UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

ESTIMATIVA DAS EXIGÊNCIAS DE TREONINA, LISINA E METIONINA+CISTINA PARA FRANGAS DE POSTURA POR MEIO DE MODELOS

Melina Aparecida Bonato
Zootecnista

2013

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Orientador: Prof[a]. Dr[a]. Nilva Kazue Sakomura

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FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS DE JABOTICABAL

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TÍTULO: ESTIMATIVA DAS EXIGÊNCIAS DE TREONINA, LISINA E METIONINA + CISTINA PARA FRANGAS DE POSTURA POR MEIO DE MODELOS

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DADOS CURRICULARES DO AUTOR

MELINA APARECIDA BONATO – filha de José Antonio Bonato e Zília Marina de Bastiani nasceu no dia 12 de outubro de 1984, na cidade de Jaú, São Paulo. Em fevereiro de 2002 ingressou no curso de Zootecnia na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista – campus de Jaboticabal, São Paulo, graduando-se em novembro de 2006. Durante o período de agosto de 2003 a julho de 2004 foi bolsista do Programa de Educação Tutorial (PET), e bolsista de iniciação científica pelo CNPq de agosto de 2004 a julho de 2006, sob orientação da Prof^{a}. Dr^{a}. Nilva Kazue Sakomura. De abril de 2007 a março de 2008 foi bolsista de Apoio Técnico pelo CNPq, sob orientação da Prof^{a}. Dr^{a}. Nilva Kazue Sakomura. Em março de 2008 iniciou o curso de Mestrado em Zootecnia pela mesma instituição, onde obteve bolsa pela FAPESP, sob mesma orientação, defendendo a dissertação em fevereiro de 2010. Em março de 2010 ingressou no curso de doutorado em Zootecnia, também na mesma instituição e sob mesma orientação, obteve bolsa pela FAPESP e defendendo esta tese em agosto de 2013.

“Não entendo. Isso é tão vasto que ultrapassa qualquer entender. Entender é sempre limitado. Mas não entender pode não ter fronteiras. Sinto que sou muito mais completa quando não entendo. Não entender, do modo como falo, é um dom. Não entender, mas não como um simples de espírito. O bom é ser inteligente e não entender. É uma benção estranha, como ter loucura sem ser doida. É um desinteresse manso, é uma doçura de burrice. Só que de vez em quando vem a inquietação: quero entender um pouco. Não demais: mas pelo menos entender que não entendo”

(Clarice Lispector)

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À Deus,

Pela saúde e inteligência. Também por ter me concedido a oportunidade de vivenciar momentos inesquecíveis com pessoas incomparáveis, que sempre me ensinaram muito.

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Esta tese é dedicada a vocês.

“A mente que se abre a uma nova ideia jamais voltará ao seu tamanho original.”
(Albert Einstein)

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SUMÁRIO

ESTIMATIVA DAS EXIGÊNCIAS DE TREONINA, LISINA E METIONINA+CISTINA PARA FRANGAS DE POSTURA POR MEIO DE MODELOS

RESUMO - O objetivo deste trabalho foi determinar as exigências de treonina para poedeiras comerciais na fase de crescimento com base nos métodos dose-resposta e fatorial. No fatorial, utilizando dados de desempenho e abate comparativo, diferentes modelos foram utilizados para estimar exigências de treonina (thr). As exigências de THR foram estimadas durante três fases: inicial (4 a 6 semanas), cria (8 a 11 semanas) e recria (13 a 16 semanas) através de três ensaios realizados separadamente. As aves foram distribuídas em um DIC, com 8 tratamentos, 6 repetições e 8 aves por unidade experimental, exceto a fase inicial, onde foram utilizadas 15 pintainhas por unidade experimental. As rações foram formuladas conforme a técnica da diluição. As variáveis mensuradas em cada fase de crescimento foram: GP (g), CR (g), consumo de thr (mg/ave/dia), CA (kg/kg), peso absoluto (g) e relativo (%) das penas, e as deposições de proteína e de gordura (g/ave/dia) no corpo livre de penas e nas penas. A máxima deposição de proteína foi 4,0 ± 0,2; 5,3 ± 0,4 e 3,5 ± 0,5 g/d, nas fases 1, 2 e 3, respectivamente. Conforme a thr na dieta diminuiu, a deposição de lipídios no corpo aumentou. A eficiência de utilização da thr foi estimada por meio da regressão linear entre o consumo e a deposição do aminoácido, e não houve diferença entre as fases avaliadas (P>0.05) sendo estimada em 85,5%. Estas respostas do crescimento do corpo e penas são importantes na determinação do nível ótimo de thr nas dietas e aves de postura em fase de crescimento. Estes dados foram utilizados em dois modelos fatoriais para estimativa da exigência de thr para o crescimento, onde no primeiro foi considerada a mantença, perda de penas, deposição do aminoácido (corpo e penas) e eficiência de utilização, e no segundo, os mesmos parâmetros, porém adicionando-se a deposição do aminoácido no ovário e oviduto. As taxas de deposição de proteína no corpo, penas e órgãos reprodutivos foram descritas pela curva de Gompertz. A taxa de crescimento do oviduto é maior que a do ovário (0,139 *vs.* 0,084 /d), porém o ovário tem um maior peso à maturidade (78,4 *vs*. 58,7 g). As taxas de maturação do corpo, corpo livre de penas e proteína do corpo foram similares (0,017/d). As exigências preditas pelos dois modelos propostos foram iguais até o início do desenvolvimento dos órgãos reprodutivos (esta idade pode mudra de acordo com a definição do usuário). Os modelos foram capazes de predizer exigencies de thr muio próximas das encontradas na literatura. Outro método utilizado para estimar as exigências de lisina (lys), metionina+cistina (met+cys) e thr foi baseado na metodologia de Goettingen. Essa metodologia permitiu estimar o máximo potencial teórico de deposição de nitrogênio (NRmaxT) nas diferentes fases, exigências de nitrogênio para mantença (NMR), eficiência de utilização (bc^{-1}) e exigências dos aminoácidos. Para isso, foram conduzidos 4 ensaios de balanço de nitrogênio onde foram utilizadas 56 aves da linhagem Dekalb White em cada fase avaliada (fase I (14 a 28 dias), II (56 a 70 dias) e III (96 a 112 dias)), distribuídas num DIC, em 5 tratamentos, 6 repetições, alojadas individualmente em gaiolas de metabolismo. Cada ensaio teve duração de 15 dias, cinco para adaptação e 10 para coleta de excretas. As rações foram formuladas pela

técnica da diluição. Todas as equações ajustadas foram significantes (P<0,01). Os NMR determinados foram 270, 303 e 348 mg/kg^{0,67}/dia, e os NRmaxT foram 3208, 2353 e 1739 mg/kg^{0,67}/dia, para cada período. Os valores de b.c^{-1} foram: 50, 90 e 100 para lys, 170, 230 e 350 para met+cys e 100, 160 e 180 para thr, nas fases I, II e III, respectivamente. Estes parâmetros foram utilizados para cálculo das exigências de acordo com 40, 50 e 60% no NRmaxT. Em geral, os valores das exigências baseados em 50% do NRmaxT foram menores que os encontrados na literatura, o que pode ser devido as características desta metodologia (modelo fatorial, método utilizado), porém foram consistentes para aves postura em fase de crescimento.

