UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

RESPOSTAS DE FRANGOS DE CORTE ÀS INGESTÕES DE METIONINA+CISTINA E TREONINA: DESEMPENHO, IMPACTO AMBIENTAL E ECONÔMICO

Daniella Carolina Zanardo Donato
Zootecnista

2013

UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

RESPOSTAS DE FRANGOS DE CORTE ÀS INGESTÕES DE METIONINA+CISTINA E TREONINA: DESEMPENHO, IMPACTO AMBIENTAL E ECONÔMICO

Daniella Carolina Zanardo Donato

Orientador: Profa. Dra. Nilva Kazue Sakomura

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Zootecnia

2013

Donato, Daniella Carolina Zanardo
D677r Respostas de frangos de corte às ingestões de metionina+cistina e treonina: desempenho, impacto ambiental e econômico / Daniella Carolina Zanardo Donato. – – Jaboticabal, 2013
xx, 88 f. ; 29 cm

Tese (doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2013
Orientadora: Nilva Kazue Sakomura
Banca examinadora: Paulo Borges Rodrigues, Nei André Arruda Barbosa, Izabelle Auxiliadora Molina de Almeida Teixeira, Antonio Sergio Ferraudo
Bibliografia

1. Aminoácidos. 2. Nível ótimo econômico. 3. Poluição ambiental. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 636.5:636.085

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação – Serviço Técnico de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.

unesp UNIVERSIDADE ESTADUAL PAULISTA
CAMPUS DE JABOTICABAL
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS DE JABOTICABAL

CERTIFICADO DE APROVAÇÃO

TÍTULO: RESPOSTAS DE FRANGOS DE CORTE ÀS INGESTÕES DE METIONINA+CISTINA E TREONINA: DESEMPENHO, IMPACTO AMBIENTAL E ECONÔMICO

AUTORA: DANIELLA CAROLINA ZANARDO DONATO
ORIENTADORA: Profa. Dra. NILVA KAZUE SAKOMURA

Aprovada como parte das exigências para obtenção do Título de DOUTOR EM ZOOTECNIA , pela Comissão Examinadora:

Profa. Dra. NILVA KAZUE SAKOMURA
Departamento de Zootecnia / Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal

Izabelle Molina
Profa. Dra. IZABELLE AUXILIADORA M. DE ALMEIDA TEIXEIRA
Departamento de Zootecnia / Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal

Prof. Dr. ANTONIO SERGIO FERRAUDO
Departamento de Ciências Exatas / Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal

Prof. Dr. NEI ANDRÉ ARRUDA BARBOSA
Evonik Degussa Brasil Ltda / São Paulo/SP

Prof. Dr. PAULO BORGES RODRIGUES
Universidade Federal de Lavras / Lavras/MG

Data da realização: 16 de dezembro de 2013.

DADOS CURRICULARES DO AUTOR

DANIELLA CAROLINA ZANARDO DONATO – filha de Mauro Donato e Maria Antonieta Zanardo Donato, nasceu no dia 23 de dezembro de 1982, na cidade de São Paulo, São Paulo. Em março de 2001 ingressou no curso de Zootecnia na Universidade Federal de Lavras, Minas Gerais. No período de abril a julho de 2004 foi monitora remunerada na disciplina de Alimentos e Alimentação, e bolsista de iniciação científica pelo CNPq de agosto de 2004 a junho de 2006, sob orientação do Prof. Dr. Paulo Borges Rodrigues. Em junho de 2006 concluiu o curso de Zootecnia, sendo distinguida em primeiro lugar no prêmio “Mérito Acadêmico”. Em março de 2007 ingressou no curso de Pós-Graduação Lato-Sensu “Especialização em Manejo de Animais Silvestres” promovido pela Faculdade de Ciências Médicas e da Saúde da Pontifícia Universidade Católica de São Paulo, tendo concluído o curso em outubro de 2007. Em julho de 2007 iniciou o curso de Mestrado em Nutrição e Produção Animal pela Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, sob orientação do Prof. Dr. Ricardo de Albuquerque, onde obteve bolsa da CAPES pelo período de março de 2008 a julho de 2009 e defendeu sua dissertação para obtenção do título de Mestre em Ciências em dezembro de 2009. Em março de 2010 ingressou no curso de Doutorado em Zootecnia pela Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista – Campus de Jaboticabal, São Paulo, sob orientação da Profª. Drª. Nilva Kazue Sakomura, onde obteve bolsa FAPESP pelo período de agosto de 2010 a março de 2013. Neste mesmo mês foi aprovada em primeiro lugar no concurso público para o cargo de Assistente de Suporte Acadêmico IV na mesma instituição, iniciando suas funções em abril. E em dezembro deste ano de 2013 defendeu esta tese para obtenção do título de Doutora em Zootecnia.

“Compreender que há outros pontos de vista é o início da sabedoria.”

(Thomas Campbell)

“Há um tempo em que é preciso abandonar as roupas usadas, que já tem a forma do nosso corpo, e esquecer os nossos caminhos, que nos levam sempre aos mesmos lugares. É o tempo da travessia e, se não ousarmos fazê-la, teremos ficado, para sempre, à margem de nós mesmos”

(Fernando Pessoa)

“Todas as vitórias ocultam uma abdicação”
(Simone de Beauvoir)

Aos meus pais, Mauro e Antonieta, aos meus avós Bruno e Marlene (*in memoriam*), José (*in memoriam*) e Jandyra, aos meus irmãos Bruno e Amanda e à minha pequena sobrinha, Alice, que são meu porto seguro e que sempre me encorajaram a batalhar pelos meus ideais e seguir em frente.

À Profa. Nilva, que me deu a oportunidade de realizar esta tese, e de continuar nesta excelente instituição, pela amizade, confiança e ensinamentos.

À todas as pessoas do Laboratório de Ciências Avícolas, por juntos formarmos uma ótima equipe de trabalho.

DEDICO

AGRADECIMENTOS

À UNESP – Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, pela possibilidade de realizar o curso de Doutorado em Zootecnia e pela possibilidade de continuar fazendo parte desta instituição, agora como funcionária.

À FAPESP – Fundação de Apoio à Pesquisa do Estado de São Paulo, pela bolsa de estudos de Doutorado e de estágio sanduíche concedidas e pelo financiamento à pesquisa.

À Profa. Dra. Nilva Kazue Sakomura pela orientação, aprendizado, amizade, e acima de tudo pela confiança e oportunidade de continuar a fazer parte desta equipe que só me faz crescer a cada dia mais.

Aos membros das bancas de qualificação (Prof. Alex, Prof. Euclides, Prof. Luciano e Dr. Edney) e de defesa (Dr. Nei, Prof. Paulo, Profa. Izabelle e Prof. Ferraudo), pela disponibilidade, colaborações, críticas sempre construtivas e ensinamentos.

Às empresas Ajinomoto, Evonik, Nutron, Poli-Nutri e Usina Santa Adélia pelas doações que tornaram possível a execução desta pesquisa.

Ao Dr. Roberto Gous, da University of KwaZulu-Natal, África do Sul, pela inestimada ajuda e todo suporte para a execução de nossos trabalhos.

Ao Dr. Philippe Lescoat, do INRA, França, que me aceitou prontamente para realizar o doutorado sanduíche em seu laboratório, mesmo que eu não tenha ido.

Ao Dr. Betrand Meda, do INRA, França, por todo seus ensinamentos, paciência e auxílio no desenvolvimento do capítulo de nitrogênio.

Aos professores Elisabeth Gonzales, Marcos Macari, Normand St. Pierre, Elisabeth Urbinati, Matheus Paranhos, Euclides Malheiros, Nilva Sakomura, Celso Pezatto e Nelson Peruzzi, pelas excelentes disciplinas ministradas e por todo o conhecimento a mim transferido.

Aos funcionários do Laboratório de Ciências Avícolas, Robson, Vicente e Izildo, por todo o auxílio na condução dos experimentos, pela amizade e atenção, e por me aceitarem tão bem como colega de trabalho.

À todos os funcionários da FCAV, em especial aos funcionários da Fábrica de Ração, da Fazenda, do Departamento de Zootecnia e da Pós-Graduação, muito obrigada por fazerem essa tese ser possível.

Aos amigos do Laboratório de Ciências Avícolas, Allan, Ana Carolina, Ana Gabriela, Ana Paula, Anchieta, Arthur, Bruna, Bruno, Camila Bueno, Camila Angélica, Cléber, Cristiane, Danilo Constant, Danilo Makino, Deise, Fernando, Flávia, Gabriel, Gabriela, Giuliana, Hanay, Hilda, Inêz, Iris, Jaqueline (Bituka), Jaqueline (Dako), Jefferson, Joyce, Juliano, Katiani, Larissa, Letícia, Marco, Melina, Michele, Miryelle, Nayara, Rafael, Rafaela, Reginaldo, Roberto e Rodrigo, muito obrigada pela amizade, colaboração, compreensão e ajuda, e em especial ao Edney, que nunca mediu esforços para me ajudar.

Aos amigos da FCAV, Leonardo (Viçosa), por ter me recebido quando eu vim prestar a prova de doutorado, Mariana (Menas), por ter me recebido sempre tão bem, e por ter me disponibilizado sua casa quando eu precisava vir prá Jaboticabal e ainda não tinha onde ficar, pela amizade, carinho e conversas sem fim; Ana, minha amiga que acabou se tornando (agora sim!) minha veterinária preferida, que sempre me salva com meus filhinhos de quatro patas, Marco (Chileno), por ter aceitado dividir o Fernando comigo; às “meninas super poderosas” Carol, Katy, Miryelle, Joyce e Paula, pelas risadas, conversas, por sempre estarem dispostas a fazer qualquer coisa e comer gordices, e à todos os demais amigos, obrigada por tudo!

Aos amigos que contribuíram de outras formas para a realização dessa tese, Claudinha, minha amiga-irmã que eu tanto amo, Daniel e Bruna (Picky), desde Lavras, sempre presentes na minha vida; Juliane, Julianne, Juliana, Larissa, Paulinha e Henry, Esther e Bruno (Papi), Elmeson (Mineiro), Babi, Bruno (Xacrete), João Paulo (Johnny, o insuportável!!), Erika, Jefferson e Julinha, e toda a galera de Piras, vocês são demais e fazem muita falta na minha vida!!!

Ao meu namorado, Fernando (Gracinha), por ser sempre tão gracinha, companheiro, querido, paciente e por me aguentar em todas as horas, principalmente nas mais difíceis, sempre me apoiando e me ajudando. Ainda bem que eu tenho você aqui comigo!

À Dirce, pela atenção, cuidado e carinho de mãe e por ser uma fonte inesgotável de temperos maravilhosos.

À toda a minha família, por todo o amor, compreensão, apoio e por me dar forças para continuar seguindo meus ideais, não importa a distância, em especial ao meu avô Bruno, que sempre acreditou em mim e no meu potencial, e à minha mãe, minha “ídola” maior, que sempre me incentivou a seguir na carreira acadêmica e me provou que ser professora é uma profissão gratificante e maravilhosa.

À Júlia (*in memoriam*), minha eterna filhinha, meu amor maior, minha querida companheira, que esteve sempre comigo aonde quer que eu fosse, com a maior alegria do mundo e sempre me dando amor incondicional. Nós fizemos tudo o que foi possível, mas sua hora chegou, cedo demais, mas chegou... **As divas são assim, como tardes de verão... Elas passam e sempre nos deixam as melhores lembranças!* * Vou te amar para sempre!

À Vitória, Luana e Asdrúbal, meus novos companheiros, por transformarem minha casa em um lar, cheio de alegria e rabinhos abanando! Ao Chico e Francis, por darem amor incondicional à minha mãe. Amo todos vocês, meus anjinhos de quatro patas!

À todos os frangos que deram suas vidas por esse trabalho (e aos que não deram também!).

E à todos aqueles que de alguma forma contribuíram para a realização dessa tese.

MUITO OBRIGADA!

SUMÁRIO

RESPOSTAS DE FRANGOS DE CORTE ÀS INGESTÕES DE METIONINA+CISTINA E TREONINA: DESEMPENHO, IMPACTO AMBIENTAL E ECONÔMICO

RESUMO – Este trabalho foi realizado com o objetivo de avaliar as respostas de frangos de corte às ingestões de metionina + cistina (MC) e treonina (THR). No primeiro estudo foram avaliadas as respostas de frangos de corte às ingestões de MC e THR. Apesar das diferenças nas repostas, foi determinada uma única eficiência de utilização dos aminoácidos para ambos os sexos, sendo 0,56 para MC e 0,73 para THR. O consumo estimado para ótima conversão alimentar foi 231 e 524 mg MC/d nas fases inicial e de crescimento, respectivamente, para ambos os sexos, e 923 e 1053 mg MC/d para fêmeas e machos, respectivamente, para a fase de terminação. Para THR, o consumo estimado foi de 184, 612 e 823 mg/d para as fases inicial, de crescimento e terminação, respectivamente, para ambos os sexos. No segundo estudo foi avaliado o impacto de ambos aminoácidos na eficiência de utilização e excreção de nitrogênio. Da mesma maneira que no primeiro estudo, foi determinada uma única eficiência de utilização dos aminoácidos para deposição de proteína para ambos os sexos, sendo 0,60 para MC e 0,59 para THR. Com relação à excreção de nitrogênio, ela aumenta exponencialmente com o aumento do consumo dos aminoácidos, sendo que a redução de uma unidade no consumo de MC ou THR acarretará na redução de 0,5% do nitrogênio excretado. Um terceiro estudo foi realizado para determinar as exigências de MC e THR para mantença e crescimento de frangos de corte e estimar o consumo ótimo econômico para máximo retorno em diferentes cenários. As exigências de MC e THR para crescimento foram 6 e 7 mg MC/g d de ganho, para as fases inicial e de crescimento, respectivamente, para ambos os sexos, e 8 e 9 mg MC/g d de ganho, para fêmeas e machos, respectivamente na fase de terminação; e 9 mg THR/g d de ganho, para ambos os sexos na fase inicial, e 8 e 9 mg THR/g d de ganho, para fêmeas e machos, respectivamente, nas fases de crescimento e terminação. Para mantença, as exigências determinadas foram 22 e 20 mg MC/kg d, para ambos os sexos, nas fases inicial e de crescimento e de terminação, respectivamente, e 26 e 22 mg THR/kg d para ambos os sexos, nas fases inicial e de crescimento, respectivamente, e 22 e 21 mg THR/kg d para fêmeas e machos, respectivamente, na fase de terminação. Utilizando estes coeficientes, foi possível determinar os consumos ótimos econômico para frangos de corte em lotes com diferentes uniformidades.

Palavras-chave: aminoácidos, avicultura, composição corporal, nível ótimo econômico, poluição ambiental

RESPONSES OF BROILER CHICKENS TO METHIONINE+CYSTEINE AND THREONINE INTAKE: PERFORMANCE, ENVIRONMENTAL AND ECONOMICAL IMPACT

ABSTRACT – The aim of this study was to evaluate the responses of broiler chickens to methionine + cysteine (MC) and threonine (THR) intake. In the first study were evaluated the responses of broiler chickens to MC and THR intake. The efficiency of utilisation of the studied amino acids was calculated through linear regression between amino acid deposition and intake, to each phase and each sex. Although the difference in responses, it was determined only one efficiency for both sexes, being 0.56 to MC and 0.73 to THR. The intake estimated for optimal feed conversion was 231 and 524 mg MC/d to initial and grower phases, respectively, for both sexes, and 923 and 1053 mg MC/d to females and males, respectively, in finisher phase. For THR, the estimated intake was 184, 612 and 823 mg/d for initial, grower and finisher phases, respectively, to both sexes. In the second study were evaluated the impact of both amino acids on efficiency of utilisation and nitrogen excretion. In the same way as in the first study, it was determined only one efficiency for both sexes, being 0.60 to MC and 0.59 to THR. Regarding the nitrogen excretion, it increases exponentially with the increase of amino acid intake, being that reducing one unit of MC or THR intake leads to a reduction of 0.5% of nitrogen excretion. A third study was conducted to determine MC and THR requirements for maintenance and growth of broilers, and to estimate the optimum economic intake to maximum profit under different scenarious. MC and THR requirements for growth were estimated as 6 and 7 mg MC/g d of gain, to initial and grower phases, respectively, for both sexes and 8 and 9 mg MC/g d of gain, to females and males, respectively, to finisher phase; and 9 mg THR/g d of gain, for both sexes in initial phase, and 8 and 9 mg THR/g d of gain, to females and males, respectively, in grower and finisher phases. The maintenance requirements were estimated as 22 and 20 mg MC/kg d, to both sexes, in initial and grower and finisher phases, respectively, and 26 and 22 mg THR/kg d to both sexes, in initial and grower phases, respectively, and 22 and 21 mg THR/kg d to females and males, respectively, in the finisher phase. Using these coefficients, it was possible to determine the optimum economic intakes to broilers in flocks with different uniformities.

Key-words: amino acids, poultry, body composition, optimum economic level, environmental pollution

LISTA DE TABELAS

Página

LISTA DE FIGURAS

CAPÍTULO 1 - CONSIDERAÇÕES GERAIS

Introdução

A proteína é considerada um dos mais importantes nutrientes na alimentação de frangos de corte, uma vez que a sua produtividade depende de uma conversão eficiente da proteína da dieta em proteína muscular (COSTA et al., 2001). Durante muitos anos, as rações para aves foram formuladas para satisfazer as necessidades de proteína bruta dos animais. Com o crescente desenvolvimento da indústria de aminoácidos idustriais, os nutricionistas passaram a formular as rações com o objetivo de satisfazer as necessidades específicas de aminoácidos essenciais. Hoje, a suplementação de aminoácidos industriais nas rações é uma prática incorporada na rotina das fábricas de rações para aves. Como consequência, os níveis de proteína bruta das dietas tiveram uma redução nas formulações em condições experimentais (ARAÚJO et al., 2004).

Dados de pesquisa (ALETOR et al., 2000; BREGENDAHL et al., 2002) confirmam que o excesso de proteína bruta consumido deprecia o desempenho das aves, eleva o custo de formulação da dieta, incrementa o calor metabólico e contribui para o aumento na excreção de nitrogênio. Alguns trabalhos (CHENG et al., 1997 a, b; CHENG et al., 1999) demonstram que o excesso de proteína na dieta aumenta as exigências das aves em aminoácidos limitantes.

Na atualidade, é recomendável formular dietas que atendam as exigências específicas para os aminoácidos essenciais, além de manter uma relação entre os aminoácidos, para evitar a perda energética da dieta e a excreção excessiva de nutrientes, em consequência do desbalanço entre os aminoácidos. Pesquisas têm sido realizadas com o objetivo de minimizar os desbalanceamentos entre os nutrientes, principalmente os aminoácidos, os quais consomem energia no processo de eliminação (ATENCIO et al., 2004). A formulação com base em aminoácidos digestíveis também permite um melhor atendimento das exigências dos animais, uma vez que não é necessário ter margens de segurança, como quando se utiliza aminoácidos totais, o que resulta em melhor desempenho das aves e redução da excreção de nitrogênio (LEMME et al., 2004).

Hurwitz et al. (1998) relataram que as exigências em aminoácidos podem ser determinadas para mantença, ganho de peso e empenamento. Pack (1995) afirmou que existem inúmeros fatores que afetam as necessidades dietéticas de aminoácidos dos frangos de corte em crescimento, tais como: energia metabolizável, proteína bruta, idade da ave, sexo e genética; sendo, dessa forma, muito difícil direcionar todas as possíveis combinações em toda a faixa de aminoácidos essenciais individualmente, porém estão se desenvolvendo proporções ideais de aminoácidos essenciais em relação à lisina, como base para calcular as especificações da dieta.

Os métodos de pesquisa para determinar as exigências ou o perfil ideal de aminoácidos compreendem a revisão de literatura, o método dose-resposta e o fatorial (SAKOMURA & ROSTAGNO, 2007). Entretanto, a grande maioria dos estudos para definir exigências dos aminoácidos tem se baseado no método dose-resposta. Este método determina os níveis nutricionais ótimos com base na resposta do desempenho animal em função dos níveis crescentes dos nutrientes na dieta. Ele também é um método bastante utilizado para fornecer componentes para os modelos fatoriais (D'MELLO, 2003), como a eficiência de utilização dos aminoácidos.

O método fatorial baseia-se no princípio que as aves necessitam dos nutrientes para a manutenção dos processos vitais e atividades, crescimento e/ou produção, levando em consideração as diferenças de peso, composição corporal, potencial de crescimento e produção das aves. Segundo Hurwitz et al. (1978), este é o método mais adequado para estabelecer as exigências nutricionais para aves, sendo a base para a elaboração de modelos matemáticos que têm como objetivo predizer as exigências das aves mantidas em diferentes condições (SAKOMURA & ROSTAGNO, 2007).

O conhecimento de como as aves respondem ao consumo de determinados aminoácidos é de fundamental importância para se determinar o consumo ótimo econômico dos mesmos, bem como permitir uma redução na excreção de nitrogênio para o ambiente, além de possibilitar a determinação das exigências destes aminoácidos com maior acurácia e precisão.

Revisão de Literatura

Metionina + Cistina

Os aminoácidos sulfurosos, metionina + cistina, são os primeiros aminoácidos limitantes em rações para aves, sendo os principais componentes da síntese proteica. Sua função primária é doar grupos metil para reações de transmetilação, especialmente na biossíntese de lipídeos e outros compostos e está envolvida no transporte de lipídeos no sangue (PATTERSON & KUNG, 1988). A metionina também serve como doadora de enxofre (PESTI et al., 1981), e é o primeiro aminoácido a ser incorporado na estrutura peptídica durante a síntese proteica, sendo que a sua deficiência pode inibir este estágio inicial (KINO & OKUMURA, 1987).

Uma dieta deficiente nesses aminoácidos reduz o ganho em peso, a eficiência alimentar, e o teor de proteína na carcaça, além de estimular o consumo de ração, contribuindo com energia adicional e, consequentemente, ocasionando acréscimo na deposição de gordura corporal (SUMMERS et al., 1992; MORAN, 1994).

Treonina

A treonina é um aminoácido nutricionalmente essencial para as aves por não poder ser sintetizada pelas mesmas. É considerada o terceiro aminoácido mais limitante, especialmente em dietas com baixa proteína bruta (KIDD & KERR, 1996; SCHUTTE, 1998), precedido dos aminoácidos sulfurosos e da lisina (LÓPEZ et al., 2001).

A treonina é encontrada em altas concentrações no coração, nos músculos, no esqueleto e sistema nervoso central. É usada em importantes processos metabólicos, tais como síntese de proteína e manutenção do “turnover” proteico corporal, formação de ácido úrico, além de auxiliar na formação do colágeno e elastina e atuar na produção de anticorpos. Ela também tem sido bem reconhecida por seu papel na mantença associada ao trato digestivo, onde tem papel importante na síntese de mucinas (SPECIAN & OLIVER, 1991; STOLL et al., 1998; VAN DER SCHOOR et al., 2002), e seu efeito com relação à maximização da produtividade

(KIDD & KERR, 1997; PENZ et al., 1997; KIDD et al., 1999; DOZIER et al., 2000; KIDD et al., 2003a; KIDD et al., 2003b).

Métodos para determinar exigências de aminoácidos das aves

O termo exigência é definido como concentração fixa de um dado nutriente na ração e, de acordo com Pack (1995), este termo muitas vezes é usado indevidamente, em parte porque sua definição não é interpretada corretamente. O autor menciona que não há exigência de um nutriente para um dado lote de animais. A questão é entender como o animal ou grupo de animais respondem ao aumento dos níveis de um nutriente e, para a decisão do nível ótimo do mesmo, deve ser considerada a avaliação econômica. Apesar de parecer óbvio, muitos pesquisadores não levam em consideração essa linha de pensamento.

Em uma população de aves, uma vez que, ao definir uma concentração fixa de um nutriente visando atender as necessidades de um lote, existe a possibilidade de super-fornecimento para aqueles indivíduos com menor potencial de resposta e sub-fornecimento de nutrientes para aqueles indivíduos com maior potencial de resposta. Com base nisso, Sakomura e Rostagno (2007) relatam que a questão mais importante é entender como uma população ou grupo de animais responde ao aumento dos níveis dos nutrientes da dieta, fornecendo-os em quantidades compatíveis com o objetivo da produção, em um dado contexto de ambiente.

Os primeiros modelos de predição das exigências de aminoácidos para aves foram desenvolvidos por Hurwitz e Bornstein (1973), para aves de postura. As exigências totais de aminoácidos foram obtidas a partir da soma das necessidades para mantença, crescimento e produção de ovos, sendo os coeficientes que expressam cada uma destas frações determinados de maneira independente. Os autores concluíram que os modelos proporcionaram estimativas menores que os valores recomendados na literatura, e atribuíram este resultado ao fato dos modelos considerarem a ineficiência de conversão dos aminoácidos dietéticos em proteínas do ovo.

Os métodos utilizados para estudar as respostas das aves aos aminoácidos essenciais baseiam-se em duas abordagens principais: dose-resposta e fatorial

(D'MELLO, 2003). O modelo dose-resposta determina as exigências com base na resposta de desempenho dos animais alimentados com dietas contendo níveis crescentes do nutriente estudado, e o fatorial se baseia no princípio de que o animal necessita de nutrientes para a manutenção dos processos vitais e atividades, crescimento e/ou produção (SAKOMURA & ROSTAGNO, 2007). Segundo Hurwitz et al. (1978), este é o método mais adequado para estabelecer as exigências nutricionais para aves, sendo a base para a elaboração de modelos matemáticos que têm como objetivo predizer as exigências das aves mantidas em diferentes condições (SAKOMURA & ROSTAGNO, 2007).

O método dose-resposta é baseado na resposta do animal ao aumento na ingestão de um determinado nutriente. Segundo Euclydes & Rostagno (2001), a adição de um nutriente limitante na ração, mantendo níveis adequados dos demais nutrientes, promove crescimento do animal até que sua exigência seja atendida. A partir daí, existirá uma faixa de estabilização no crescimento e, em seguida, dependendo do nutriente, poderá ocorrer uma perda de peso do animal.

A variação resultante do acréscimo de um nutriente na ração, partindo de níveis baixos até níveis elevados, pode ser descrito em quatro fases distintas: a) inicial ou mantença – o acréscimo do nutriente garante apenas a sobrevivência do animal, sendo os níveis insuficientes para permitir o crescimento; b) resposta – os animais começam a apresentar crescimento, melhor eficiência alimentar, até um nível no qual estabiliza a produção; c) estável - os níveis do nutriente não apresentam resposta à produção ou toxidez proveniente do excesso. Embora para o animal possa ser considerada uma fase ótima, do ponto de vista econômico esses níveis não são recomendáveis; d) tóxico **–** o nível elevado do nutriente pode causar redução na produção em consequência de efeitos, tais como interação, antagonismos, etc (SAKOMURA & ROSTAGNO, 2007).

O método dose-resposta, por ser prático e de fácil execução, tem sido a base para a elaboração de tabelas de exigências nutricionais como *Nutrient Requirements of Poultry* (NRC, 1994) e Tabelas Brasileiras para Aves e Suínos (ROSTAGNO et al. 2000; ROSTAGNO et al. 2005; ROSTAGNO et al., 2011). Entretanto, fatores como ambiente e genética afetam a determinação das exigências, dificultando o estabelecimento dos níveis nutricionais, sendo necessário repetir as pesquisas em diferentes condições para melhor definição das exigências. Este método é aplicado em ensaios de alimentação, nos quais são avaliados o desempenho do animal. Para a condução dos ensaios, são necessários conhecimentos relacionados ao delineamento experimental, dietas, variáveis avaliadas e análises dos dados (SAKOMURA, 2005; SAKOMURA & ROSTAGNO, 2007).

