant, it is of note that the diets with the higher CP worked bets. This would suggest that the higher CP is serving as a safety net for other essential and, possibly, non-essential AA that do not have a formulated set minimum. The lack of formulated set minimums for these other essential AA is related to limited research base confirmation of what these ratios should be and the lack of commercially available and cost effective sources of these AA. In the case of nonessential AA, the question remains of how clearly we know these are nonessential at all potential CP concentrations.

When crystalline L-Val and L-Ile were supplemented in PRG 3 and PRG 4 (with a concurrent reduction in CP), BW gain and FCR were improved over that of than birds fed the PRG 1. No treatment differences were detected when commercial cuts were compared. Therefore, we can conclude that the PRG 1 limited bird growth due to, in part, a feed formulation strategy that did not set a digestible Val and Ile ratios to digestible Lys. Also, it seems that supplementing L-Val and L-Ile to ratios that are suggested in the current literature [3, 4, 22] produced competitive field results.

Recommendations for dietary Lys in broiler diets have been increasing in the last decades [22, 27-33]. As broilers continue to be selected for increased breast meat, a higher concentration of dietary Lys than the one that maximizes performance is expected, as this essential AA is in high proportion in breast meat [2, 34]. However, a 5% increase in digestible Lys, maintaining ideal AA ratios, did not translated into either performance or parts yield improvements. One exception occurred from 36 to 43 d, a period in which breast muscles are growing at a higher rate when compared to other broiler chicken muscles [35], which eventually led to improvement in the cumulative FCR for the entire grow-out period. Therefore, it seems that the recommended digestible Lys values [22] used in the control PRG were satisfactory to optimize live performance and carcass yields. An opportunity to maximize growth still exists during the finisher phase, which needs further economic evaluation before the adoption of a decision to increase digestible Lys recommendations.

CONCLUSIONS AND APPLICATIONS

1. Formulation of diets having traditional recommendations of digestible Lys with Val and Ile to Lys ratios of 0.77 and 0.67, without restricting CP, led to the best results overall in performance and fat pad deposition; therefore, if the goal is to reduce CP, similar results should be achieved by adding L-Ile, with further reductions in protein if L-Val is also added.
2. Increasing diet digestible Lys by 5% when compared with traditional recommendations led to gains in growth and FCR only if fed from placement to the finisher feeding phase.

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Acknowledgments

Authors want to thank Conselho Nacional de Pesquisa (CNPq; Brasilia, DF, Brazil) and Ajinomoto Biolatina (São Paulo, Brazil) for grants that supported this study.

Table 1. Diet formulation using CP and amino acid (AA) restrictions with or without a 5% increase in digestible lysine fed to Cobb 500 male broilers from 1 to 7 d of age[1]

Item	PRG 1	PRG 1 - 5%	PRG 2	PRG 2 - 5%	PRG 3	PRG 3 - 5%	PRG 4	PRG 4 - 5%
Ingredients								
Corn, 8.3%	57.05	57.31	48.41	44.33	53.48	50.05	56.08	52.94
Soybean meal, 46.1%	37.01	36.64	44.52	47.90	40.06	42.89	37.78	40.35
Soybean Oil	2.05	1.95	3.79	4.55	2.73	3.36	2.17	2.74
Limestone	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04
Dicalcium Phosphate	1.07	1.07	1.01	0.98	1.05	1.03	1.07	1.05
Sodium Bicarbonate	0.51	0.57	0.13	0.01	0.36	0.26	0.47	0.38
Salt	0.16	0.12	0.42	0.50	0.27	0.33	0.19	0.25
DL-Methionine, 99%	0.36	0.41	0.30	0.32	0.33	0.36	0.35	0.38
L-Lysine HCl, 78%	0.30	0.39	0.07	0.05	0.21	0.21	0.27	0.28
L-Threonine, 98.5%	0.14	0.19	0.04	0.04	0.10	0.11	0.13	0.14
Choline Chloride , 60%	0.08	0.08	0.04	0.03	0.07	0.05	0.07	0.06
Premix[2]	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
L-Valine, 96.5%	-	-	-	-	0.07	0.08	0.11	0.12
L-Isoleucine, 98.5%	-	-	-	-	-	-	0.04	0.04
FML Nutrient composition, % (Analyzed)								
AME$_n$, kcal/kg	2,960							
Ca	0.92							
Av. P	0.46							
Na	0.22							
DEB, mEq/kg[3]	250							
CP	22.4 (22.2)	22.40 (22.3)	24.8 (24.5)	26.0 (26.2)	23.4 (23.6)	24.5 (24.4)	22.7 (22.7)	23.7 (23.5)
Digestible Lys	1.324	1.390	1.324	1.390	1.324	1.390	1.324	1.390
Digestible TSAA	0.953	1.001	0.953	1.001	0.953	1.001	0.953	1.001
Digestible Thr	0.861	0.904	0.861	0.904	0.861	0.903	0.861	0.904
Digestible Val	0.905	0.899	1.019	1.070	1.019	1.070	1.019	1.070
Digestible Ile	0.839	0.833	0.958	1.011	0.887	0.931	0.887	0.931
Digestible Leu	1.699	1.689	1.857	1.927	1.763	1.821	1.714	1.767
Digestible Arg	1.409	1.398	1.619	1.712	1.494	1.572	1.430	1.501
Digestible Trp	0.239	0.237	0.277	0.294	0.254	0.268	0.243	0.256
Total Lys	1.43 (1.40)	1.49 (1.47)	1.44 (1.48)	1.51 (1.55)	1.43 (1.45)	1.50 (1.50)	1.43 (1.44)	1.50 (1.50)
Total TSAA	1.03 (1.02)	1.08 (1.07)	1.04 (1.03)	1.09 (1.08)	1.04 (1.03)	1.09 (1.08)	1.03 (1.03)	1.08 (1.08)
Total Thr	0.97 (0.98)	1.01 (1.02)	0.98 (0.98)	1.03 (1.02)	0.97 (0.96)	1.02 (1.02)	0.97 (0.95)	1.01 (1.00)
Total Val	1.02 (1.01)	1.01 (0.99)	1.15 (1.15)	1.20 (1.23)	1.14 (1.14)	1.19 (1.19)	1.13 (1.13)	1.19 (1.18)
Total Ile	0.93 (0.91)	0.92 (0.91)	1.06 (1.06)	1.12 (1.13)	0.98 (0.99)	1.03 (1.05)	0.98 (0.99)	1.02 (1.04)
Total Leu	1.84 (1.85)	1.83 (1.84)	2.02 (2.00)	2.09 (2.07)	1.91 (1.92)	1.98 (1.99)	1.86 (1.88)	1.92 (1.94)

Total Arg	1.49 (1.50)	1.48 (1.49)	1.71 (1.77)	1.81 (1.82)	1.58 (1.58)	1.66 (1.65)	1.51 (1.53)	1.59 (1.60)
Total Trp	0.26 (0.26)	0.26 (0.25)	0.31 (0.30)	0.32 (0.32)	0.28 (0.27)	0.30 (0.30)	0.27 (0.26)	0.28 (0.28)

[1] PRG 1 = CP restriction to 22.4, 21.1, 19.8, and 18.4%, respectively, in the 4 age periods with minimum digestible AA to digestible Lys set only for TSAA and Thr; PRG 2 = CP not restricted whereas minimum ratios of digestible AA to digestible Lys were extended to include Val (0.77) and Ile (0.67); PRG 3 = restrictions were as in PRG 2 with L-Val supplementation; PRG 4 = restrictions were the same as in PRG 3 but with L-Ile supplementation; PRG – 5% were diets with a 5% increase in digestible Lys, but maintaining the same digestible AA to digestible Lys ratios.

[2] Composition per kg of feed: vit. A, 8,000 UI; vit. D_3, 2,000 UI; vit. E, 30 UI; vit. K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; choline chloride 60%, 800 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; sodium monensin 40%, 120 mg, and avilamycin, 10 mg (Elanco Animal Health, Greenfield, IN); phytase (HiPhos GT 1000, DSM Nutritional Products Inc. (Parsippany, NJ), 100 mg.

[3] Dietary electrolytic balance. (Na + K – Cl), mEq/kg diet.