Palavras-chave: eficiência, deposição de proteína, balanço de nitrogênio

THREONINE, LYSINE AND METIONINE+CYSTINE REQUIREMENTS FOR PULLETS USING MODELS

ABSTRACT - The aim of this study was to determine threonine requirements for pullets in the growth period based on the dose-response and factorial methods. In a factorial method, using performance data and comparative slaughter, different models were used to estimate threonine (thr) requirements. These requirements were estimated for three phases: I (4 to 6 weeks), II (8 to 11 weeks) and III (13 to 16 weeks). The birds were distributed in a completely randomized with 8 treatments, 6 replicates of e8 birds each, except the phase I, where we used 15 chicks each. The diets were formulated according to the dilution technique. Variables measured were: BWG (g), FI (g), thr intake (mg/bird/day), FC (kg/kg), absolute (g) and relative weight (%) of feathers, deposition of protein and fat (g/bird/day) in body feather free and feathers. The maximum growth rate for protein was 4.0 ± 0.2, 5.3 ± 0.4 and 3.5 ± 0.5 g/d, for phases I, II and III, respectively. The efficiency of utilization of thr was estimated by linear regression between amino acid intake and deposition, and was the same for all periods, at 0.85 ± 0.1 mg/mg. As dietary thr content decreased the amount of body lipid deposition increased. With this information it is possible to determine the daily requirement for thr for the potential growth of body and feather protein in growing pullets. These data were used in two factorial models to estimate the thr requirement for growth, where in the first model was considered the maintenance, loss of feathers, the amino acid deposition (body and feathers) and efficiency of utilization, and in the second one, the same parameters but by adding the amino acid deposition in the ovary and the oviduct. The potential rates of body and feather protein growth are described by the Gompertz growth curve. The rate of growth of the oviduct appears to be considerably faster than that of the ovary (0.139 vs. 0.084 /d) but the ovary attains a higher mature weight (78.4 vs. 58.7 g). The rates of maturing of the body, feather-free body and body protein were shown to be similar at 0.017/d. The requirements predicted by the two models remain the same until the growth of the reproductive organs is initiated, the age at which this occurs being defined by the user. The models predict realistic thr requirements for pullets growing at their potential from hatching to sexual maturity. Another method used to estimate the requirements of lysine (lys), methionine+cystine (met+cys) and thr was based on Goettingen approach. This methodology allows us to estimate the maximum theoretical potential of nitrogen deposition (NRmaxT) in different ages, nitrogen maintenance requirement (NMR), efficiency of utilization (b.c-1) and aminoacids requirements. For this, were conducted 4 nitrogen balance trials, where 56 Dekalb White pullets were used in each period (period I (14 to 28 d), II (56 to 70 d) and III (96 to 112 d)), distributed in a completely randomized design, with 5 treatments and 6 replications, housed individually in metabolism cages. Each experiment period during 15 days, 5 as adaptation and 10 for excreta collection. The diets were formulated by dilution technique. The NMR determined were 270, 303 and 348 mg/kg0.67/day, and NRmaxT were 3208, 2353 and 1739 mg/kg0.67/day, for each period. The b.c-1 values were: 50, 90 and 100 for lys, 170, 230 and 350 for met+cys and 100, 160 and 180 for threonine, for periods I, II and III, respetively. These

parameters were used to calculeted the aminoacids requirements considering 40, 50 and 60% of NRmaxT. In general, the based on 50% of the NRmaxT values are lower than the recommended values, which is due the methodology applied in this study (factorial model, type of approach), although the requirements are consistent for pullets.

Keywords: efficiency, protein deposition, nitrogen balance

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. Introdução

O crescimento acelerado e a alta taxa de deposição de proteínas corporais figuram entre as principais características das linhagens modernas de corte e postura. Do ponto de vista nutricional, é imprescindível o fornecimento adequado de nutrientes, especialmente de aminoácidos que são necessários para a síntese e deposição de proteínas corporais.

O método tradicionalmente utilizado para estimar as exigências de aminoácidos das aves tem sido o dose-resposta, que se baseia nas respostas de desempenho de aves alimentadas com dietas contendo níveis crescentes de um aminoácido (SAKOMURA e ROSTAGNO, 2007).

Segundo Baker e Han (1994), Rostagno et al. (1999), Lemme (2005), Rostagno et al. (2005) e Sakomura e Rostagno (2007) o nível ótimo de aminoácidos nas rações das aves pode variar em função de fatores como genética, idade, sexo, temperatura ambiente, níveis nutricionais, fontes de energia e proteína, desafios imunológicos e manejo. Assim, as estimativas das exigências de aminoácidos, obtidas pelo método dose-resposta, podem ser aplicadas somente para condições idênticas àquelas em que as exigências foram estabelecidas (OVIEDO-RONDÓN e WALDROUP, 2002). Além disso, o método dose-resposta pode superestimar as exigências de aminoácidos, pois pode considerar o ganho de peso do animal como um todo, não separando peso de gordura e de proteína depositada, sendo que não há demandas de aminoácidos para deposição e mantença do tecido adiposo.

Outro método que pode ser utilizado para estimar as exigências é o fatorial, que se fundamenta no princípio de que as aves necessitam de aminoácidos para a manutenção dos processos vitais, crescimento e/ou produção, sendo a base para a elaboração dos modelos matemáticos de predição das exigências (SAKOMURA e ROSTAGNO, 2007). Segundo Hurwitz et al. (1978), este é o método mais adequado para estabelecer as exigências nutricionais das aves, pois considera as diferenças no peso, composição corporal e potencial de crescimento.

A determinação das exigências para a mantença e das eficiências de utilização dos aminoácidos para crescimento constituem a base para a elaboração de modelos matemáticos que buscam descrever a relação entre o consumo de aminoácidos e sua utilização para manutenção e deposição da proteína corporal, permitindo simular as respostas de aves mantidas sob diferentes condições de criação. Vários estudos foram conduzidos para determinar exigências de mantença (LEVEILLE e FISHER, 1959; HRUBY, 1998; EDWARDS et al., 1999; SAKOMURA e COON, 2003; BROWN et al., 2006; NONIS e GOUS, 2008; SIQUEIRA, 2009), de crescimento e eficiência de utilização para lisina em aves (EDWARDS et al., 1999; FATUFE et al., 2004; BRITO, 2007; SIQUEIRA, 2009). Na estimativa da exigência dos demais aminoácidos, devido à ausência de informações, o conceito de proteína ideal tem sido utilizado.

Entretanto, para estimar exigências de forma precisa integrando o método fatorial em modelos de crescimento é necessário também conhecer os parâmetros de mantença e eficiência de utilização dos demais aminoácidos. Adicionalmente, da mesma forma que para o método fatorial a maior parte dos estudos no método dose resposta estima exigência para lisina. Dessa forma, tendo em vista a escassez de publicações acerca dos aminoácidos que não a lisina, este trabalho teve como objetivo estimar as exigências de treonina, lisina e metionina+cistina com base nos métodos dose-resposta e fatorial e elaborar modelos de predição das exigências deste aminoácido para aves de postura em fase de crescimento.

1.1. Principais aminoácidos limitantes em dietas para aves

1.1.1. Treonina

Geralmente define-se que o nível protéico ótimo de uma dieta é aquele suficiente para atender as exigências de aminoácidos para mantença, crescimento e/ou produção de ovos das aves. Entretanto, é necessário que a ordem dos aminoácidos limitantes nas dietas esteja bem definida, para que os excessos ou deficiências sejam evitados.

A ordem dos aminoácidos essenciais limitantes nas dietas para aves tem sido estudada por várias décadas, existindo considerável número de publicações que afirmam que os primeiros aminoácidos limitantes para a maioria das dietas das aves

são os aminoácidos sulfurosos (metionina e cistina), seguidos da lisina e da treonina (VIEIRA e BERRES, 2007).