Segundo Santomá (1991), o método fatorial constitui a base para a estimativa das exigências por meio de modelos matemáticos que levam em consideração a ingestão, retenção e a eficiência de deposição do aminoácido, além do peso corporal e da produção.

Ele é baseado no princípio de que a exigência em energia ou nutrientes do animal é a quantidade a ser fornecida para sua manutenção, crescimento proteico, engorda e produção. O método fatorial também representa uma ferramenta para a compreensão do metabolismo energético e proteico dos animais, importante para os estudos de modelagem, os quais visam a definição de um sistema adequado para a produção.

O modelo de Reading, matematicamente descrito por Curnow (1973) tem uma forma sigmoidal característica, ou seja, a curva considera a resposta de um indivíduo como modelo fatorial simples e, em seguida, deriva a resposta integrada média da população a partir de um grande número de respostas individuais.

A curva de resposta resultante da população é definida por sete parâmetros: produção máxima media g/dia (Emax); variância na produção máxima (σ^2 Emax); peso corporal médio kg (W); variância de peso corporal (σ^2 W), correlação entre a produção e o peso corporal (rEW) e duas constantes que representam a quantidade de aminoácidos necessários por unidade de produção (a; mg/gE) e por unidade de peso corporal (b; mg/kgW).

A ingestão de aminoácido ótima para lucro máximo é calculado pela seguinte equação: A ótimo (mg/dia) = aEmax + bW + x $\sqrt{(a^2.\sigma^2 \text{ Emax} + b^2.\ \sigma^2 \text{ W} + 2\text{abrEW}\ \sigma\text{Emax}.\ \sigma\text{W})}$, onde X é desvio padrão da distribuição normal da média, que é excedido com uma probabilidade ak de uma extremidade; e K é custo marginal de um mg de aminoácido consumido pelo valor marginal de um g de carne produzido.

Para ambas metodologias é fundamental que se tenha uma ampla variação nas respostas obtidas, desde respostas na fase próxima à mantença até a fase estável, e que estas respostas sejam em função do aminoácido estudado. Uma das vantagens em utilizar a técnica da diluição de dietas para se obter os tratamentos é que ela fornece respostas acuradas das aves ao incremento do aminoácido, uma vez que, diluindo-se sequencialmente uma dieta concentrada em aminoácidos com uma dieta isenta dos mesmos, o balanço de aminoácidos se mantém constante entre os tratamentos, confirmando que as respostas são em função do aminoácido limitante que está sendo testado, e não de imbalanços ou antagonismo (GOUS, 1980), como pode acontecer quando se utiliza a técnica da suplementação, que consiste da adição de um único aminoácido a ser testado em uma dieta basal.

Excreção de nitrogênio

Com o crescimento da produção comercial de frangos de corte e a sua intensificação, tem aumentado também a produção de dejetos e, portanto a emissão de poluentes, em mesma escala. Atualmente há uma preocupação dos pesquisadores em buscar alternativas que visam minimizar a emissão destes poluentes para o ambiente.

Algumas regiões de vários Estados do Brasil têm problemas graves relacionados com a aglomeração da produção animal, sendo o solo e a água os alvos de maior preocupação (RODRIGUES et al., 2007). Com esse mesmo problema, a União Europeia já implementou leis que visam minimizar a poluição ambiental, especialmente no que diz respeito à emissão de nitrogênio.

O nitrogênio excretado, originário da proteína da dieta, é o principal responsável pela poluição ambiental nos grandes complexos avícolas (MORSE, 1995), sendo que essa excreção aumenta com a idade das aves, uma vez que a eficiência alimentar diminui (KUBENA et al., 1973). Por esta razão, a manipulação da dieta através da redução do teor de proteína e a suplementação com aminoácidos sintéticos, tem sido considerada um procedimento para controlar a quantidade de nitrogênio excretado pelas aves.

Nos galpões de frangos de corte a proteína não digerida é depositada na cama como resíduo e é quebrada pelas bactérias em nitrogênio amoniacal (amônia – NH_3 e íon amônio – NH_4^+). O impacto desses compostos nitrogenados gerados pelas produções animais sobre o ciclo do nitrogênio é considerado como uma das questões mais críticas que afeta a vida na Terra (NEUMANN et al., 2011).

Altos níveis de gás NH_3 estão associados a elevado estresse respiratório, tanto das aves quanto dos funcionários no aviário (GATES, 2000). No entanto, o nitrogênio depositado na cama segue os destinos que são dados a esta, podendo se tornar um elemento poluente. No solo, o nitrogênio é transformado em nitrato, causando a acidificação do mesmo. Quando dissolvido na água, o excesso de nitrogênio favorece o desenvolvimento desordenado de algas, cuja decomposição consome o oxigênio nela dissolvido. A esse processo é dado o nome de eutrofização, que compromete o crescimento de espécies aquáticas, como peixes, crustáceos, etc (PENZ Jr. et al., 1999).

O óxido nitroso (N_2O), um dos mais importantes gases de efeito estufa, é formado através dos processos de nitrificação e desnitrificação durante a estocagem, compostagem e sua aplicação como fertilizante (SOMMER & DAHL, 1999). Este gás na troposfera absorve a radiação térmica e contribui para o efeito estufa da atmosfera. Ele ainda contribui para a diminuição da camada de ozônio na estratosfera, a qual protege a biosfera dos efeitos nocivos da radiação ultra violeta (MOSIER et al., 1998).

Portanto, o conhecimento das respostas dos animais ao consumo de aminoácidos, bem como a determinação do consumo para ótimo desempenho, são essenciais para o contínuo crescimento e desenvolvimento da indústria avícola, e servem também como medidas mitigadoras para reduzir a poluição ambiental advinda da excreção de nitrogênio.

OBJETIVOS

Objetivo geral

Avaliar as respostas de crescimento, deposição de nutrientes e excreção de nitrogênio das aves aos níveis crescentes de metionina + cistina e treonina na dieta.

Objetivos específicos

1. Determinar as eficiências de utilização de metionina + cistina e treonina para deposição de aminoácidos e proteína;
2. Determinar o consumo de metionina + cistina e treonina para frangos de corte machos e fêmeas para ótima resposta nas fases inicial (1 a 14 dias), de crescimento (15 a 28 dias) e terminação (29 a 42 dias de idade);
3. Estimar os parâmetros do modelo de Reading para predizer as exigências com base na mantença e ganho de peso e variabilidade na população;
4. Determinar o consumo ótimo econômico de metionina + cistina e treonina para frangos de corte machos e fêmeas para ótima resposta nas fases inicial (1 a 14 dias), de crescimento (15 a 28 dias) e terminação (29 a 42 dias de idade) em diferentes cenários.

Referências Bibliográficas

ALETOR, V. A.; HAMID, I. I.; NIESS, E.; PFEFFER, E. Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole-body composition and efficiencies nutrient utilisation. **Journal Science Food Agriculture**, v. 80, p. 547-554, 2000.

ARAÚJO, L. F.; JUNQUEIRA, O. M.; ARAÚJO, C. S. S. Redução do nível protéico da dieta, através da formulação baseada em aminoácidos digestíveis. **Ciência Rural**, v. 34, n. 4, p. 1197-1201, 2004.

ATENCIO, A.; ALBINO, L. F. T.; ROSTAGNO, H. S.; VIEITES, F. M. Exigências de metionina+cistina para frangos de corte machos em diferentes fases de criação. **Revista Brasileira de Zootecnia**, v. 33, n. 5, p. 1152-1166, 2004.

BREGENDAHL, K.; SELL, J. L.; ZIMMERMAN, D. R. Effect of low-protein diets on growth performance and body composition of broiler chicks. **Poultry Science**, v. 81, p. 1156-1167, 2002.

CHENG, T. K.; HAMRE, M. L.; COON, C. N. Effect of constant and cyclic environmental temperatures, dietary protein and amino acids levels on broiler performance. **Journal of Applied Poultry Research**, v. 8, p. 426-439, 1999.

CHENG, T. K.; HAMRE, M. L.; COON, C. N. Effect of environmental temperature, dietary protein and energy levels on broiler performance. **Journal of Applied Poultry Research**, v. 6, p. 1-17, 1997a.

CHENG, T. K.; HAMRE, M. L.; COON, C. N. Responses of broilers to dietary protein levels and amino acid supplementation to low protein diets at various environmental temperatures. **Journal of Applied Poultry Research**, v. 6, p. 18-33, 1997b

COSTA, F. G. P.; ROSTAGNO, H. S.; ALBINO, L. F. T.; GOMES, P. C.; TOLEDO, R. S.; VARGAS JR., J. G. Níveis dietéticos de proteína bruta para frangos de corte de 1 a 21 e 22 a 42 dias de idade. **Revista Brasileira de Zootecnia,** v. 30, p. 1498-1505, 2001.

CURNOW, R.N. A smooth population response curve based on an abrupt threshold and plateau model for individuals. **Biometrics**, v.29, p.1-10, 1973.

D'MELLO, J. P. F. Responses of growing poultry to amino acids. In: D'MELLO, J. P. F. **Amino acid in animal nutrition**. 2nd ed. Wallingford: CABI Publishing, p. 237-264, 2003.

DOZIER III, W. A.; MORAN Jr., E. T.; KIDD, M. T. Threonine requirement for broiler males from 42 to 56 days of age. **Journal of Applied Poultry Research**, v. 9, p. 214-222, 2000.

EUCLYDES, R. F.; ROSTAGNO, H. S. Estimativas dos níveis nutricionais via experimentos de desempenho. Nutrição de Aves e Suínos. In: **Workshop Latino-Americano Ajinomoto Biolatina**, 1., 2001, Foz do Iguaçu. Anais… Foz do Iguaçu. p. 77-88, 2001.

GATES, R. S. Poultry diet manipulation to reduce output of pollutants to environment. In: **Simpósio Sobre Resíduos da Produção Avícola**. 2000, Concórdia. Embrapa. Anais... Concórdia, p. 62-74, 2000.

GOUS, R.M. (1980) An improved method for measuring the response of broiler chickens to increasing dietray concentrations of an amino acid. In: **Proceedings** of European Poultry Conference. World's Poultry Science Association, Hamburg/Germany, pp.32–39.

HURWITZ, S., SKLAN, D., BARTOV, I. New formal approaches to the determination of energy and amino acid requeriments of chicks. **Poultry Science**, v. 57, p. 197-205, 1978.

HURWITZ, S.; BORNSTEIN, S. The protein and amino acid requirement of laying hens: suggest models for calculations. **Poultry Science**, v. 52, p. 1124-1134, 1973.

HURWITZ, S.; SKLAN, D.; TALPAZ, H.; PLAVNIK, I.. The effect of dietary protein level on the lysine and arginine requirements of growing chickens. **Poultry Science**, v. 77, p. 689-696, 1998.

KIDD, M. T., KERR, B. J. L-threonine for poultry: A review. **Journal of Applied Poultry Research**, v. 5, p. 358-367, 1996.

KIDD, M. T.; BARBER, S. J.; VIRDEN, W. S.; DOZIER III, W. A. CHAMBLEE, D. W; WIERNUSZ, C. Threonine responses of male cobb finishing broilers in differing environmental conditions. **Journal of Applied Poultry Research**, v. 12, p. 115-123, 2003a.

KIDD, M. T.; KERR, B. J. Threonine responses in commercial broilers at 30 to 42 days. **Journal of Applied Poultry Research**, v. 6, p. 362-367, 1997.

KIDD, M. T.; LERNER, S. P.; ALLARD, J. P.; RAO, S. K.; HALLEY, J. T. Threonine needs of finishing broilers: growth, carcass, and economic responses. **Journal of Applied Poultry Research**, v. 8, p. 160-169, 1999.

KIDD, M. T.; ZUMWALT, C. D.; BARBER, S. J.; DOZIER III, W. A.; CHAMBLEE, D. W.; WIERNUSZ, C. Threonine responses of female cobb 500 broilers from days 42 to 56. **Journal of Applied Poultry Research**, v. 12, p. 130-136, 2003b.

KINO, K.; OKUMURA, J.-I. Whole-body protein turnover in chicks fed control, histidine, or methionine plus cysteine-free diets. **Poultry Science**, v. 66, p. 1392-1397, 1987.

KUBENA, L. F.; REECE, F. N.; MAY, J. D. Nutritive properties of broiler excreta as influenced by environmental temperature, collection interval, age of broilers and diet. **Poultry Science**, v. 52, p. 1700-1703, 1973.

LEMME, A.; RAVINDRAN, V.; BRYDEN, W. L. Ileal digestibility of amino acids in feed ingredients for broilers. **World's Poultry Science Journal**, v. 60, p. 423-438, 2004

LÓPEZ, R. M.; MÉNDEZ, T. J.; GONZÁLEZ, E. A.; ARNEZCUA, C. M. Necesidades de treonina en pollos sometidos a dos calendarios de vacunación. **Veterinária México**, v. 32, n. 3, p. 189-194, 2001.

MORAN Jr., E. T. Response of broiler strains differing in body fat to inadequate methionine: live performance and processing yields. **Poultry Science**, v. 73, p. 1116-1126, 1994.

MORSE, D. Environmental considerations of livestock producers. **Journal of Animal Science**, v. 73, n. 9, p. 2733-2740, 1995.

MOSIER, A.R.; DUXBURY, J.M.; FRENEY, J.R.; HEINEMEYER, O. AND MINAMI, K. Assessing and mitigating N_2O emissions from agricultural soils. **Climatic Change**, v.40, p.7-38, 1998.

NATIONAL RESEARCH COUNCIL. **Nutrient requirements of poultry**. 9th.ed. Washington, D.C.: National Academy of Science, 154 p., 1994.

NEUMANN, K.; VERBURG, P. H.; ELBERSEN, B.; STEHFEST, E.; WOLTJER, G. B. Multi-scale scenarios of spatial-temporal dynamics in the European livestock sector. **Agriculture, Ecosystems and Environment**, v. 140, p. 88-101, 2011.

PACK, M. Proteína ideal para frangos de corte. Conceitos e posição atual. In: **Conferência APINCO de ciência e tecnologia avícolas**, 1995, Curitiba. Anais… Campinas: FACTA, p.95-110, 1995.

PATTERSON, J. A.; KUNG Jr., L. Metabolism of DL-methionine and methionine analogues by rumen microorganisms. **Journal of Dairy Science**, v. 71, p. 3292-3301, 1988.

PENZ Jr., A. M.; COLNAGO, G. L.; JENSEN, L. S. Threonine supplementation of practical diets for 3 to 6-wk-old broilers. **Journal of Applied Poultry Research**, v. 6, p. 355-361, 1997.

PENZ JR., A. M.; MEINERZ, C. E. T.; MAGRO, N. Efeito da nutrição na quantidade e qualidade dos dejetos suínos. In: **Reunião Anual da Sociedade Brasileira de Zootecnia**, 36. Anais… Porto Alegre: SBZ, 1999.

PESTI, G. M.; BENEVENGA, N. J.; HARPER, A. E.; SUNDE, M.L. The effects of high dietary protein and nitrogen levels on the preformed methyl group requirement and methionine-induced growth depression in chicks. **Poultry Science**, v. 60, p. 425-432, 1981.

RODRIGUES, P. B.; BRITO, J. A. G.; SILVA, E. L.; NASCIMENTO, G. A. J. Manejo da dieta para reduzir o impacto ambiental da excreção de nutrientes na avicultura. In: **VII Seminário de Aves e Suínos – AveSui Regiões**, 2007, Belo Horizonte. Anais... Belo Horizonte, 2007.

ROSTAGNO, H. S.; ALBINO, L. F. T.; DONZELE, J. L.; GOMES, P. C.; OLIVEIRA, R. F. M.; LOPES, D. C.; FERREIRA, A. S.; BARRETO, S. L. T.; EUCLIDES, R. F.

Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3a ed. Universidade Federal de Viçosa, Viçosa, Minas Gerais/Brazil, 2011.

ROSTAGNO, H. S.; ALBINO, L. F. T.; DONZELE, J. L.; GOMES, P. C.; OLIVEIRA, R. F. M.; LOPES, D. C.; FERREIRA, A. S.; BARRETO, S. L. T. **Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais**. 2a ed. Universidade Federal de Viçosa, Viçosa, Minas Gerais/Brazil, 2005.

ROSTAGNO, H. S.; ALBINO, L. F. T.; DONZELE, J. L.; GOMES, P.C.; FERREIRA, A. S.; LOPES, D. C.; OLIVEIRA, R. F. M.. **Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais**. Viçosa: UFV, Departamento de Zootecnia, 2000.

SAKOMURA, N. K. Uso de modelos para estimar as exigências nutricionais de aves. In: **II Simpósio internacional sobre exigências nutricionais de aves e suínos**, 2005, Viçosa-MG. Anais... Viçosa-MG: UFV, p. 253-292, 2005.

SAKOMURA, N. K.; ROSTAGNO, H. S. **Métodos de pesquisa em nutrição de monogástricos**. Funep, Jaboticabal, São Paulo/Brazil, 2007.

SANTOMÁ, G. Necesidades proteicas de las gallinas ponedoras. In: De Blas, C., Mateos, G.G. (Ed.). **Nutricion y alimentación de gallinas ponedoras**. Madrid: Muldi Prensa, p. 71-114, 1991.

SCHUTTE, J. B. The ideal amino acid profile for laying hens and broiler chicks. In: **Proceedings of the 1998 Arkansas Nutrition Conference**, 1998, Fayetteville, AR. Proceedings… Fayetteville, AR., p. 33-39, 1998.

SOMMER, S.G. AND DAHL, P. Nutrient and Carbon balance during the composting of litter. **Journal of Agricultural Engineering Research**, v.74, p.145-153, 1999.

SPECIAN, R. D.; OLIVER, M. G. Functional biology of intestinal goblet cells. **American Journal of Physiology**, v. 260, p. C183-C193, 1991.

STOLL, B.; HENRY, J.; REEDS, P. J.; YU, H.; JAHOOR, F.; BURRIN, D. G.. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. **The Journal of Nutrition**, v. 128, p. 606–614, 1998.

SUMMERS, J. D.; SPRATI, D.; ATKINSON, J. L. Broiler weight gain and carcass composition when fed diets varying in amino acid balance, dietary energy and protein level. **Poultry Science**, v. 71, p. 263-273, 1992.

VAN DER SCHOOR, S. R. D.; REEDS, P. J.; STOLL, B.; HENRY, J. F.; ROSENBERGER, J. R.; BURRIN, D. G.; VAN GOUDOEVER, J. B. The high metabolic cost of a functional gut. **Gastroenterology**, v. 123, p. 1931-1940, 2002.

CAPÍTULO 2 - RESPONSES OF BROILERS TO METHIONINE+CYSTEINE AND THREONINE INTAKE

DONATO, D.C.Z., SAKOMURA, N.K.

Animal Science Department, Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil; sakomura@fcav.unesp.br

Abstract

The objective of this research was to measure responses of broilers to methionine+cysteine and to threonine intake, to determine their efficiencies of utilisation for growth and their intake for optimum response in the initial, grower and finisher phases. Six dose-responses trials were conducted with males and females Cobb broilers in these three rearing phases. Measurements included body weight gain, digestible amino acid intake, feed conversion ratio, the proportion of feathers in the body weight and the amino acid and lipid deposition in the body and feathers. The efficiency of utilisation of the amino acids studied was calculated by linear regression of amino acid deposition and the amino acid intake for each phase. In despite of the differences on responses between sexes and phases, the efficiency of utilisation remained unchanged, being 0.56 and 0.73 for methionine+cysteine and threonine, respectively. The methionine+cysteine intakes estimated for optimum feed conversion ratio were 231 and 524 mg/d in the initial and grower phases, respectively, for both males and females, and 923 and 1053 mg/d for females and males, respectively, in finisher phase. For threonine these intakes were 184, 612 and 823 mg/d in initial, grower and finisher phases, for both males and females.

Keyword: amino acids, dose-response method, efficiency of utilisation

Introduction

To maintain the increasing production and productivity of broilers, in addition to health care and management, nutrition is of great importance. All nutrients should be supplied in sufficient quantities so that nutrient deficiencies do not occur, as these lead to low productivity, and excesses should be avoided as these lead to an increased cost of production, the excretion of waste products into the environment and losses in productivity.

Protein is considered one of the most important nutrients in the feed of broilers, since their productivity depends mainly on an efficient conversion of feed protein into muscle protein (Costa et al*.*, 2001). However, now that amino acids have being produced on a commercial scale by industries, nutritionists have reduced levels of dietary crude protein and now formulate diets in order to meet the specific needs for essential amino acids. So, it is of importance to know how broilers respond to the intake of specific amino acids so that the optimum economic intakes of these amino acids can be determined. Maintenance requirements and the efficiency of utilisation of amino acids necessary for protein deposition are essential to the development of models to estimate amino acid responses (Edwards et al., 1997, Edwards et al., 1999).

Methionine + cysteine (MC) and threonine (THR) stand out as two of the first three limiting essential amino acids. Methionine has a specific role in protein synthesis, being the first amino acid to be incorporated into the peptide structure, and its deficiency may inhibit this early stage (Kino and Okumura, 1987). THR has its importance in various metabolic processes such as protein synthesis, maintenance of body protein turnover, role in antibody production, as well as in the maintenance associated with the digestive tract, where it plays an important role in the synthesis of mucins.

There are two main approaches for studying the response of poultry to essential amino acids: the dose-response method, which measures the response in performance to increasing intakes of the nutrient under test; and the factorial method, which is based on the principle that the animal needs amino acids to maintain vital processes and activities, as well as for growth and/or production (Sakomura and Rostagno, 2007).

The dose-response method has traditionally been used to study the responses of poultry to increasing concentrations of amino acids in the diet. It is useful as a means of providing components for the factorial models (D'Mello, 2003a) such as the efficiency of amino acid utilisation, and also to evaluate the factors that influence this efficiency, thereby contributing to the development of generalized models of nutrient responses.

This paper describes the studies conducted at the Laboratory of Poultry Sciences, FCAV / UNESP - Jaboticabal that have been aimed at assessing responses of broilers to increasing levels of MC and THR in the diet, their efficiencies of utilisation for growth and their estimated intakes for optimum responses in initial, grower and finisher phases.

Material and methods

Six trials were conducted at the Poultry Science Laboratory of Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal Campus in São Paulo, Brazil, designed to measure the response of broiler chickens during three rearing phases (1-14, 15-28 and 29-42 d) to dietary amino acids.

Five hundred and sixty male and female broilers (totalling 1,120 broilers per trial and 3360 broilers per amino acid studied) were distributed in a completely random design in each trial, according to a 7 x 2 factorial (7 levels of dietary amino acids and 2 sexes) totalling 14 treatments, with 4 replicates. Each experimental unit comprised 20 birds. Cobb 500® broilers were used in all the trials. At the beginning of each trial, birds were individually weighed and distributed such that each experimental unit had homogeneous weight.

The experimental diets were formulated using the dilution technique. A high protein summit diet was formulated to contain approximately 1.2 times the digestible MC and THR levels suggested by Rostagno et al. (2005) for broilers during the respective phases, and all other essential amino acids were set at a minimum of 1.4 times their suggested levels. These summit diets were diluted sequentially with isoenergetic, protein-free diets (N free) (Fisher and Morris, 1970) (Table 2-1), to create a range of feeds increasing in the studied amino acids content (MC: 1-14 d: 3.05 to 10.88; 15-28 d: 2.72 to 9.79, and 29-42 d: 2.50 to 9.03 g MC/kg) (THR: 1-14 d: 1.49 to 9.96; 15-28 d: 1.30 to 8.86, and 29-42 d: 1.20 to 8.17 g THR/kg).

To verify whether the studied amino acid was limiting in the dilution series, a pilot trial to each amino acid was conducted with 30 male Cobb broilers from 1 to 14 days for MC and from 7 to 21 days for THR. They were distributed in a completely random design in two treatments in each trial, being the lowest studied levels for each amino acid and the control treatment. This treatment had the same nutritional composition as the lowest treatment in the dilution series, but it was supplemented with the test amino acid to reach the same level of the second treatment. BGW and FCR were evaluated.

Digestible amino acid contents of the summit diets were determined through a digestibility trial using the method described by Sakomura and Rostagno (2007). Total amino acid concentrations in the diets and excreta were determined by high performance liquid chromatography (HPLC).

Body weight gain (BWG, g/bird d), digestible amino acid intake (MCi or THRi, mg/d, calculated from the feed intake), feed conversion ratio (FCR, g/g) and the proportion of feathers in the body weight (PF) were calculated from the measurements of body weight and feed intake made during each rearing phase. The deposition of protein and lipid (g/d) in the feather-free body and in the feathers was determined using the comparative slaughter technique. The number of birds sampled at the beginning of each phase was 6, whilst 2 birds of each experimental unit were sampled at the end of each phase of the trials.

The amino acid content of protein in the feather-free body and in the feathers was measured in birds sampled at the beginning and end of each phase and were analyzed by HPLC from which the amount of amino acids deposited during each phase was calculated.

Table 2-1. Composition (g/kg) and analyzed nutrient content (g/kg) of the summit and N-free diets used in each phase of the trial

Ingredients (%)	Diets		
	MC Summit	THR Summit	N-Free
Soybean meal (45%)	510	510	-
Maize	339	339	-
Soybean oil	65.0	65.0	100
Corn gluten meal (60%)	37.3	36.8	
Dicalcium phosphate	20.3	20.3	27.0
Limestone	9.45	9.45	5.07
Salt	4.38	4.38	5.13
DL- Methionine (99%)	3.28	5.12	-
L-Lysine (78.5%)	4.89	4.87	-
L-Threonine (99%)	2.01	0.33	-
L-Valine (96.5%)	1.40	1.40	-
Choline chloride (60%)	1.00	1.00	1.00
L-Arginine (98.5%)	0.54	0.53	
Mineral Premix[1]	0.50	0.50	0.50
Vitamin Premix[2]	0.50	0.50	0.50
Potassium chloride	-	-	11.4
Starch	-	-	428
Sugar	-	-	150
Rice Husk	-	-	150
Anticoccidiostatic[3]	0.50	0.50	0.50
Growth promotor[4]	0.05	0.05	0.05
Antioxidant[5]	0.10	0.10	0.10
Inert[6]	-	-	121
Nutrients (g/kg)			
Metabolizable energy (kcal/kg)[7]	3050	3050	3050
Crude Protein	294	298	8.90
Methionine+Cysteine[8]	9.04	10.8	-
Methionine[8]	5.82	7.48	-
Lysine[8]	15.8	17.1	-
Tryptophan[8]	3.14	2.81	-
Threonine[8]	10.2	9.99	-
Arginine[8]	16.1	16.7	-
Valine[8]	12.3	14.1	-
Isoleucine[8]	9.70	11.3	-
Leucine[8]	21.2	23.1	-
Phenylalanine[8]	12.9	13.7	-
Calcium	10.0	10.0	10.0
Sodium	2.20	2.20	2.20
Available phosphorus	5.00	5.00	5.00

[1]Content/kg of product: Mn = 150,000 mg, Fe = 100,000 mg, Zn = 100,000 mg, Cu= 16,000 mg, I = 1,500 mg, [2]Content/kg of product: Folic acid = 1000 mg, pantothenic acid = 15,000 mg, Niacin = 40,000 mg, Biotin = 60 mg, vit B1 = 1,800 mg, vit, B12 = 12,000 mg, vit, B2 = 6,000 mg, vit, B6 = 2,800 mg, vit D3 = 2,000,000 UI, vit E = 15,000 mg, vit, K3 = 1,800 mg, Se = 300 mg, antioxidant = 500 mg, [3]coxistac, [4]Zinc bacitracin, [5]BHT, [6]Wash sand, [7]Predicted value, [8]Digestible amino acid composition,

Dilution scheme (summit:N-free): MC - Initial phase: 0.28:0.72; 0.40:0.60; 0.52:0.48; 0.64:0.36; 0.76:0.24; 0.88:0.12; 1.00:0.00; Grower phase: 0.25:0.75; 0.36:0.64; 0.47:0.53; 0.58:0.42; 0.68:0.32; 0.79:0.21; 0.90:0.10; Finisher phase: 0.23:0.77; 0.33:0.67; 0.43:0.57; 0.53:0.47; 0.63:0.37; 0.73:0.27; 0.83:0.17. THR – Initial phase: 0.15:0.85; 0.40:0.60; 0.52:0.48; 0.64:0.36; 0.76:0.24; 0.88:0.12;

1.00:0.00; Grower phase: 0.13:0.87; 0.35:0.65; 0.46:0.54; 0.57:0.43; 0.67:0.33; 0.78:0.22; 0.89:0.11; Finisher phase: 0.12:0.88; 0.33:0.67; 0.42:0.58; 0.52:0.48; 0.62:0.38; 0.72:0.28; 0.82:0.18.