Table 2. Diet formulation using CP and amino acid (AA) restrictions with or without a 5% increase in digestible lysine fed to Cobb 500 male broilers from 8 to 21 d of age[1]

Item	PRG 1	PRG 1 - 5%	PRG 2	PRG 2 - 5%	PRG 3	PRG 3 - 5%	PRG 4	PRG 4 - 5%
Ingredients								
Corn, 8.3%	59.58	59.78	53.35	49.53	57.51	54.34	59.69	56.77
Soybean meal, 46.1%	33.92	33.58	39.35	42.47	35.70	38.31	33.78	36.16
Soybean Oil	3.05	2.95	4.30	5.01	3.43	4.02	2.96	3.49
Limestone	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Dicalcium Phosphate	0.89	0.89	0.84	0.82	0.87	0.85	0.89	0.87
Sodium Bicarbonate	0.42	0.48	0.15	0.04	0.33	0.24	0.43	0.35
Salt	0.20	0.19	0.38	0.49	0.26	0.32	0.19	0.24
DL-Methionine, 99%	0.31	0.36	0.26	0.29	0.29	0.32	0.31	0.34
L-Lysine HCl, 78%	0.26	0.35	0.09	0.08	0.20	0.20	0.26	0.27
L-Threonine, 98.5%	0.11	0.16	0.04	0.04	0.09	0.09	0.11	0.12
Choline Chloride , 60%	0.07	0.07	0.05	0.04	0.07	0.05	0.07	0.06
Premix[2]	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
L-Valine, 96.5%	-	-	-	-	0.06	0.07	0.09	0.10
L-Isoleucine, 98.5%	-	-	-	-	-	-	0.03	0.04
FML Nutrient composition, % (Analyzed)								
AME_n, kcal/kg	3,050							
Ca	0.84							
Av. P	0.42							
Na	0.21							
DEB, mEq/kg[3]	230							
CP	21.1 (21.2)	21.1 (21.0)	22.9 (23.0)	24.0 (24.0)	21.7 (21.6)	22.7 (22.6)	21.1 (21.2)	22.0 (21.9)
Digestible Lys	1.217	1.278	1.217	1.278	1.217	1.278	1.217	1.278
Digestible TSAA	0.876	0.920	0.876	0.920	0.876	0.920	0.876	0.920
Digestible Thr	0.791	0.831	0.791	0.831	0.791	0.831	0.791	0.831
Digestible Val	0.854	0.849	0.937	0.984	0.937	0.984	0.937	0.984
Digestible Ile	0.787	0.782	0.873	0.922	0.815	0.856	0.815	0.856
Digestible Leu	1.624	1.615	1.739	1.803	1.661	1.715	1.620	1.669
Digestible Arg	1.319	1.309	1.470	1.557	1.368	1.440	1.314	1.380
Digestible Trp	0.223	0.221	0.250	0.266	0.232	0.245	0.222	0.234
Total Lys	1.32 (1.33)	1.38 (1.38)	1.32 (1.34)	1.39 (1.41)	1.32 (1.31)	1.38 (1.38)	1.31 (1.30)	1.38 (1.37)
Total TSAA	0.95 (0.96)	1.00 (1.00)	0.96 (0.95)	1.01 (1.01)	0.95 (0.97)	1.00 (1.01)	0.95 (0.97)	1.00 (0.99)
Total Thr	0.89 (0.89)	0.93 (0.94)	0.90 (0.99)	0.94 (0.96)	0.89 (0.89)	0.94 (0.95)	0.89 (0.88)	0.93 (0.95)
Total Val	0.96 (0.97)	0.96 (0.95)	1.05 (1.06)	1.11 (1.11)	1.05 (1.04)	1.10 (1.09)	1.04 (1.04)	1.10 (1.10)
Total Ile	0.87 (0.86)	0.86 (0.85)	0.96 (0.97)	1.02 (1.03)	0.90 (0.91)	0.95 (0.96)	0.90 (0.89)	0.94 (0.94)
Total Leu	1.76 (1.75)	1.75 (1.77)	1.89 (1.90)	1.96 (1.96)	1.80 (1.82)	1.86 (1.87)	1.75 (1.76)	1.81 (1.80)

Total Arg	1.40 (1.40)	1.39 (1.39)	1.56 (1.57)	1.65 (1.67)	1.45 (1.46)	1.53 (1.54)	1.39 (1.37)	1.46 (1.48)
Total Trp	0.25 (0.24)	0.24 (0.24)	0.28 (0.28)	0.29 (0.29)	0.26 (0.25)	0.27 (0.26)	0.25 (0.24)	0.26 (0.25)

[1] PRG 1 = CP restriction to 22.4, 21.1, 19.8, and 18.4%, respectively, in the 4 age periods with minimum digestible AA to digestible Lys set only for TSAA and Thr; PRG 2 = CP not restricted whereas minimum ratios of digestible AA to digestible Lys were extended to include Val (0.77) and Ile (0.67); PRG 3 = restrictions were as in PRG 2 with L-Val supplementation; PRG 4 = restrictions were the same as in PRG 3 but with L-Ile supplementation; PRG – 5% were diets with a 5% increase in digestible Lys, but maintaining the same digestible AA to digestible Lys ratios.

[2] Composition per kg of feed: vit. A, 8,000 UI; vit. D_3, 2,000 UI; vit. E, 30 UI; vit. K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; choline chloride 60%, 800 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; sodium monensin 40%, 120 mg, and avilamycin, 10 mg (Elanco Animal Health, Greenfield, IN); phytase (HiPhos GT 1000, DSM Nutritional Products Inc. (Parsippany, NJ), 100 mg.

[3] Dietary electrolytic balance. (Na + K – Cl), mEq/kg diet.

Table 3. Diet formulation using different CP and amino acid (AA) restrictions with or without a 5% increase in digestible lysine fed to Cobb 500 male broilers from 22 to 35 d of age[1]

Item	PRG 1	PRG 1 - 5%	PRG 2	PRG 2 - 5%	PRG 3	PRG 3 - 5%	PRG 4	PRG 4 - 5%
Ingredients								
Corn, 8.3%	61.86	62.08	59.01	55.57	62.11	59.26	63.82	61.20
Soybean meal, 46.1%	30.96	30.65	33.44	36.25	30.72	33.07	29.21	31.36
Soybean Oil	4.26	4.18	4.83	5.47	4.19	4.71	3.82	4.29
Limestone	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Dicalcium Phosphate	0.69	0.69	0.67	0.65	0.69	0.67	0.71	0.69
Sodium Bicarbonate	0.24	0.29	0.11	0.07	0.25	0.17	0.32	0.25
Salt	0.30	0.26	0.38	0.45	0.29	0.35	0.24	0.29
DL-Methionine, 99%	0.25	0.29	0.23	0.24	0.25	0.27	0.26	0.28
L-Lysine HCl, 78%	0.19	0.27	0.12	0.10	0.20	0.20	0.25	0.25
L-Threonine, 98.5%	0.07	0.11	0.04	0.04	0.08	0.08	0.09	0.10
Choline Chrolide, 60%	0.07	0.07	0.06	0.05	0.07	0.06	0.08	0.07
Premix[2]	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
L-Valine, 96.5%	-	-	-	-	0.04	0.05	0.07	0.08
L-Isoleucine, 98.5%	-	-	-	-	-	-	0.02	0.03
FML Nutrient composition, % (Analyzed)								
AME_n, kcal/kg	3,150							
Ca	0.76							
Av. P	0.38							
Na	0.20							
DEB, mEq/kg[3]	200							
CP	19.8 (19.9)	19.8 (19.9)	20.6 (20.3)	21.6 (21.6)	19.8 (19.8)	20.6 (20.6)	19.3 (19.3)	20.1 (20.0)
Digestible Lys	1.095	1.150	1.095	1.150	1.095	1.150	1.095	1.150
Digestible TSAA	0.788	0.828	0.788	0.828	0.788	0.828	0.788	0.828
Digestible Thr	0.712	0.747	0.712	0.748	0.712	0.747	0.712	0.747
Digestible Val	0.805	0.800	0.843	0.886	0.843	0.886	0.843	0.886
Digestible Ile	0.738	0.732	0.777	0.821	0.734	0.771	0.734	0.771
Digestible Leu	1.551	1.543	1.603	1.661	1.546	1.594	1.514	1.558
Digestible Arg	1.232	1.222	1.301	1.379	1.225	1.290	1.183	1.242
Digestible Trp	0.208	0.206	0.220	0.234	0.206	0.218	0.199	0.210
Total Lys	1.19 (1.20)	1.24 (1.26)	1.19 (1.18)	1.25 (1.24)	1.19 (1.20)	1.25 (1.24)	1.18 (1.17)	1.24 (1.23)
Total TSAA	0.86 (0.85)	0.90 (0.90)	0.86 (0.85)	0.91 (0.90)	0.86 (0.87)	0.90 (0.90)	0.86 (0.87)	0.90 (0.90)
Total Thr	0.81 (0.82)	0.84 (0.86)	0.81 (0.81)	0.85 (0.86)	0.81 (0.84)	0.85 (0.88)	0.80 (0.81)	0.84 (0.85)
Total Val	0.91 (0.92)	0.90 (0.91)	0.95 (0.97)	1.00 (0.99)	0.94 (0.96)	0.99 (0.99)	0.94 (0.94)	0.99 (0.99)
Total Ile	0.81 (0.83)	0.81 (0.82)	0.86 (0.86)	0.91 (0.90)	0.81 (0.80)	0.85 (0.84)	0.81 (0.78)	0.85 (0.85)
Total Leu	1.68 (1.67)	1.67 (1.66)	1.74 (1.70)	1.80 (1.79)	1.67 (1.69)	1.73 (1.72)	1.64 (1.65)	1.69 (1.70)

Total Arg	1.31 (1.32)	1.30 (1.30)	1.38 (1.39)	1.46 (1.46)	1.30 (1.29)	1.37 (1.36)	1.26 (1.25)	1.32 (1.33)
Total Trp	0.23 (0.22)	0.23 (0.22)	0.24 (0.23)	0.26 (0.25)	0.23 (0.22)	0.24 (0.24)	0.22 (0.21)	0.23 (0.22)

[1] PRG 1 = CP restriction to 22.4, 21.1, 19.8, and 18.4%, respectively, in the 4 age periods with minimum digestible AA to digestible Lys set only for TSAA and Thr; PRG 2 = CP not restricted whereas minimum ratios of digestible AA to digestible Lys were extended to include Val (0.77) and Ile (0.67); PRG 3 = restrictions were as in PRG 2 with L-Val supplementation; PRG 4 = restrictions were the same as in PRG 3 but with L-Ile supplementation; PRG – 5% were diets with a 5% increase in digestible Lys, but maintaining the same digestible AA to digestible Lys ratios.