A treonina foi descoberta em 1935 por W. C. Rose e logo depois foi considerada um aminoácido essencial para aves (KIDD e KERR, 1996). É considerada o terceiro aminoácido limitante nas dietas, tendo grande importância para mantença e por participar em altas concentrações na constituição da proteína endógena em relação a outros aminoácidos essenciais (FULLER, 1991). Em ensaios de metabolismos com aves, ao se corrigir as perdas de proteínas endógenas dos aminoácidos, obtêm-se maiores valores para a digestibilidade dos aminoácidos essenciais, sobretudo, a treonina, o triptofano e a arginina (CHUNG e BAKER, 1992).

Atualmente, com a disponibilidade comercial de aminoácidos sintéticos como a L-treonina, com preços acessíveis, tem possibilitado a redução dos níveis de proteína bruta com concomitante redução dos custos das dietas, trazendo benefícios econômicos e ambientais para a atividade avícola (KIDD e KERR, 1996). Contudo, é de extrema importância obter estimativas precisas das exigências desses aminoácidos, para que as necessidades das aves sejam supridas, maximizando desempenho produtivo e a lucratividade.

Em razão de sua importância em dietas práticas para aves e suínos, a treonina tem sido mais estudada, principalmente em virtude da escassez de informações sobre seus efeitos na nutrição animal e das condições em que sua suplementação nas rações seria benéfica aos animais (SARAIVA et al., 2006).

A treonina, ao contrário da maioria dos aminoácidos não pode ser sintetizada a partir de outros aminoácidos, porém a partir dela outros aminoácidos podem ser sintetizados, como a glicina e a serina. A DL-Treonina, segundo Kidd e Kerr (1996), pode fornecer apenas 25% da treonina que o animal irá aproveitar (pois têm 4 isômeros, D, L, D-allo e L-allo), assim somente a L-treonina tem valor biológico significativo.

Os esqueletos de carbono resultantes do catabolismo dos aminoácidos, geram piruvato para obtenção de energia ou produção de glicose e glicina para o metabolismo (acetil - CoA, creatina, serina, ácido úrico, sais biliares, etc). Baker at al. (1972), avaliaram o efeito da treonina em uma dieta livre de glicina e serina e

verificaram que o excesso de treonina na dieta pode suprir a deficiência de ambos, porém o contrário não é verdadeiro.

No tecido intestinal, os aminoácidos podem ser utilizados principalmente de 3 formas: incorporados às proteínas, convertidos em outros aminoácidos, substratos metabólicos e intermediários via transaminação, e completamente oxidados em CO_2. Nos dois primeiros caminhos, os aminoácidos podem ser depositados e reciclados dependendo da necessidade do corpo e de funções biológicas. No caso da treonina, a incorporação em secreções endógenas (mucina) que serão fermentadas no intestino grosso representam as perdas. O mesmo acontece se a treonina é completamente oxidada em CO_2 pelas células da mucosa. É o aminoácido em maior concentração na mucina e nos anticorpos, sendo que sua deficiência pode comprometer o funcionamento do sistema digestivo e imunológico e reduzir sua disponibilidade para síntese de proteína muscular (STOLL, 2006).

A secreção, reciclagem e perda da mucina intestinal tem o maior impacto na exigência de treonina, e isto talvez contribua com um gasto de energia do organismo, aumentando as necessidades energéticas do mesmo (STOLL, 2006).

1.1.2. Lisina

A lisina é um aminoácido essencial cujas exigências nutricionais estão intimamente relacionadas com o potencial de deposição de proteína corporal, sendo considerada o segundo aminoácido limitante para frangos de corte quando dietas “práticas” são utilizadas. Por estes e outros motivos, foi eleita o aminoácido referência para a aplicação do conceito de “proteína ideal”, que preconiza o estabelecimento das concentrações dos aminoácidos nas dietas com base em relações fixas com a lisina. Desse modo, alterações na concentração de lisina das dietas resultarão em modificações nas exigências de todos os outros aminoácidos essenciais, o que salienta a importância da obtenção de estimativas acuradas e precisas das exigências de lisina para frangos de corte (SIQUEIRA, 2009).

A lisina, considerada o segundo aminoácido limitante para aves, está envolvida principalmente na síntese de proteínas musculares, e participa em menores proporções de outros processos metabólicos (BAKER e HAN, 1994). A lisina é considerada como aminoácido referência em dietas formuladas pelo conceito de proteína ideal por ser o primeiro aminoácido limitante na maioria das dietas para

suínos e o segundo, depois dos aminoácidos sulfurosos, para aves. Além disso, este aminoácido encontra-se disponível na forma cristalina para ser utilizada nas rações práticas dos animais e sua análise laboratorial é simples e direta.

O metabolismo do D- e L-lisina foi estudado em frangos de corte usando isótopos estáveis 14C e 15N, e diferentemente do que acontece em ratos, as aves ativamente metabolizam D-lisina e L-pipecolato, isto indica que a D-lisina é degradada via conversão para pipecolato, α-aminoadipato, α-cetoadipato, e eventualmente para o CO_2. O átomo de α-nitrogênio da D-lisina é primeiro removido do esqueleto de carbono por esta via. Já a L-lisina é metabolizada por duas vias alternativas que convergem em α-aminoadipato. A principal via que resulta na retenção do átomo de α-nitrogênio da L-lisina em α-aminoadipato, inclui sacaropina como um precursor de α-aminoadipato e é, portanto, idêntica ao catabolismo de L-lisina em ratos. Embora a sacaropina seja indicada como um intermediário na degradação de L-lisina, uma grande percentagem da dose injetada por via intramuscular é excretada inalterada na urina (GROVE e ROGHAIR, 1971).

Embora o principal destino da lisina dietética seja a síntese de proteína corporal, a exigência metabólica de lisina também inclui a síntese carnitina e a oxidação obrigatória. A mais importante função da carnitina é no transporte de ácidos graxos de cadeia longa para dentro da mitocôndria para b-oxidação e subsequente produção de energia via ciclo do ácido cítrico. A biossíntese de carnitina envolve a metilação de lisina ligadas a proteínas, após isto a carnitina é libertada da proteína (VAZ e WANDERS, 2002). Embora a carnitina tenha uma função metabólica importante, resultados com ratos indicam que 1% da lisina dietética é realmente convertida a carnitina, quando a lisina é não limitante na dieta (TAMPHAICHITR et al., 1971; TAMPHAICHITR e BROQUIST, 1973). No entanto, este estudo também mostrou que, com uma dieta limitante em lisina, houve um declínio nos níveis extra-hepáticos de carnitina (TAMPHAICHITR et al., 1971) e, portanto, apesar da síntese de carnitina utilizar apenas uma pequena quantidade de lisina, é influenciada pela ingestão desta.

As respostas de frangos de corte aos níveis de lisina das dietas vêm sendo estudadas há décadas, e embora exista considerável número de publicações acerca deste tema, as recomendações dos diferentes estudos são amplamente

contrastantes. Este cenário é resultante da diversidade de fatores que influenciam a determinação das exigências de lisina, destacando-se aqueles de caráter metodológico.

1.1.3. Metionina+cistina

A metionina+cistina é o primeiro aminoácido limitante para aves que recebem dietas à base de milho e soja, ingredientes que compõem as dietas tradicionais de aves no Brasil. Os níveis de metionina+cistina na dieta podem ser afetados pelos níveis de colina, lisina e arginina (CHAMRUSPOLLERT, 2001).