The efficiency of utilisation of MC and THR was obtained by linear regression of the amino acid deposited in the whole body (feather-free body + feathers) on digestible amino acid intake. The amino acid intake for growth was calculated by subtracting the maintenance requirement from the total consumed. The amino acid requirement for maintenance used in the calculations were those measured by Bonato et al. (2011), namely, 24 mg of MC/kg$^{0.75}$/d and 50 mg of THR/kg$^{0.75}$/d. To ensure that only the linear portion of the response was used for this regression the lowest four levels of MC and THR were used.

Variance analisys residuals were tested for homoscedasticity of variance, normality and independence and being the assumptions satisfied to null hypostesis, a statistical analysis of variance was conducted on each of the variables measured. Linear response plateau and quadratic polynomial models were fitted to the data. To estimate the optimum amino acid intake both models were used, since the point where the quadratic curve first intersects the plateau of the broken line is considered “a realistic and objective estimate of the requirement for a population of animals” (Baker, 2003). This point was obtained using the equation: $L = \beta_0 * AAi^2 + \beta_1 * AAi + \beta_2$, being AAi equals to MC or THR intake, L is the maximum (or minimum) response determined by the linear response plateau model, β_0 is the quadratic regression coefficient, which indicates change in the parable inclination (if $\beta_0>0$, the parable is up; if $\beta_0<0$, the parable is down), β_1 is the linear regression coefficient, which represents the inclination angle and β_2 is the intercept with y axis.

To express optimum amino acids intakes as concentrations in the diet, the mean feed intakes observed in the three highest levels of each trial were used, these being 34, 77 and 160 g/d for females and 36, 85 and 183 g/d for males, during the initial, grower and finisher phases, respectively, for MC trial and 29, 91 and 144 g/d for females and 30, 101 and 173 g/d for males, during the initial, grower and finisher phases, respectively, for THR trial.

Results

Results of the pilot trials confirmed that MC and THR were the limiting amino acids in the respective dilution series. The addition of industrial MC and THR to the diet with the lowest contents of these amino acids improved both BWG (7.79 g/bird d vs 5.18 g/bird d for MC and 30.05 g/bird d vs 27.55 g/bird d for THR) and FCR (2.65 g/g vs 2.98 g/g for MC and 2.28 g/g vs 2.45 g/g for THR).

In MC trial gender influenced (Table 2-2) the responses in BWG after the second phase (Table 2-3), but with FCR this effect was only significant in the last phase. The reduction to 0.19 of the maximum MC intake in the initial phase reduced BWG to 0.36 of the maximum response. For grower and finisher phases, reducing MC intake to approximately 0.32 of the maximum, regardless of sex, reduced BWG to 0.48 and 0.54 of the maximum gain for both males and females, respectively, in the grower phase and to 0.65 and 0.57 of the maximum gain for both males and females, in the finisher phase. These responses are illustrated in Figure 2-1.

Table 2-2. Variance analysis results of the variables body weight gain (BWG), feed conversion ratio (FCR), proportion of feathers (PF), body protein deposition (BPd) and body lipid content (BLc) of the methionine+cysteine trial

Statistic	Variables				
	BWG	FCR	PF	BPd	BLc
		Initial			
F MC	<0.01	<0.01	0.80	<0.01	<0.01
F Sex	<0.01	<0.01	<0.01	<0.01	<0.01
F MC*Sex	0.61	0.68	0.17	0.34	<0.01
		Grower			
F MC	<0.01	<0.01	<0.01	<0.01	<0.01
F Sex	<0.01	0.10	<0.01	0.16	<0.01
F MC*Sex	<0.01	0.13	0.17	<0.05	0.42
		Finisher			
F MC	<0.01	<0.01	<0.05	<0.01	<0.01
F Sex	<0.01	0.52	<0.01	<0.01	<0.01
F MC*Sex	<0.01	<0.01	0.53	<0.01	<0.05

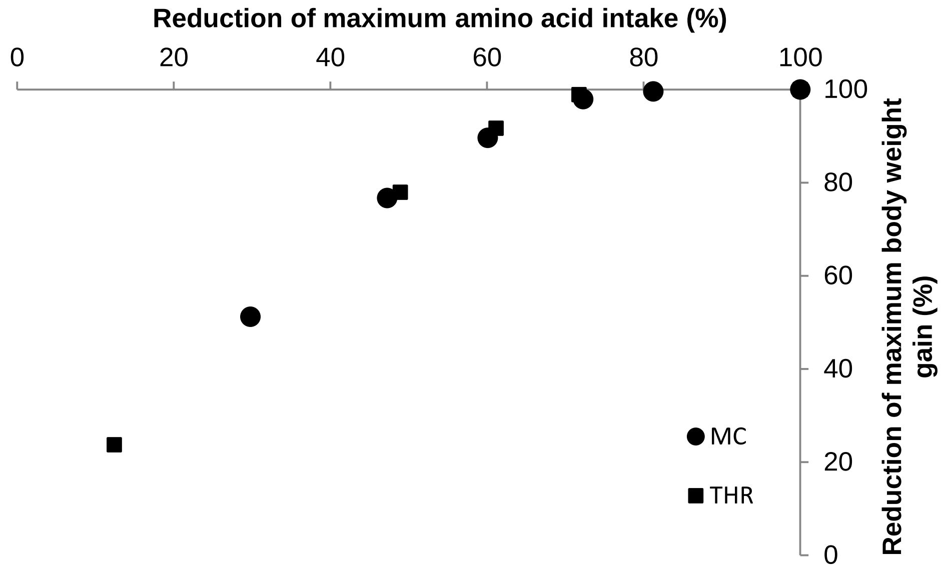

Figure 2-1. Reduction in maximum body weight gain of male broilers as a function of methionine+cysteine (MC) and threonine (THR) intake expressed as a proportion of maximum intake for the total period (1 – 42 days)

Gender differences in the responses in BWG in the THR trial (Tables 2-4 and 2-5) are apparent in all phases, but no such effect was observed in FCR. A reduction to 0.06 of the maximum THR intake resulted in a reduction to 0.13 of maximum BWG in the initial phase, regardless of sex. In the grower and finisher phases, reducing THR intake to approximately 0.13 of the maximum reduced BWG to 0.20 of maximum gain, regardless of sex during grower phase and to 0.30 and 0.28 of maximum gain for males and females, respectively, in the finisher phase.

The analyzed amino acid contents of the feather-free body and feathers were, respectively, 29 and 54 g MC/kg and 36 and 45 g THR/kg.

In Tables 2-6 and 2-7 the rates of protein deposition are presented together with the body lipid contents of broilers fed MC and THR.

Table 2-3. Body weight gain (BWG; g/bird d), feed conversion ratio (FCR; g/g) and proportion of feathers (PF) of broiler males and females in response to intakes of methionine+cysteine (MCi, mg/bird d) during three rearing phases (means ± SE)

Levels	Females				Males			
	MCi	BWG	FCR	PF	MCi	BWG	FCR	PF
Initial								
2.53	53	9.3 ± 1.10	2.2 ± 0.03	0.369 ± 0.03	60	10.7 ± 0.39	2.2 ± 0.01	0.299 ± 0.04
3.62	107	17.7 ± 1.37	1.7 ± 0.01	0.381 ± 0.03	124	20.9 ± 0.34	1.6 ± 0.01	0.268 ± 0.01
4.70	162	24.8 ± 0.47	1.4 ± 0.01	0.306 ± 0.05	171	26.5 ± 0.19	1.4 ± 0.01	0.338 ± 0.06
5.79	205	28.0 ± 0.46	1.3 ± 0.01	0.391 ± 0.04	214	29.3 ± 0.15	1.3 ± 0.01	0.340 ± 0.02
6.87	237	28.1 ± 0.32	1.2 ± 0.01	0.393 ± 0.03	248	30.7 ± 0.33	1.2 ± 0.01	0.279 ± 0.05
7.96	271	28.1 ± 0.39	1.2 ± 0.03	0.464 ± 0.03	282	30.8 ± 0.39	1.2 ± 0.01	0.275 ± 0.03
9.04	289	27.2 ± 0.50	1.2 ± 0.01	0.359 ± 0.05	302	29.0 ± 0.42	1.2 ± 0.02	0.313 ± 0.04
RMSE[1] (27 d.f.)[2]		1.52	0.04	0.72		0.66	0.02	0.77
Grower								
2.26	194	30.4 ± 0.52	2.8 ± 0.01	0.414 ± 0.02	197	30.4 ± 0.54	2.9 ± 0.03	0.311 ± 0.02
3.25	293	43.4 ± 0.84	2.1 ± 0.01	0.404 ± 0.02	314	46.5 ± 0.99	2.1 ± 0.03	0.309 ± 0.04
4.25	381	51.6 ± 0.92	1.7 ± 0.01	0.377 ± 0.02	411	56.1 ± 0.90	1.7 ± 0.02	0.317 ± 0.02
5.24	456	54.8 ± 0.67	1.6 ± 0.01	0.452 ± 0.03	498	62.5 ± 0.24	1.5 ± 0.01	0.451 ± 0.02
6.15	495	56.7 ± 0.70	1.4 ± 0.02	0.423 ± 0.01	537	62.0 ± 0.51	1.4 ± 0.02	0.363 ± 0.05
7.14	557	57.5 ± 0.73	1.4 ± 0.01	0.549 ± 0.02	611	65.1 ± 0.63	1.3 ± 0.02	0.394 ± 0.04
8.14	601	56.5 ± 0.59	1.3 ± 0.01	0.463 ± 0.05	661	63.1 ± 2.04	1.3 ± 0.02	0.443 ± 0.01
RMSE[1] (27 d.f.)[2]		1.45	0.03	0.53		1.99	0.04	0.64
Finisher								
2.08	377	56.5 ± 0.85	3.2 ± 0.01	0.515 ± 0.02	427	59.8 ± 1.02	3.4 ± 0.05	0.495 ± 0.02
2.98	548	72.5 ± 1.77	2.5 ± 0.04	0.468 ± 0.03	646	83.9 ± 1.09	2.6 ± 0.03	0.427 ± 0.03
3.89	693	81.4 ± 1.99	2.2 ± 0.02	0.505 ± 0.01	796	94.2 ± 2.85	2.2 ± 0.03	0.427 ± 0.02
4.79	823	86.2 ± 1.44	2.0 ± 0.01	0.567 ± 0.01	945	101.4 ± 1.81	1.9 ± 0.02	0.473 ± 0.01
5.70	943	87.2 ± 0.79	1.9 ± 0.02	0.499 ± 0.04	1078	103.7 ± 1.07	1.8 ± 0.02	0.453 ± 0.02
6.60	1062	87.0 ± 1.17	1.9 ± 0.01	0.555 ± 0.03	1213	105.7 ± 1.93	1.7 ± 0.02	0.512 ± 0.03
7.50	1153	86.4 ± 2.15	1.8 ± 0.04	0.553 ± 0.03	1331	105.1 ± 2.86	1.7 ± 0.04	0.440 ± 0.03
RMSE[1] (27 d.f.)[2]		3.07	0.05	0.52		3.91	0.06	0.50

[1]RMSE: Residual mean square error; [2]d.f.: degrees of freedom

Table 2-4. Variance analysis results of the variables body weight gain (BWG), feed conversion ratio (FCR), proportion of feathers (PF), body protein deposition (BPd) and body lipid content (BLc) of the threonine trial

Statistics	Variable				
	BWG	FCR	PF	BPd	BLc
Initial					
F THR	<0.01	<0.01	<0.01	<0.01	<0.01
F Sex	0.11	0.92	0.67	<0.01	<0.01
F THR*Sex	<0.05	0.83	<0.05	<0.01	<0.05
Grower					
F THR	<0.01	<0.01	<0.01	<0.01	<0.01
F Sex	<0.01	0.23	<0.01	<0.01	<0.05
F THR*Sex	<0.01	0.23	<0.01	0.26	<0.01
Finisher					
F THR	<0.01	<0.01	<0.05	<0.01	<0.01
F Sex	<0.01	0.54	0.45	<0.01	<0.01
F THR*Sex	<0.01	0.99	0.07	<0.05	0.38

Table 2-5. Body weight gain (BWG; g/bird d), feed conversion ratio (FCR; g/g) and proportion of feathers (PF) of broiler males and females in response to intakes of threonine (THRi, mg/bird d) during three rearing phases (means ± SE)

	Initial							
	Females				Males			
Levels	THRi	BWG	FCR	PF	THRi	BWG	FCR	PF
1.50	16	2.6 ± 0.20	4.3 ± 0.15	0.404 ± 0.13	18	2.9 ± 0.09	4.2 ± 0.06	0.313 ± 0.08
4.00	117	15.1 ± 0.59	1.9 ± 0.02	0.766 ± 0.07	113	14.5 ± 0.85	1.9 ± 0.02	0.503 ± 0.04
5.20	160	18.9 ± 0.42	1.6 ± 0.05	0.726 ± 0.10	159	18.3 ± 0.39	1.7 ± 0.03	0.551 ± 0.06
6.40	185	19.9 ± 0.79	1.5 ± 0.04	0.586 ± 0.05	203	21.5 ± 0.59	1.5 ± 0.02	0.609 ± 0.03
7.59	221	21.3 ± 0.30	1.4 ± 0.02	0.624 ± 0.04	220	20.6 ± 0.35	1.4 ± 0.01	0.802 ± 0.04
8.79	252	21.2 ± 0.53	1.4 ± 0.02	0.547 ± 0.03	265	22.3 ± 0.72	1.4 ± 0.01	0.681 ± 0.07
9.99	266	19.4 ± 0.87	1.4 ± 0.02	0.689 ± 0.04	291	22.0 ± 0.77	1.3 ± 0.01	0.772 ± 0.10
RMSE[1] (d.f.)[2]		1.15	0.12	1.47		1.19	0.06	1.28
	Grower							
	Females				Males			
Levels	THRi	BWG	FCR	PF	THRi	BWG	FCR	PF
1.30	86	11.8 ± 0.40	5.6 ± 0.01	0.266 ± 0.04	103	13.3 ± 0.19	6.0 ± 0.33	0.336 ± 0.03
3.50	377	45.3 ± 0.26	2.4 ± 0.01	0.332 ± 0.03	444	48.8 ± 1.13	2.6 ± 0.06	0.256 ± 0.02
4.60	479	55.0 ± 0.71	1.9 ± 0.01	0.351 ± 0.01	522	59.6 ± 0.72	1.9 ± 0.01	0.295 ± 0.02
5.70	567	58.1 ± 0.25	1.7 ± 0.01	0.359 ± 0.01	626	64.7 ± 1.62	1.7 ± 0.02	0.293 ± 0.03
6.70	629	57.8 ± 0.45	1.6 ± 0.01	0.467 ± 0.02	706	67.5 ± 0.76	1.6 ± 0.01	0.383 ± 0.04
7.79	713	59.2 ± 1.23	1.5 ± 0.01	0.478 ± 0.02	803	68.3 ± 0.80	1.5 ± 0.02	0.331 ± 0.03
8.89	776	57.6 ± 0.40	1.5 ± 0.01	0.470 ± 0.01	850	64.7 ± 0.49	1.5 ± 0.01	0.345 ± 0.03
RMSE[1] (d.f.)[2]		1.23	0.08	0.49		1.84	0.26	0.54
	Finisher							
	Females				Males			
Levels	THRi	BWG	FCR	PF	THRi	BWG	FCR	PF
1.20	163	25.0 ± 1.32	5.5 ± 0.25	0.445 ± 0.01	189	28.7 ± 1.44	5.5 ± 0.31	0.460 ± 0.02
3.30	554	67.0 ± 2.59	2.5 ± 0.09	0.455 ± 0.02	666	84.1 ± 1.47	2.4 ± 0.04	0.445 ± 0.02
4.20	689	78.9 ± 0.65	2.1 ± 0.04	0.457 ± 0.02	848	95.7 ± 1.44	2.1 ± 0.02	0.401 ± 0.01
5.20	833	86.7 ± 3.60	1.9 ± 0.06	0.387 ± 0.06	964	101.0 ± 1.44	1.8 ± 0.02	0.472 ± 0.01
6.20	923	85.5 ± 2.61	1.7 ± 0.03	0.487 ± 0.02	1112	107.7 ± 0.67	1.7 ± 0.01	0.468 ± 0.01
7.19	1048	85.5 ± 1.84	1.7 ± 0.02	0.436 ± 0.01	1261	107.8 ± 3.06	1.6 ± 0.02	0.466 ± 0.02
8.19	1128	83.0 ± 1.09	1.7 ± 0.03	0.493 ± 0.01	1359	102.6 ± 2.22	1.6 ± 0.01	0.510 ± 0.01
RMSE[1] (d.f.)[2]		4.36	0.21	0.54		3.64	0.23	0.28

[1]RMSE: Residual mean square error; [2]d.f.: degrees of freedom

In general, in all phases, the deposition of protein increased with amino acid intake until it reached a plateau (Figure 2-2). The rate of deposition of each amino acid (MC, THR) was calculated for each treatment during each phase of the experiment taking account of the content of amino acids in the protein of body and feathers. In despite of the differences in rates of deposition between genders and phases, the efficiency of utilisation of MC and THR did not differ, being 0.56 (MCd = 28.01 + 0.56MCi; R^2 = 0.94) for MC and 0.73 (THRd = 10.87 + 0.73THRi; R^2 = 0.91) for THR.

Table 2-6. Body protein deposition (BPd; g/bird d) and body lipid content (BLc; g/kg) of broiler males and females as a function of methionine+cysteine intake (MCi; mg/bird d) (means ± SE)

Levels	Females MCi	Females BPd	Females BLc	Males MCi	Males BPd	Males BLc
Initial						
2.53	53	1.25 ± 0.16	177 ± 5.82	60	1.59 ± 0.07	142 ± 5.79
3.62	107	2.67 ± 0.24	155 ± 2.63	124	3.25 ± 0.07	128 ± 1.03
4.70	162	3.57 ± 0.10	133 ± 1.87	171	3.92 ± 0.15	118 ± 1.30
5.79	205	4.30 ± 0.13	101 ± 1.72	214	4.57 ± 0.04	102 ± 3.82
6.87	237	4.85 ± 0.08	62.7 ± 0.92	248	5.02 ± 0.19	60.8 ± 5.22
7.96	271	4.69 ± 0.05	58.3 ± 4.16	282	4.82 ± 0.17	55.9 ± 1.19
9.04	289	4.71 ± 0.06	55.4 ± 3.44	302	4.70 ± 0.05	42.4 ± 1.47
RMSE[1] (27 d.f.)[2]		0.26	6.64		0.24	6.83
Grower						
2.26	194	1.25 ± 0.16	177 ± 5.82	197	1.59 ± 0.07	142 ± 5.79
3.25	293	2.67 ± 0.24	155 ± 2.63	314	3.25 ± 0.07	128 ± 1.03
4.25	381	3.57 ± 0.10	133 ± 1.87	411	3.92 ± 0.15	118 ± 1.30
5.24	456	4.30 ± 0.13	101 ± 1.72	498	4.57 ± 0.04	102 ± 3.82
6.15	495	4.85 ± 0.08	62.7 ± 0.92	537	5.02 ± 0.19	60.8 ± 5.22
7.14	557	4.69 ± 0.05	58.3 ± 4.16	611	4.82 ± 0.17	55.9 ± 1.19
8.14	601	4.71 ± 0.06	55.4 ± 3.44	661	4.70 ± 0.05	42.4 ± 1.47
RMSE[1] (27 d.f.)[2]		0.40	7.10		0.54	12.46
Finisher						
2.08	377	1.25 ± 0.16	177 ± 5.82	427	1.59 ± 0.07	142 ± 5.79
2.98	548	2.67 ± 0.24	155 ± 2.63	646	3.25 ± 0.07	128 ± 1.03
3.89	693	3.57 ± 0.10	133 ± 1.87	796	3.92 ± 0.15	118 ± 1.30
4.79	823	4.30 ± 0.13	101 ± 1.72	945	4.57 ± 0.04	102 ± 3.82
5.70	943	4.85 ± 0.08	62.7 ± 0.92	1078	5.02 ± 0.19	60.8 ± 5.22
6.60	1062	4.69 ± 0.05	58.3 ± 4.16	1213	4.82 ± 0.17	55.9 ± 1.19
7.50	1153	4.71 ± 0.06	55.4 ± 3.44	1331	4.70 ± 0.05	42.4 ± 1.47
RMSE[1] (27 d.f.)[2]		0.89	11.92		1.64	9.80

[1]RMSE: Residual mean square error; [2]d.f.: degrees of freedom

Body lipid content decreased as amino acid intake increased (Tables 2-6 and 2-7). The responses in body lipid to increasing amino acid intake over the whole period (1 – 42 d) for each amino acid are shown in Figure 2-3. Birds fed MC-limiting feeds, at the end of 42 d, had less body fat than those birds fed THR-limiting feeds.

Table 2-7. Body protein deposition (BPd; g/bird d) and body lipid content (BLc; g/kg) of broiler males and females as a function of threonine intake (THRi; mg/bird d) (means ± SE)

Levels	Females			Males		
	THRi	BPd	BLc	THRi	BPd	BLc
Initial						
1.50	16	0.40 ± 0.08	154 ± 13.16	18	0.40 ± 0.03	181 ± 13.67
4.00	117	2.83 ± 0.09	135 ± 6.59	113	2.46 ± 0.16	175 ± 3.95
5.20	160	3.42 ± 0.10	138 ± 5.40	159	3.21 ± 0.03	149 ± 0.30
6.40	185	3.41 ± 0.09	114 ± 1.71	203	3.86 ± 0.04	132 ± 1.02
7.59	221	3.72 ± 0.11	111 ± 4.13	220	4.06 ± 0.09	120 ± 3.66
8.79	252	3.40 ± 0.07	90.5 ± 1.42	265	3.88 ± 0.12	95.7 ± 5.54
9.99	266	3.47 ± 0.19	83.2 ± 2.93	291	4.08 ± 0.14	78.3 ± 2.65
RMSE[1] (d.f.)[2]		0.22	10.75		0.21	10.11
Grower						
1.30	86	1.31 ± 0.17	282 ± 6.24	103	1.51 ± 0.03	248 ± 12.65
3.50	377	6.78 ± 0.30	183 ± 8.00	444	7.13 ± 0.21	214 ± 9.31
4.60	479	8.60 ± 0.23	172 ± 1.46	522	8.85 ± 0.20	180 ± 7.59
5.70	567	8.74 ± 0.12	161 ± 2.86	626	9.61 ± 0.19	140 ± 3.56
6.70	629	9.22 ± 0.20	145 ± 1.36	706	10.29 ± 0.08	131 ± 7.39
7.79	713	9.62 ± 0.29	128 ± 3.82	803	10.11 ± 0.22	101 ± 4.85
8.89	776	9.51 ± 0.23	111 ± 1.93	850	9.87 ± 0.18	111 ± 7.48
RMSE[1] (d.f.)[2]		0.45	7.54		0.34	14.84
Finisher						
1.20	163	5.48 ± 0.28	344 ± 14.53	189	4.95 ± 0.65	330 ± 11.26
3.30	554	12.28 ± 0.27	294 ± 16.39	666	14.03 ± 0.57	278 ± 15.55
4.20	689	14.84 ± 0.21	252 ± 3.25	848	15.54 ± 0.59	251 ± 8.58
5.20	833	14.75 ± 0.77	237 ± 12.47	964	17.26 ± 0.93	185 ± 5.49
6.20	923	16.13 ± 0.40	192 ± 9.78	1112	18.51 ± 0.59	183 ± 3.91
7.19	1048	14.95 ± 0.59	229 ± 27.65	1261	18.82 ± 1.05	221 ± 9.60
8.19	1128	15.01 ± 0.47	192 ± 4.44	1359	18.34 ± 0.67	157 ± 6.76
RMSE[1] (d.f.)[2]		0.94	26.73		1.48	18.37

[1]RMSE: Residual mean square error; [2]d.f.: degrees of freedom

The fitted equations relating body weight gain, body protein deposition and body fat content to amino acid intake are presented in Tables 2-8 and 2-9 for MC and THR respectively. Also shown in the tables are, body weight gain, body protein deposition and body lipid content at the optimum intake, as well as the calculated optimum intakes of methionine + cysteine and threonine when measured in males and females in the initial, grower and finisher phases. The estimated intake for maximum BPd are closer to those estimated to maximal BWG. However, the estimated requirement for minimum BLc are higher than those for maximal BWG and BPd, this difference being more pronounced for the older birds.

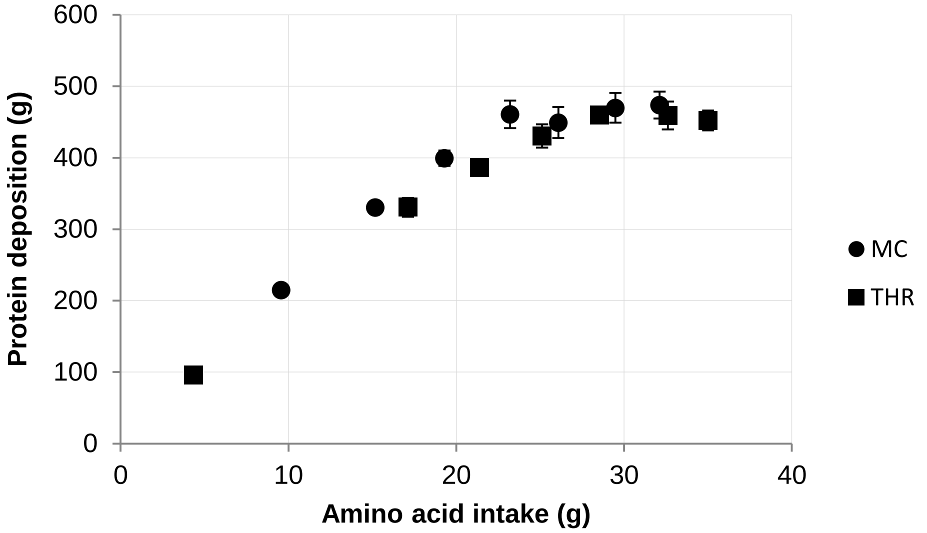

Figure 2-2. Protein deposition in male broilers after 42 d as a function of methionine+cysteine (MC) and threonine (THR) intake.