[2] Composition per kg of feed: vit. A, 8,000 UI; vit. D_3, 2,000 UI; vit. E, 30 UI; vit. K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; choline chloride 60%, 800 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; sodium monensin 40%, 120 mg, and avilamycin, 10 mg (Elanco Animal Health, Greenfield, IN); phytase (HiPhos GT 1000, DSM Nutritional Products Inc. (Parsippany, NJ), 100 mg.

[3] Dietary electrolytic balance. (Na + K – Cl), mEq/kg diet.

Table 4. Diet formulation using CP and amino acid (AA) restrictions with or without a 5% increase in digestible lysine fed to Cobb 500 male broilers from 36 to 43 d of age[1]

Item	PRG 1	PRG 1 - 5%	PRG 2	PRG 2 - 5%	PRG 3	PRG 3 - 5%	PRG 4	PRG 4 - 5%
Ingredients								
Corn, 8.3%	66.00	66.21	64.00	60.92	66.52	63.70	67.63	65.25
Soybean meal, 46.1%	27.26	26.94	28.97	31.53	26.75	29.10	25.79	27.74
Soybean Oil	4.20	4.13	4.61	5.19	4.13	4.61	3.84	4.28
Limestone	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Dicalcium Phosphate	0.47	0.47	0.45	0.43	0.47	0.45	0.48	0.46
Sodium Bicarbonate	0.19	0.24	0.11	0.01	0.21	0.13	0.27	0.20
Salt	0.31	0.28	0.37	0.44	0.30	0.36	0.26	0.31
DL-Methionine, 99%	0.21	0.25	0.20	0.21	0.21	0.23	0.22	0.24
L-Lysine HCl, 78%	0.19	0.26	0.134	0.13	0.20	0.20	0.24	0.24
L-Threonine, 98.5%	0.06	0.10	0.04	0.04	0.07	0.07	0.08	0.09
Choline Chrolide, 60%	0.08	0.09	0.08	0.07	0.08	0.08	0.09	0.08
Premix[2]	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
L-Valine, 96.5%	-	-	-	-	0.03	0.04	0.05	0.06
L-Isoleucine, 98.5%	-	-	-	-	-	-	0.02	0.02
FML Nutrient composition, % (Analyzed)								
AME_n, kcal/kg	3,200							
Ca	0.66							
Av. P	0.33							
Na	0.19							
DEB, mEq/kg[3]	180							
CP	18.4 (18.3)	18.4 (18.4)	19.0 (18.8)	19.9 (19.7)	18.3 (18.5)	19.1 (19.0)	18.0 (17.9)	18.7 (18.6)
Digestible Lys	1.006	1.056	1.006	1.056	1.006	1.056	1.006	1.056
Digestible TSAA	0.724	0.760	0.724	0.760	0.724	0.760	0.724	0.760
Digestible Thr	0.654	0.686	0.654	0.686	0.654	0.686	0.654	0.686
Digestible Val	0.748	0.743	0.775	0.813	0.775	0.813	0.775	0.813
Digestible Ile	0.678	0.673	0.706	0.746	0.674	0.708	0.674	0.708
Digestible Leu	1.471	1.464	1.508	1.561	1.466	1.509	1.440	1.480
Digestible Arg	1.127	1.118	1.176	1.247	1.119	1.179	1.086	1.140
Digestible Trp	0.189	0.187	0.198	0.210	0.188	0.198	0.182	0.191
Total Lys	1.09 (1.07)	1.14 (1.14)	1.09 (1.09)	1.15 (1.15)	1.09 (1.09)	1.15 (1.13)	1.09 (1.09)	1.14 (1.16)
Total TSAA	0.79 (0.78)	0.83 (0.82)	0.79 (0.79)	0.83 (0.82)	0.79 (0.78)	0.83 (0.82)	0.79 (0.78)	0.83 (0.81)
Total Thr	0.74 (0.75)	0.78 (0.79)	0.75 (0.74)	0.78 (0.80)	0.74 (0.77)	0.78 (0.77)	0.74 (0.75)	0.78 (0.80)
Total Val	0.84 (0.85)	0.84 (0.83)	0.87 (0.87)	0.92 (0.90)	0.87 (0.85)	0.91 (0.90)	0.87 (0.85)	0.91 (0.92)
Total Ile	0.75 (0.74)	0.74 (0.73)	0.78 (0.77)	0.82 (0.81)	0.74 (0.73)	0.78 (0.77)	0.74 (0.74)	0.78 (0.80)
Total Leu	1.59 (1.60)	1.58 (1.59)	1.63 (1.64)	1.69 (1.70)	1.58 (1.60)	1.63 (1.62)	1.55 (1.57)	1.60 (1.61)

Total Arg	1.20 (1.21)	1.19 (1.18)	1.25 (1.26)	1.32 (1.34)	1.19 (1.21)	1.25 (1.24)	1.15 (1.14)	1.21 (1.23)
Total Trp	0.21 (0.21)	0.21 (0.20)	0.22 (0.21)	0.23 (0.23)	0.21 (0.20)	0.22 (0.20)	0.20 (0.19)	0.21 (0.20)

[1] PRG 1 = CP restriction to 22.4, 21.1, 19.8, and 18.4%, respectively, in the 4 age periods with minimum digestible AA to digestible Lys set only for TSAA and Thr; PRG 2 = CP not restricted whereas minimum ratios of digestible AA to digestible Lys were extended to include Val (0.77) and Ile (0.67); PRG 3 = restrictions were as in PRG 2 with L-Val supplementation; PRG 4 = restrictions were the same as in PRG 3 but with L-Ile supplementation; PRG – 5% were diets with a 5% increase in digestible Lys, but maintaining the same digestible AA to digestible Lys ratios.

[2] Composition per kg of feed: vit. A, 8,000 UI; vit. D_3, 2,000 UI; vit. E, 30 UI; vit. K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; choline chloride 60%, 800 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; sodium monensin 40%, 120 mg, and avilamycin, 10 mg (Elanco Animal Health, Greenfield, IN); phytase (HiPhos GT 1000, DSM Nutritional Products Inc. (Parsippany, NJ), 100 mg.

[3] Dietary electrolytic balance. (Na + K – Cl), mEq/kg diet.

Table 5. Growth performance of broilers fed diets formulated using different CP and amino acid (AA) restrictions with or without 5% increase in digestible lysine[1]

	1 to 7 d		8 to 21 d		22 to 35 d		36 to 43 d		1 to 35 d		1 to 43 d	
Treatment	BWG[3], g	FCR [4]	BWG, g	FCR	BWG, g	FCR	BWG, g	FCR	BWG, g	FCR	BWG, g	FCR
Feeding Program[2]												
PRG 1	146 ^{b}	1.154 ^{b}	806 ^{b}	1.408 ^{b}	1,399 ^{ab}	1.751 ^{b}	775	2.001	2,324 ^{b}	1.599 ^{b}	3,105 ^{b}	1.694 ^{b}
PRG 2	155 ^{a}	1.098 ^{a}	844 ^{a}	1.343 ^{a}	1,383 ^{b}	1.743 ^{b}	797	2.019	2,379 ^{a}	1.560 ^{a}	3,167 ^{a}	1.669 ^{a}
PRG 3	149 ^{ab}	1.121 ^{ab}	810 ^{b}	1.384 ^{b}	1,425 ^{a}	1.721 ^{a}	789	2.031	2,375 ^{a}	1.576 ^{ab}	3,169 ^{a}	1.681 ^{ab}
PRG 4	150 ^{ab}	1.143 ^{b}	815 ^{b}	1.384 ^{b}	1,415 ^{a}	1.737 ^{ab}	776	2.014	2,352 ^{ab}	1.584 ^{ab}	3,130 ^{ab}	1.687 ^{ab}
SEM (n=18)	1.9	0.0115	8.0	0.0113	8.9	0.0088	12.0	0.0226	12.7	0.0075	16.1	0.0069
Digestible Lys												
Control	149	1.138	820	1.385	1,407	1.746	777	2.021	2,356	1.584	3,131	1.691 ^{b}
Control plus 5%	150	1.124	818	1.375	1,404	1.730	792	2.012	2,359	1.575	3,154	1.675 ^{a}
SEM (n=36)	1.3	0.0082	5.7	0.0080	6.3	0.0062	8.5	0.0160	9.0	0.0053	11.4	0.0049
Main effect (*P*-value)												
Feeding Program	0.004	0.002	0.004	0.001	0.004	0.031	0.467	0.815	0.008	0.003	0.007	0.028
Digestible Lys	0.658	0.185	0.801	0.323	0.730	0.074	0.178	0.664	0.817	0.196	0.125	0.014
Interaction	0.307	0.298	0.857	0.881	0.260	0.259	0.027	0.026	0.985	0.997	0.239	0.832

$^{a–b}$ Means not sharing a common superscript within a column differ ($P<0.05$).

[1] Means of 18 and 36 replicates of 25 birds per pen, respectively, for feeding program and digestible Lys.