A metionina é normalmente suplementada na dieta na forma seca de DL-Metionina (DL-Met; 99%) ou como DL-Metionina líquida hidróxi análoga (MMTBA, contendo 88% de substância ativa) (KALBANDE, 2009). Como possuem os isômeros D e L, portanto, podem ser convertidas a L-metionina para serem utilizadas na síntese de proteína ou no metabolismo intermediário (ROMBOLA, 2008).

A Metionina pode ser classificada como glicogênica porque é metabolizada em ácido pirúvico através da succinil-CoA, e é convertida em S-adenosil metionina por uma reação dependente de ATP. Ela funciona como um importante doador de grupo metil no organismo, necessários para a biossíntese de inúmeras substâncias fundamentais para o perfeito funcionamento do organismo (BAKER, 1991), como creatina, carnitina, poliaminas (importante em tecidos que estão sintetizando proteínas), epinefrina (adrenalina), colina e melatonina (hormônio produzido pela glândula pineal), e é também um regulador de divisão celular (CORRÊA et al., 2006). Após a desmetilação, a homocisteína é formada e subseqüentemente metabolizada através de duas vias: uma é a via de recuperação envolvendo sua re-síntese em metionina pela homocisteína metiltransferase na presença de vitamina B12. O outro caminho se segue a partir da cistationina em cisteína após receber o esqueleto de carbono da serina. A homoserina resultante é decomposta em succinil-Coa e então metabolizada em ácido pirúvico (AJINOMOTO, 2009).

Um importante aspecto da relação entre a proteína e a metionina é a habilidade dos dois atuarem como lipotróficos, ajudando a produzir carnes mais magras (KALBANDE, 2009). Talvez, a metionina haja como um agente lipotrófico através de seu papel no balanço protéico ou como um doador de grupo metil e seu

envolvimento na colina, betaína, ácido fólico e no metabolismo da vitamina B (YOUNG et al., 1955).

A adição de metionina sintética em excesso à ração pode ser metabolizada em componentes altamente tóxicos, como o metilpropionato, podendo afetar o desempenho das aves (BENDER, 1975). Já uma dieta deficiente em metionina reduz o ganho de peso, a eficiência alimentar e o teor de proteína na carcaça, além de estimular o consumo de ração, contribuindo com energia adicional e, conseqüentemente, ocasionando acréscimo na deposição de gordura corporal (SUMMERS et al., 1992; MORAN, 1994).

A exigência fisiológica existe tanto para a metionina quanto para a cisteína ou cistina. Quando a cist(e)ína está deficiente e a metionina em excesso, é muito provável que a metionina se converta em cist(e)ína. E este catabolismo tem duas funções: remover o excesso de metionina (como já citado é extremamente tóxico), e suprir a deficiência de cist(e)ína (GRABER e BAKER, 1971).

A cistina, por sua vez, tem função especial na estrutura de proteínas como insulina, imunoglobulinas e queratina, sendo esta intimamente relacionada ao processo de empenamento (BAKER, 1991). As penas são estruturas queratinizadas, ricas em cistina, arginina e aminoácidos de cadeia ramificada, cuja principal função é recobrir o corpo das aves protegendo-as das intempéries e auxiliando na termorregulação corporal (LEESON e SUMMERS, 1997).

A suplementação de cistina na dieta, não afeta o desempenho de aves de linhagem de empenamento lento, no entanto, o nível de 0,38% melhora o desempenho de aves de empenamento rápido. Isso mostra a importância da cistina para o empenamento (KALINOWSKI et al., 2003).

As formulações de rações têm sido realizadas considerando-se esses aminoácidos em conjunto (metionina+cistina). O fornecimento adequado de aminoácidos sulfurosos para as aves é de extrema importância para a formação e renovação das penas, além disso, esses aminoácidos influenciam outros processos metabólicos importantes.

A grande maioria dos estudos que estabeleceram as exigências ou relações ideais dos aminoácidos para as aves foi realizada com base no método dose-resposta, avaliando principalmente o ganho de peso e a conversão alimentar.

Entretanto, sabe-se que a composição do ganho das aves pode variar em função de fatores dietéticos e ambientais, apresentando diferentes deposições de proteína, gordura, água e cinzas. Assim, modelos de predição das exigências de aminoácidos fundamentados nas eficiências de utilização dos nutrientes e nos potenciais de deposição protéica, podem ser ferramentas importantes para predizer com maior acurácia as respostas das aves criadas sob diferentes condições.

1.2. Modelos matemáticos para predizer as exigências nutricionais das aves

Modelos de predição das exigências de aminoácidos das aves elaborados com base no método fatorial são bastante escassos na literatura. Segundo Siqueira (2009) os primeiros modelos de predição das exigências de aminoácidos para aves foram desenvolvidos por Hurwitz et al. (1973), para aves de postura. As exigências totais de aminoácidos foram obtidas à 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. Posteriormente, Hurwitz et al. (1983), baseando-se na composição da carcaça e penas de perus na fase de crescimento e em experimentos com balanço de nitrogênio, elaboraram um modelo para estimar as necessidades de aminoácidos baseado no somatório das exigências de mantença, ganho de peso da carcaça sem penas e ganho em penas.

Martin et al. (1994), basearam-se no modelo de crescimento proposto por Emmans (1981), e elaboraram um modelo que fraciona os componentes de mantença e crescimento do corpo em proporções específicas para o corpo depenado e para as penas, partindo-se do pressuposto que a composição em aminoácidos das penas difere acentuadamente da composição do corpo depenado (EMMANS e FISHER, 1986), e, além disso, considerando que as características de crescimento das penas diferem em relação ao crescimento do resto do corpo, sendo afetadas por fatores como: potencial genético, sexo e idade da ave.

Segundo Sakomura e Rostagno (2007), este método fraciona a exigência total em manutenção, crescimento e produção, sendo expresso por: Caa = AAm + AAc, onde Caa é o consumo do aminoácido (exigência), AAm, é a exigência de aminoácido para a mantença e AAc é a demanda de aminoácido para retenção

proteína corporal, que é compreendida em deposição corporal do aminoácido dividida pela sua eficiência de utilização. O AAm é dependente do peso, da composição corporal e da temperatura ambiente, AAc depende do potencial genético para deposição de proteína corporal, e o Caa sofrem influências ambientais e genéticas.

A exigência de aminoácidos para o crescimento pode ser definida com base na composição de aminoácidos da proteína corporal e nas eficiências de utilização dos aminoácidos da dieta para a deposição corporal. As eficiências de utilização dos aminoácidos podem ser obtidas em ensaios dose-resposta pela regressão linear da deposição corporal dos aminoácidos em função da ingestão dos mesmos (SAKOMURA, 2005). Desta forma, o coeficiente angular ou de inclinação da reta representa a eficiência de utilização do aminoácido, ou seja, a proporção do aminoácido ingerido que foi depositado no corpo da ave (SAKOMURA e ROSTAGNO, 2007).

Uma nova metodologia vem sendo desenvolvida baseada no modelo fatorial, dose-resposta e ensaios de balanço de nitrogênio (SAMADI e LIEBERT, 2006a,b; SAMADI e LIEBERT, 2007a; SAMADI e LIEBERT, 2008; LIEBERT, 2008). Este método é baseado em modelos matemáticos não lineares utilizando-se de relações matemáticas entre as variáveis nitrogênio ingerido (NI), nitrogênio excretado (NEX) e máximo teórico de retenção diária de nitrogênio (NRmaxT), sendo estas informações obtidas a partir de ensaios de balanço de nitrogênio com aves. Esta metodologia demonstra ser de fácil execução, inerente ao método dose-resposta e permite o fracionamento das exigências, assim como no método fatorial.