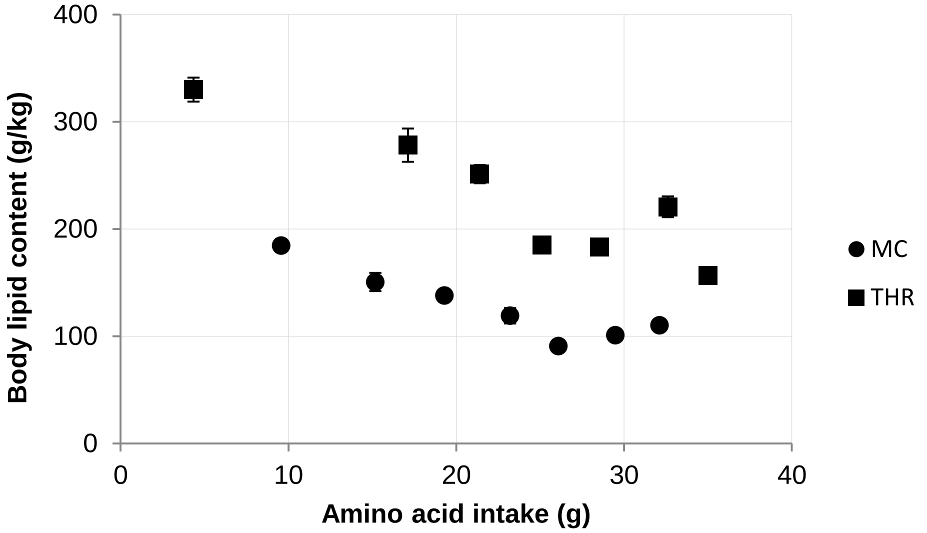

Figure 2-3. Body lipid content of male broilers after 42 days as a function of methionine+cysteine (MC) and threonine (THR) intake.

Other aspects of interest are the optimum responses predicted by the models. BWG, BPd and FCR are quite close to MC and THR in all phases, being closer with advancing age. But the optimum response in BLc, as expected, was lower for males in all phases and to both amino acids, and was much greater for THR, as already observed in THR responses.

Table 2-8. Fitted equations describing responses to methionine + cysteine, body weight gain (BWG, g/d), feed conversion ratio (FCR, g/g), body protein deposition (BPd, g/d) and body lipid content (BLc, g/kg) at optimum intake, and optimum intakes (MCi)

Variable	Equation	Optimum response	MCi†	MCi§
	Initial Phase			
	Females			
BWG	$27.85 = -0.0005*MCi^2 + 0.25*MCi - 2.68$	27.9	216	6.35
FCR	$1.22 = 0.00002*MCi^2 - 0.01*MCi + 2.75$	1.22	222	6.53
BPd	$4.75 = -0.00006*MCi^2 + 0.04*MCi - 0.53$	4.75	268	7.88
BLc	$57.71 = 0.0004*MCi^2 - 0.68*MCi + 219.20$	57.7	279	8.21
	Males			
BWG	$29.94 = -0.0005*MCi^2 + 0.25*MCi - 2.93$	29.9	230	6.39
FCR	$1.19 = 0.00002*MCi^2 - 0.01*MCi + 2.84$	1.19	236	6.56
BPd	$4.78 = -0.00006*MCi^2 + 0.04*MCi - 0.48$	4.78	245	6.81
BLc	$31.50 = 0.001*MCi^2 - 0.92*MCi + 214.80$	31.5	344	9.56
	Grower Phase			
	Females			
BWG	$56.38 = -0.0002*MCi^2 + 0.23*MCi - 6.14$	56.4	486	6.31
FCR	$1.42 = 0.000009*MCi^2 - 0.01*MCi + 4.49$	1.42	500	6.49
BPd	$9.97 = -0.00002*MCi^2 + 0.03*MCi - 0.19$	9.97	510	6.62
BLc	$61.03 = 0.0002*MCi^2 - 0.45*MCi + 265.10$	61.0	620	8.05
	Males			
BWG	$63.17 = -0.0002*MCi^2 + 0.23*MCi - 8.15$	63.2	543	6.39
FCR	$1.38 = 0.000007*MCi^2 - 0.01*MCi + 4.43$	1.38	546	6.42
BPd	$10.42 = -0.00002*MCi^2 + 0.03*MCi - 0.90$	10.4	564	6.64
BLc	$39.82 = 0.0002*MCi^2 - 0.45*MCi + 258.10$	39.8	810	9.53
	Finisher Phase			
	Females			
BWG	$86.69 = -0.00008*MCi^2 + 0.17*MCi - 6.18$	86.7	853	5.33
FCR	$1.87 = 0.000003*MCi^2 - 0.006*MCi + 5.05$	1.87	923	5.77
BPd	$14.97 = -0.00002*MCi^2 + 0.03*MCi - 1.16$	15.0	822	5.14
BLc	$132.00 = 0.0001*MCi^2 - 0.33*MCi + 320.29$	132	986	6.16
		Males		
BWG	$104.00 = -0.00008*MCi^2 + 0.18*MCi - 3.69$	104	1033	5.64
FCR	$1.80 = 0.000003*MCi^2 - 0.006*MCi + 5.63$	1.80	1053	5.75
BPd	$17.89 = -0.00001*MCi^2 + 0.04*MCi - 3.42$	17.9	1063	5.81
BLc	$90.00 = 0.0001*MCi^2 - 0.38*MCi + 340.15$	90.0	1161	6.34

† mg/bird d; § g/kg

Table 2-9. Fitted equations describing responses to threonine, body weight gain (BWG, g/d), feed conversion ratio (FCR, g/g), body protein deposition (BPd, g/d) and body lipid content (BLc, g/kg) at optimum intake, and optimum intakes (THRi)

Variable	Equation	Optimum response	THRi†	THRi§
		Initial Phase		
		Females		
BWG	$20.45 = -0.0004*THRi^2 + 0.18*THRi – 0.27$	20.5	204	7.03
FCR	$1.44 = 0.00007*THRi^2 – 0.03*THRi + 4.71$	1.44	180	6.21
BPd	$3.49 = -0.00007*THRi^2 + 0.03*THRi – 0.08$	3.49	182	6.28
BLc	$88.71 = 0.001*THRi^2 – 0.58*THRi + 180.60$	88.7	277	9.55
		Males		
BWG	$21.59 = -0.0003*THRi^2 + 0.16*THRi + 0.23$	21.6	228	7.60
FCR	$1.45 = 0.00006*THRi^2 – 0.03*THRi + 4.57$	1.45	186	6.20
BPd	$3.97 = -0.00005*THRi^2 + 0.03*THRi – 0.17$	3.97	235	7.83
BLc	$65.08 = 0.001*THRi^2 – 0.75*THRi + 223.80$	65.1	344	11.47
		Grower Phase		
		Females		
BWG	$58.18 = -0.0001*THRi^2 + 0.18*THRi – 2.92$	58.2	600	6.59
FCR	$1.66 = 0.00001*THR^2 – 0.02*THR + 6.85$	1.66	552	6.07
BPd	$9.27 = -0.00002*THRi^2 + 0.03*THRi – 0.98$	9.27	624	6.86
BLc	$116.90 = 0.0001*THRi^2 – 0.35*THRi + 308.03$	117	777	8.54
		Males		
BWG	$66.71 = -0.0001*THRi^2 + 0.18*THRi – 4.23$	66.7	743	7.36
FCR	$1.56 = 0.00001*THRi^2 – 0.02*THRi + 7.42$	1.56	668	6.61
BPd	$9.96 = -0.00002*THRi^2 + 0.03*THRi – 1.27$	9.96	722	7.15
BLc	$105.00 = 0.0003*THRi^2 – 0.53*THRi + 270.63$	105	909	9.00
		Finisher Phase		
		Females		
BWG	$85.18 = -0.00009*THRi^2 + 0.18*THRi – 1.54$	85.2	884	6.14
FCR	$1.76 = 0.000006*THRi^2 – 0.01*THRi + 7.12$	1.76	823	5.72
BPd	$15.21 = -0.00002*THRi^2 + 0.03*THRi + 0.86$	15.2	835	5.80
BLc	$199.80 = 0.000009*THRi^2 – 0.17*THRi + 374.44$	200	1077	7.48
		Males		
BWG	$104.80 = -0.00007*THRi^2 + 0.18*THRi – 1.79$	105	1070	6.18
FCR	$1.69 = 0.000004*THRi^2 – 0.009*THRi + 7.06$	1.69	1017	5.88
BPd	$18.56 = -0.00001*THRi^2 + 0.03*THRi + 0.25$	18.6	1220	7.05
BLc	$187.60 = 0.00002*THRi^2 – 0.17*THRi + 368.39$	188	1218	7.04

† mg/bird d; § g/kg

Discussion

In this study, based on the responses of broilers to MC and THR intake, the efficiency of utilisation of these amino acids was measured and their intakes required to maximize performance was estimated.

The technique of diet formulation, dilution or supplementation, affects directly the responses of animals to the amino acids intake. Gous and Morris (1985) favoured the use of the dilution technique for dose-response experiments involving individual amino acids, since it favors the expression of the productive potential of the poultry. According to Gous (1980), when this technique is used, the balance of amino acids remains constant between treatments so that the responses are the consequence of the limiting amino acid being tested without the response being confounded by imbalances or antagonisms. The responses obtained in this study appeared to be a true reflection of changes in the intake of the amino acids under test. That the responses were to the amino acids under test was confirmed with the positive response obtained to the supplementation of the lowest level of each of the amino acids with the industrial form of the amino acid.

The results of protein deposition and body lipid content presented in this paper confirm the reports of Leclercq (1998) and Gous (1998, 2007), that increasing the content of a limiting amino acid in the diet enhances deposition of protein and reduces the body lipid content in broilers, thereby changing the composition of the body weight gain. But this statement is only valid for birds fed diets formulated by the dilution technique. According to D’Mello (2003a), when comparing the dilution and graded supplementation techniques, it is possible to observe contrasting effects on carcass fat content of chicks fed levels of individual amino acids, and is important to consider methodological aspects when interpreting carcass fat responses to amino acid intake. This is due to the fact that the birds have a genetic potential for growth and protein deposition, and offering them a diet with the same energy content, but deficient in protein or amino acid, they will consume more feed in an attempt to reach their potential growth, consuming more energy than necessary and depositing more fat (Emmans, 1981, 1989; Kidd et al., 1996).

Results of the THR response trial are in accordance with those found by Kidd et al. (1996), being that even the highest levels of this amino acid were not sufficient to decrease body lipid content, especially when compared to the results observed in the MC trial. This lack of effect of THR on carcass fat has been reported previously (Leclercq, 1998; Kidd et al., 1999; Dozier et al., 2000a,b; 2001). The abdominal fat depot is suggested to be the least sensitive response criterion for dietary THR, but not for MC (Dozier, 2000b). This fact is related to the utilisation of THR by broilers, since this amino acid is heavily involved in feather formation, although the percentage of THR in the whole carcass is similar to that of feathers (Fisher et al., 1981, Stilborn et al., 1997). Once assimilated in feathers, there is no turnover of THR for reutilisation in protein synthesis (Dozier, 2000a). On the other hand, MC acts as a lipotropic agent (Andi, 2012), playing a role in carnitine synthesis, which stimulates the oxidative metabolism of lipids (Schutte et al., 1997), thus reducing abdominal fat (Andi, 2012) and, consequently, body lipid content.

For the reason cited above, protein or amino acid deposition was used to calculate the efficiency of utilisation of each amino acid, rather than body weight gain. In the present study, the contents of MC and THR measured in the feather-free body and feathers are consistent with the literature (Sklan and Noy, 2004; Stilborn et al., 2010; Silva, 2012).

Some authors suggest that the efficiencies of utilisation are different for each amino acid and that differences must be due to species and genotype (Edwards et al. 1999; Fatufe et al., 2004). Other authors report that these factors and some others like age, sex, environmental temperature, immunological stress and diet nutritional composition exert their effects by affecting feed intake, and not specifically the amino acids utilisation efficiency (D'Mello, 2003a).

In the case of poultry, feathers grow at a different rate to that of the rest of the body (Emmans, 1989; Gous et al., 1999) and also the rates differ between sexes, since birds are feather-sexable, thus females have feather growth rates greater than males (Marcato et al. 2009). Therefore, it was expected that there would be differences in the efficiency of utilisation of amino acids, especially MC, between phases and between the sexes. However, no differences were observed for any of the amino acids studied, since the slopes of the lines were similar for both sexes, as well as in the different growth phases. Therefore we determined a single efficiency of utilisation to describe the average daily requirement of these amino acids.

Heger and Frydrych (1989) reported that the relative concentration of the amino acid in the diet is the main factor that affects the efficiency of utilisation, the efficiency being higher when the amino acid is deficient, and the increased consumption of the limiting amino acid makes its efficiency of utilisation decrease, because a greater proportion of this amino acid is allocated to alternative metabolic processes which do not deposit protein. For this reason, we used only the results of the four most deficient levels of MC and THR to obtain their efficiencies of utilisation. We obtained similar values to those found in the literature for MC and intermediate value for THR (Edwards et al. 1997; Edwards and Baker, 1999; Stilborn et al., 2010).

Edwards et al. (1997) also reported that the efficiencies of utilisation of individual amino acids are different, as observed in this study. One explanation for this difference is that essential amino acids have different degradation rates. When studying THR requirements for maintenance in the chick, Edwards et al. (1997) found negative THR retention for the groups that received diets with 0.05, 0.10 and 0.15 of the ideal requirements, and an efficiency of THR utilisation of 0.82. In another study, Edwards and Baker (1999) observed only positive retention in chicks fed graded levels of MC. But in this case, the efficiency of utilisation was only 0.52, a reflection of the diverse functions of methionine beyond its role in protein synthesis (D'Mello, 2003b).

The MC intakes for maximum response reported here are in accordance with the findings of some other authors. The intake to maximize FCR exceeded that for maximizing body weight gain (Schutte and Pack, 1995; Baker et al., 1996; Mack et al., 1999), suggesting that at marginal deficiencies of methionine birds consume more feed to meet their requirement. Chamruspollert et al. (2002) have also reported a higher methionine intake to maximize protein synthesis in broiler chicks when compared to that for maximum growth, as found in this trial, suggesting that methionine should be supplemented more in initial diets, to reach the maximum protein synthesis and maximum lean body yield, but in grower and finisher phases, as these requirements are very close to each other.

However, the intake of THR to maximize body weight gain exceeded that for maximum FCR. This result is contrary to the findings of Kidd and Kerr (1997) and Baker et al. (2002) who found that the intake required to maximize BWG and FCR was the same. On the other hand, Mack et al. (1999) and Ahmadi and Golian (2010) found that intake of THR had to be higher to maximize FCR than that required to maximize BWG. This could be due to the formulation technique used in the present trial, which resulted in less change in carcass composition over the range of THR intakes than in the case of MC. More energy is required for fat deposition than for protein deposition (Wallis, 1999) which may also explain the difference in intake of THR required to maximize body weight gain and protein gain, especially for female broilers which deposited more fat than males.

In conclusion, the efficiency of utilisation of MC and THR, for both male and female broilers are, respectively, 0.56 and 0.73. The MC intakes for optimum FCR were 231 mg/d (7.00 g/kg) and 524 mg/d (4.68 g/kg) in the initial and grower phases, for both males and females, and 923 mg/d (5.27 g/kg) and 1053 mg/d (5.40 g/kg) for females and males, respectively, in the finisher phase. THR intakes were 184 mg/d (5.58 g/kg), 612 mg/d (5.46 g/kg) and 823 mg/d (4.45 g/kg) in the initial, grower and finisher phases, respectively, for both males and females.

The knowledge of how broilers respond to incremental intakes of amino acids enables the nutritionist to define optimum economic intakes of different amino acids, but to convert these into dietary concentrations for feed formulation purposes the expected food intake of broilers needs first to be predicted.

Acknowledgements

To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support to this project and scholarship.

References

AHMADI, H. AND GOLIAN, A. (2010) The integration of broiler chicken threonine responses data into neural network models. *Poultry Science* 89, 2535-2541.

ANDI, M.A. (2012) Effects of additional DL-methionine in broiler starter diet on blood lipids and abdominal fat. *African Journal of Biotechnology* 11, 7579-7581.

BAKER, D.H. (2003) Ideal amino acid patterns for broiler chicks. In: D'Mello, J.P.F. *Amino acid in animal nutrition*. 2nd edn. CABI Publishing, Wallingford, Oxon/UK, pp. 223- 236.

BAKER, D.H.; BATAL, A.B.; PARR, T.M., AUGSPURGER, N.R. AND PARSONS, C.M. (2002) Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine and valine for chicks during the second and third week of life. *Poultry Science* 81, 485-494.

BAKER, D.H.; FERNANDEZ, S.R.; WEBEL, D.M. AND PARSONS, C.M. (1996) Sulfur amino acid requirement and cysteine replacement value of broiler chicks during the period three to six weeks posthatching. *Poultry Science* 75, 737–742.

BONATO, M.A.; SAKOMURA, N.K.; SIQUEIRA, J.C.; FERNANDES, J.B.K. AND GOUS, R.M. (2011) Maintenance requirements for methionine and cysteine, and threonine for poultry. *South African Journal of Animal Science* 41, 209-222.

CHAMRUSPOLLERT, M.; PESTI, G.M. AND BAKALLI, R.I. (2002) Determination of the methionine requirement of male and female broiler chicks using an indirect amino acid oxidation method. *Poultry Science* 81, 1004-1013.

COSTA, F.G.P.; ROSTAGNO, H.S.; ALBINO, L.F.T.; GOMES, P.C.; TOLEDO, R.S. AND VARGAS JR., J.G. (2001) Níveis Dietéticos de Proteína Bruta para Frangos de Corte de 1 a 21 e 22 a 42 Dias de Idade. *Revista Brasileira de Zootecnia* 30, 1498-1505.

D'MELLO, J.P.F. (2003a) Responses of growing poultry to amino acids. In: D'MELLO, J.P.F. *Amino acid in animal nutrition*. 2nd edn. CABI Publishing, Wallingford, Oxon/UK, pp. 237- 264.

D'MELLO, J.P.F. (2003b) Amino acids as multifunctional molecules. In: D'MELLO, J.P.F. *Amino acid in animal nutrition*. 2nd edn. CABI Publishing, Wallingford, Oxon/UK, pp. 1- 14.

DOZIER III, W.A.; MORAN JR., E.T. AND KIDD, M.T. (2000a) Responses of fast- and slow-feathering male broilers to dietary threonine during 42 to 56 days of age. *Journal of Applied Poultry Research*. 9, 460-467.

DOZIER III, W.A.; MORAN JR., E.T. AND KIDD, M.T. (2000b) Threonine requirement for broiler males from 42 to 56 days of age. *Journal of Applied Poultry Research* 9, 214-222.

DOZIER III, W.A.; MORAN JR., E.T. AND KIDD, M.T. (2001) Male and female broiler responses to low and adequate dietary threonine on nitrogen and energy balance. *Poultry Science* 80, 926-930.

EDWARDS, H.M. AND BAKER D. (1999) Maintenance sulfur amino acid requirements of young chicks and efficiency of their use for accretion of whole-body sulfur amino acids and protein. *Poultry Science* 78, 1418-1423.

EDWARDS, H.M.; BAKER, D.H.; FERNANDEZ, S.R. AND PARSONS, C.M. (1997) Maintenance threonine requirement and efficiency of its use for accretion of whole-body threonine and protein in young chicks. *British Journal of Nutrition* 78, 111-119.

EDWARDS, H.M.; FERNANDEZ, S.R. AND BAKER, D.H. (1999) Maintenance lysine requirement and efficiency of using lysine for accretion of whole-body lysine and protein in young chicks. *Poultry Science* 78, 1412-1417.

EMMANS, G.C. (1981) A model of the growth and feed intake of ad libitum fed animals, particularly poultry. In: Hillyer, G.M.; Whittemore, C.T. and Gunn, R.G. *Computers in Animal Production*. British Society of Animal Production. Edinburgh/UK, pp. 106 – 110.

EMMANS, G.C. (1989) The growth of turkeys. In: Nixey, C. and Grey, T.C. *Recent Advances in Turkey Science*. Butterworths, London/UK, pp. 135 – 166.

FATUFE, A.A.; TIMMBER, R. AND RODEHUTSCORD, M. (2004) Response to lysine in composition of body weight gain and efficiency of lysine utilisation of growing male chickens from two genotypes. *Poultry Science* 83, 1314- 1324.

FISHER, C. AND MORRIS, T.R. (1970) The determination of the methionine requirements of laying pullets by a diet dilution technique. *British Poultry Science* 11, 67-82.

FISHER, M.L.; LEESON, S.; MORRISON, W.D. AND SUMMERS, J.D. (1981) Feather growth and feather composition of broiler chickens. *Canadian Journal of Animal Science* 61, 769-773.

GOUS, R.M. (1980) An improved method for measuring the response of broiler chickens to increasing dietray concentrations of an amino acid. In: *Proceedings of European Poultry Conference.* World's Poultry Science Association, Hamburg/Germany, pp.32–39.

GOUS, R.M. (1998) Making progress in the nutrition of broilers. *Poultry Science* 77, 111-117.

GOUS, R.M. (2007) Methodologies for modeling energy and amino acid responses in poultry. *Revista Brasileira de Zootecnia* 36, 263-275 (Suplemento).

GOUS, R.M. AND MORRIS, T.R. (1985) Evaluation of a diet dilution technique for measuring the response of broiler chickens to increasing concentrations of lysine. *British Poultry Science* 26, 147-161.

GOUS, R.M.; MORAN JR., E.T.; STILBORN, H.R.; BRADFORD, G.D. AND EMMANS, G.C. (1999) Evaluation of the paramenters needed to describe the overall growth, the chemical growth, and the growth of feathers and breast muscles of broilers. *Poultry Science* 78, 812-821.

HEGER, J. AND FRYDRYCH, Z. (1989) Efficiency of utilisation of amino acids. In: Friedman, M. *Absorption and utilisation of amino acids***.** CRC Press, Boca Raton, Florida/USA, pp. 31- 56.

KIDD, M.T. AND KERR, B.J. (1997) Threonine responses in commercial broilers at 30 to 42 days. *Journal of Applied Poultry Research* 6, 362-367.

KIDD, M.T.; KERR, B.J.; FIRMAN, J.D. AND BOLING, S.D. (1996) Growth and carcass characteristics of broilers fed low-protein, threonine-supplemented diets. *Journal of Applied Poultry Research* 5, 180-190.

KIDD, M.T.; LERNER, S.P.; ALLARD, J.P.; RAO, S.K. AND HALLEY, J.T. (1999) Threonine needs of finishing broilers: An evaluation of growth, carcass, and economic responses. *Journal of Applied Poultry Research* 8, 160–169.

KINO, K. AND OKUMURA, J.-I. (1987) Whole-body protein turnover in chicks fed control, histidine, or methionine plus cysteine-free diets. *Poultry Science* 66, 1392-1397.

LECLERCQ, B. (1998) Specific effects of lysine on broiler production: comparison with threonine and valine. *Poultry Science* 77, 118-123.

MACK, S.; BERCOVICI, D.; DEGROOTE, G.; LECLERCQ, B.; LIPPENS, M.; PACK, M.; SCHUTTE, J.B. AND VAN CAUWENBERGHE, S. (1999) Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. *British Poultry Science* 40, 257–265.

MARCATO, S.M.; SAKOMURA, N.K.; FERNANDEZ, J.B.K.; NASCIMENTO, D.C.N.; FURLAN, R.L. AND PIVA, G.H. (2009) Crescimento e deposição de nutrientes nas penas, músculo, ossos e pele de frangos de corte de duas linhagens comerciais. *Ciência e Agrotecnologia* 33, 1159-1168.

ROSTAGNO, H.S.; ALBINO, L.F.T.; DONZELE, J.L.; GOMES, P.C.; OLIVEIRA, R.F.M.; LOPES, D.C.; FERREIRA, A.S. AND BARRETO, S.L.T. (2005) *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais*. 2nd edn. Universidade Federal de Viçosa, Viçosa, Minas Gerais/Brazil.

SAKOMURA, N.K. AND ROSTAGNO, H.S. (2007) *Métodos de pesquisa em nutrição de monogástricos*. Funep, Jaboticabal, São Paulo/Brazil.

SCHUTTE, J.B. AND PACK, M. (1995) Sulfur amino acid requirement of broiler chicks from fourteen to thirty eight days of age. 1.Performance and carcass yield. *Poultry Science* 74, 480–487.

SCHUTTE, J.B.; DE JONG, J.; SMINK, W. AND PACK, M. (1997) Replacement value of betaine for DL-methionine in male broiler chicks. *Poultry Science* 76, 321-325.

SILVA, E.P. (2012) Modelos de crescimento e das respostas de frangas de postura submetidas a diferentes ingestões de aminoácidos sulfurados. Phd thesis. Universidade Estadual Paulista “Júlio de Mesquita Filho”, Jaboticabal, São Paulo, Brazil.

SKLAN, D. AND NOY, Y. (2004) Catabolism and deposition of amino acids in growing chicks: effect of dietary supply. *Poultry Science* 83, 952 - 961.

STILBORN, H.L.; MORAN JR., E.T.; GOUS, R.M. AND HARRISON, M.D. (1997) Effect of age on feather amino acid content in two broiler strain crosses and sexes. *Journal of Applied Poultry Research* 6, 205-209.

STILBORN, H.L.; MORAN JR., E.T.; GOUS, R.M. AND HARRISON, M.D. (2010) Influence of age on carcass (feather-free) amino acid content for two broiler strain-crosses and sexes. *Journal of Applied Poultry Research* 19, 13-23.

WALLIS, I.R. (1999) Dietary supplements of methionine increase breast meat yield and decrease abdominal fat in growing broiler chickens. *Australian Journal of Experimental Agriculture* 39, 131-141.

CAPÍTULO 3 - IMPACT OF DIETARY METHIONINE+CYSTEINE AND THREONINE ON EFFICIENCY OF NITROGEN UTILISATION IN BROILERS AND EXCRETION TO ENVIRONMENT

DONATO, D.C.Z.; SAKOMURA, N.K.

[1]Animal Science Department, Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil;
sakomura@fcav.unesp.br

Abstract

There is a concern of researchers to seek alternatives that aim to minimize the emission of pollutants into the environment. Nitrogen (N) is one of the most critical pollutants and to find ways to reduce N excretion by the animals is of extremely importance. The objectives of this research was to evaluate the responses of N excretion (NE) and N deposition (ND) in broilers to methionine+cysteine and threonine intake and determine the efficiency of utilisation of both amino acids for protein deposition. Six trials were conducted to measure the NE and ND in broiler chickens during three rearing phases to dietary amino acid. The efficiency of utilisation of the amino acids studied was calculated by linear regression of body protein deposition and the amino acid intake. In despite of the differences between sexes and phases, the efficiency of utilisation remained unchanged, being 0.60 and 0.59 for methionine+cysteine and threonine, respectively. The rate of NE has exponential behavior, increasing with amino acid intake. At the amino acid intake where protein deposition is maximal, NE is 50% lower than the maximum excretion (reached at maximum amino acid intake). For each unit of MC or THR intake reduction there is a reduction on NE of 0.5%.

Keywords: amino acids, environmental pollution, poultry

Introduction

There is a growing concern of researchers to seek alternatives that aim to minimize the emission of pollutants into the environment, since there is an intensification and regional conglomeration trend in livestock which increase these pollutants emission (OENEMA et al., 2007).