[2] PRG 1 = CP restriction to 22.4, 21.1, 19.8, and 18.4%, respectively, in the 4 age periods with minimum digestible AA to digestible Lys set only for TSAA and Thr; PRG 2 = CP not restricted whereas minimum ratios of digestible AA to digestible Lys were extended to include Val (0.77) and Ile (0.67); PRG 3 = restrictions were as in PRG 2 with L-Val supplementation; PRG 4 = restrictions were the same as in PRG 3 but with L-Ile supplementation; PRG – 5% were diets with a 5% increase in digestible Lys, but maintaining the same digestible AA to digestible Lys ratios.

[3] BW gain.

[4] Values are corrected for the weight of dead birds.

Table 6. Interaction for BW gain (BWG) and FCR of broilers fed diets formulated using different CP and amino acid (AA) restrictions with or without 5% increase in digestible lysine from 36 to 43 d[1, 2]

	Digestible Lys			
	BWG[4], g		FCR[5]	
Feeding Program[3]	Control	Control plus 5%	Control	Control plus 5%
PRG 1	791$^{a, x}$	758$^{b, x}$	1.969$^{a, x}$	2.034$^{b, y}$
PRG 2	767$^{a, y}$	827$^{a, x}$	2.078$^{b, y}$	1.960$^{a, x}$
PRG 3	770$^{a, x}$	808$^{ab, x}$	2.040$^{b, x}$	2.022$^{ab, x}$
PRG 4	778$^{a, x}$	775$^{ab, x}$	1.998$^{ab, x}$	2.031$^{b, x}$

[a,b] Means not sharing a common superscript within a column differ ($P < 0.05$)

[x,y] Means not sharing a common superscript within a row differ ($P < 0.05$)

[1] Means of 9 replicates of 25 birds per pen (SEM = 17.0)

[2] PRG 1 = CP restriction to 22.4, 21.1, 19.8, and 18.4%, respectively, in the 4 age periods with minimum digestible AA to digestible Lys set only for TSAA and Thr; PRG 2 = CP not restricted whereas minimum ratios of digestible AA to digestible Lys were extended to include Val (0.77) and Ile (0.67); PRG 3 = restrictions were as in PRG 2 with L-Val supplementation; PRG 4 = restrictions were the same as in PRG 3 but with L-Ile supplementation; PRG – 5% were diets with a 5% increase in digestible Lys, but maintaining the same digestible AA to digestible Lys ratios.

[3] Values are corrected for the weight of dead birds.

Table 7. Carcass, abdominal fat, and yields of the commercial cuts of broilers fed diets formulated using CP and amino acid (AA) restrictions with or without a 5% increase in digestible lysine, %[1]

Treatment	Carcass[3]	Abdominal fat	Breast fillets[4]	Tenders	Thighs	Drumsticks	Wings	Cage
Feeding Program[2]								
PRG 1	80.5	2.11 ^{a}	25.6	5.43	18.2	12.5	9.46	22.4
PRG 2	80.8	1.85 ^{b}	25.7	5.44	18.3	12.5	9.53	22.3
PRG 3	80.4	1.96 ^{ab}	25.8	5.45	18.1	12.5	9.52	22.4
PRG 4	80.1	2.02 ^{ab}	25.4	5.37	18.2	12.4	9.43	22.1
SEM (n=18)	0.29	0.151	0.24	0.046	0.12	0.10	0.064	0.15
Digestible Lysine Levels								
Control	80.4	2.00	25.7	5.41	18.2	12.5	9.48	22.3
Control plus 5%	80.1	1.97	25.6	5.42	18.2	12.4	9.49	22.3
SEM (n=36)	0.20	0.107	0.17	0.032	0.08	0.07	0.046	0.11
Main effect (*P*-value)								
Feeding Program	0.287	0.011	0.652	0.626	0.748	0.587	0.232	0.294
Digestible Lysine	0.189	0.603	0.188	0.634	0.588	0.535	0.789	0.958
Interaction	0.333	0.564	0.695	0.300	0.660	0.532	0.994	0.237

[a–b] Means not sharing a common superscript within a column differ ($P<0.05$).

[1] Means of 9 replicates of 25 birds per pen (SEM = 0.0320)

[2] PRG 1 = CP restriction to 22.4, 21.1, 19.8, and 18.4%, respectively, in the 4 age periods with minimum digestible AA to digestible Lys set only for TSAA and Thr; PRG 2 = CP not restricted whereas minimum ratios of digestible AA to digestible Lys were extended to include Val (0.77) and Ile (0.67); PRG 3 = restrictions were as in PRG 2 with L-Val supplementation; PRG 4 = restrictions were the same as in PRG 3 but with L-Ile supplementation; PRG – 5% were diets with a 5% increase in digestible Lys, but maintaining the same digestible AA to digestible Lys ratios.

[3] Eviscerated carcass as a percentage of body weight, whereas cuts are proportions of the carcass.

[4] Skinless boneless *Pectoralys major*.

CAPÍTULO III[1]

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[1] Artigo submetido na revista Animal Feed Science and Technology em Outubro de 2014 e aceito em Abril de 2015.

Performance and meat production from broiler chickens fed diets formulated with variable crude protein restrictions supplemented or not with L-valine and L-isoleucine

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ABSTRACT

A total of 4,800 one day (d) old Cobb × Cobb 500 male broilers was used in a study with the objective of evaluating dietary programs formulated with different degrees of restriction in crude protein (CP) and the supplementation of L-valine and L-isoleucine. Four feeding programs (PRG) reflecting different strategies to deliver amino acids (AA) to broilers were used, respectively from d 1 to 7, 8 to 21, 22 to 35 and 36 to 42, as follow: PRG 1, CP restricted to 224, 211, 198 and 184 g/kg with minimum digestible AA to lysine (Lys) ratios only set for total sulfur AA (TSAA) (0.72) and threonine (Thr) (0.65); PRG 2, as PRG 1 without CP restriction with AA ratios to Lys extended to valine (Val) (0.77) and isoleucine (Ile) (0.67); PRG 3, as PRG 2 supplemented with L-Val; PRG 4, as PRG 3 supplemented with L-Ile. Replications of the 4 PRG provided from 1 to 21 d were further divided into the 4 respective PRG given from 22 to 42 d. The study was conducted as a completely randomized block design with 2 time blocks (experimental replication). In each experimental block there were 24 replications per treatment from 1 to 21 d, whereas 12 replications were used from 22 to 42 d. Overall, there were no effects for time block or neither for the interaction between PRG fed in the periods of 1 to 21 and 22 to 42 d. Performance and processing results demonstrated benefits of formulating diets using Val and Ile minimum ratios. Besides, body weight gain was higher when L-Val and L-Ile were supplemented together (PRG 4); on the other hand, feed conversion ratio (FCR) was better using the same ratios of Val and Ile without their synthetic sources. Carcass yield data was not affected by any PRG regardless of the period fed with the exception of the proportion of abdominal fat that was significantly reduced when broilers were fed diets with minimum ratios of Val and Ile without supplementation with their synthetic sources. In conclusion, formulation of diets for broilers without establishing a minimum CP level but using AA ratios to Lys as in PRG 2, 3 and 4, allowed live performance and carcass yield results that were competitive. Using L-Val and L-

Ile led to improved FCR and, therefore, this possibility should be explored worldwide such that production of broiler meat may become more efficient.

Key words: amino acid, broiler, L-isoleucine, L-valine

Abbreviations: amino acids, AA; apparent metabolizable energy, AME; body weight gain, BWG; crude protein, CP; days, d; digestible, dig.; feed conversion ratio, FCR; feed intake, FI; feeding program, PRG; isoleucine, Ile; lysine, Lys; methionine, Met; soybean meal, SBM; threonine, Thr; total sulfur amino acids, TSAA; valine, Val.

1. Introduction

Broiler feeds are usually formulated using linear least cost formulation software and frequently set at a minimum concentration of crude protein (CP) in a conservative strategy to have essential and non-essential amino acid (AA) intakes safeguarded. Providing dietary AA for broilers using commercial available synthetic sources [L-Lysine (L-Lys), DL-Methionine (DL-Met) and L-Threonine (L-Thr) as well as Methionine analogue] allowed nutritionists to achieve competitive broiler field performance and meat yield (Hill and Kim, 2013; Kobayashi et al., 2013). Formulating diets with further CP reduction is possible with the utilization of other synthetic AA if commercially available at competitive prices. Examples are L-valine (L-Val), the fourth limiting AA for broilers in maize-soybean diets, and L-isoleucine (L-Ile), in many situations the fifth limiting, followed by arginine (Kidd et al., 2004a; Corzo et al., 2007, 2010; Berres et al., 2010b; Tavernari et al., 2013).

The use of the ideal protein concept has become a worldwide used strategy to formulate broiler feeds. This approach targets to reduce the absorbed AA amount that are in relative excess compared to lysine (Lys), thus avoiding excess oxidation, decreasing metabolic costs and improving AA balance (Lemme, 2003; Vieira and Angel, 2012).

Protein is a costly nutrient in commercial poultry diets. The increasing cost of feed ingredients and the competition with the biofuel industry for some plant feedstuffs, such as maize and soy oil, indicates a scenario of increased costs for feed ingredients in the coming years. Starting in 2007, the prices of the major crops used in animal production have increased dramatically in real terms reaching a peak in 2008 and then declining in 2009 and 2010, followed by sharp increases again in 2011 (Rosegrant et al., 2012). Therefore, reducing CP through the increased use of synthetic AA can help to maintain competitive meat production while minimizing the impact of the increased cost and volatility of protein sources.