Desta forma, torna-se possível modelar a os efeitos da maior ou menor ingestão de ração e deposição proteína e predizer um plano nutricional conforme a meta traçada pelas empresas nos seus diferentes sistemas de produção. Estudos realizados para determinar os parâmetros acima descritos para poedeiras em fase de crescimento são inexistentes. Desta forma o objetivo deste trabalho foi estimar as exigências de treonina, lisina e metionina+cistina com base nos métodos dose-resposta e fatorial e elaborar modelos de predição das exigências deste aminoácido para aves de postura em fase de crescimento.

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CAPÍTULO 2 – THE RESPONSE TO DIETARY THREONINE IN GROWING PULLETS

Abstract

This study aimed to provide information on the response of laying-type pullets to dietary threonine using a summit dilution method. Three trials were conducted during the phases I (4 to 6 weeks), II (8 to 11 weeks) and III (13 to 16 weeks), using Dekalb White pullets distributed in a completely randomized design, with 8 dietary threonine contents. In phase I, threonine content ranged from 2.3 to 7.6 mg/g, in phase II from 1.7 to 5.5, and in phase III, 1.4 to 4.7 mg threonine/g feed). Measurements were made of body weight gain, feed intake, threonine intake and the deposition of protein and lipid in the feather-free body and in the feathers. Linear regressions were fitted to all the data falling below the break-point defined by the broken stick regression, to estimate the efficiency of utilization of threonine. The maximum growth rate for protein was 4.0 ± 0.2, 5.3 ± 0.4 and 3.5 ± 0.5 g/d, for phases I, II and III, respectively. The efficiency of utilization of dietary threonine for threonine deposition in each period was the same, at 0.85 ± 0.1 mg/mg. As dietary threonine content decreased the amount of body lipid deposition increased. By defining the rates of growth of body and feather protein, and calculating the amount of THR required each day to meet these requirements, taking account of the efficiency of utilisation of THR for protein growth, and including an amount to meet the requirement for THR to maintain body and feather protein, it is possible to determine the daily requirement for THR to enable a pullet to grow at its potential.

Keywords: dose-response method, protein and lipid deposition, efficiency of utilization

1. Introduction

Studying protein and lipid deposition in body and feathers, and amino acid efficiency utilization, it's possible to know the potential growth of each strain. With this information, models to predict optimum amounts of amino acids to pullets during the growing period can be fitted. There aren't many studies about growing pullets in the literature, and this phase is very important for the formation of a bird that will lay eggs for a long period.

Previous studies (Silva *et al*., 2000a,b,c; Silva *et al*., 2009a,b; D'Agostini *et al*., 2012) determined requirement for each amino acid, whereas there is no such thing for a population of birds given the wide variation in potential and actual growth rates in a population, instead that by measuring and publishing responses to an amino acid it is possible to obtain useful information, such as the efficiency of utilisation of the amino acid for protein growth; to determine whether these efficiencies change during growth or whether the same efficiency can be used throughout; and that the effects of amino acid supply on the growth of body and feather protein and body lipid can be useful in describing the consequences of marginal and gross deficiencies of amino acids on growth.

The method used in this paper was the dose-response using threonine (THR) as an amino acid reference (limiting in diets) to know the birds response (Emmans, 1981; Martin *et al*., 1994). According to Martin *et al*. (1994), this method is related to the fact that a bird has a potential growth rate of free lipid that seeks to attain; and that it seeks to attain a particular degree of fatness; and these assumptions are dependent on the genotype and the stage of maturity of the bird. All these assumptions will impact on egg production, and for a proper production is reached, proper sanity, correct management and adequate nutrition in the growth and pre-laying phase are largely responsible for the correct development of the birds, being the uniformity of the flock of fundamental importance (Silva, 2012). The uniformity in the development of reproductive organs depends largely on meeting the amino acid requirements.

THR is considered to be the third limiting amino acid in maize/soybean diets, being important for maintenance and in the formation of endogenous protein (Fuller, 1991). It is the amino acid in higher concentrations in mucin and antibodies, and its deficiency may impair the functioning of the immune and digestive system and reduce its availability for muscle protein synthesis (Stoll, 2006).

Considering all these information, this study was aimed to provide useful information on the response of pullets to dietary THR using the dose-response method to measure responses in three periods of growth.

2. Material and methods

Three trials were conducted at the Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Departamento de Zootecnia, Jaboticabal, São Paulo, Brazil, designed to measure the response of pullets during three phases of growth to dietary threonine, phase I from 4 to 6 weeks, phase II from 8 to 11 weeks, and phase III from 13 to 16 weeks. A total of 1488 Dekalb White pullets were used in the trials. They were distributed, in a completely randomized design, to 8 treatments (dietary levels of THR) with each treatment being replicated 6 times, using 15 birds per replication in phase I and 8 birds in phases II and III.

Prior to the start of each experimental period the birds received diets formulated according to the requirements recommended by Rostagno *et al.* (2005). Management of the pullets and the lighting programme followed to the recommendations of the Dekalb White supplier.

At the beginning of each phase the birds were standardized according to body weight and distributed to cages with a floor space of 0.053 m^2/bird in phase I and 0.113 m^2/bird in phases II and III.

The experimental diets were formulated using a dilution technique. A high protein summit diet was formulated containing approximately 1.3, 1.3 and 1.4 times the digestible THR levels suggested by Rostagno *et al*. (2005) for pullets during the phases I, II and III, respectively, with the minimum levels of all other essential amino acids being set at 1.5 times (for phases I and II) and 1.6 times (for phase III) their suggested levels. These summit diets were diluted sequentially with isoenergetic, protein-free diets (N free) (Fisher and Morris, 1970), as shown in Table 1, to create a range of feeds increasing in THR content (THRc) (phase I: 2.5 to 8.2; phase II: 1.8 to 5.9, and phase III: 1.5 to 5.1 mg digestible THR/g feed.)

Table 1. Composition (g/kg) of the basal diets used in each phase of the trial together with the analysed nutrient content of each diet