The European Union has already implemented laws regulating environmental pollution, especially with regard to the emission of nitrogen (N) compounds, judged as one of the most critical pollutants, which affect life on earth in various ways (NEUMANN et al., 2011). According to Velthof et al. (2013), this includes pollution of groundwater due to nitrate leaching, eutrophication of surface water, soil acidification, global warming and stratospheric ozone destruction, caused by the oxidation of nitrate (N_2O) through the reaction $O(^1D) + N_2O \rightarrow 2NO$ in the stratosphere (CRUTZEN & EHHALT, 1977), and also impacts on human health and plants.

Approximately 52% of the N excreted in animal housing systems is used as plant nutrient, whereas 48% is lost to the environment (OENEMA et al., 2007). So, finding ways to reduce N excretion by the animals is of extreme importance, and reducing the dietary crude protein content and supplying the correct amount of essential amino acids, increasing the N efficiency utilisation by the animals, seems to be an effective measure.

Researches have shown that is possible to formulate diets with low crude protein level and with amino acid profile closer to broilers requirement without compromising performance (CAUWENBERGHE & BURNHAM, 2001; Si, 2004; FARIA FILHO et al., 2005; GOMIDE et al., 2011). Body protein deposition depends on broilers' maximum genetic potential and an excess of protein or an amino acid imbalance in the diet can promote an excessive amino acid load on bloodstream to be metabolized, requiring extra spending energy, which is diverted from production to N excretion processes in the synthesis of uric acid (ALETOR et al., 2000). Formulating diets with amino acid on a digestible basis is a way to reduce dietary crude protein and amino acids. The knowledge on how broilers respond to amino acid intake and its efficiency of utilisation to protein deposition can also allow the reduction of nitrogen excretion.

The objective of this research was to evaluate the nitrogen excretion (NE) and nitrogen deposition (ND) by males and females broilers to dietary methionine+cysteine and threonine, and determine the efficiency of utilisation of both amino acids for protein deposition.

Material & Methods

Birds and Housing

Six trials were conducted at the Poultry Science Laboratory of Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal Campus in São Paulo, Brazil, designed to measure the response of broiler chickens during three rearing phases (1-14, 15-28 and 29-42 d) to dietary amino acids.

Five hundred and sixty male and female broilers (totaling 1,120 broilers per trial and 3360 broilers per amino acid studied) were distributed in a completely random design in each trial, according to a 7 × 2 factorial (7 levels of dietary amino acids and 2 sexes) totaling 14 treatments, with 4 replicates. Each experimental unit comprised 20 birds. Cobb 500® broilers were used in all the trials. At the beginning of each trial, birds were individually weighed and distributed such that each experimental unit had homogeneous weight.

Experimental Diets

The experimental diets were formulated using the dilution technique. A high protein summit diet was formulated to contain approximately 1.2 times the digestible MC and THR levels suggested by Rostagno et al. (2005) for broilers during the respective phases, and all other essential amino acids were set at a minimum of 1.4 times their suggested levels. These summit diets were diluted sequentially with isoenergetic, protein-free diets (N free) (FISHER & MORRIS, 1970) (Table 3-1), to create a range of feeds increasing in the studied amino acids content (MC: 1-14 d: 3.05 to 10.88; 15-28 d: 2.72 to 9.79, and 29-42 d: 2.50 to 9.03 g MC/kg) (THR: 1-14 d: 1.49 to 9.96; 15-28 d: 1.30 to 8.86, and 29-42 d: 1.20 to 8.17 g THR/kg).

Table 3-1. Composition (g/kg) and analyzed nutrient content (g/kg) of the summit and N-free diets used in each phase of the trial

Ingredients (%)	Diets		
	MC Summit	THR Summit	N-Free
Soybean meal (45%)	510	510	-
Maize	339	339	-
Soybean oil	65.0	65.0	100
Corn gluten meal (60%)	37.3	36.8	
Dicalcium phosphate	20.3	20.3	27.0
Limestone	9.45	9.45	5.07
Salt	4.38	4.38	5.13
DL- Methionine (99%)	3.28	5.12	-
L-Lysine (78.5%)	4.89	4.87	-
L-Threonine (99%)	2.01	0.33	-
L-Valine (96.5%)	1.40	1.40	-
Choline chloride (60%)	1.00	1.00	1.00
L-Arginine (98.5%)	0.54	0.53	
Mineral Premix[1]	0.50	0.50	0.50
Vitamin Premix[2]	0.50	0.50	0.50
Potassium chloride	-	-	11.4
Starch	-	-	428
Sugar	-	-	150
Rice Husk	-	-	150
Anticoccidiostatic[3]	0.50	0.50	0.50
Growth promotor[4]	0.05	0.05	0.05
Antioxidant[5]	0.10	0.10	0.10
Inert[6]	-	-	121
Nutrients (g/kg)			
Metabolizable energy (kcal/kg)[7]	3050	3050	3050
Crude Protein	294	298	8.90
Methionine+Cysteine[8]	9.04	10.8	-
Methionine[8]	5.82	7.48	-
Lysine[8]	15.8	17.1	-
Tryptophan[8]	3.14	2.81	-
Threonine[8]	10.2	9.99	-
Arginine[8]	16.1	16.7	-
Valine[8]	12.3	14.1	-
Isoleucine[8]	9.70	11.3	-
Leucine[8]	21.2	23.1	-
Phenylalanine[8]	12.9	13.7	-
Calcium	10.0	10.0	10.0
Sodium	2.20	2.20	2.20
Available phosphorus	5.00	5.00	5.00

[1]Content/kg of product: Mn = 150,000 mg, Fe = 100,000 mg, Zn = 100,000 mg, Cu= 16,000 mg, I = 1,500 mg, [2]Content/kg of product: Folic acid = 1000 mg, pantothenic acid = 15,000 mg, Niacin = 40,000 mg, Biotin = 60 mg, vit B1 = 1,800 mg, vit, B12 = 12,000 mg, vit, B2 = 6,000 mg, vit, B6 = 2,800 mg, vit D3 = 2,000,000 UI, vit E = 15,000 mg, vit, K3 = 1,800 mg, Se = 300 mg, antioxidant = 500 mg, [3]coxistac, [4]Zinc bacitracin, [5]BHT, [6]Wash sand, [7]Predicted value, [8]Digestible amino acid composition,

Dilution scheme (summit:N-free): MC - Initial phase: 0.28:0.72; 0.40:0.60; 0.52:0.48; 0.64:0.36; 0.76:0.24; 0.88:0.12; 1.00:0.00; Grower phase: 0.25:0.75; 0.36:0.64; 0.47:0.53; 0.58:0.42; 0.68:0.32; 0.79:0.21; 0.90:0.10; Finisher phase: 0.23:0.77; 0.33:0.67; 0.43:0.57; 0.53:0.47; 0.63:0.37; 0.73:0.27; 0.83:0.17. THR – Initial phase: 0.15:0.85; 0.40:0.60; 0.52:0.48; 0.64:0.36; 0.76:0.24; 0.88:0.12;

1.00:0.00; Grower phase: 0.13:0.87; 0.35:0.65; 0.46:0.54; 0.57:0.43; 0.67:0.33; 0.78:0.22; 0.89:0.11; Finisher phase: 0.12:0.88; 0.33:0.67; 0.42:0.58; 0.52:0.48; 0.62:0.38; 0.72:0.28; 0.82:0.18.

To verify whether the responses were due to studied amino acid limitation in the dilution series, a pilot trial to each amino acid was conducted with 30 male Cobb broilers from 1 to 14 days for MC and from 7 to 21 days for THR. They were distributed in a completely random design in two treatments in each trial, being the lowest studied levels for each amino acid and the control treatment. This treatment had the same nutritional composition as the lowest treatment in the dilution series, but it was supplemented with the test amino acid to reach the same level of the second treatment. BWG and FCR were evaluated.

Measures and Chemical Analysis

Nitrogen intake (NI, g/bird/d) was calculated from the feed intake (NI = FI×Ndiet/100). Nitrogen deposition (ND, g/bird/d) was determined using the comparative slaughter technique, where 6 birds at the beginning of each phase and 2 birds of each experimental unit at the end of each phase were slaughtered and feather-free body and feathers were analyzed by Kjeldahl method, from which the amount of nitrogen deposited during each phase was calculated. Nitrogen excretion (NE, g/bird/d) was calculated as the difference between the nitrogen intake and the deposition (NE = NI – ND). It was also evaluated the ratio ND/NI and NE/ND.

Statistical Analyses

All statistical analyses were performed in SAS 9.1 software (2009) using PROC NLIN. A statistical analysis of variance was conducted on each of the variables measured.

In order to estimate the rate of NE an exponential regression model $Y=a.e^{b.X}$ (Eq. 1) (Samadi & Liebert, 2006; 2007) was fitted to NE data in function of amino acid intake for each studied phase and sex, where Y is NE (g/bird/d), a is the intercept, b is the rate of excretion and X is the amino acid intake (mg/bird/d). This model has a biological interpretation for NE, since NE increases with amino acid intake, and increases exponentially after ND reaches the plateau. When amino acid intake is null the *a* coefficient represents the NE, in this case, the endogenous losses of N.

To determine the maximum rate of protein deposition in each phase and for each sex was fitted a linear response plateau model Y=L+u(R-X) if X<R; if X>=R, then Y=L (Eq. 2), where Y is protein deposition (g/bird/d), L is maximum protein deposition (g/bird/d), u is the slope, which is the coefficient of response to amino acid intake, and the inverse of the efficiency of utilisation of amino acids for protein deposition, R is the break point and X is the amino acid intake (mg/bird/d).

To test the hypotheses null – the coefficients are similar to both sexes and all phases; and alternative – the coefficients are different at least for one sex and/or phase, the *a* and *b* coefficients estimated by Eq. 1 and *u* coefficients estimated by Eq. 2 for each phase and sex were compared using a likelihood ratio test for non-linear models described by Regazzi (2003).

Results

Pilot Trial

Results of the pilot trials confirmed that MC and THR were the limiting amino acids in the respective dilution series. The addition of industrial MC and THR to the feed with the lowest contents of these amino acids improved both BWG (7.79 g/bird/d vs 5.18 g/bird/d for MC and 30.05 g/bird/d vs 27.55 g/bird/d for THR) and FCR (2.65 g/g vs 2.98 g/g for MC and 2.28 g/g vs 2.45 g/g for THR).

Nitrogen Intake, Excretion and Deposition

The results of analysis of variance of the variables NI, ND, NE, ND/NI and NE/ND of the MC trial are shown in Table 3-2. The mean nitrogen intake, deposition and excretion by broilers subjected to increasing dietary levels of MC are presented in Table 3-3 and in Table 3-4 are presented the nitrogen deposition and nitrogen intake (ND/NI) and nitrogen excretion and nitrogen deposition (ND/NI) ratios. Figure 3-1 illustrates nitrogen deposited and excreted (and the sum of both is the nitrogen intake) for male broilers from one to 42 days of age, in function of MC intake.

In Table 3-2 it is observed an interaction between sex and treatment in grower phase for all evaluated variables, except NE/ND. In finisher phase the interaction was not significant only for NE in MC trial.

Table 3-2. Variance analysis results of the variables nitrogen intake (NI), nitrogen deposition (ND), nitrogen excretion (NE), ND/NI ratio and NE/ND ratio of the methionine+cysteine trial

Statistic	Variables				
	NI	ND	NE	ND/NI	NE/ND
			Initial		
F MC	<0.01	<0.01	<0.01	<0.01	<0.01
F Sex	<0.01	<0.01	0.28	0.06	0.32
F MC*Sex	0.86	0.32	0.19	0.06	0.10
			Grower		
F MC	<0.01	<0.01	<0.01	<0.01	<0.01
F Sex	<0.01	0.17	<0.01	<0.01	<0.01
F MC*Sex	<0.01	<0.05	<0.05	<0.05	0.08
			Finisher		
F MC	<0.01	<0.01	<0.01	<0.01	<0.01
F Sex	<0.01	<0.01	<0.01	<0.01	0.13
F MC*Sex	<0.01	<0.01	0.97	<0.01	<0.05

In Table 3-3 it is observed that in all phases and for both sexes NI, ND and NE increased with dietary amino acid, but ND increased until reached a plateau (Figure 3-1), whereas NE increases exponentially, as shown in Table 3-8. Although the differences between sexes in NE for MC in grower phase, both parameters of exponential fitted equations were the same, according to the methodology described by Regazzi (2003).

The ratio ND/NI (Table 3-4), as expected, decreases as the level of MC in the diet, and the MC intake, increases, since the efficiency of amino acid utilisation for nitrogen deposition is higher when the amino acid is deficient.

Also as expected, the ratio NE/ND (Table 3-4) in the MC trial increases as the level of amino acid in the diet increases. With the knowledge that there is a genetic limit for nitrogen deposition, after this limit the nitrogen excretion will increase exponentially with the amino acid intake.

In Table 3-5 it is possible to observe an interaction between sex and treatment in initial phase only for NI and ND in THR trial. In grower phase there was interaction only for NI, and in finisher phase the interaction was not significant for NE and ND/NI and NE/ND in THR trial.

As for MC, NI, ND and NE increased with dietary THR increase (Table 3-6) in all phases and for both sexes, reaching the same ND plateau (Figure 3-2), and the same exponential behavior of NE, as shown in Table 3-8. As observed in Figures 3-1 and 3-2, NE can exceed 50% of NI (the sum of ND and NE).

Table 3-3. Average nitrogen intake (NI, g/bird/d), deposition (ND, g/bird/d) and excretion (NE, g/bird/d) of broiler males and females in response to dietary methionine+cysteine (MC, g/kg) during three rearing phases

	Females	Males	Females	Males	Females	Males
MC	NI		ND		NE	
Initial Phase (1-14 days)						
2.53	0.247 ± 0.06	0.280 ± 0.02	0.200 ± 0.05	0.255 ± 0.02	0.047 ± 0.02	0.025 ± 0.01
3.62	0.502 ± 0.07	0.584 ± 0.02	0.426 ± 0.08	0.521 ± 0.02	0.076 ± 0.01	0.063 ± 0.02
4.70	0.763 ± 0.03	0.804 ± 0.01	0.571 ± 0.23	0.627 ± 0.05	0.192 ± 0.05	0.177 ± 0.05
5.79	0.962 ± 0.02	1.005 ± 0.01	0.689 ± 0.04	0.731 ± 0.01	0.273 ± 0.04	0.274 ± 0.02
6.87	1.111 ± 0.02	1.167 ± 0.02	0.776 ± 0.02	0.803 ± 0.06	0.335 ± 0.02	0.363 ± 0.05
7.96	1.273 ± 0.05	1.324 ± 0.02	0.750 ± 0.02	0.771 ± 0.05	0.522 ± 0.04	0.553 ± 0.04
9.04	1.356 ± 0.03	1.417 ± 0.04	0.754 ± 0.02	0.751 ± 0.02	0.603 ± 0.04	0.666 ± 0.05
RMSE[1] (27 d.f.)[2]	0.04	0.02	0.04	0.04	0.03	0.04
Grower Phase (14-28 days)						
2.26	0.910 ± 0.03	0.924 ± 0.02	0.843 ± 0.09	0.760 ± 0.05	0.067 ± 0.06	0.164 ± 0.06
3.25	1.378 ± 0.04	1.474 ± 0.04	1.197 ± 0.08	1.174 ± 0.04	0.181 ± 0.05	0.300 ± 0.01
4.25	1.788 ± 0.06	1.929 ± 0.03	1.389 ± 0.06	1.400 ± 0.10	0.399 ± 0.06	0.529 ± 0.08
5.24	2.141 ± 0.04	2.341 ± 0.01	1.468 ± 0.05	1.667 ± 0.10	0.673 ± 0.03	0.674 ± 0.10
6.15	2.324 ± 0.04	2.521 ± 0.02	1.545 ± 0.03	1.579 ± 0.07	0.780 ± 0.07	0.941 ± 0.07
7.14	2.617 ± 0.07	2.872 ± 0.04	1.722 ± 0.02	1.690 ± 0.05	0.936 ± 0.02	1.182 ± 0.08
8.14	2.825 ± 0.07	3.103 ± 0.10	1.644 ± 0.06	1.736 ± 0.14	1.181 ± 0.11	1.367 ± 0.07
RMSE[1] (27 d.f.)[2]	0.05	0.04	0.06	0.09	0.07	0.07
Finisher Phase (28-42 days)						
2.08	1.769 ± 0.05	2.004 ± 0.05	1.557 ± 0.04	1.410 ± 0.05	0.213 ± 0.02	0.594 ± 0.05
2.98	2.572 ± 0.06	3.033 ± 0.05	2.030 ± 0.13	2.172 ± 0.16	0.542 ± 0.12	0.861 ± 0.19
3.89	3.257 ± 0.11	3.739 ± 0.13	2.378 ± 0.09	2.590 ± 0.19	0.879 ± 0.19	1.150 ± 0.07
4.79	3.864 ± 0.12	4.440 ± 0.10	2.404 ± 0.11	2.668 ± 0.22	1.460 ± 0.10	1.741 ± 0.15
5.70	4.431 ± 0.11	5.063 ± 0.07	2.411 ± 0.19	2.715 ± 0.32	2.020 ± 0.13	2.347 ± 0.27
6.60	4.990 ± 0.12	5.696 ± 0.12	2.390 ± 0.05	2.924 ± 0.12	2.600 ± 0.14	2.818 ± 0.11
7.50	5.414 ± 0.04	6.254 ± 0.09	2.425 ± 0.27	2.939 ± 0.19	2.989 ± 0.26	3.315 ± 0.14
RMSE[1] (27 d.f.)[2]	0.09	0.09	0.15	0.20	0.15	0.16

[1]RMSE: Residual mean square error; [2]d.f.: degrees of freedom

The ratio ND/NI for THR (Table 3-7) showed the same behavior as for MC, decreasing as the level of THR in the diet, and the THR intake, increases. Comparing both amino acids, it is possible to observe that ND/NI ratio was a little higher in MC than in THR trial, especially at lower levels, showing that the nitrogen deposition in MC trial was higher.

Table 3-4. Average nitrogen deposition and intake ratio and nitrogen excretion and deposition ratio of broiler males and females in response to dietary methionine+cysteine (MC, g/kg) during three rearing phases (all data)

MC	Females	Males	Females	Males
	ND/NI		NE/ND	
	Initial Phase (1-14 days)			
2.53	0.805 ± 0.09	0.909 ± 0.04	0.253 ± 0.13	0.101 ± 0.05
3.62	0.846 ± 0.04	0.892 ± 0.03	0.184 ± 0.06	0.122 ± 0.04
4.70	0.750 ± 0.05	0.780 ± 0.06	0.338 ± 0.09	0.288 ± 0.10
5.79	0.716 ± 0.04	0.727 ± 0.01	0.400 ± 0.07	0.375 ± 0.03
6.87	0.698 ± 0.02	0.688 ± 0.05	0.433 ± 0.04	0.458 ± 0.10
7.96	0.590 ± 0.01	0.582 ± 0.04	0.696 ± 0.04	0.723 ± 0.11
9.04	0.556 ± 0.02	0.531 ± 0.02	0.801 ± 0.07	0.888 ± 0.09
RMSE[1] (27 d.f.)[2]	0.04	0.04	0.08	0.08
	Grower Phase (14-28 days)			
2.26	0.924 ± 0.07	0.823 ± 0.06	0.087 ± 0.08	0.220 ± 0.09
3.25	0.868 ± 0.04	0.796 ± 0.01	0.154 ± 0.05	0.256 ± 0.02
4.25	0.777 ± 0.03	0.725 ± 0.05	0.289 ± 0.05	0.383 ± 0.09
5.24	0.686 ± 0.02	0.712 ± 0.04	0.459 ± 0.04	0.408 ± 0.08
6.15	0.665 ± 0.02	0.627 ± 0.03	0.506 ± 0.05	0.598 ± 0.07
7.14	0.659 ± 0.02	0.589 ± 0.02	0.520 ± 0.05	0.701 ± 0.07
8.14	0.582 ± 0.03	0.559 ± 0.03	0.720 ± 0.09	0.793 ± 0.09
RMSE[1] (27 d.f.)[2]	0.04	0.04	0.06	0.08
	Finisher Phase (28-42 days)			
2.08	0.880 ± 0.01	0.704 ± 0.02	0.137 ± 0.01	0.422 ± 0.04
2.98	0.789 ± 0.04	0.717 ± 0.06	0.270 ± 0.07	0.402 ± 0.12
3.89	0.731 ± 0.05	0.692 ± 0.03	0.372 ± 0.09	0.447 ± 0.06
4.79	0.622 ± 0.02	0.605 ± 0.04	0.609 ± 0.06	0.659 ± 0.11
5.70	0.544 ± 0.03	0.536 ± 0.06	0.844 ± 0.11	0.880 ± 0.18
6.60	0.479 ± 0.02	0.509 ± 0.02	1.089 ± 0.07	0.966 ± 0.07
7.50	0.448 ± 0.05	0.470 ± 0.03	1.253 ± 0.24	1.133 ± 0.11
RMSE[1] (27 d.f.)[2]	0.04	0.04	0.11	0.11

[1]RMSE: Residual mean square error; [2]d.f.: degrees of freedom

The ratio NE/ND for THR (Table 3-7), also increased as the level of THR in the diet increased. When comparing both amino acids, it is observed higher results for THR than for MC. For the lower amino acid levels this was expected, since the first level of the THR trial was lower than of the MC trial. For the higher amino acids levels, over the maximum nitrogen deposition, this fact shows a lower efficiency of amino acid utilisation for nitrogen deposition of THR in comparison to MC.

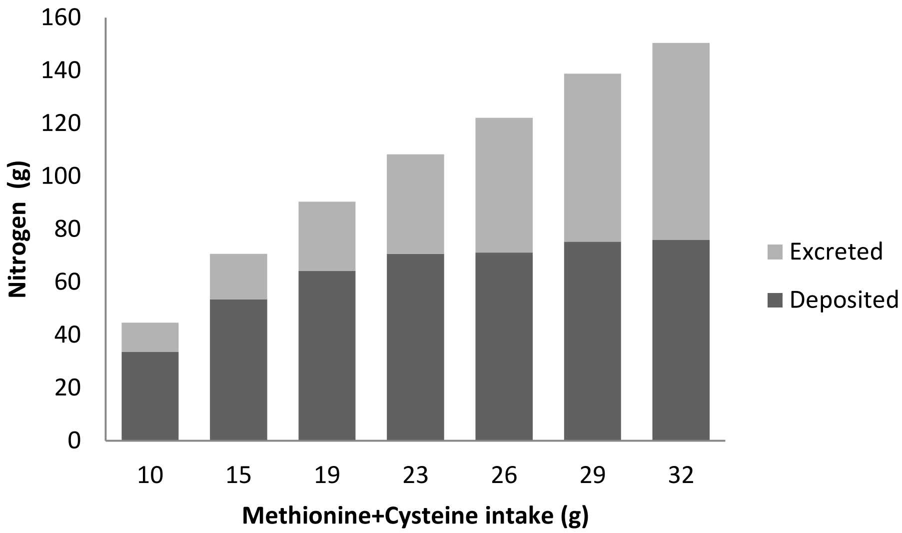

Figure 3-1. Total nitrogen deposition and excretion (g/bird) in male broilers from 1 to 42 days of age as a function of total methionine+cysteine intake (g/bird).

Table 3-5. Variance analysis results of the variables nitrogen intake (NI), nitrogen deposition (ND), nitrogen excretion (NE), ND/NI ratio and NE/ND ratio of the threonine trial

Statistic	Variables				
	NI	ND	NE	ND/NI	NE/ND
		Initial			
F THR	<0.01	<0.01	<0.01	<0.01	<0.01
F Sex	<0.01	<0.01	0.88	0.49	0.06
F THR*Sex	<0.05	<0.01	0.41	0.30	0.21
		Grower			
F THR	<0.01	<0.01	<0.01	<0.01	<0.01
F Sex	<0.01	<0.01	<0.01	<0.05	<0.05
F THR*Sex	<0.01	0.28	0.06	0.79	0.80
		Finisher			
F THR	<0.01	<0.01	<0.01	<0.01	<0.01
F Sex	<0.01	<0.01	<0.01	0.09	0.29
F THR*Sex	<0.01	<0.01	0.21	0.27	0.38

According to the fitted equations (Table 3-8), the rate of NE decreases with age, while the endogenous losses, increases. When expressed as kilogram of body weight, considering the average weight of each phase (0.164, 0.843 and 2.017kg for initial, grower and finisher phases, respectively), the endogenous losses are 0.177, 0.099 and 0.138 for MC and 0.231, 0.239 and 0.108 for THR, for initial, grower and finisher phases, respectively.