Commercial implementation of L-Val and L-Ile in poultry feeds is still limited because of insufficient information on the effects of these synthetic AA supplements in different feeding programs throughout the broilers life. Therefore, generating data on live broiler performance and proportions of meat cuts using diets with or without synthetic L-Val and L-Ile is necessary if nutritionists are to use them effectively in feed formulation. Ratios of digestible (dig.) valine (Val) and isoleucine (Ile) to dig. Lys that optimizes growth has been suggested to be between 0.75 to 0.77 and 0.65 to 0.67, respectively (Kidd et al., 2004a; Corzo et al., 2009, 2010; Berres et al., 2010a,b; Corrent and Bartett, 2011; Tavernari et al., 2013). However, optimal ratios of dig. Val and/or dig. Ile to dig. Lys for carcass optimization has not been clearly defined.

The objective of the present study was to evaluate the effects of feeding programs formulated using least cost linear formulation, restricting CP or not, and supplementing L-Val and L-Ile, on live performance and processing yield of the commercial cuts of major importance.

2. Material and methods

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

2.1. General bird husbandry

Twenty five day-old male Cobb × Cobb 500 slow feathering broiler chickens were placed in 1.65 m × 1.65 m floor pens (9.2 birds per m^2) with new rice hulls as bedding. Each pen was equipped with a tube feeder and a bell drinker. Average temperature was 32 ºC at placement, which was reduced by 1 ºC every 2 d to provide comfort throughout the study through the use of thermostatically controlled heaters, fans and foggers. Lighting was continuous until 7 d of age, with a 14L:10D cycle used afterwards. Birds had *ad libitum* access to water and mash feeds.

2.2. Dietary treatments

Experimental feeds in each period considered are presented in Tables 1 to 4. Least cost linear formulation was used to formulate diets based on previously analyzed maize and soybean meal (SBM) for AA (method L257; Official Journal of The European Community, 1998). Feeds were formulated using digestible AA calculated by multiplying analyzed total AA by a coefficient of digestibility (Rostagno et al., 2011).

Four feeding phases were used: pre starter (1-7 d), starter (8-21 d), grower (22-35 d), and finisher (36-42 d). The minimum ratios of digestible AA to Lys were 0.72 total sulfur amino acids (TSAA), 0.65 threonine (Thr), 1.08 arginine (Arg), 0.17 (tryptophan) Trp, and 1.07 leucine (Leu) for all diets. Experimental diets met or exceeded the recommendations of Rostagno et al. (2011) for all nutrients except CP and AA. Supplementation of AA was carried out using synthetic DL-Met (990 g/kg), L-Lys HCl (780 g/kg), L-Thr (985 g/kg), L-Val (965 g/kg), and L-Ile (985 g/kg). The experimental treatments were composed by feeding programs (PRG) resulting from the use of four CP and AA restriction strategies. In PRG 1, CP was restricted to 224, 211, 198 and 184 g/kg in the pre starter, starter, grower, and finisher phases, respectively; however, minimum AA to Lys ratios were only set for TSAA and Thr. In PRG 2, CP was not restricted while minimum ratios of AA to Lys were extended to include Val (0.77) and Ile (0.67). In PRG 3, feed formulation was carried out as for PRG 2 but with L-Val supplementation (0.67, 0.55, 0.45, and 0.37 g/kg, in the pre starter, starter, grower, and finisher phases diets, respectively). In PRG 4, feed formulation was similar to PRG 3 but both with L-Val and L-Ile supplementation (1.15 and 0.48, 0.96 and 0.42, 0.82 and 0.37, and 0.69 and 0.33 g/kg in the pre starter, starter, grower, and finisher phases, respectively).

Birds were placed in 192 floor pens of 2.8 m^2 (experimental units having 25 birds at the beginning of the experiment). Two time blocks were used in the present study (experimental replication in time). There were 4 PRG that were replicated 24 times per block from 1 to 21 d of age (pre starter and starter phases). Starting on day 22 through the end of the study, the 24 replicates were equally divided into 6 others, which were then fed to one of the 4 PRG from 22 to 42 d of age (grower and finisher phases). Therefore, from 22 to 42 d, there were 16 treatments, each replicated 6 times per block. The final design had 2 time blocks and 48 replicates per treatment from 1 to 21 d of age and 12 replicates from 22 to 42 d of age.

2.3. Measurements

Live performance was evaluated using pen bird weights and feed intake (FI) at 7, 21, 35 and 42 d of age. Feed conversion ratio (FCR) was corrected for the weight of dead birds. Pens were checked daily for dead birds. At 42 d of age, 6 birds per pen were randomly taken from each pen after being fasted for 8 h. These birds were individually weighed, electrically stunned with 45 V for 3 s, bled for 3 min through a jugular vein cut, followed by scalding at 60 ºC for 45 s and plucked. Evisceration was done manually and carcasses were chilled in ice for 3 h and then hung for 3 min to draw off excess water. Carcasses were weighed and abdominal fat was removed and weighed. Trained industry personnel performed the carcass cuts obtaining deboned breast meat (*Pectoralys major* plus *Pectoralys minor*) with skin as well as bone-in thighs, drumsticks, wings and cages, which were immediately weighed. Carcass yield was expressed relative to live weight, whereas abdominal fat and carcass cuts were expressed as a proportion of carcass weight.

2.4. Statistical analyses

The study was conducted as a completely randomized block design with a time block (experimental replication). Treatments were 4 PRG with 48 replications from 1 to 21 d, and 16 PRG with 12 replications from 22 to 42 d. From 22 to 42 d, treatments were arranged in a 4 × 4 factorial (4 PRG from 1-21 d × 4 PRG from 22-42 d) in split-plot where the PRG from 1 to 21 d was the principal plot and the PRG used from 22 to 42 d the subplot. Mortality, carcass and cut yields were analyzed after arcsine transformation $[(\% \text{ mortality}/100) + 0.05]^{0.5}$.

Data was analyzed using a mixed model as follow:

$$Y_{ijkl} = \mu + b_i + \alpha_j + (b\alpha)_{ij} + \beta_k + (\alpha\beta)_{jk} + e_{ijkl}$$

Where:

Y_{ijkl} = response variable

μ = general mean

b_i = effect of experimental repetition - block (random variable)

α_j = effect of principal plot (feeding programs 1-21 days)

$(b\alpha)_{ij}$ = principal plot error (random variable)

β_k = effect of subplot (feeding programs 22-42 days)

$(\alpha\beta)_{jk}$ = effect of interaction

ε_{ijkl} effect of random error

Data normality and homoscedasticity were verified using the Shapiro-Wilk test (Shapiro, 1965). Normal and homogeneous data were analyzed using the Mixed Models with the PROC MIXED procedure of SAS (SAS Institute, Cary, North Carolina). The factors PRG from 1 to 21 d and 22 to 42 d were considered fixed effects. Restricted maximum likelihood (REML) analysis (Gilmour et al., 1995) showed that the best Akaike information criteria (Littell et al., 1998) were obtained when experiment replication (block) was fitted as a random effect. Means were compared using least square means comparisons and were separated by the PDIFF procedure of SAS (version 9.2) when main effect differences or their interaction were detected. Significance was accepted at the $P \leq 0.05$ level and mean differences were separated using Tukey's HSD test.

3. Results

No statistical differences for any of the studied variables were detected between experiment replications (block). Slight deviations were observed between analyzed and formulated CP and AA in the feeds (Tables 1 to 4). Growth performance and carcass yield are shown in Tables 5 to 7.

There were no statistical differences in mortality between any treatments (data not shown). Cumulative mortality were 1.15 % at 21 d, and 2.74 % at 42 d. Growth performance data from 1 to 21 d of age are shown in Table 5. Broilers fed PRG 1 had lower body weight gain (BWG) when compared with the other PRG, and higher FCR ($P<0.001$), than birds fed PRG 2 and 3. In this period, FCR was worse in birds fed PRG 1, best for those fed PRG 2, and intermediate for PRG 3 ($P<0.001$); however, FCR was not different between broilers fed PRG 1, 3 and 4. Birds fed PRG 4 had the highest FI, whereas those fed PRG 2 had the lowest

FI ($P<0.001$) and those fed PRG 1 and 3 were intermediate and not different to any other PRG.

There were no interactions between PRG fed in the two periods on growth performance from 22 to 42 d of age (Table 6) or in the cumulative data obtained at the end of the study. There were no effects of the PRG fed from 1 to 21 d on BWG from 22 to 42 d. However, birds fed PRG 4 from 1 to 21 d exhibited higher cumulative BWG (1 to 42 d) than birds fed all other PRG ($P<0.05$). Effects of PRG from 22 to 42 d were found with birds from PRG 4 having higher BWG in this period, as well as in the cumulative BWG from 1 to 42 d ($P<0.05$), when compared to birds fed PRG 1. However, BWG in this period for birds fed PRG 2 and 3 was not different from those of birds fed PRG 1 and 4.