Ingredients	Diets					
	Phase I		Phase II		Phase III	
	Summit	N Free	Summit	N Free	Summit	N Free
Maize	555	-----	622	-----	669	-----
Soybean meal	391	-----	223	-----	157	-----
Starch	-----	543	-----	509	-----	540
Rice Husk	-----	120	-----	148	-----	150
Sugar	-----	120	-----	150	-----	120
Sand	-----	118	-----	100	-----	100
Wheat bran	-----	-----	87.0	-----	111	-----
Soybean oil	13.3	50.0	30.0	50.0	30.0	50.0
Dicalcium phosphate	17.4	23.6	15.0	21.2	10.6	16.8
Limestone	10.5	6.60	9.90	4.70	12.2	6.60
Salt	4.60	4.20	3.50	3.60	3.30	3.40
Potassium choride	-----	11.5	-----	11.3	-----	10.9
DL- Methionine (98 %)	3.50	-----	2.60	-----	2.00	-----
L-Lysine (78.5 %)	2.60	-----	2.40	-----	2.30	-----
L-Tryptophan (93.5 %)	-----	-----	0.40	-----	0.50	-----
L-Valine (98 %)	-----	-----	0.80	-----	0.50	-----
L-Isoleucine (98 %)	-----	-----	1.00	-----	0.80	-----
Vitamin/Mineral Premix[1]	1.50	1.50	1.50	1.50	1.50	1.50
Choline Chloride	0.70	0.70	0.70	0.70	0.70	0.70
Antioxidant	0.10	0.10	0.10	0.10	0.10	0.10
Nutrients						
Metabolizable energy[4] (MJ/kg)	12.1	12.1	12.1	12.1	12.1	12.1
Crude Protein[2]	223	6.70	180	6.70	153	6.70
Lysine[2,3]	14.0	-----	9.93	-----	8.32	-----
Methionine + cystine[2,3]	10.3	-----	8.05	-----	6.95	-----
Methionine[2,3]	6.70	-----	5.12	-----	4.27	-----
Tryptophan[2,3]	2.83	-----	2.57	-----	2.25	-----
Threonine[2,3]	8.15	-----	5.94	-----	5.10	-----
Arginine[2,3]	15.9	-----	11.2	-----	9.38	-----
Valine[2,3]	10.5	-----	8.53	-----	7.20	-----
Isoleucine[2,3]	9.89	-----	7.90	-----	6.55	-----
Leucine[2,3]	19.4	-----	15.2	-----	13.7	-----
Phenylalanine + tyrosine[2,3]	11.8	-----	8.59	-----	7.37	-----
Calcium	9.40	9.40	8.30	8.30	8.00	8.00
Sodium	1.80	1.80	1.60	1.60	1.50	1.50
Avaliable phosphorus	4.40	4.40	3.90	3.90	3.10	3.10
Potassium	8.70	8.70	6.60	6.60	5.70	5.70
Crude fibre	30.7	47.5	30.6	58.7	30.0	59.4

[1]Content/kg - vit. A = 8.000.000IU, vit. D3 = 2.300.000IU, vit. E = 12.350g, vit. B1 = 2.400mg, vit. B2 = 5.950mg, vit. B6 = 2.500mg, vit. B12 = 12.000mcg, Niacin = 38.000mg, pantothenic acid = 12.000mg, vit. K3 = 1.800mg, folic acid = 950 mg, selenium = 300mg, antioxidant = 250mg, Biotin = 60mg, manganese = 200.000mg, iron = 100.000mg, zinc = 160.000mg, copper = 16.000mg, iodine = 1.500mg.

[2]Values analyzed by HPLC.

[3]Digestible amino acid composition determinated by trials with cecectomized roosters.

[4] Predicted value calculated according to Rostagno *et al.* (2005).

To confirm that THR was limiting in each series an additional treatment was included in the design in which the feed with the lowest level of THR was supplemented with 0.86 g, 0.62 g or 0.53 g of L-THR/kg in phases I, II and III, respectively. A significantly greater response in growth or feed conversion to this treatment compared with the unsupplemented feed would indicate that all feeds in the dilution series were limiting in THR.

A digestibility trial was conducted to determine the digestible amino acid contents of the summit diets used in each phase using the method describle by Sakomura and Rostagno (2007). Cecectomized roosters (6 per treatment) were fed 40 g of feed by intubation after 48 hours of fasting. Excreta collection continued for 48 hours after feeding. In order to quantify the metabolic and endogenous losses of amino acids in the body a N-free feed was fed to 6 roosters. Amino acid contents were quantified in samples of diets and excreta that were hydrolyzed with 6N HCl during 24 hours. The amino acid released in the hydrolysis were separated by high performance liquid chromatography (HPLC) reverse phase and detected by U.V. at 254 nm.

Body weight gain (BWG, g), feed intake (FI, g), digestible THR intake (THRi, mg/day) and feed conversion (FC, kg/kg) were calculated from the measurements of body weight and feed intake made during each phase of growth. The absolute (g) and relative weights (% of body weight) of feathers, deposition of protein and lipid (g/d) in the feather-free body and in the feathers was determined by the comparative slaughter technique using representative samples of birds at the beginning and end of each phase. The number of birds sampled at the beginning of each phase was 18, whilst 96 birds were sampled at the end of each phase of the trial (two birds from each of the six replications of eight treatments).

The THR content (THRc) of protein in the feather-free body and in the feathers of the birds sampled at the beginning and end of each phase was analyzed by high performance liquid chromatography (HPLC) from which the amount of THR deposited during each phase was calculated. The analysis of crude protein, ether extract and dry matter proceeded according to AOAC (1990).

Regressions were adjusted on each of the variables measured or calculated, including FI, BWG, FC, body protein deposition (BPd, g/d), feather protein deposition (FPd, g/d), protein (body plus feathers) deposition (g/d), body lipid deposition (BLd, g) and THR deposition (THRd, mg/d) in order to determine the means and standard errors for each variable.

A broken stick regression describle by Robbins *et al.* (1979) was fitted to determine the maximum rate of deposition of protein in each phase: $Y = Rmax + U . (R – X) + Є$, in this equation, Y is the response variable (BPd or THRd), X is the independent variable (THRi) associated to Y; R_{max} is the maximum expected value for deposition (BPd or THRd); U is the slope of the function; R is the breakpoint in the horizontal axis, this parameter represents the point where for a intake X the response is maximal; and Є is aleatory error based on normality assumptions. Linear regressions were then fitted to all the data falling below the break-point defined by the broken stick regression in each phase, in order to estimate the coefficient of response to THR intake, this being the inverse of the efficiency of utilisation of THR for protein growth. Statistical analyses were performed in SAS 9.1 software (2009) using PROC NLIN and in Genstat (2009) for analysis of simple linear regression with groups (where the slopes and intercepts from each phase are compared to each other).

3. Results

Mean responses to the feeds varying in threonine content are given in Table 2 for each of the three phases of the trial. The addition of synthetic THR to the feed with the lowest level of THR resulted in higher FI, BWG, BPd and FPd and lower FC and BLd in all three phases than the feed with the lowest THR content, confirming that THR was first-limiting in the feeds used in this trial.

On the three or four levels with the highest THR content, feed intake was relatively stable (Table 2) but as the THR content declined further, feed intake decreased, falling to 93% of the maximum in phase I of the trial, and to 81% and 77% in phases II and III, respectively. The effect on body weight gain (Table 2) was considerably more severe, with the gains on feeds with the lowest THR contents falling to 48, 12 and 9% of the highest gains achieved. Feed conversion efficiency reflected these differences in food intake and performance (Table 2), the values on feeds with the lowest THR content being 52, 18 and 11% of the highest FCE’s recorded, for phases I, II and III, respectively.

In all three phases, protein deposition increased with increasing intake of THR (calculated by FI multiplied by THRc) up to a maximum value, this being reached on 165 ± 8.2 mg THR/d in phase I, 230 ± 25.8 mg/d in phase II and 168 ± 41.5 mg/d in phase III as measured by the broken stick equations fitted in each phase. The maximum growth rates achieved in each of the phases were 4.00 ± 0.11, 5.28 ± 0.40 and 3.51 ± 0.49 g/d. The

intercepts differed between phases, these being 0.09 ± 0.41; -0.51 ± 0.32 and -0.68 ± 0.28 for the three phases respectively showing that the requirement for maintenance changes with age. Whereas the linear slope of the responses to THR in each phase was 0.023 ± 0.002, 0.028 ± 0.006 and 0.018 ± 0.005, respectively, there was no difference ($P<0.05$) between the slopes of the lines (0.024 ± 0.003) when these were compared using simple linear regression with groups (phases). Thus the efficiency of utilisation of THR for protein deposition as measured here was (1/0.024) = 0.414. These responses are illustrated in Figure 1 in which protein deposition (body plus feathers, g/d) for each of the three phases has been regressed against THR intake. Note the common slope in each phase of growth.