Table 3-6. Nitrogen intake (NI, g/bird/d), deposition (ND, g/bird/d) and excretion (NE, g/bird/d) of broiler males and females in response to dietary threonine (THR, g/kg) during three rearing phases

THR	Females	Males	Females	Males	Females	Males
	NI		ND		NE	
Initial Phase (1-14 days)						
1.50	0.071 ± 0.01	0.077 ± 0.01	0.053 ± 0.01	0.058 ± 0.01	0.018 ± 0.01	0.019 ± 0.01
4.00	0.505 ± 0.04	0.486 ± 0.05	0.440 ± 0.02	0.394 ± 0.05	0.065 ± 0.05	0.092 ± 0.02
5.20	0.691 ± 0.02	0.684 ± 0.01	0.541 ± 0.03	0.514 ± 0.01	0.151 ± 0.05	0.170 ± 0.01
6.40	0.798 ± 0.03	0.874 ± 0.03	0.545 ± 0.03	0.618 ± 0.01	0.252 ± 0.02	0.256 ± 0.02
7.59	0.953 ± 0.02	0.951 ± 0.03	0.596 ± 0.03	0.649 ± 0.03	0.357 ± 0.03	0.302 ± 0.01
8.79	1.087 ± 0.03	1.144 ± 0.07	0.544 ± 0.02	0.620 ± 0.04	0.542 ± 0.03	0.524 ± 0.07
9.99	1.148 ± 0.09	1.256 ± 0.07	0.556 ± 0.06	0.652 ± 0.04	0.592 ± 0.04	0.604 ± 0.08
RMSE[1] (27 d.f.)[2]	0.04	0.05	0.03	0.03	0.03	0.04
Grower Phase (14-28 days)						
1.30	0.370 ± 0.01	0.444 ± 0.04	0.210 ± 0.05	0.242 ± 0.01	0.160 ± 0.04	0.203 ± 0.04
3.50	1.626 ± 0.01	1.916 ± 0.15	1.085 ± 0.10	1.141 ± 0.07	0.541 ± 0.10	0.776 ± 0.11
4.60	2.068 ± 0.05	2.251 ± 0.06	1.370 ± 0.07	1.416 ± 0.06	0.698 ± 0.03	0.834 ± 0.06
5.70	2.447 ± 0.02	2.703 ± 0.12	1.399 ± 0.04	1.538 ± 0.06	1.048 ± 0.04	1.164 ± 0.17
6.70	2.713 ± 0.06	3.047 ± 0.05	1.475 ± 0.06	1.646 ± 0.03	1.238 ± 0.11	1.402 ± 0.06
7.79	3.075 ± 0.11	3.465 ± 0.04	1.540 ± 0.09	1.618 ± 0.07	1.535 ± 0.08	1.847 ± 0.09
8.89	3.346 ± 0.06	3.668 ± 0.04	1.522 ± 0.07	1.579 ± 0.06	1.825 ± 0.09	2.089 ± 0.09
RMSE[1] (27 d.f.)[2]	0.06	0.08	0.07	0.06	0.08	0.10
Finisher Phase (28-42 days)						
1.20	0.705 ± 0.04	0.814 ± 0.02	0.532 ± 0.12	0.595 ± 0.05	0.173 ± 0.10	0.219 ± 0.07
3.30	2.388 ± 0.05	2.875 ± 0.13	1.964 ± 0.09	2.245 ± 0.18	0.424 ± 0.08	0.630 ± 0.21
4.20	2.971 ± 0.08	3.659 ± 0.12	2.396 ± 0.05	2.486 ± 0.19	0.575 ± 0.06	1.174 ± 0.20
5.20	3.592 ± 0.08	4.160 ± 0.11	2.482 ± 0.06	2.761 ± 0.30	1.096 ± 0.14	1.400 ± 0.23
6.20	3.982 ± 0.24	4.796 ± 0.06	2.581 ± 0.13	2.961 ± 0.19	1.401 ± 0.13	1.834 ± 0.24
7.19	4.520 ± 0.28	5.438 ± 0.23	2.392 ± 0.19	3.144 ± 0.25	2.129 ± 0.31	2.363 ± 0.33
8.19	4.866 ± 0.25	5.861 ± 0.20	2.402 ± 0.15	2.935 ± 0.21	2.464 ± 0.40	2.926 ± 0.20
RMSE[1] (27 d.f.)[2]	0.18	0.14	0.12	0.21	0.21	0.22

[1]RMSE: Residual mean square error; [2]d.f.: degrees of freedom

Table 3-7. Average nitrogen deposition and intake ratio and nitrogen excretion and deposition ratio of broiler males and females in response to dietary threonine (THR, g/kg) during three rearing phases (all data)

THR	Females	Males	Females	Males
	ND/NI		NE/ND	
	Initial Phase (1-14 days)			
1.50	0.745 ± 0.15	0.756 ± 0.03	0.381 ± 0.25	0.325 ± 0.06
4.00	0.876 ± 0.08	0.809 ± 0.05	0.150 ± 0.11	0.240 ± 0.08
5.20	0.783 ± 0.06	0.752 ± 0.01	0.283 ± 0.10	0.331 ± 0.02
6.40	0.684 ± 0.03	0.707 ± 0.02	0.465 ± 0.06	0.414 ± 0.03
7.59	0.625 ± 0.03	0.682 ± 0.02	0.603 ± 0.07	0.466 ± 0.04
8.79	0.501 ± 0.02	0.543 ± 0.04	0.998 ± 0.09	0.849 ± 0.14
9.99	0.484 ± 0.02	0.521 ± 0.04	1.072 ± 0.10	0.931 ± 0.16
RMSE[1] (27 d.f.)[2]	0.07	0.03	0.13	0.09
	Grower Phase (14-28 days)			
1.30	0.564 ± 0.12	0.547 ± 0.06	0.831 ± 0.34	0.844 ± 0.21
3.50	0.667 ± 0.06	0.596 ± 0.03	0.507 ± 0.13	0.680 ± 0.09
4.60	0.662 ± 0.02	0.629 ± 0.02	0.512 ± 0.05	0.591 ± 0.06
5.70	0.572 ± 0.02	0.571 ± 0.05	0.750 ± 0.05	0.761 ± 0.14
6.70	0.544 ± 0.03	0.540 ± 0.01	0.843 ± 0.11	0.852 ± 0.05
7.79	0.501 ± 0.02	0.467 ± 0.02	1.000 ± 0.09	1.145 ± 0.10
8.89	0.455 ± 0.02	0.430 ± 0.02	1.203 ± 0.11	1.326 ± 0.10
RMSE[1] (27 d.f.)[2]	0.06	0.03	0.16	0.12
	Finisher Phase (28-42 days)			
1.20	0.752 ± 0.15	0.732 ± 0.08	0.378 ± 0.33	0.377 ± 0.14
3.30	0.822 ± 0.03	0.782 ± 0.07	0.217 ± 0.05	0.286 ± 0.12
4.20	0.807 ± 0.02	0.680 ± 0.05	0.240 ± 0.02	0.478 ± 0.11
5.20	0.694 ± 0.03	0.663 ± 0.06	0.442 ± 0.06	0.518 ± 0.14
6.20	0.649 ± 0.02	0.618 ± 0.05	0.542 ± 0.04	0.625 ± 0.13
7.19	0.530 ± 0.05	0.572 ± 0.05	0.897 ± 0.17	0.759 ± 0.16
8.19	0.496 ± 0.06	0.501 ± 0.03	1.036 ± 0.23	1.003 ± 0.12
RMSE[1] (27 d.f.)[2]	0.07	0.06	0.17	0.13

[1]RMSE: Residual mean square error; [2]d.f.: degrees of freedom

Protein Deposition

Although there is no interaction between sex and level of MC or THR for ND in some of the phases studied, the linear response plateau model for protein deposition (PD) in function of amino acid intake was fitted separately (Tables 3-9 and 3-10, respectively), so it was possible to determine the maximum PD and also evaluate the efficiency of amino acids utilisation for PD for each sex. Comparing the slopes (*u* coefficients) for each sex and phase for each amino acid through the likelihood ratio test, it was observed that there was no difference between them. Thus, the efficiency of MC utilisation for PD was 1/0.0166 = 0.60, and for THR 1/0,0170 = 0.59. It is important to note that the efficiency of protein deposition is the slope (*u*) of the broken line model, being evaluated with the amino acid levels under those related to the maximum protein deposition. The fitted linear response plateau model for each amino acid are illustrated in Figures 3-3 and 3-4, for MC and THR, respectively.

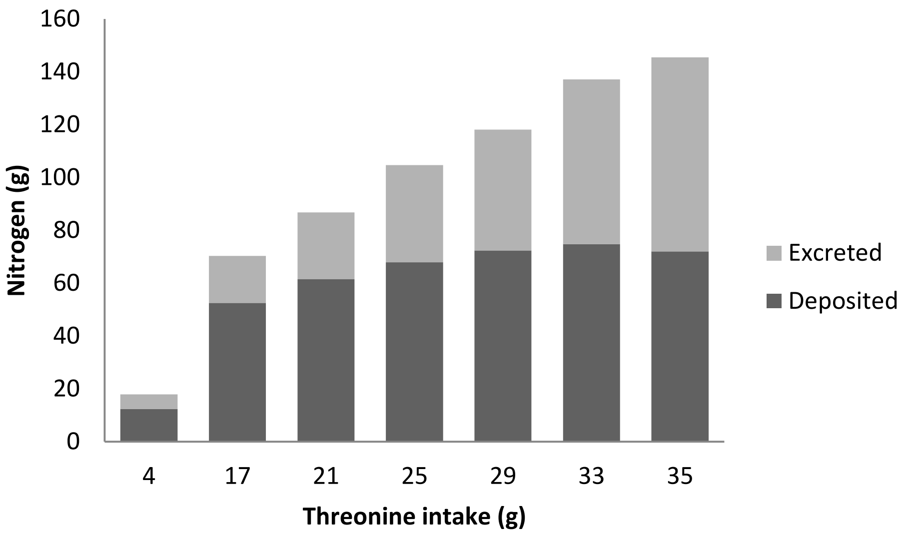

Figure 3-2. Total nitrogen deposition and excretion (g/bird) in male broilers from 1 to 42 days of age as a function of total threonine intake (g/bird).

Table 3-8. Fitted equations to describe nitrogen excretion (NE; g/bird/d) responses to methionine+cysteine (MCi; mg/bird/d) and threonine (THRi; mg/bird/d) intake by broilers in initial, grower and finisher phases

Amino acid	Phase	Equation	R^2
MC	Initial	$NE = 0.0291 \times e^{0.0104 \times MCi}$	0.99
	Grower	$NE = 0.0834 \times e^{0.00432 \times MCi}$	0.99
	Finisher	$NE = 0.2791 \times e^{0.00194 \times MCi}$	0.98
THR	Initial	$NE = 0.0379 \times e^{0.00983 \times THRi}$	0.98
	Grower	$NE = 0.2018 \times e^{0.00279 \times THRi}$	0.99
	Finisher	$NE = 0.2170 \times e^{0.00195 \times THRi}$	0.99

The plateau (maximum body PD) estimated for MC and THR are quite close, being closer with advancing age.

Body Protein Deposition vs. Nitrogen Excretion

When plotting the body protein deposition and the nitrogen excretion against amino acid intake (Figures 3-5, 3-6 and 3-7), it was observed in initial and grower phases (Figures 3-5 and 3-6, respectively) that the differences in maximum PD between males and females for THR, and for both amino acids in finisher phase (Figure 3-7) reflects in differences in NE.

Table 3-9. Slope (u), maximum protein deposition (L, g/bird/day) and methionine+cysteine intake (MCi, mg/bird/d) breakpoint (R) estimated values determined by fitting linear response plateau model to body protein deposition (PD) PD= L+u(R - MCi) for females and males broilers in initial, grower and finisher phases

Phase	Sex	Slope (u)	Maximum protein deposition (L)	MCi breakpoint (R)	R^2
Initial	Females	-0.0166 ± 0.0008	4.75 ± 0.28	239.0 ± 21.6	0.98
	Males		4.85 ± 0.25	233.1 ± 20.7	0.95
Grower	Females		10.23 ± 0.25	486.3 ± 21.6	0.90
	Males		10.43 ± 0.25	512.0 ± 21.6	0.93
Finisher	Females		14.97 ± 0.22	727.8 ± 22.2	0.73
	Males		18.08 ± 0.24	960.1 ± 24.1	0.83

It has been observed in grower phase (Figure 3-6) that although the protein deposition is higher for MC, the NE is lower, but in finisher phase (Figure 3-7) the contrary occurs, there was a higher protein deposition for THR, and lower NE.

Table 3-11 shows the NE amounts estimated by the equations presented in Table 3-8, considering the amino acid intake for maximum PD (presented in Tables 3-9 and 3-10) for MC and THR.

It is also important to note that, for maximum protein deposition, the relation between this deposition (converted into nitrogen) and nitrogen excretion are the same, regardless of sex, being 2.1, for both amino acids.

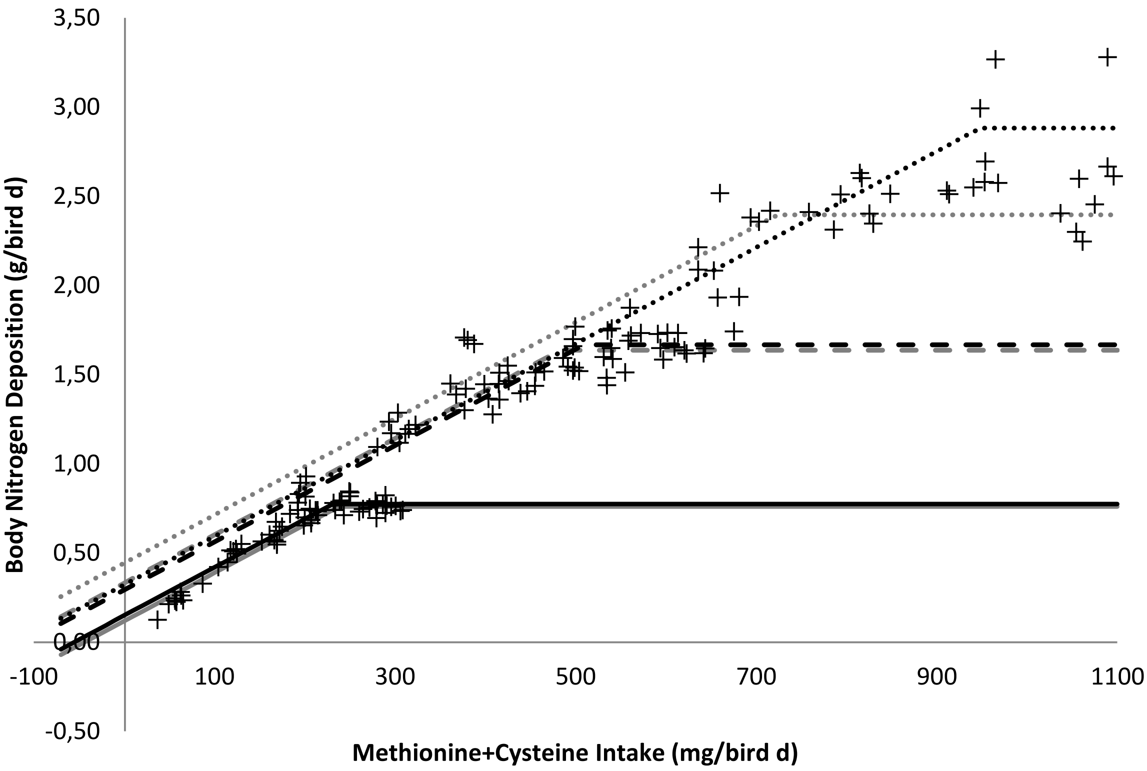

Figure 3-3. Body protein deposition in function of methionine+cysteine intake by broiler chickens in initial (males ─── and females ───), grower (males ‒ ‒ ‒ and females ‒ ‒ ‒) and finisher phases (males ········ and females ········); observed data (+).

Table 3-10. Slope (u), maximum protein deposition (L, g/bird/day) and threonine intake (THRi, mg/bird/d) breakpoint (R) estimated values determined by fitting linear response plateau model to body protein deposition (PD) PD= L+u(R - THRi) for females and males broilers in initial, grower and finisher phases

Phase	Sex	Slope (u)	Maximum protein deposition (L)	THRi breakpoint (R)	R^2
Initial	Females		3.50 ± 0.18	173.7 ± 16.0	0.95
	Males		4.00 ± 0.20	212.5 ± 16.0	0.98
Grower	Females	-0.0170 ± 0.0004	9.27 ± 0.18	532.7 ± 16.7	0.97
	Males		9.96 ± 0.18	598.3 ± 16.6	0.98
Finisher	Females		15.39 ± 0.18	735.4 ± 17.3	0.96
	Males		18.76 ± 0.21	1009.7 ± 18.3	0.93

Discussion

In this research was evaluated the responses of NE and ND by males and females broilers to dietary methionine+cysteine and threonine in initial, grower and finisher phases, and it was determined the efficiency of utilisation of both amino acids to body protein deposition.

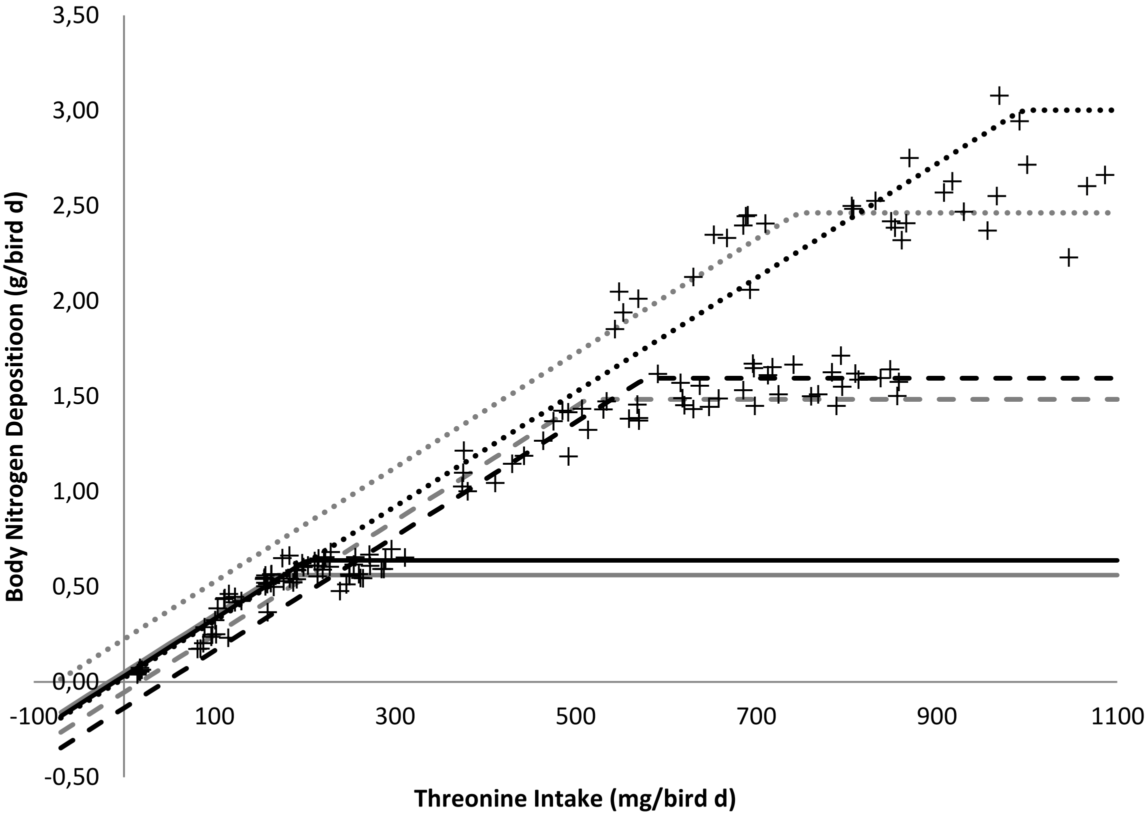

Figure 3-4. Body protein deposition in function of threonine intake by broiler chickens in initial (males ▬▬ and females ▬▬), grower (males ‒ ‒ ‒ and females ‒ ‒ ‒) and finisher phases (males ········ and females ········); observed data (+).

Proteins and amino acids are fundamental on nutritional and metabolic aspects for broiler chickens, since they are related to organism process such as the synthesis of structural tissues, the muscles (FARIA FILHO et al., 2006). It is well known that body protein deposition depends on the birds' genetic potential, thus excess of dietary crude protein or amino acid is unable to increase this potential. When the diet is formulated with excess of crude protein or amino acids it can lead to decrease in productivity. This occurs because the excess of N is catabolized as uric acid, and there is an energetic cost in this process (ALETOR et al., 2000). It is necessary 4 mols of ATP to incorporate one amino acid in protein chain whereas to excrete one amino acid it is needed from 6 up to 18 mols of ATP, depending on N content in the amino acid (MACLEOD, 1997). Besides the energy costs, the decrease in productivity is also observed from the N deposition efficiency point of view. Above a given threshold (PDmax) the birds are not able to use efficiently the N in the diet and it is excreted. The diets with excess crude protein or amino acids levels have higher costs, but the PD and BWG of the birds are the same and NE will be higher and cause environmental problems.

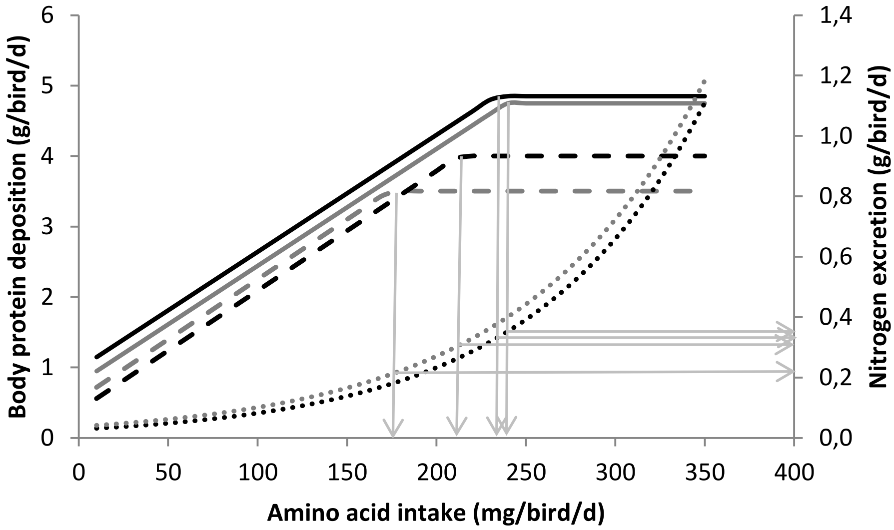

Figure 3-5. Body protein deposition (BPd; g/bird/d) and nitrogen excretion (NE; g/bird/d) by broilers in function of methionine+cysteine (BPd males —— and females ——; NE ·······) or threonine (BPd males – – – and females – – – ; NE ·······) intake (mg/bird/d) in initial phase.

According to Sklan and Noy (2004), the carcass accretion is determined by the most limiting amino acid, until some maximal rate is achieved, whereby another amino acid may become limiting. This amino acid limitation is assured when dilution technique is used to formulate experimental diets, since the balance of amino acid remains the same, what cannot be assured when the graded supplementation technique is used (GOUS & MORRIS, 1985).

This explains why the efficiency of amino acid utilisation is better when all amino acids are at or slightly below, but not above their requirement for protein accretion and maintenance (CORZO et al., 2005). This fact gives us support to use the slope of linear response plateau model to determine the efficiency of utilisation of amino acid for protein deposition. After the maximum protein deposition is reached, the efficiency of utilisation of amino acid decrease, so less amino acid is catabolized, and the excessive N is excreted, and the rate of NE increases. Using the first derivative of the fitted equations to predict NE is possible to verify that the rate of NE for MC and THR intake decreased 92, 87 and 80% from the maximum rate (at maximum amino acid intake observed) to the minimum (at minimum amino acid intake observed), in initial, grower and finisher phases, respectively. The rate of NE for MC and THR intakes at maximum body protein deposition represented approximately 50% of the maximum rate. These values (92, 87 and 80%) also confirmed that ND efficiency is decreasing with increasing age. This means that late stages of rearing are the most important when dealing with NE, since the feed conversion ratio is higher, and also the feed intake, therefore if amino acid intake is not optimal, NE will increase.

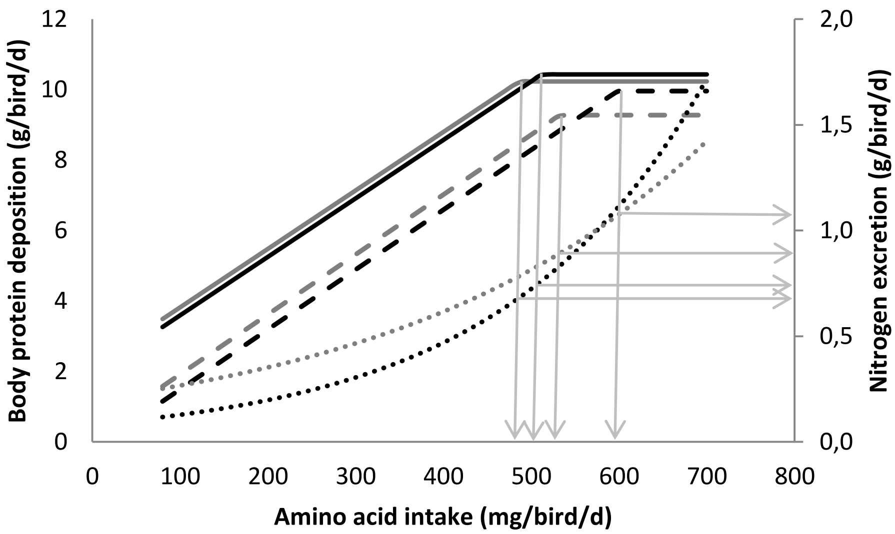

Figure 3-6. Body protein deposition (BPd; g/bird/d) and nitrogen excretion (NE; g/bird/d) by broilers in function of methionine+cysteine (BPd males ——— and females ——— ; NE ·······) or threonine (BPd males – – – and females – – – ; NE ·······) intake (mg/bird/d) in grower phase.

Concerning the endogenous nitrogen losses, according to Cowieson et al. (2009) the measurement of these losses and better understanding the factors that influence them are important to provide information that improve the efficiency of protein and amino acid utilisation, what leads to reduce nitrogen excretion. Sibbald (1981) observed an increase in endogenous losses of N with increasing age of cockerels, as observed in our trials. When expressed as per kilogram of body weight these losses decrease. According to the author, this fact is expected because older birds are heavier and their greater fat reserves are used as source of energy to maintain life during fasting.

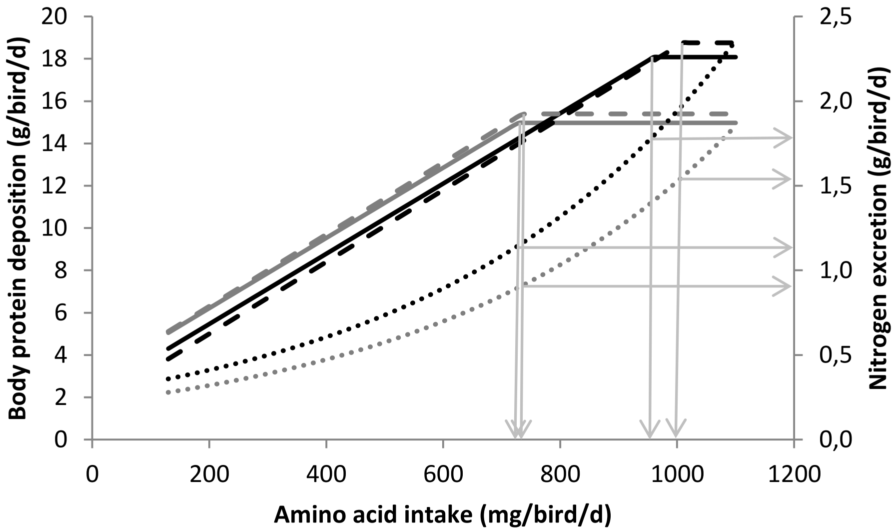

Figure 3-7. Body protein deposition (BPd; g/bird/d) and nitrogen excretion (NE; g/bird/d) by broilers in function of methionine+cysteine (BPd males —— and females —— ; NE······) or threonine (BPd males – – – and females – – – ; NE·······) intake (mg/bird/d) in finisher phase.

Table 3-11. Nitrogen excretion (g/bird/d) by broilers fed methionine+cysteine (MC) or threonine (THR) levels for maximum body protein deposition

Phase	MC		THR	
	Females	Males	Females	Males
Initial	0.349	0.329	0.208	0.306
Grower	0.682	0.762	0.892	1.071
Finisher	1.145	1.798	0.910	1.554

It was expected the amino acid utilisation efficiency for protein deposition to be different for MC and THR, since essential amino acids have different degradation rates (EDWARDS et al., 1997); and between sex and phases, since the nutrient requirements are higher during initial growth phases, which is associated with muscle mass and/or nitrogen deposition (NAMAZU et al., 2008). However, in our study only one efficiency was determined for MC and THR, and they are very similar to each other.

Using the data estimated in this trial for maximum protein deposition for MC and THR and replacing them in the NE equations, also presented in this paper, it was determined an average of 33% of NE arising from feed, considering the amino acid intake for maximum protein deposition. This value is lower than that reported by Coufal et al. (2006) of 43.6%, and Applegate et al. (2003) of 39.8%. According to Coufal et al. (2006) differences in feed N content, feed consumptions rates, bird size at marketing and carcass N values used in the calculations could explain these differences, also the methodology employed and the amino acids profile can be mentioned as a factor that affects this result. Using standard diets, with normal crude protein level, De Boer et al. (2000) reported 55% of NE arising from diets, which shows the lower efficiency of nutrient utilisation, and also an environmental problem. Therefore, it is important supply amino acids at animals requirements for growth.