There were no effects of PRG fed from 1 to 21 d on FCR in the period of 22 to 42 d as well as in the cumulative from 1 to 42 d. However, PRG fed from 22 to 42 d of age affected FCR in this period as well as the cumulative FCR from 1 to 42 d ($P<0.01$). It was observed that birds fed PRG 2 from 22 to 42 d had better FCR ($P<0.05$) in the same period of age when compared to those fed PRG 1, but birds fed PRG 3 and 4 could not be statistically separated from those fed PRG 1 and 2. Birds fed PRG 2 also had better FCR from 1 to 42 d ($P<0.05$) when compared to birds fed PRG 1 and 4, whereas birds fed PRG 3 had no statistical difference to all other PRG.

No differences were observed in FI from 22 to 42 d or in the cumulative FI from 1 to 42 d for any PRG fed from 1 to 21 d. Considering the PRG fed from 22 to 42 d, birds fed PRG 4 had higher FI ($P<0.05$) in this same period, as well as in the cumulative period from 1 to 42 d, when compared to PRG 2 and 3. No differences, however, were observed when compared to birds fed PRG 1.

Carcass yield data are shown in Table 7. There was no interaction between PRG fed from 1 to 21 d and 22 to 42 d. For most carcass responses, there were no effects of PRG, regardless of the period in which they were fed. One exception was PRG 2 from 22 to 42 d that produced the lowest abdominal fat when compared to all other PRG ($P<0.05$).

4. Discussion

Dietary AA allowances that maximize broiler economic productivity depend not only on performance and proportion of carcass components but also on the mix and market price of products being sold as well as on ingredient availability and costs. Dietary AA concentrations that maximize growth, FCR and breast meat yield are different for each of these parameters. For instance, optimum essential AA concentrations are highest for breast meat than for whole carcass yield or BWG (Moran and Bilgili, 1990; Schutte and Pack, 1995; Aleator et al., 2000; Vieira et al., 2004). Breast meat generally is sold for a higher price than other carcass components; however, differences exist between markets worldwide, which may impact the profitability of the mix of cuts produced. Therefore, AA density in the feeds depends on the desired market weight, product mix, broiler live cost, and is also dependent on broiler genetic potential (Vieira and Angel, 2012). Regardless of the feeding strategy, supplementation of broiler feeds with the mainly available synthetic AA (Met, Lys and Thr) reduces production costs without compromising growth performance and meat yields. The most notable reduction in cost that results from supplementing with synthetic AA is obtained by lowering SBM dietary inclusions, which is the most widely used protein source by poultry producers. Constant increases in SBM market prices have occurred over the last decade, recently elevating its value by 163.33 % (Index Mundi, 2013).

The use of the fourth and fifth limiting synthetic AA (L-Val and L-Ile) in maize-SBM feeds (Corzo et al., 2007; Berres et al., 2010b) seems to be inevitable. As with synthetically produced Met, Lys and Thr, those 2 AA, as likely as others that will follow in the future, will likely reach a competitive market price and therefore, are expected to produce economic returns in diets with lower percentages of SBM. Based on the presented results, the importance of using L-Val and L-Ile as synthetic sources of AA to increase Val and Ile to the ratios suggested by Rostagno et al. (2011) is evident, as these AA sources allowed for competitive performance (PRG 4) when compared to PRG 1, which was notably deficient, but showed similar performance when compared to PRG 2.

Requirements of AA derived by dose-response trials depend not only on the amount of dietary CP, its quality and availability for growth, but also on how the dietary protein is balanced in the test diets (Temim et al., 2000; Eits et al., 2003; Berres et al., 2011). Recent studies have demonstrated that supplementation with L-Val and L-Ile allow for growth and processing performance of broilers comparable to industry standards (Corzo et al., 2007, 2009; Berres et al., 2010b, 2011). In the present study, the highest BWG and lowest FCR obtained were for birds fed PRG 2, 3 and 4. Even without statistical differences between them, their performance data can be related to the minimum ratios of Val and Ile to Lys established in PRG 2 without supplementation with L-Val and L-Ile. However, when L-Val and L-Ile were supplemented in PRG 3 and 4, which led to a reduction in CP, BWG and FCR were not different when compared to birds fed PRG 2. It is possible to conclude that PRG 1 imposed a limitation in growth due to a strategy of feed formulation that did not take into account the adequate ratios of Val and Ile to Lys. The study also evidenced that supplementing L-Val and L-Ile to reach suggested ideal ratios (Corzo et al., 2007; Berres et al., 2010a; Rostagno et al., 2011) produced competitive growth results.

Feed formulation of broiler diets, without taking into account Val and Ile ideal ratios, leads to poorer broiler performance when compared with broilers fed diets formulated with higher levels of Val and Ile derived from a higher CP diet. Using synthetic sources of Val and Ile in commercial broiler feeds will depend on the market prices of these synthetic AA when compared with Val and Ile contents in ingredients such as SBM.

4. Conclusion

Supplementing broiler diets with synthetic L-Val and L-Ile results in broiler growth performance and meat yields that are similar to that obtained with diets having similar dig. Val and Ile levels derived from non-synthetic sources, therefore, allowing for a reduction in the amounts of SBM and other protein feedstuffs used to produce these diets.

Acknowledgments

Authors wish to thank Conselho Nacional de Pesquisa (CNPq) for scholarship grants and Ajinomoto Biolatina, São Paulo, Brazil, for the funds supporting this study.

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Table 1
Ingredient composition and chemical analysis of the experimental pre starter diets (g/kg) formulated using different limiting AA restrictions from 1 to 7 d of age[a]

Item	PGR 1	PGR 2	PGR 3	PGR 4
Ingredient				
Maize (78 g/kg)	563.29	474.87	523.72	558.06
Soybean meal (451 g/kg)	376.59	453.34	410.59	380.33
Soybean oil	21.78	39.53	29.37	21.93
Limestone	10.38	10.37	10.38	10.38
Dicalcium phosphate	10.67	10.04	10.39	10.64
Sodium bicarbonate	3.94	0.14	2.26	3.76
Sodium chloride	2.39	4.98	3.53	2.51
DL-Methionine (990 g/kg)	3.61	3.01	3.35	3.59
L-Lysine HCl (780 g/kg)	2.93	0.62	1.91	2.82
L-Threonine (985 g/kg)	1.37	0.38	0.93	1.32
Choline chloride (600 g/kg)	0.75	0.42	0.60	0.73
Premix[b]	2.30	2.30	2.30	2.30
L-Valine (965 g/kg)	-	-	0.67	1.15
L-Isoleucine (935 g/kg)	-	-	-	0.48
Nutrient composition, g/kg or as described[c]				
Apparent metabolizable energy, MJ/kg	12.39	12.39	12.39	12.39
Calcium	9.2	9.2	9.2	9.2
Available phosphorus	4.6	4.6	4.6	4.6
Sodium	2.2	2.2	2.2	2.2
DEB, mEq/kg[d]	240	240	240	240
Crude protein	224.0 (223.0)	248.7 (248.3)	235.4 (234.3)	226.3 (226.7)
Digestible lysine	13.2	13.2	13.2	13.2
Digestible total sulfur amino acids	9.5	9.5	9.5	9.5
Digestible threonine	8.6	8.6	8.6	8.6
Digestible valine	9.0	10.2	10.2	10.2
Digestible isoleucine	8.3	9.5	8.9	8.9
Total lysine	14.3 (14.4)	14.4 (14.5)	14.3 (14.1)	14.3 (14.1)
Total sulfur amino acids	10.3 (10.3)	10.4 (10.0)	10.4 (9.9)	10.3 (10.0)
Total threonine	9.7 (9.5)	9.7 (10.1)	9.7 (9.6)	9.7 (10.2)
Total valine	10.2 (10.1)	11.5 (11.8)	11.4 (11.2)	11.3 (10.7)
Total isoleucine	9.2 (9.2)	10.5 (10.8)	9.8 (9.6)	9.7 (9.2)

[a] PRG 1 – CP restricted and minimum AA to Lys set only for TSAA and Thr; PRG 2 - CP not restricted while minimum ratios of AA to Lys were extended to Val (0.77) and Ile (0.67); PRG 3 - restrictions were as in PRG 2 with L-Val supplementation; PRG 4 - restrictions were the same as in PRG 3 but with L-Ile supplementation.

[b] Contributed per kilogram of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 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; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; sodium monensin 40%, 120 mg/kg (Elanco Animal Health, Greenfield, IN), avilamycin, 10 mg/Kg (Elanco Animal Health, Greenfield, IN); and phytase, 100 mg/kg (HiPhos GT 1000, DSM Nutritional Products Inc., Parsippany, NJ).

[c] Values in parentheses were determined by chemical analysis.

[d] Dietary electrolytic balance (Na + K – Cl), mEq/kg of the diet.