Table 2. The mean responses in feed intake (FI), body weight gain (BWG), feed conversion efficiency (FCE), feather-free body protein deposition (BPd), feather protein deposition (FPd) and body lipid deposition (BLd) of pullets to dietary threonine (THRc) in phases I (4-6), II (8-11) and III (13-16 weeks)

THRc g/kg	FI g/d	BWG g/d	FCE g/kg	BPd[1] g/d	FPd[1] g/d	BLd[2] g/kg
Phase I (4 to 6 weeks)						
2.5	23.6	6.21	262	0.87	0.62	87.1
3.2[3]	23.9	6.81	285	1.26	0.77	78.9
3.3	25.2	7.85	312	1.27	0.88	61.7
4.1	25.4	9.49	373	1.60	1.17	52.2
4.9	25.5	10.5	413	1.71	1.25	28.4
5.7	25.5	11.5	450	2.03	1.49	21.2
7.3	25.5	12.8	495	2.39	1.71	19.3
8.2	25.4	12.9	508	2.21	1.72	17.6
RMS[4] (34 d.f.)[5]	0.047	0.092	0.004	0.070	0.017	3.257
Phase II (8 to 11 weeks)						
1.8	35.2	1.61	50.3	0.40	0.14	69.3
2.3[3]	37.3	3.31	82.6	0.69	0.29	66.8
2.4	39.1	3.60	82.6	0.81	0.54	62.9
3.0	41.6	8.45	189	1.75	1.12	51.8
3.6	41.8	8.10	187	1.87	1.24	48.4
4.2	42.3	9.30	207	2.28	1.54	40.2
5.4	43.6	13.4	288	2.80	2.27	36.0
5.9	42.3	12.9	280	3.03	1.97	36.2
RMS (31 d.f.)	8.568	4.400	7.640	0.941	0.150	12.97
Phase III (13 to 16 weeks)						
1.5	36.9	0.95	23.4	0.24	0.07	74.4
1.9[3]	37.1	1.79	58.5	0.55	0.16	71.0
2.0	40.2	3.47	84.0	1.08	0.53	65.4
2.5	44.4	7.30	150	1.46	0.64	58.4
3.1	47.4	7.78	152	1.53	0.82	54.1
3.6	47.0	9.35	188	1.78	1.38	50.0
4.6	48.2	10.8	206	2.12	1.55	45.2
5.1	48.0	9.98	202	2.25	1.32	45.7
RMS (33d.f.)	14.08	3.310	26.02	1.182	0.264	23.57

[1]Crude protein deposited during the experimental period estimted by the diffrence between initial reference slaughter and final slaughter. [2]Body lipid deposition (g/kg) in feather-free body of birds sampled at end of each phase. [3]Aditional level to determine whether threonine was the limiting amino acid (level one plus 0.86 g, 0.62 g or 0.53 g of L-THR/kg in each phase). [4]RMS: Residual mean square. [5]df: degrees of freedom.

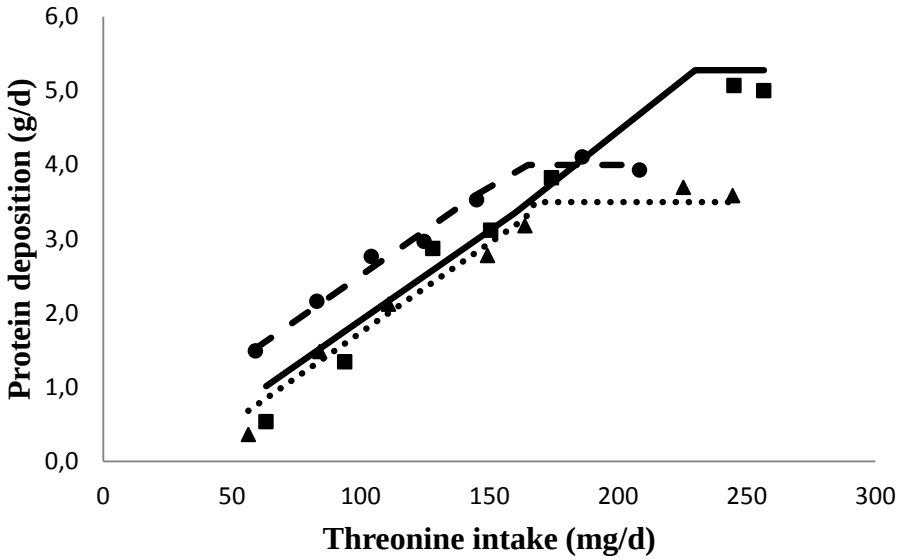

Figure 1. The response in protein (body plus feathers) deposition (g/d) to threonine intake (mg/d) for pullets in phases I (4-6 weeks, ●), 2 (8-11 weeks, ■) and 3 (13-16 weeks, ▲).

The analysed THR contents of body and feather protein were 37 and 45; 37 and 43; and 39 and 43 mg/g protein, for phases I, II and III, respectively, the mean THR contents of body and feather protein thus being 37.7 and 43.8 mg/g respectively. These values were used to calculate the rates of THR deposition by pullets on each of the dietary treatments in each phase of the trial. As with protein deposition, the maximum rates of threonine deposition were reached on different daily amounts of dietary THR, namely, 164 ± 4.08, 200 ± 15.7 and 108 ± 11.7 mg/d. Similarly, when the linear portion of the response in each period was interrogated, the rate of increase in THR deposition differed marginally in each phase (0.934 (± 0.09), 0.966 (± 0.23) and 0.752 (± 0.19) mg/mg) but when the data were combined using simple linear regression with groups, the slopes were found not to differ and the common slope for all periods was 0.855 ± 0.13 mg/mg. The intercepts differed significantly; being 16.8 ± 14.7, -7.1 ± 11.6 and -29.1 ± 10.3 for phases I, II and III, respectively.

As the dietary THR content decreased the amount of body lipid deposition increased (Table 2) so that by the end of each phase of the trial those pullets on the lowest THR contents were fatter than pullets reared on the higher THR contents. The responses in lipid deposition at the end of each phase of the trial are illustrated in Figure 2.

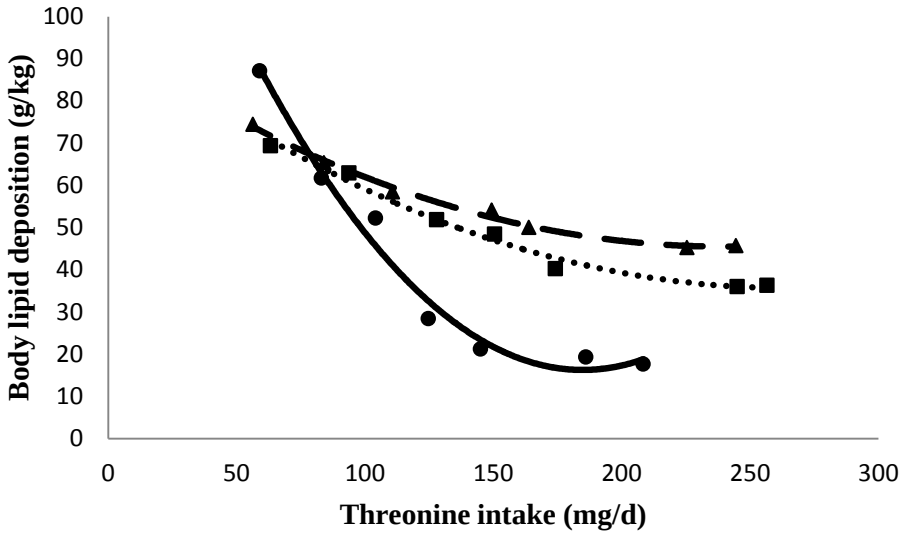

Figure 2. Body lipid deposition at the end of each phase as a function of threonine content in the diet, in phases I (4-6 weeks,●), II (8-11 weeks, ■) and III (13-16 weeks, ▲).