Several researches (CAUWENBERGHE & BURNHAM, 2001; SI, 2004; FARIA FILHO et al., 2005) evaluated the NE in function of dietary crude protein and found for each decrease of 1% on dietary crude protein leads to a decrease from 7 (FARIA FILHO et al., 2005) up to 10% in NE (CAUWENBERGHE & BURNHAM, 2001; SI, 2004). Concerning amino acid reduction, it is shown in this paper, that the decrease in NE is exponential, and age dependent. In average, for each unit of MC or THR intake reduction there will be a reduction on NE of 0.5%. This excretion reduction seems to be minimal, but it need to be considered that it is about only one amino acid, and if applied on a commercial basis, there is a big impact. For example, in a facility with 30,000 broilers reared from 1 to 42 days, with an excess of 10 mg/bird/d of MC over the requirement for maximum protein deposition would increase the nitrogen excretion about 44.0 kg.

It is possible to reduce NE improving body protein deposition by matching more closely the dietary amino acid to requirement of broilers. Considering that the world population is increasing and also the food consumption, and the actual tendencies, for a social demand for sustainable productions, mitigation measures are needed to avoid, or at least reduce, the impact of the intensification and conglomeration of livestock. Therefore, it is important to know the responses of animals concerning nitrogen excretion and nitrogen/protein deposition to dietary amino acids, and also know how the adjustment to the correct amino acid intake can influence the environmental questions.

Acknowledgements

To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support to this project and scholarship.

References

ALETOR, V. A.; HAMID, I. I.; NIESS, E.; PFEFFER, E. Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole-body composition and efficiencies nutrient utilisation. **Journal Science Food Agriculture**, v. 80, p. 547-554, 2000.

APPLEGATE, T. J.; POTTURI, L. P. V.; ANGEL, R. Model for estimating poultry manure nutrient excretion: A mass balance approach. In: **Animal, Agricultural and Food Processing Wastes.** Proceedings of the Ninth International Symposium, American Society of Agricultural Engineers, St. Joseph, MI. p. 296–302, 2003.

CAUWENBERGHE, S. V.; BURNHAM, D. New developments in amino acid protein nutrition of poultry, as related to optimal performance and reduced nitrogen excretion. In: **European Symposium on Poultry Nutrition**. 2001, Blankenberge, Belgium. Proceedings…Blankenberge, Belgium, p. 141-149, 2001.

CORZO, A.; FRITTS, C. A.; KIDD, M. T.; KERR, B. J. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. **Animal Feed Science and Technology**, v. 118, p. 319-327, 2005.

COUFAL, C. D.; CHAVEZ, C.; NIEMEYER, P. R.; CAREY, J. B. Nitrogen emissions from broilers measured by mass balance over eighteen consecutive flocks. **Poultry Science**, v. 85, p. 384-391, 2006.

COWIESON, A. J.; BEDFORD, M. R.; SELLE, P. H.; RAVINDRAN, V. Phytate and microbial phytase: implications for endogenous nitrogen losses and nutrient availability. **World's Poultry Science Journal**, v. 65, p. 401-417, 2009.

CRUTZEN, P.J.; EHHALT, D.H. Effects of nitrogen fertilizers and combustion on the stratospheric ozone layer. **Ambio**, v. 6, p. 112-117, 1977.

DE BOER, I. J. M.; VAN DER TOGT, P. L.; GROSSMAN, M; KWAKKEL, R. P. Nutrient flows for poultry production in the Netherlands. **Poultry Science**, v. 79, p. 172-179, 2000.

EDWARDS, H. M.; BAKER, D. H.; FERNANDEZ, S. R.; PARSONS, C. M. Maintenance threonine requirement and efficiency of its use for accretion of whole-body threonine and protein in young chicks. **British Journal of Nutrition**, v. 78, p. 111-119, 1997.

FARIA FILHO, D. E.; ROSA, P. S.; FIGUEIREDO, D. F.; DAHLKE, F.; MACARI, M.; FURLAN, R. L. Dietas de baixa proteína no desempenho de frangos criados em diferentes temperaturas. **Pesquisa Agropecuária Brasileira**, v. 41, n. 1, p. 101-106, 2006.

FARIA FILHO, D. E.; ROSA, P. S.; VIEIRA, B. S.; MACARI, M.; FURLAN, R. L. Protein levels and environmental temperature effects on carcass characteristics, performance, and nitrogen excretion of broiler chickens from 7 to 21 days of age. **Brazilian Journal of Poultry Science**, v. 7, n. 4, p. 247-253, 2005.

FISHER, C.; MORRIS, T. R. The determination of the methionine requirements of laying pullets by a diet dilution technique. **British Poultry Science**, v. 11, p. 67-82, 1970.

GOMIDE, E. M.; RODRIGUES, P. B.; BERTECHINI, A. G.; FREITAS, R. T. F.; FASSANI, E. J.; REIS, M. P.; RODRIGUES, N. E. B.; ALMEIDA, E. C. Rações com níveis reduzidos de proteína bruta, cálcio e fósforo com fitase e aminoácidos para frangos de corte. **Revista Brasileira de Zootecnia**, v. 40, n. 11, p .2405-2414, 2011.

GOUS, R. M.; MORRIS, T. R. Evaluation of a diet dilution technique for measuring the response of broiler chickens to increasing concentrations of lysine. **British Poultry Science**, v. 26, p. 147-161, 1985.

KIDD, M. T., ZUMWALT, C. D., CHAMBLEE, D. W., CARDEN, M. L., BURNHAM, D. J. Broiler growth and carcass responses to diets containing l-threonine versus diets containing threonine from intact protein sources. **Journal of Applied Poultry Research**, v. 11, p. 83–89, 2002.

MACLEOD, M. G. Effects of amino acid balance and energy:protein ratio on energy and nitrogen metabolism in male broiler chickes. **British Poultry Science**, v. 38, n. 4, p. 405-411, 1997.

NAMAZU, L. B.; KOBASHIGAWA, E.; ALBUQUERQUE, R.; SCHAMMASS, E. A.; TAKEARA, P.; TRINDADE NETO, M. A. Lisina digestível e zinco quelado para frangos de corte machos: desempenho e retenção de nitrogênio na fase pré-inicial. **Revista Brasileira de Zootecnia**, v. 37, n. 9, p. 1634-1640, 2008.

NEUMANN, K.; VERBURG, P. H.; ELBERSEN, B.; STEHFEST, E.; WOLTJER, G. B. Multi-scale scenarios of spatial-temporal dynamics in the European livestock sector. **Agriculture, Ecosystems and Environment**, v. 140, p. 88-101, 2011.

OENEMA, O.; OUDENDAG, D.; VELTHOF, G. L. Nutrient losses from manure management in the European Union. **Livestock Science**, v. 112, p. 261-272, 2007.

REGAZZI, A. J. Teste para verificar a igualdade de parâmetros e a identidade de modelos de regressão não-linear. **Revista Ceres**, v .287, n. 50, p. 9-26, 2003.

ROSTAGNO, H. S.; ALBINO, L. F. T.; DONZELE, J. L.; GOMES, P. C.; OLIVEIRA, R. F. M.; LOPES, D. C.; FERREIRA, A. S.; BARRETO, S. L. T. **Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais**. 2nd edn. Universidade Federal de Viçosa, Viçosa, Minas Gerais/Brazil, 2005.

SAMADI; LIEBERT, F. Estimation of nitrogen maintenance requirements and potential for nitrogen deposition in fast-growing chickens depending on age and sex. **Poultry Science**, v. 85, p. 1421-1429, 2006.

SAMADI; LIEBERT, F. Threonine requirement of slow-growing male chickens depends on age and dietary efficiency of threonine utilisation. **Poultry Science**, v. 86, p. 1140-1148, 2007.

SAS Institute. **SAS user's guide: statistics**. Cary: Statistical Analysis System Institute, 2009.

SI, J.; FRITTS, C. A.; WALDROUP, P. W.; BURNHAM, D. J. Effects of excess methionine from meeting needs for total sulfur amino acids on utilisation of diets low in crude protein by broiler chicks. **Journal of Applied Poultry Research**, v. 13, p. 579-587, 2004.

SIBBALD, I. R. Metabolic plus endogenous energy and nitrogen losses of adult cockerels: the correction used in the bioassay for true metabolizable energy. **Poultry Science**, v. 60, p. 805-811, 1981.

SKLAN, D.; NOY, Y. Catabolism and deposition of amino acids in growing chicks: Effect of dietary supply. **Poultry Science**, v. 83, p. 952-961, 2004.

VELTHOF, G. L.; LESSCHEN, J. P.; WEBB, J.; PIETRZAK, S.; MIATKOWSKI, Z.; PINTO, M.; OENEMA, O. The impact of the Nitrates Directive on nitrogen emissions from agriculture in the EU-27 during 2000-2008. **Science of the Total Environment**, IN PRESS xxx, p.xxx-xxx, 2013. Disponível em: < http://dx.doi.org/10.1016/j.scitotenv.2013.04.058>

CAPÍTULO 4 - MODEL TO ESTIMATE METHIONINE+CYSTEINE AND THREONINE REQUIREMENTS FOR BROILER CHICKENS FOCUSED ON ECONOMIC ASPECTS

DONATO, D.C.Z.; SAKOMURA, N.K.

[1]Animal Science Department, Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil;
sakomura@fcav.unesp.br

Abstract

To evaluate the benefits of a high amino acid density diet, nutritionists need to take into account the amino acids costs and meat prices. The objective of this research was to estimate methionine+cysteine and threonine requirements of broiler chickens for maintenance and for body weight gain, and use these coefficients to determine the optimum economic intakes to maximum profits in different scenarious. Six trials were conducted with broiler chickens during three rearing phases to evaluate responses of body weight, body weight gain, and amino acid intake to dietary amino acid levels. The data were fitted by Reading Model. The requirements of MC and THR for body weight gain and maintenance were determined for both sexes and to the three phases. For growth it was estimated ranging from 6 to 9 mg MC/g d of gain to females and males and in the three evaluated phases; and about 9 mg THR/g d of gain, for both sexes in the three phases. For maintenance the requirement was estimated ranging from 20 to 22 mg MC/kg d, to both sexes, in initial and grower and finisher phases, and from 21 to 26 mg THR/kg d to both sexes, in the three evaluated phases. Considering the standard deviations of body weight and body weight gain, and the amino acids costs and meat price, it was determined the optimum economic amino acid intake to various scenarious. It can be concluded that the coefficients determined in this trial are consistent and can be applied in Reading Model to determine amino acid intake to various scenarious of amino acids costs and meat price.

Keywords: amino acids, economic evaluation, Reading Model

Introduction

It is well known that nutrition represents about 70% of broiler production costs. Protein is the second most expensive nutrient in the diet, and in addition, the nitrogen excretion due to oversupply of protein and amino acids, is an environmental problem that greatly affects the broiler production.

For more than a decade nutritionists have been reducing the protein level of broilers' diets and supplementing it with industrial amino acids, specially methionine+cysteine (MC), lysine and threonine (THR), the most limiting amino acids in a corn and soybean meal based diet, in an attempt to avoid reduction in live performance, but most of them ignore economic factors (EITS et al., 2005). In dose-response trial is determined what level dietary protein can be reduced and which amino acids need to be supplied, the broiler requirement for each amino acid, and how these factors affect broiler performance and carcass quality (EITS et al., 2003).

Corzo et al. (2005) shows the benefits of feeding broilers with high amino acid density diets, but the same author affirms that the economic aspects of this strategy depends on ingredient costs and meat prices at a particular time and for a particular market. On the other hand, reducing dietary amino acid levels can reduce feed costs without affecting body weight gain, however it can decrease carcass yield, breast meat accretion, and increase feed conversion (BARTOV & PLAVNIK, 1998; KIDD et al., 2005; AFROUZIYEH et al., 2011).

Poultry industry look for a way to reduce production costs and market better quality products (TALPAZ et al., 1988), thus nutrient requirements should be established based on monetary returns rather on maximum animal performance (GAHL et al., 1995; PACK & SCHUTTE, 1995). Also, uniformity is an important measure that needs to be taken into account when optimizing feed, since poor uniformity reduces revenue and increase waste. According to Behre & Gous (2008), a marginally deficient feed in amino acid will negatively affect uniformity, leading the broilers to overconsume energy to different extents (EMMANS, 1981, 1989; CORZO et al., 2004). In addition, the consumers are demanding for uniformity of whole broilers and cuts (BEHRE & GOUS, 2008).

Therefore, a model to estimate amino acid requirements of broilers should achieve their potential growth and be flexible enough to consider other scenarious (GOUS, 1998). The Reading Model (FISHER et al., 1973) seems to be a good tool to estimate amino acid requirements, since it is a factorial model, which takes into account maintenance and growth requirements, and economic related factors.

The objective of this study was to estimate MC and THR requirements of broiler chickens for maintenance and for body weight gain, and apply these coefficients in the Reading Model to determine the optimum economic intakes to maximum profits in different scenarious.

Material & Methods

Six trials were conducted at the Poultry Science Laboratory of Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal Campus in São Paulo, Brazil, designed to measure the response of broiler chickens during three rearing phases (1-14, 15-28 and 29-42 d) to dietary amino acids.

Five hundred and sixty male and female broilers (totaling 1,120 broilers per trial and 3,360 broilers per amino acid studied) were distributed in a completely random design in each trial, according to a 7 x 2 factorial (7 levels of dietary amino acids and 2 sexes) totaling 14 treatments, with 4 replicates, with 20 birds each. Cobb 500® broilers were used in all the trials. At the beginning of each trial, birds were individually weighed and distributed such that each experimental unit had homogeneous weight.

The experimental diets were formulated using the dilution technique. A high protein summit diet was formulated to contain approximately 1.2 times the digestible MC and THR levels suggested by Rostagno et al. (2005) for broilers during the respective phases, and all other essential amino acids were set at a minimum of 1.4 times their suggested levels. These summit diets were diluted sequentially with isoenergetic, protein-free diets (N free) (FISHER AND MORRIS, 1970) (Table 4-1), to create a range of feeds increasing in the studied amino acids content (MC: 1-14 d: 3.05 to 10.88; 15-28 d: 2.72 to 9.79, and 29-42 d: 2.50 to 9.03 g MC/kg) (THR: 1-14 d: 1.49 to 9.96; 15-28 d: 1.30 to 8.86, and 29-42 d: 1.20 to 8.17 g THR/kg).

Table 4-1. Composition (g/kg) and analyzed nutrient content (g/kg) of the summit and N-free diets used in each phase of the trial

Ingredients (%)	Diets		
	MC Summit	THR Summit	N-Free
Soybean meal (45%)	510	510	-
Maize	339	339	-
Soybean oil	65.0	65.0	100
Corn gluten meal (60%)	37.3	36.8	
Dicalcium phosphate	20.3	20.3	27.0
Limestone	9.45	9.45	5.07
Salt	4.38	4.38	5.13
DL- Methionine (99%)	3.28	5.12	-
L-Lysine (78.5%)	4.89	4.87	-
L-Threonine (99%)	2.01	0.33	-
L-Valine (96.5%)	1.40	1.40	-
Choline chloride (60%)	1.00	1.00	1.00
L-Arginine (98.5%)	0.54	0.53	
Mineral Premix[1]	0.50	0.50	0.50
Vitamin Premix[2]	0.50	0.50	0.50
Potassium chloride	-	-	11.4
Starch	-	-	428
Sugar	-	-	150
Rice Husk	-	-	150
Anticoccidiostatic[3]	0.50	0.50	0.50
Growth promotor[4]	0.05	0.05	0.05
Antioxidant[5]	0.10	0.10	0.10
Inert[6]	-	-	121
Nutrients (g/kg)			
Metabolizable energy (kcal/kg)[7]	3050	3050	3050
Crude Protein	294	298	8.90
Methionine+Cysteine[8]	9.04	10.8	-
Methionine[8]	5.82	7.48	-
Lysine[8]	15.8	17.1	-
Tryptophan[8]	3.14	2.81	-
Threonine[8]	10.2	9.99	-
Arginine[8]	16.1	16.7	-
Valine[8]	12.3	14.1	-
Isoleucine[8]	9.70	11.3	-
Leucine[8]	21.2	23.1	-
Phenylalanine[8]	12.9	13.7	-
Calcium	10.0	10.0	10.0
Sodium	2.20	2.20	2.20
Available phosphorus	5.00	5.00	5.00

[1]Content/kg of product: Mn = 150,000 mg, Fe = 100,000 mg, Zn = 100,000 mg, Cu= 16,000 mg, I = 1,500 mg, [2]Content/kg of product: Folic acid = 1000 mg, pantothenic acid = 15,000 mg, Niacin = 40,000 mg, Biotin = 60 mg, vit B1 = 1,800 mg, vit, B12 = 12,000 mg, vit, B2 = 6,000 mg, vit, B6 = 2,800 mg, vit D3 = 2,000,000 UI, vit E = 15,000 mg, vit, K3 = 1,800 mg, Se = 300 mg, antioxidant = 500 mg, [3]coxistac, [4]Zinc bacitracin, [5]BHT, [6]Wash sand, [7]Predicted value, [8]Digestible amino acid composition,

Dilution scheme (summit:N-free): MC - Initial phase: 0.28:0.72; 0.40:0.60; 0.52:0.48; 0.64:0.36; 0.76:0.24; 0.88:0.12; 1.00:0.00; Grower phase: 0.25:0.75; 0.36:0.64; 0.47:0.53; 0.58:0.42; 0.68:0.32; 0.79:0.21; 0.90:0.10; Finisher phase: 0.23:0.77; 0.33:0.67; 0.43:0.57; 0.53:0.47; 0.63:0.37; 0.73:0.27; 0.83:0.17. THR – Initial phase: 0.15:0.85; 0.40:0.60; 0.52:0.48; 0.64:0.36; 0.76:0.24; 0.88:0.12;

1.00:0.00; Grower phase: 0.13:0.87; 0.35:0.65; 0.46:0.54; 0.57:0.43; 0.67:0.33; 0.78:0.22; 0.89:0.11; Finisher phase: 0.12:0.88; 0.33:0.67; 0.42:0.58; 0.52:0.48; 0.62:0.38; 0.72:0.28; 0.82:0.18.

To verify whether the studied amino acid was limiting in the dilution series, a pilot trial to each amino acid was conducted with 30 male Cobb broilers from 1 to 14 days for MC and from 7 to 21 days for THR. They were distributed in a completely random design in two treatments in each trial, being the lowest studied levels for each amino acid and the control treatment. This treatment had the same nutritional composition as the lowest treatment in the dilution series, but it was supplemented with the test amino acid to reach the same level of the second treatment. BGW and FCR were evaluated.

Digestible amino acid contents of the summit diets were determined in a digestibility trial using the method described by Sakomura and Rostagno (2007). Total amino acid concentrations in the diets and excreta were determined by high performance liquid chromatography (HPLC).

Body weight (BW g/bird), body weight gain (BWG, g/bird d), feed intake (g/bird d) and digestible amino acid intake (MCi or THRi, mg/d, calculated from the feed intake) were calculated from the measurements of body weight and feed intake made during each rearing phase.

All the variables had their residuals tested for normality and homoscedasticity using a Cramer - Von Mises and Levene test. Data were analyzed using SAS PROC GLM 9.2 (SAS, 2009).

The data tested amino acid intake (*I*, mg/day), body weight gain (*BWG*, g/day), and mean body weight (*W*, kg) were used to calculate the parameters *a* and *b*. The Reading Model was fitted according to the equation:

$$AA_{opt}\left(\frac{mg}{day}\right) = a \times BWG_{max} + b \times W + \sqrt{[a^2 \times \sigma BWG_{max} + b^2 \times \sigma^2 W]} \times \mathrm{z}$$

Where *a* is the amino acid requirement per body weight gain unit (mg/g d), BWG_{max} is maximum body weight gain mean (g/day), *b* is the amino acid requirement per body weight unit (mg/kg d), σBWG_{max} is the standard deviation of maximum body weight gain (g/day), σW is the standard deviation of body weight (kg), and z is the standard deviation of the normal distribution associated to the product of the relation

$a \times k$; being k the marginal cost of one mg of industrial amino acid (US$/mg)/ marginal revenue of one g of meat produced (US$/g).

The maximum BWG was calculated by adding three times the standard deviation to the mean BWG of the flock (MORRIS & BLACKBURN, 1982), since it has a normal distribution, and so the bird is genetically capable of achieving this production.

Constant *a* (mg/g) was determined by the equation:

$$a = [I - (b \times W)]/BWG$$

And constant *b* (mg/kg) was determined by the equation:

$$b = [I - (a \times BWG)]/W$$

To confirm that the values of the coefficients *a* and *b* were consistent, simulations with 750 individuals generated randomly in an excel spreadsheet were made, and the mean responses of BWG was compared to the responses observed in the trials.

To estimate the requirements of flocks with different uniformities simulations with the same population cited above were made, varying the uniformity of the flock. The uniformities studied were 95% (high), 80% (medium) and 65% (low), which affected the standard deviation of maximum body weight gain and standard deviation of body weight.

Results

Results of the pilot trials confirmed that MC and THR were the limiting amino acids in the respective dilution series. The addition of industrial MC and THR to the feed with the lowest contents of these amino acids improved both BWG (7.79 g/bird d vs 5.18 g/bird d for MC and 30.05 g/bird d vs 27.55 g/bird d for THR) and FCR (2.65 g/g vs 2.98 g/g for MC and 2.28 g/g vs 2.45 g/g for THR).

The mean feed intakes, amino acid intakes and body weight gains of broilers fed increasing dietary levels of MC are presented in Table 4-2 and of THR in Table 4-3.

In both cases, the levels of the amino acids influenced both feed intake and body weight gain. The maximum feed intakes could be observed between the second and the forth levels of the studied amino acids, being that higher differences were observed between the first and second studied levels for the threonine trial.

For BWG is possible to observe that a plateau is reached between the forth and fifth levels of each amino acid. The maximum response of both amino acids are very closer to each other, representing thereby the potential of the strain. In threonine trial, since the first level was more deficient than the one in methionine+cysteine trial, the responses observed for this level were lower than in MC trial.

For both amino acids, in growth and finisher phases, the feed intake and BWG for male broilers were higher than for females.

In Tables 4-4 and 4-5 are presented the estimated parameters *a* and *b* of Reading Model, for MC and THR, respectively, for each sex, in the three evaluated phases, as well as the mean values of *BW* (kg), BWG_{max} (g/day) and the standard deviation of the normal distribution of the mean (*z*).

There is a small increase in the parameter *a*, the requirement for growth, in MC trial with the phases, which is not observed in THR trial. Concerning the parameter *b*, the requirement for maintenance, in the MC trial the values estimated to the finisher phase is a little lower than to the other phases, but in the THR trial, this value decreases from initial to grower phase, and then was kept constant.

Using this parameters some simulations were made varying the uniformity of the flock (what affects the standard deviation of BWG and W) in an attempt to determine the MC (Table 4-6) and THR (Table 4-7) requirements for a high production individual (MC or THR high), average production individual (MC or THR average) and low production individual (MC or THR low).

The current marginal costs of the two amino acids in this study were US$ 5.49/kg for DL-Methionine and US$ 4.12/kg for L-Threonine (quoted on January 09th, 2014). The current price of broiler chicken meat was US$ 2.12/kg (quoted on January 09th, 2014). From these values k (g/mg) was calculated by dividing the price of one mg of amino acid by the price of one g of chicken meat, being 0.00258 and 0.00194 for MC and THR, respectively. Considering this prices scenario, and also the uniformity variability of the flock, the optimum economic amino acid intake was estimated for both MC and THR (Tables 4-6 and 4-7, respectively), to reach the requirement of 95% of the population.

Table 4-2. Average feed intake (FI), methionine+cysteine intake (MCi) and body weight gain (BWG) of broilers females and males subjected to dietary levels of methionine+cysteine (MC levels) during initial, grower and finisher phases

MC levels (g/kg)	FI (g/bird/day)		MCi (mg/bird/day)		BWG (g/bird/day)	
	F	M	F	M	F	M
Initial Phase						
0.253	20.78	23.58	52.60	59.69	9.34	10.71
0.362	29.56	34.37	106.90	124.28	17.69	20.87
0.470	34.56	36.42	162.45	171.22	24.76	26.46
0.579	35.41	36.97	204.85	213.91	28.00	29.31
0.687	34.42	36.15	236.51	248.35	28.10	30.66
0.796	34.06	35.42	270.93	281.79	28.12	30.77
0.904	31.94	33.38	288.76	301.73	27.20	29.03
Grower Phase						
0.226	85.71	86.99	193.71	196.60	30.43	30.45
0.325	90.13	96.42	293.33	313.79	43.43	46.48
0.425	89.60	96.65	380.68	410.66	51.58	56.13
0.524	86.92	95.04	455.74	498.32	54.82	62.47
0.615	80.49	87.29	494.78	536.60	56.66	61.99
0.714	78.00	85.61	557.08	611.42	57.54	65.12
0.814	73.92	81.19	601.43	660.59	56.51	63.08
Finisher Phase						
0.208	181.16	205.13	376.67	426.51	56.47	59.79
0.298	183.57	216.43	547.63	645.65	72.54	83.93
0.389	178.35	204.78	693.28	796.04	81.43	94.19
0.479	171.70	197.30	822.63	945.29	86.16	101.38
0.570	165.63	189.24	943.31	1077.77	87.19	103.71
0.660	160.98	183.75	1062.35	1212.60	86.96	105.71
0.750	153.62	177.44	1152.64	1331.35	86.45	105.09

Table 4-3. Average feed intake (FI), threonine intake (THRi) and body weight gain (BWG) of broilers females and males subjected to dietary levels of threonine (THR levels) during initial, grower and finisher phases

THR levels (g/kg)	FI (g/bird/day)		THRi (mg/bird/day)		BWG (g/bird/day)	
	F	M	F	M	F	M
Initial Phase						
0.150	10.96	11.98	16.43	17.95	2.58	2.85
0.400	29.31	28.17	117.15	112.60	15.13	14.53
0.520	30.83	30.52	160.21	158.59	18.94	18.26
0.640	28.91	31.70	184.88	202.72	19.94	21.54
0.759	29.09	29.02	220.94	220.38	21.32	20.55
0.879	28.64	30.15	251.88	265.13	21.16	22.30
0.999	26.63	29.14	266.06	291.17	19.39	21.97
Grower Phase						
0.130	66.05	79.30	85.81	103.02	11.80	13.34
0.350	107.75	127.00	376.86	444.19	45.25	48.84
0.460	104.28	113.50	479.33	521.72	54.95	59.60
0.570	99.57	109.99	567.16	626.48	58.13	64.72
0.670	93.94	105.50	628.94	706.38	57.80	67.50
0.779	91.45	103.05	712.78	803.26	59.21	68.32
0.889	87.21	95.61	775.66	850.31	57.59	64.73
Finisher Phase						
0.120	136.23	157.29	163.36	188.62	25.01	28.73
0.330	167.88	202.09	553.62	666.43	66.96	84.15
0.420	164.11	202.11	688.77	848.26	78.93	95.71
0.520	160.23	185.60	832.63	964.44	86.73	100.98
0.620	148.98	179.43	923.03	1111.70	85.46	107.67
0.719	145.64	175.21	1047.89	1260.66	85.51	107.84
0.819	137.66	165.82	1128.04	1358.75	83.00	102.62

Table 4-4. Average body weight (*W*), maximum body weight gain mean (BWG_{max}), methionine+cysteine requirements determined by Reading Model (*a* and *b*) and standard deviation of the normal distribution of the mean (*z*) of broilers females and males in initial, grower and finisher phases

Phase	Sex	*W* (kg)	BWG_{Max} (g/day)	*a* (mg/g d)	*b* (mg/kg d)	*z*
Initial	F	0.240	28	6	22	1.80
	M	0.255	30	6	22	1.80
Grower	F	0.635	56	7	22	1.73
	M	0.670	63	7	22	1.73
Finisher	F	1.685	87	8	20	1.61
	M	2.000	105	9	20	1.61

a = methionine+cysteine requirement per g of body weight gain; *b* = methionine+cysteine maintenance requirement per kg body weight.