Table 2

Ingredient composition and chemical analysis of the experimental starter diets (g/kg) formulated using different limiting AA restrictions from 8 to 21 d of age[a]

Item	PGR 1	PGR 2	PGR 3	PGR 4
Ingredient				
Maize (78 g/kg)	585.27	524.62	564.45	594.35
Soybean meal (451 g/kg)	348.63	401.26	366.40	340.05
Soybean oil	32.40	44.57	36.28	29.81
Limestone	9.63	9.62	9.63	9.63
Dicalcium phosphate	8.79	8.36	8.65	8.87
Sodium bicarbonate	2.95	0.34	2.07	3.38
Sodium chloride	2.81	4.59	3.41	2.52
DL-Methionine (990 g/kg)	3.08	2.67	2.94	3.15
L-Lysine HCl (780 g/kg)	2.42	0.84	1.89	2.68
L-Threonine (985 g/kg)	1.04	0.37	0.82	1.16
Choline chloride (600 g/kg)	0.68	0.46	0.61	0.72
Premix[b]	2.30	2.30	2.30	2.30
L-Valine (965 g/kg)	-	-	0.55	0.96
L-Isoleucine (935 g/kg)	-	-	-	0.42
Nutrient composition, g/kg or as described[c]				
Apparent metabolizable energy, kcal/kg	12.77	12.77	12.77	12.77
Calcium	8.4	8.4	8.4	8.4
Available phosphorus	4.2	4.2	4.2	4.2
Sodium	2.1	2.1	2.1	2.1
DEB, mEq/kg[d]	220	220	220	220
Crude protein	211.0 (209.2)	229.0 (229.3)	218.1 (217.7)	210.2 (208.9)
Digestible lysine	12.2	12.2	12.2	12.2
Digestible total sulfur amino acids	8.8	8.8	8.8	8.8
Digestible threonine	7.9	7.9	7.9	7.9
Digestible valine	8.6	9.4	9.4	9.4
Digestible isoleucine	7.9	8.7	8.2	8.2
Total lysine	13.2 (12.8)	13.2 (12.9)	13.2 (13.2)	13.1 (12.9)
Total sulfur amino acids	9.5 (9.4)	9.6 (9.0)	9.5 (9.1)	9.5 (9.5)
Total threonine	8.9 (9.4)	9.0 (9.5)	8.9 (9.6)	8.9 (9.2)
Total valine	9.7 (9.5)	10.5 (10.6)	10.5 (10.3)	10.4 (10.2)
Total isoleucine	8.7 (8.5)	9.6 (9.4)	9.0 (8.9)	9.0 (8.9)

[a] PRG 1 – CP restricted and minimum AA to Lys set only for TSAA and Thr; PRG 2 - CP not restricted while minimum ratios of AA to Lys were extended to Val (0.77) and Ile (0.67); PRG 3 - restrictions were as in PRG 2 with L-Val supplementation; PRG 4 - restrictions were the same as in PRG 3 but with L-Ile supplementation.

[b] Contributed per kilogram of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 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; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; sodium monensin 40%, 120 mg/kg (Elanco Animal Health, Greenfield, IN), avilamycin, 10 mg/Kg (Elanco Animal Health, Greenfield, IN); and phytase, 100 mg/kg (HiPhos GT 1000, DSM Nutritional Products Inc., Parsippany, NJ).

[c] Values in parentheses were determined by chemical analysis.

[d] Dietary electrolytic balance (Na + K – Cl), mEq/kg of the diet.

Table 3

Ingredient composition and chemical analysis of the experimental grower diets (g/kg) formulated using different limiting AA restrictions from 22 to 35 d of age[a]

Item	PGR 1	PGR 2	PGR 3	PGR 4
Ingredient				
Maize (78 g/kg)	612.65	558.90	591.82	618.25
Soybean meal (451 g/kg)	313.75	360.40	331.59	308.30
Soybean oil	43.45	54.24	47.39	41.67
Limestone	8.84	8.83	8.84	8.84
Dicalcium phosphate	6.88	6.50	6.73	6.92
Sodium bicarbonate	2.44	0.13	1.56	2.71
Sodium chloride	2.91	4.49	3.51	2.72
DL-Methionine (990 g/kg)	2.77	2.40	2.63	2.82
L-Lysine HCl (780 g/kg)	2.40	1.00	1.87	2.57
L-Threonine (985 g/kg)	0.96	0.36	0.73	1.03
Choline chloride (600 g/kg)	0.65	0.45	0.58	0.68
Premix[b]	2.30	2.30	2.30	2.30
L-Valine (965 g/kg)	-	-	0.45	0.82
L-Isoleucine (935 g/kg)	-	-	-	0.37
Nutrient composition, g/kg or as described[c]				
Apparent metabolizable energy, kcal/kg	13.19	13.19	13.19	13.19
Calcium	7.6	7.6	7.6	7.6
Available phosphorus	3.8	3.8	3.8	3.8
Sodium	2.0	2.0	2.0	2.0
DEB, mEq/kg[d]	200	200	200	200
Crude protein	198.0 (195.8)	213.0 (211.9)	204.1 (203.7)	197.0 (196.6)
Digestible lysine	11.3	11.3	11.3	11.3
Digestible total sulfur amino acids	8.1	8.1	8.1	8.1
Digestible threonine	7.4	7.4	7.4	7.4
Digestible valine	8.0	8.7	8.7	8.7
Digestible isoleucine	7.3	8.0	7.6	7.6
Total lysine	12.2 (12.0)	12.3 (12.4)	12.3 (12.0)	12.2 (11.8)
Total sulfur amino acids	8.9 (9.0)	8.9 (8.4)	8.9 (8.4)	8.8 (8.0)
Total threonine	8.3 (8.8)	8.4 (8.9)	8.3 (8.7)	8.3 (8.5)
Total valine	9.0 (8.9)	9.8 (10.3)	9.8 (10.2)	9.7 (9.6)
Total isoleucine	8.1 (7.8)	8.9 (9.2)	8.4 (8.8)	8.3 (8.2)

[a] PRG 1 – CP restricted and minimum AA to Lys set only for TSAA and Thr; PRG 2 - CP not restricted while minimum ratios of AA to Lys were extended to Val (0.77) and Ile (0.67); PRG 3 - restrictions were as in PRG 2 with L-Val supplementation; PRG 4 - restrictions were the same as in PRG 3 but with L-Ile supplementation.

[b] Contributed per kilogram of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 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; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; sodium monensin 40%, 120 mg/kg (Elanco Animal Health, Greenfield, IN), avilamycin, 10 mg/Kg (Elanco Animal Health, Greenfield, IN); and phytase, 100 mg/kg (HiPhos GT 1000, DSM Nutritional Products Inc., Parsippany, NJ).

[c] Values in parentheses were determined by chemical analysis.

[d] Dietary electrolytic balance (Na + K – Cl), mEq/kg of the diet.

Table 4

Ingredient composition and chemical analysis of the experimental finisher diets (g/kg) formulated using different limiting AA restrictions from 36 to 42 d of age[a]

Item	PGR 1	PGR 2	PGR 3	PGR 4
Ingredient				
Maize (78 g/kg)	654.91	597.76	624.35	647.72
Soybean meal (451 g/kg)	275.21	324.81	301.53	280.94
Soybean oil	42.69	54.16	48.63	43.57
Limestone	7.95	7.94	7.95	7.95
Dicalcium phosphate	4.63	4.23	4.42	4.58
Sodium bicarbonate	2.63	0.17	1.33	2.35
Sodium chloride	2.65	4.33	3.54	2.84
DL-Methionine (990 g/kg)	2.56	2.17	2.35	2.52
L-Lysine HCl (780 g/kg)	2.66	1.17	1.87	2.49
L-Threonine (985 g/kg)	0.99	0.35	0.65	0.92
Choline chloride (600 g/kg)	0.82	0.61	0.71	0.80
Premix[b]	2.30	2.30	2.30	2.30
L-Valine (965 g/kg)	-	-	0.37	0.69
L-Isoleucine (935 g/kg)	-	-	-	0.33
Nutrient composition, g/kg or as described[c]				
Apparent metabolizable energy, MJ/kg	13.40	13.40	13.40	13.40
Calcium	6.6	6.6	6.6	6.6
Available phosphorus	3.3	3.3	3.3	3.3
Sodium	1.9	1.9	1.9	1.9
DEB, mEq/kg[d]	185	185	185	185
Crude protein	184.0 (183.2)	200.0 (198.5)	192.7 (193.4)	186.5 (186.0)
Digestible lysine	10.6	10.6	10.6	10.6
Digestible total sulfur amino acids	7.6	7.6	7.6	7.6
Digestible threonine	6.9	6.9	6.9	6.9
Digestible valine	7.4	8.2	8.2	8.2
Digestible isoleucine	6.7	7.5	7.1	7.1
Total lysine	11.5 (11.4)	11.5 (11.9)	11.5 (11.4)	11.5 (11.3)
Total sulfur amino acids	8.3 (7.9)	8.4 (7.8)	8.3 (7.8)	8.3 (8.0)
Total threonine	7.8 (7.6)	7.9 (8.1)	7.8 (8.2)	7.8 (7.9)
Total valine	8.4 (8.1)	9.2 (9.0)	9.2 (9.2)	9.1 (8.6)
Total isoleucine	7.4 (6.9)	8.2 (8.1)	7.8 (7.8)	7.8 (7.4)

[a] PRG 1 – CP restricted and minimum AA to Lys set only for TSAA and Thr; PRG 2 - CP not restricted while minimum ratios of AA to Lys were extended to Val (0.77) and Ile (0.67); PRG 3 - restrictions were as in PRG 2 with L-Val supplementation; PRG 4 - restrictions were the same as in PRG 3 but with L-Ile supplementation.

[b] Contributed per kilogram of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 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; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; sodium monensin 40%, 120 mg/kg (Elanco Animal Health, Greenfield, IN), avilamycin, 10 mg/Kg (Elanco Animal Health, Greenfield, IN); and phytase, 100 mg/kg (HiPhos GT 1000, DSM Nutritional Products Inc., Parsippany, NJ).