Table 4-5. Average body weight (*W*), maximum body weight gain mean (BWG_{max}), threonine requirements determined by Reading Model (*a* and *b*) and standard deviation of the normal distribution of the mean (*z*) of broilers females and males in initial, grower and finisher phases

Phase	Sex	*W* (kg)	BWG_{Max} (g/day)	*a* (mg/g d)	*b* (mg/kg d)	*z*
Initial	F	0.190	21	9	26	1.76
	M	0.200	22	9	26	1.76
Grower	F	0.740	58	8	22	1.81
	M	0.830	66	9	22	1.76
Finisher	F	1.690	85	8	22	1.81
	M	1.960	106	9	21	1.76

a = threonine requirement per g of body weight gain; *b* = threonine maintenance requirement per kg body weight.

Table 4-6. Standard deviation of maximum body weight gain mean (σBWG_{max}), of body weight (σW), methionine+cysteine requirement for broilers with different production levels and optimal economic methionine+cysteine intake (MC opt) for females and males broilers in initial, grower and finisher phases considering different production uniformity and birds potential

Phase	Sex	Uniformity[1]	σ BWG_{max} (g/day)	σ *W* (kg)	MC high[2] (mg/bird d)	MC average[3] (mg/bird d)	MC low[4] (mg/bird d)	MC opt.[5]	MC opt.[6]
Initial	F	High	1.4	0.024	199	173	147	192	5.65
		Medium	5.6	0.048	281	175	71	274	8.06
		Low	9.8	0.084	364	182	0	298	8.76
	M	High	1.5	0.026	213	185	157	205	5.69
		Medium	6.0	0.051	297	186	74	263	7.31
		Low	10.5	0.089	396	201	6	327	9.08
Grower	F	High	2.8	0.064	467	407	346	446	5.79
		Medium	11.2	0.127	650	408	165	570	7.40
		Low	19.6	0.223	856	432	8	693	9.00
	M	High	3.15	0.067	524	456	387	504	5.93
		Medium	12.6	0.134	733	461	188	640	7.53
		Low	22.1	0.235	941	463	0	781	9.19
Finisher	F	High	4.35	0.169	841	733	625	801	5.01
		Medium	17.4	0.337	1172	742	311	1016	6.35
		Low	30.5	0.590	1471	718	0	1234	7.71
	M	High	5.25	0.200	1130	984	837	1081	5.91
		Medium	21.0	0.400	1557	972	388	1352	7.39
		Low	36.8	0.700	2014	991	0	1623	8.87

[1] Uniformity of the flock: high = 95%, medium = 80% and low = 65%; [2] high production; [3] average production; [4] low production; [5]mg/bird d; [6] g/kg

It is possible to observe, for both amino acids, that the range of requirements for individuals with high, average and low production in a high uniformity flock is very small when comparing to a medium uniformity flock, and this range is even higher in a low uniformity flock.

Regardless of the uniformity of the flock, the amino acid requirement for an individual with average production does not change, while the requirement for a high production individual increases the double from a high uniformity to a low uniformity flock. The opposite occurs to a low production individual, the requirement decreases to zero, when the uniformity becomes worse.

Considering that the optimal economic intake was fixed to reach the requirement of 95% of the population, the estimated values are consistent, being in all cases higher than the requirement for an average production individual, and lower than the requirement for a high production individual.

Table 4-7. Standard deviation of maximum body weight gain mean (σBWG_{max}), of body weight (σW), threonine requirement for broilers with different production levels and optimal economic threonine intake (THR opt) for females and males broilers in initial, grower and finisher phases considering different production uniformity and birds potential

Phase	Sex	Uniformity[1]	σ BWG_{max} (g/day)	σ W (kg)	THR high[2] (mg/bird d)	THR average[3] (mg/bird d)	THR low[4] (mg/bird d)	THR opt.[5]	THR opt.[6]
Initial	F	High	1.1	0.019	223	194	165	214	7.38
		Medium	4.2	0.038	312	195	78	275	9.48
		Low	7.4	0.067	401	196	0	335	11.6
	M	High	1.1	0.020	234	204	173	224	7.47
		Medium	4.4	0.040	328	206	83	286	9.53
		Low	7.7	0.070	417	203	0	342	11.4
Grower	F	High	2.9	0.074	551	480	408	531	5.84
		Medium	11.6	0.148	764	477	190	677	7.44
		Low	20.3	0.259	979	477	0	842	9.25
	M	High	3.3	0.083	705	613	521	675	6.68
		Medium	13.2	0.166	984	617	250	866	8.57
		Low	23.1	0.291	1247	604	0	1037	10.3
Finisher	F	High	4.3	0.169	822	716	611	793	5.51
		Medium	17.0	0.338	1142	721	300	1011	7.02
		Low	29.8	0.592	1460	723	0	1244	8.64
	M	High	5.3	0.196	1141	994	846	1094	6.32
		Medium	21.2	0.392	1585	995	404	1403	8.11
		Low	37.1	0.686	2040	1007	0	1693	9.79

[1] Uniformity of the flock: high = 95%, medium = 80% and low = 65%; [2] high production; [3] average production; [4] low production; [5] mg/bird d; [6] g/kg

Table 4-8. Optimum economic methionine+cysteine intake (mg/bird d) according to different uniformity productions and different *k* values for broilers females and males in initial, grower and finisher phases

Phase	Uniformity[1]	*k* cost ratio[2]		
		0.00193	0.00258	0.00322
		Females		
Initial	High	192	192	191
	Medium	250	274	243
	Low	311	298	295
Grower	High	448	446	445
	Medium	577	570	562
	Low	712	693	681
Finisher	High	803	801	1199
	Medium	1036	1016	991
	Low	1253	1234	1205
		Males		
Initial	High	205	205	204
	Medium	269	263	260
	Low	328	327	318
Grower	High	505	504	499
	Medium	651	640	629
	Low	798	781	771
Finisher	High	1083	1081	1076
	Medium	1386	1352	1344
	Low	1688	1623	1603

[1] Uniformity of the flock: high = 95%, medium = 80% and low = 65%; [2] k cost ratio (US$/mg of DL-Methionine/US$/g of chicken meat) actual, 25% above and below

A range of cost ratios (k) representing 25% above and below the current value for each amino acid (US$/mg) or broiler meat (US$/kg) was used to calculate the optimum economic intakes of MC and THR for both sexes, in each phase and considering the same three uniformity production (95, 80 and 65%). The results are given in Tables 4-8 and 4-9, respectively. The lowest is the uniformity of the flock, the highest is the difference in optimal economic amino acid intake estimated for each price scenario. The same behavior is observed for the phases, the eldest are the birds, the highest are the differences.

Table 4-9. Optimum economic threonine intake (mg/bird d) according to different uniformity productions and different *k* values for broilers females and males in initial, grower and finisher phases

Phase	Uniformity[1]	*k* cost ratio[2]		
		0.00145	0.00194	0.00242
		Females		
Initial	High	215	214	213
	Medium	277	275	269
	Low	343	335	328
Grower	High	533	531	530
	Medium	690	677	670
	Low	850	842	818
Finisher	High	795	793	787
	Medium	1027	1011	1000
	Low	1246	1244	1210
		Males		
Initial	High	226	224	223
	Medium	292	286	282
	Low	356	342	343
Grower	High	679	675	673
	Medium	869	866	846
	Low	1088	1037	1028
Finisher	High	1103	1094	1090
	Medium	1427	1403	1373
	Low	1751	1693	1666

[1] Uniformity of the flock: high = 95%, medium = 80% and low = 65%; [2] k cost ratio (US$/mg of L-Threonine/US$/g of chicken meat) actual, 25% above and below

Discussion

In this research, the data were fitted to Reading Model to estimate MC and THR requirements of broiler chickens and to determine the optimum economic intakes to maximum profit in different scenarious.

Regarding the FI, it was possible to confirm the pattern described by Emmans (1981, 1989) and Kidd et al. (1996), that the birds receiving the diets with lower levels of amino acids have a greater intake in an attempt to reach their potential growth. But even consuming more, or as much as, only the birds that received diets above the fourth level of amino acid could reach their potential growth. In what concerns the FI of the birds receiving the lowest levels of MC and THR, this level was so deficient, that even an increase in intake couldn't avoid the amino acid deficiency, and also, since the metabolizable energy content was not deficient, these birds were consuming much more energy than their requirement, what regulates their consumption.

To fit the Reading Model it is important to have low levels of amino acid intake, where the responses are linear, and higher levels of amino acid intake, where the responses rises in a curvilinear fashion until a plateau is reached (FISHER et al., 1973), which represents the maximum BWG as observed in this research.

The Reading Model, is a model that has some advantages over other approaches to curve fitting. This model considers the response of an individual and the variation among individuals in a flock, what leads to estimate coefficients with biological meaning. Also, since the variation of individuals about their response mean is taken into account, these coefficients can be used to different flocks, with different conditions, as body weight and also in different environments (FISHER et al., 1973).

Another important advantage about this model is that it makes possible to determine an optimum economic nutrient intake in several economic scenario. According to Kleyn & Gous (1988), the optimum economic intake is determined when any increase in output (body weight gain) compensates the increase in cost of supplying an extra unit of nutrient intake.

According to Fisher et al. (1973), it is critical to consider values for *a* and *b* coefficients, although there are some information in literature that could be considered. However, according to this author, it is more satisfactory to get these values by fitting the model to data from experimental studies.

The comparison of the mean BWG responses obtained through simulations with those observed in the trials, confirmed the consistence of coefficients *a* and *b* determined in this study. According to Fisher et al. (1973), when a simulation is made, in order to minimize the errors, a sample size of about 500 individuals is required to adjust smooth and realistic curves, though using 50% more individuals can assure a good adjustment of the curves.

The coefficient *a* (mg/g) determined in this trial for MC and THR are close to that reported by Clark et al. (1982) for lysine, of 13 mg/g of gain. Although the author worked with a different amino acid, this comparison can be made, since this coefficient has a biological meaning. According to these authors and to Gous and

Morris (1985), the coefficient *a* is related to the concentration of the amino acid under test in the composition of the body weight gain. So, it is likely the requirement of lysine for BWG be higher than those for MC or THR, once lysine is the major amino acid in body protein, while MC and THR have close concentrations among them.

According to Talpaz et al. (1986), the requirement of amino acids for maintenance (g/d) is proportional to metabolic body weight, which increases along the Gompertz curve with age. Threonine maintenance requirements determined in this study are higher than MC, what is consistent, since THR is the most important amino acid in the maintenance component. In a nitrogen balance trial with sexual mature Cobb and Bovans White roosters, Bonato et al. (2011) determined closer maintenance requirements for MC (19mg/kg d) regardless of the strain, when comparing to those determined in this trial (20 – 22mg/kg d). The same authors also determined THR requirements for maintenance, but for this amino acid the authors reported lower value (17 mg/kg d) regardless of the strain, and higher value (32 mg/kg d) for Cobb strain. The values determined in this trial (21 – 26 mg/kg d) are in the range reported by these authors.

In what concerns the uniformity of the flock, it was shown in this paper that the requirements for individuals with high or low production change with the uniformity. According to Peak et al. (2000), birds receiving a higher nutrition quality have better overall uniformity, and this uniformity is improved when sexes are fed separately, since females and males have different growth rates (MARCATO et al., 2009). A good uniformity of flock is important especially when broilers achieve the market age. Depending on which market they will be sold, a special care should be taken. Processing the carcass to sell it on parts needs high uniformity of the flock, since the equipments are not able to process a wide range of body weights simultaneously, and also in this market, controlling the size and quality of parts is essential (Peak et al., 2000). Also, the premium that the producers receive for high breast meat percentage indicates that high amino acid density in the diet worth economically (PACK & SCHUTTE, 1995).

By using the Reading Model, it is possible to estimate the “additional amount of amino acid worth feeding” (FISHER et al., 1973), which in addition with the flock mean requirement is the optimal economic. This amount is the difference between the requirement of the individual with average production and the requirement of the individual with sustainable maximum output (KLEYN & GOUS, 1988). This model takes into consideration the decrease of efficiency of amino acids utilisation after the maximum output is reached. This decrease in efficiency should be considered when estimating requirements for optimum economic returns (GAHL et al., 1995). Dozier et al. (2006) reported that increases in chicken meat prices affect more the improvement in dietary amino acid levels than variations in diet cost. In the Reading Model, the *k* value (cost ratio) changes in the same way if the marginal cost of the amino acid increases or if the meat price decreases, since it works with "cost/benefit" ratio. But, if we consider that flocks with better uniformity fed high amino acid density diets will receive extra premium for breast meat, than chicken meat prices will truly affect more the improvement in dietary amino acid levels.

According to Pack & Schutte (1995), when dealing with cheap amino acids the optimum economic requirement is close to the maximum response, as observed to THR which requirement was closer to maximum response (individual with high production) than MC. However, when the cost of the amino acid is high, meeting the requirements of the flock may be a problem, which can be overcame by reducing the level of amino acids to the level of production dictated by the first-limiting amino acid and keeping the amino acid balance constant (MCDONALD & MORRIS, 1985). Fisher et al. (1973); Gous (1998) and Afrouziyeh et al. (2011) stated that the requirements of animals should be seeing as variable, so prediction equations could be used to estimate requirements in any economic conditions. Also, Gous (1998) reported that keeping the amino acid requirements fixed is impossible to evaluate the responses on growth, feed intake or carcass composition of birds, neither apply cost-benefit analysis, since meat and feed prices chance (AFROUZIYEH et al., 2011), or evaluate how to change these requirements according to genotypes available to the broiler industry.

In conclusion MC and THR requirements for growth were estimated as 6 and 7 mg MC/g d of gain, to initial and grower phases, respectively, for both sexes and 8 and 9 mg MC/g d of gain, to females and males, respectively, to finisher phase; and 9 mg THR/g d of gain, for both sexes in initial phase, and 8 and 9 mg THR/g d of gain, to females and males, respectively, in grower and finisher phases. The maintenance requirements were estimated as 22 and 20 mg MC/kg d, to both sexes, in initial and grower and finisher phases, respectively, and 26 and 22 mg THR/kg d to both sexes, in initial and grower phases, respectively, and 22 and 21 mg THR/kg d to females and males, respectively, in the finisher phase. Using these coefficients, it was possible to determine the optimum economic intakes to broilers in flocks with different uniformities.

Nutritionists need a model that enables to estimate requirements for maximum profit considering the economic scenario and other aspects inherent of each flock. The Reading Model is a strategic tool that can be applied in any economic and productive scenario, and the coefficients determined in this trial are consistent and can be applied in all circumstances to estimate economic levels of amino acids.

Acknowledgements

To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support to this project and scholarship.

References

AFROUZIYEH, M.; SHIVAZAD, M.; CHAMANI, M.; DASHTI, G.; AND AMIRDAHRI, S. (2011) Use of nonlinear programming to determine the economically optimal energy density in laying hens diet during phase. **Journal of Applied Poultry Research**, v.20, p.50-55.

BARTOV, I. AND PLAVNIK, I. (1998) Moderate Excess of Dietary Protein Increases Breast Meat Yield of Broiler Chicks. **Poultry Science**, v.77, p.680–688.

BERHE, E.T. AND GOUS, R.M. (2008) Effect of dietary protein content on growth, uniformity and mortality of two commercial broiler strains. **South African Journal of Animal Science**, v.38, p.293-302.

BONATO, M.A.; SAKOMURA, N.K.; SIQUEIRA, J.C.; FERNANDES, J.B.K. AND GOUS, R.M. (2011) Maintenance requirements for methionine and cysteine, and threonine for poultry. **South African Journal of Animal Science**, v.41, p.209-222.

CLARK, F.A.; GOUS, R.M. AND MORRIS, T.R. (1982) Response of broiler chickens to well-balanced protein mixtures. **British Poultry Science**, v.23, p.433-446.

CORZO, A., MCDANIEL, C.D., KIDD, M.T., MILLER, E.R., BOREN, B.B. AND FANCHER, B.I. (2004) Impact of dietary amino acid concentration on growth, carcass yield, and uniformity of broilers. **Australian Journal of Agriculture Research**, v.55, p.1133-1138.

CORZO, A.; KIDD, M.T.; BURNHAM, D.J.; MILLER, E.R.; BRANTON, S.L.; AND GONZALEZ-ESQUERRA, R. (2005) Dietary Amino Acid Density Effects on Growth and Carcass of Broilers Differing in Strain Cross and Sex. **Journal of Applied Poultry Research**, v.14, p.1–9.

DOZIER III, W.A.; GORDON, R.W.; ANDERSON, J.; KIDD, M.T.; CORZO, A. AND BRANTON, S.L. (2006) Growth, Meat Yield, and Economic Responses of Broilers Provided Three- and Four-Phase Schedules Formulated to Moderate and High Nutrient Density During a Fifty-Six-Day Production Period. **Journal of Applied Poultry Research**, v.15, p.312–325.

EITS, R.M.; GIESEN, G.W.J.; KWAKKEL, R.P.; VERSTEGEN, M.W.A. AND DEN HARTOG, L.A. (2005): Dietary balanced protein in broiler chickens. 2. An economic analysis, **British Poultry Science**, v.46, p.310-317.

EITS, R.M.; KWAKKEL, R.P.; VERSTEGEN, M.W.A. AND EMMANS, G.C. (2003) Responses of broiler chickens to dietary protein: effects of early life protein nutrition on later responses. **British Poultry Science**, v.44, p.398 — 409

EMMANS, G.C. (1981) A model of the growth and feed intake of ad libitum fed animals, particularly poultry. In: HILLYER, G.M.; WHITTEMORE, C.T. AND GUNN, R.G. **Computers in Animal Production**. British Society of Animal Production. Edinburgh/UK, pp. 106 – 110.

EMMANS, G.C. (1989) The growth of turkeys. In: NIXEY, C. AND GREY, T.C. **Recent Advances in Turkey Science**. Butterworths, London/UK, pp. 135 – 166.

FISHER, C. AND MORRIS, T.R. (1970) The determination of the methionine requirements of laying pullets by a diet dilution technique. **British Poultry Science**, v.11, p.67-82.

FISHER, C.; MORRIS, T.R. AND JENNINGS, R.C. (1973): A model for the description and prediction of the response of laying hens to amino acid intake. **British Poultry Science**, v.14, p.469-484.

GAHL, M.J.; CRENSHAW, T.D. AND BENEVENGA, N.J. (1995) Diminishing returns in weight, nitrogen, and lysine gain of pigs fed six levels of lysine from three supplemental sources. **Journal of Animal Science**, v.72, p.3177-3187

GOUS, R.M. (1998) Making Progress in the Nutrition of Broilers. **Poultry Science**, v.77, p.111–117.

GOUS, R.M. AND MORRIS, T.R. (1985) Evaluation of a diet dilution technique for measuring the response of broiler chickens to increasing concentrations of lysine. British Poultry Science, v.26, p.147-161.

KIDD, M.T.; CORZO, A.; HOEHLER, D.; MILLER, E.R. AND DOZIER, III, W.A. (2005) Broiler Responsiveness (Ross × 708) to Diets Varying in Amino Acid Density. **Poultry Science**, v.84, p.1389–1396

KIDD, M.T.; KERR, B.J.; FIRMAN, J.D. AND BOLING, S.D. (1996) Growth and carcass characteristics of broilers fed low-protein, threonine-supplemented diets. **Journal of Applied Poultry Research**, 5, 180-190.

KLEYN, F.J. AND GOUS, R.M. (1988) A Mathematical Model for the Formulation of Optimal Amino Acid and Energy Concentrations in Feeds for Laying Hens. **Agricultural Systems**, v.26, p.65-76.

MARCATO, S.M.; SAKOMURA, N.K.; FERNANDEZ, J.B.K.; NASCIMENTO, D.C.N.; FURLAN, R.L. AND PIVA, G.H. (2009) Crescimento e deposição de nutrientes nas penas, músculo, ossos e pele de frangos de corte de duas linhagens comerciais. **Ciência e Agrotecnologia**, v.33, p.1159-1168.

MCDONALD, M.W. AND MORRIS, T.R. (1985) Quantitative review of optimum amino acid intakes for young laying pullets. **British Poultry Science**, v.26, p.253-264.

MORRIS, T.R. AND BLACKBURN, H.A. (1982). The shape of the response curve relating protein intake to egg output for flocks of laying birds. **British Poultry Science**, v.23, p.405-424.

PACK, M. AND SCHUTTE, J.B. (1995) Sulfur Amino Acid Requirement of Broiler Chicks from Fourteen to Thirty-Eight Days of Age. 2. Economic Evaluation. **Poultry Science**, v.74, p.488-493.

PEAK, S.D.; WALSH, T.J.; BENTON, C.E.; BRAKE, J. AND VAN HORNE, P.L.M. (2000) Effects of two planes of nutrition on performance and uniformity of fours trains of broiler chicks. **Journal of Applied Poultry Research**, v.9, p.185-194.

ROSTAGNO, H.S.; ALBINO, L.F.T.; DONZELE, J.L.; GOMES, P.C.; OLIVEIRA, R.F.M.; LOPES, D.C.; FERREIRA, A.S. AND BARRETO, S.L.T. (2005) **Tabelas brasileiras para aves e suínos**: composição de alimentos e exigências nutricionais. 2nd edn. Universidade Federal de Viçosa, Viçosa, Minas Gerais/Brazil.

SAKOMURA, N.K. AND ROSTAGNO, H.S. (2007) **Métodos de pesquisa em nutrição de monogástricos**. Funep, Jaboticabal, São Paulo/Brazil.

SAS Institute. (2009) **SAS user's guide: statistics**. Cary: Statistical Analysis System Institute.

TALPAZ, H.; DE LA TORRE, J.R.; SHARPE, P.J.H. AND HURWITZ, S. (1986) Dynamic Optimization Model for Feeding of Broilers. **Agricultural Systems**, v.20, p.121-132.

TALPAZ, H.; HURWITZ, S.; DE LA TORRE, J.R. AND SHARPE, P.J.H. (1988) Economic Optimization of a Growth Trajectory for Broilers. **American Journal of Agricultural Economics**, v.70, p.382-390.

CAPÍTULO 5 - IMPLICAÇÕES

Atualmente a carne de frango está entre os alimentos de origem animal mais consumidos no mundo, sendo uma proteína de alta qualidade e de baixo custo para o consumidor, se comparada às outras carnes. E, futuramente, será uma das únicas fontes de proteína animal, juntamente com a carne de peixe, produzida para consumo humano, em virtude do crescente aumento demográfico populacional, escassez de espaço para a criação de bovinos e suínos, bem como a necessidade de se produzir proteína animal em abundância e no menor espaço de tempo possível para suprir a demanda. Sendo assim, urge que a correta nutrição destas aves, com dietas balanceadas, que fornecem todos os nutrientes necessários e em quantidade que atinja sua exigência é de fundamental importância para que se tenha uma boa produtividade no setor avícola.

Desta forma, a fim de se aprimorar a dieta balanceada das aves, foram expostas nesta tese as respostas das aves às ingestões de metionina + cistina e treonina, ressaltando-se as deposições de proteína e de gordura corporais, bem como o consumo ótimo de ambos os aminoácidos determinado pelo método dose-resposta. Essas respostas informam ao produtor até que quantidade é viável fornecer estes aminoácidos às aves, além de informar o que acontece na composição corporal das mesmas quando o aminoácido é fornecido além ou aquém da quantidade ótima. Porém, sabe-se que o método dose-resposta possui a limitação de determinar respostas que são válidas apenas para as condições em que foram conduzidos os ensaios, então, foram determinadas também as eficiências de utilização destes aminoácidos para deposição corporal de aminoácidos e proteína e também as exigências de ambos os aminoácidos tanto para ganho de peso, como para mantença, os quais podem ser utilizadas em modelos fatoriais, permitindo assim ao produtor a determinação do consumo ótimo destes aminoácidos em qualquer situação.

O crescimento da avicultura traz consigo vários problemas ambientais, tais como desmatamento de florestas para plantio de grãos e cereais, bem como para expansão das áreas de criação de aves e poluição decorrente dos dejetos gerados, que muitas vezes não são tratados de maneira adequada antes de serem aplicados como fertilizantes, ou são simplesmente descartados.

Visando minimizar o problema da poluição ambiental, foi investigado no presente trabalho qual a contribuição dos aminoácidos metionina + cistina e treonina na emissão de compostos nitrogenados pelas aves, onde se constatou que a correta nutrição aminoacídica, sem excessos e sem “margens de segurança”, permite às aves expressar seu potencial de crescimento e deposição proteica, portanto sem afetar a produtividade, reduzindo a excreção de nitrogênio. Foram determinados os consumos ótimos dos aminoácidos para máxima deposição protéica, sendo que o fornecimento além desta quantidade será descartado pela ave, e gerará um custo duplo ao produtor, o do fornecimento na dieta, e a excreção para o ambiente. Foram determinadas também as eficiências de utilização dos aminoácidos para deposição protéica, que poderão ser utilizadas em modelos fatoriais para se determinar o consumo ótimo em qualquer situação de criação das aves. Foram apresentadas também as curvas de excreção de nitrogênio, mostrando que quanto maior é o consumo dos aminoácidos, maior é a excreção de nitrogênio para o ambiente, e que, qualquer redução que se faça no sobrefornecimento dos aminoácidos para as aves, não afetará o desempenho das mesmas e diminuirá significativamente a excreção deste elemento poluente. Logicamente, além de adotar esta medida, o produtor pode também adotar outras, tanto nutricionais, como a utilização de enzimas para obter uma melhor digestibilidade da dieta, reduzindo a excreção não só de compostos nitrogenados, mas também de outros elementos poluentes, como o fósforo, e também de manejo, como o correto tratamento (compostagem) da cama de frango para ser utilizada como fertilizante e o correto descarte das aves mortas. A união destas medidas trará benefícios ao produtor no aspecto econômico, como também ambiental, podendo preservar a área de produção de uma maneira mais efetiva.

A correta suplementação dos aminoácidos nas dietas das aves, além de proporcionar às aves um ótimo desempenho, e minimizar a excreção de nitrogênio, garante também ao produtor a redução no custo das dietas, evitando o desperdício de nutrientes. Porém, os preços dos aminoácidos e da carne de frango são variáveis, de acordo com a oferta e a demanda, portanto a relação custo/benefício é flutuante. Sendo assim, o nível ótimo econômico não pode ser um valor fixo, ele também deve variar com essa flutuação. Outro fator que afeta o nível ótimo econômico é a uniformidade do lote, que nem sempre é ótima. Assim, foram estimados os parâmetros do modelo de Reading, através do qual é possível predizer o consumo ótimo econômico com base na flutuação da relação custo/benefício e variabilidade da população, que pode ser aplicado nas mais diversas situações. Nesta tese estão expostas algumas situações, com variação nos preços dos aminoácidos e da carne, na produtividade do lote e também na uniformidade do mesmo. Porém, mais do que isto, está descrito como trabalhar com este modelo, de maneira que o produtor pode aplica-lo às suas condições, e então determinar qual é o nível ótimo-econômico para a sua produção.

Considerando que a produção de frangos de corte continuará crescente, as informações constantes nesta tese auxiliarão os produtores a manter uma boa produtividade, com redução de excreção de compostos nitrogenados, produzir proteína animal de boa qualidade, com menor custo ao consumidor e obter um máximo retorno econômico, obtendo-se, então, uma produção sustentável, que incorpore as melhores alternativas possíveis para minimizar os impactos ambientais e sociais.