[c] Values in parentheses were determined by chemical analysis.

[d] Dietary electrolytic balance (Na + K – Cl), mEq/kg of the diet.

Table 5
Growth performance of broilers from 1 to 21 d of age

Treatment[a]	Body weight gain (g)	Feed conversion ratio[b]	Feed intake (g)
PRG 1	890^{b}	1.390^{c}	$1{,}237^{ab}$
PRG 2	910^{a}	1.346^{a}	$1{,}225^{b}$
PRG 3	904^{a}	1.369^{b}	$1{,}238^{ab}$
PRG 4	913^{a}	1.378^{bc}	$1{,}258^{a}$
SEM (n = 48)	6.0	0.0074	14.5
P-value	<0.001	<0.001	<0.001

Means not sharing a common superscript within a column differ (P<0.05).

[a] PRG 1 - CP restricted to 224, 211 g/kg, respectively from 1 to 7 d and 8 to 21 d of age, respectively with minimum AA to Lys set only for TSAA (0.72) and Thr (0.65); PRG 2 - CP not restricted while minimum ratios of AA to Lys were used also for Val (0.77) and Ile (0.67); PRG 3 - restrictions were as in PRG 2 but L-Val supplementation was allowed; PRG 4 - restrictions were the in PRG 3 but L-Ile supplementation was allowed.

[b] Values represent the feed conversion ratio adjusted for the weight of dead birds.

Table 6

Growth performance of broilers fed diets formulated using different limiting AA restrictions from 1 to 42 days of age[a]

	22 to 42 d			1 to 42 d		
Treatment[b]	Body weight gain (g)	Feed conversion ratio	Feed intake (g)	Body weight gain (g)	Feed conversion ratio	Feed intake (g)
1 to 21 d						
PRG 1	2,233	1.716	3,831	3,123^{b}	1.612	5,036
PRG 2	2,217	1.729	3,833	3,126^{b}	1.601	5,009
PRG 3	2,217	1.718	3,810	3,122^{b}	1.605	5,011
PRG 4	2,248	1.708	3,841	3,161^{a}	1.606	5,078
22 to 42 d						
PRG 1	2,213^{b}	1.729^{b}	3,827^{ab}	3,115^{b}	1.614^{b}	5,031^{ab}
PRG 2	2,232^{ab}	1.705^{a}	3,807^{b}	3,139^{ab}	1.596^{a}	5,011^{b}
PRG 3	2,223^{ab}	1.715^{ab}	3,813^{b}	3,125^{ab}	1.604^{ab}	5,016^{b}
PRG 4	2,247^{a}	1.721^{ab}	3,869^{a}	3,152^{a}	1.610^{b}	5,076^{a}
SEM (n = 24)	14.4	0.0145	4.41	19.1	0.0111	57.4
Main effect (P-value)						
Period 1 to 21 d	0.132	0.118	0.658	0.043	0.341	0.058
Period 22 to 42 d	0.021	0.018	0.005	0.013	0.005	0.015
Interaction	0.871	0.058	0.146	0.474	0.247	0.346

Means not sharing a common superscript within a column for each period differ ($P<0.05$).

[a] Means of 12 replicates of 25 birds per pen.

[b] PRG 1 - CP restricted to 224, 211, 198 and 184 g/kg, respectively in the pre starter, starter, grower, and finisher phases, respectively, with minimum AA to Lys set only for TSAA (0.72) and Thr (0.65); PRG 2 - CP not restricted while minimum ratios of AA to Lys were used also for Val (0.77) and Ile (0.67); PRG 3 - restrictions were as in PRG 2 but L-Val supplementation was allowed; PRG 4 - restrictions were the same as in PRG 3 but L-Ile supplementation was allowed.

Table 7

Carcass, abdominal fat and yields of the commercial cuts (g/kg) of broilers fed diets formulated using different limiting AA restrictions from 1 to 42 days of age[a]

Treatment[b]	Carcass[c]	Abdominal fat	Breast fillet[d]	Tender	Thigh	Drumstick	Wing	Cage
1 to 21 d								
PRG 1	798	17.7	261	55.0	178	121	95.9	227
PRG 2	798	17.2	261	55.0	178	121	96.1	226
PRG 3	798	17.0	263	55.0	178	121	96.5	227
PRG 4	799	17.6	262	55.3	178	121	95.6	226
22 to 42 d								
PRG 1	800	17.7^{a}	262	55.1	177	120	95.6	226
PRG 2	800	16.6^{b}	260	55.4	179	122	96.1	227
PRG 3	799	17.6^{a}	262	55.5	177	121	96.4	226
PRG 4	797	17.7^{a}	262	54.9	178	121	95.9	226
SEM (n = 24)	2.1	0.45	2.1	0.53	0.9	1.1	0.7	1.2
Main effect (P-value)								
Period 1 to 21 d	0.954	0.456	0.925	0.784	0.973	0.999	0.637	0.753
Period 22 to 42 d	0.076	0.041	0.713	0.599	0.127	0.103	0.738	0.935
Interaction	0.900	0.440	0.094	0.057	0.550	0.621	0.363	0.177

Means not sharing a common superscript within a column for each period differ ($P<0.05$).

[a] Means of 12 replicates of 6 birds sampled per pen.

[b] PRG 1 - CP restricted to 224, 211, 198 and 184 g/kg, respectively in the pre starter, starter, grower, and finisher phases, respectively, with minimum AA to Lys set only for TSAA (0.72) and Thr (0.65); PRG 2 - CP not restricted while minimum ratios of AA to Lys were used also for Val (0.77) and Ile (0.67); PRG 3 - restrictions were as in PRG 2 but L-Val supplementation was allowed; PRG 4 - restrictions were the same as in PRG 3 but L-Ile supplementation was allowed.

[c] Eviscerated carcass as a percentage of body weight, whereas cuts are proportions of the carcass.

[d] Skinless boneless *Pectoralys major*.

CAPÍTULO IV

CONSIDERAÇÕES FINAIS

A suplementação com L-Val e L-Ile permitiu obter resultados similares em desempenho e rendimento de carcaça às dietas com maior conteúdo de PB e suplementada com os AA sintéticoss usuais (DL-Met, L-Lis e L-Tre). Portanto, a suplementação de L-Val e L-Ile em dietas para frangos de corte, se torna uma prática viável para redução de PB visando, principalmente, a redução dos custos de formulação devido a redução da inclusão do farelo de soja e sua alta variabilidade do preço no mercado mundial nos últimos anos, principalmente devido a concorrência com a indústria de biocombustível.

O aumento em 5% da Lis digestível melhorou a conversão alimentar acumulada dos frangos de corte aos 43 dias, com isso, a formulação de rações utilizando um valor de Lis digestível superior ao já utilizado pela indústria avícola, dependerá, portanto, da avaliação da viabilidade econômica em um ambiente que demonstre seus benefícios.

A utilização de diferentes programas alimentares, independente do valor de PB, durante o período de 1 a 21 dias não afeta o desempenho zootécnico e o rendimento de carcaça de frangos de corte, desde que sejam utilizados programas alimentares com alto valor de PB ou com suplementação sintética de Val e Ile na fase subsequente.

Portanto, reduzir a PB através da suplementação com os cinco primeiros AA limitantes na forma sintética, mantém a produção de carne avícola competitiva e minimizar o impacto do aumento do custo das fontes de proteicas, sendo os lucros resultantes da melhoria do desempenho a campo e do rendimento de carne ao, se utilizar dietas com alta densidade de AA, altamente dependentes do custo da ração e do preço de mercado da carne de frango.

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VITA

Daniel José Antoniol Miranda, primogênito de José Geraldo Miranda e Martha Maria Antoniol Miranda (*in memoriam*), nasceu em Visconde do Rio Branco, MG, em 13 de setembro de 1984. Cursou o ensino fundamental na Escola Estadual Galdino Leocádio, no distrito de Vilas Boas, Guiricema, MG. Concluiu o ensino médio na Escola Estadual Prefeito Antônio Arruda em Guiricema, MG. Em 2003 ingressou no Curso de Zootecnia da Universidade Federal Rural do Rio de Janeiro, RJ, obtendo o Grau de Zootecnista em outubro de 2008. Iniciou, em março de 2009, o curso de mestrado na área de Nutrição Animal, no Programa de Pós-Graduação em Zootecnia da Universidade Federal de Minas Gerais, Belo Horizonte, MG, sob a orientação do professor Leonardo José Camargos Lara desenvolvendo trabalho de dissertação sobre o efeito da granulometria do milho e o valor de energia metabolizável em rações peletizadas para frangos de corte. Obteve o título de mestre em Zootecnia em fevereiro de 2011. No ano de 2011, ingressou no curso de Doutorado em Produção Animal pelo Programa de Pós-Graduação em Zootecnia pela Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, desenvolvendo o trabalho de tese sobre a suplementação de aminoácidos de cadeia ramificada em dietas com redução proteica para frangos de corte. Realizou pesquisas durante 12 meses no Departamento de Animal Science na Universidade de Purdue, EUA, através do programa doutorado-sanduíche da CAPES, no período de outubro de 2013 a outubro de 2014, trabalhando com perdas endógenas de aminoácidos para suínos em crescimento e frangos de corte. Foi submetido à banca de defesa de Tese em março de 2015 pela Universidade Federal do Rio Grande do Sul em Porto Alegre, RS.