To determine to what extent the proportions of feather and body protein varied during growth and according to the amount of dietary THR in the feed, the amounts of body and feather protein at the end of each phase of the trial were used to calculate the proportion of feather protein to body protein and the results are presented in Table 3. The relationship between the proportion of protein in feathers and in the body at the end of each phase was analyzed by linear regression, with the phases as factors, using simple linear regression with groups. The slopes of these regressions (BPc and FPc) were the same between phases and could be represented by one value only, namely, 0.0248 ± 0.0036.

Table 3. Threonine content in the diet (THRc), feathers protein content (FPc) and body protein content (BPc) at the end of each phase, and their relationship (FPc/BPc)

Phase I (4 to 6 weeks)								
THRc (g/kg)	2.5	3.2	3.3	4.1	4.9	5.7	7.3	8.2
FPc[1] (g)	12.5	14.8	16.1	20.1	21.1	24.6	27.7	27.9
BPc[2] (g)	26.4	33.3	32.1	36.6	36.7	42.7	47.8	45.2
FPc/BPc[3]	0.47	0.44	0.50	0.55	0.58	0.58	0.58	0.62
Phase II (8 to 11 weeks)								
THRc (g/kg)	1.8	2.3	2.4	3.0	3.6	4.2	5.4	5.9
FPc (g)	30.7	33.9	35.9	44.3	44.8	49.1	55.5	56.2
BPc (g)	70.9	77.1	74.0	88.6	86.7	92.3	89.8	105.6
FPc/BPc	0.43	0.44	0.49	0.50	0.52	0.53	0.62	0.53
Phase III (13 to 16 weeks)								
THRc (g/kg)	1.5	2.0	2.0	2.5	3.1	3.6	4.6	5.1
FPc (g)	54.6	56.8	59.1	66.4	69.0	76.8	79.2	75.9
BPc (g)	112	115	124	133	134	137	142	144
FPc/BPc	0.49	0.49	0.48	0.50	0.52	0.56	0.56	0.53

[1]Feather protein content - calculated as the sum of the protein content in the feathers at the beginning of each phase (3.75; 27.5 and 57.5 g, respectively for phases I, II and III) and the daily gain in each phase. [2]Body protein content - calculated as the sum of the protein content in the body feather free at the beginning of each phase (14.3; 60.4 and 112g, respectively for phases I, II and III) and the daily gain in each phase. [3]FPc/BPc - (Feather protein content/body protein content).

4. Discussion

The objective of the research reported here was to measure the response of growing pullets during three phases of growth to dietary THR with a view to determining the efficiency of utilisation of THR for body and feather protein growth, and to determine whether this efficiency would change as they became older and approached sexual maturity. The three phases used in the trial represented an early phase, when the degree of maturity of the pullets (u, body protein at time *t*/mature body protein) was only 0.116; the middle phase represented the period closer to that of maximum growth (u = 0.282) and the latter period represented the phase just prior to the development of sexual maturity, when growth rate is slowing down (u = 0.497). The mature body protein was estimated by Gompertz as 0.261 kg. Feathers grow at a different rate to the body (Emmans, 1987) so it would be expected that the relative growth rates of body and feather protein would differ in each of these periods, but because the THR content of body protein is so similar to that of feather protein, these relative differences would not have influenced the response as much as might some other amino acids

whose concentrations in body and feather protein differ markedly, as is the case with lysine and cysteine (Silva et al., 2013).

The values found in the literature for THR content in the body and the feathers range from 33.9 to 40.2 mg/g for the feather-free body (Saunders *et al*., 1997, Williams *et al.,* 1954; Stilborn *et al*., 2010; Silva, 2012), and from 42.0 to 44.9 mg/g for feathers (Fisher *et al*., 1981; Stilborn *et al.,* 1997; Silva, 2012). The THR contents of body and feather protein determined in this trial (37.7 and 43.8 mg/g respectively) are similar to these published values and could therefore confidently be used to calculate the deposition of threonine in the body and feathers.

Of importance in measuring the efficiency of utilisation of THR for protein deposition is to ensure that the linear portion of the response curve is identified and used for this purpose. We used the broken stick model to identify the point at which no further response to THR intake was obtained, and then used all points below this intake to measure the regression coefficient, which was then compared using simple linear regression with groups, the groups in this case being the three periods during which measurements of response were made.

Also of importance was the confirmation that all feeds used in the three periods were first-limiting in THR, as indicated by the significantly improved response in food intake, growth and FCE when synthetic THR was added to the feed with the lowest THR content in each of the response periods. This is one of the major advantages to the use of the summit dilution technique compared with the graded supplementation technique as in the latter technique it cannot be guaranteed that the amino acid under test remains first limiting in all the feeds in the dilution series, nor is it likely that this would be the case (Gous and Morris, 1985). The efficiencies of utilisation of THR in the three periods under test could be regarded as being accurately estimated using the method applied here.

The age of the pullets did not alter the regression coefficient reflecting the rate of protein deposition with increasing THR intake, the value being 0.024 ± 0.003 when data from the three periods were combined. The efficiency of utilisation of THR for protein deposition remained at 41.4% over all periods. According to Edwards et at. (1997), studing young chicks, THR concentration in the whole-body protein accreted was constant 4.41 mg (0.441) at all levels of THR intake, and this value is slightly higher than that found in this paper. Although this efficiency is relatively low, and according to Stoll (2006) amino acids can be deposited and recycled by the body for purposes pf growth or other biological functions (as

incorporation into protein and conversion via transamination into other amino acids, metabolic substrates and biosynthetic intermediates), therefore, in the case of some amino acids, namely threonine and cysteine, incorporation into endogenous secretions that are fermented in the large intestine represents a nutritional loss. Likewise, if the amino acids are completely oxidized to CO_2 by the mucosal cells, this is also a nutritional loss, especially in the case of essential amino acids. This low efficiency also possibly because of the imbalanced nature of the amino acids in the feed, the efficiency with which dietary THR was converted to THR in the body and feathers was considerably higher, with 0.855 ± 0.13 mg of dietary THR being converted to each mg of body and feather protein.

The increase in body lipid deposition as the THR content in the feed was reduced has been reported previously for broilers (Burnham *et al*., 1992) where the summit dilution technique was also applied. The highest lipid content recorded in this trial (87 g/kg, Table 2) is considerably lower than that reported for broilers in the above paper (190 g/kg, with the lowest lipid content in that trial being 100 g/kg). These birds seem to be able to deposit more fat in the first phase than in the other two phases (II and III), but this may be due to the range of THRc in this phase. The theory of food intake of Emmans (1981; 1987) predicts that birds will get fat when offered a feed limiting in protein or an amino acid because they will overconsume energy in an attempt to consume sufficient of the limiting nutrient in the feed in an endeavour to grow at their potential. The extent to which energy can be overconsumed depends on the amount of lipid that can be stored in the body: if the genotype is such that a large amount of lipid can be stored, then the bird will be capable of consuming relatively more of an imbalanced feed than one whose ability to fatten is limited genetically. The range of food intakes measured in this trial was relatively small, and in no instances did the food intake on marginally deficient feeds increase above that of the pullets on the highest level of THR, whereas in the Burnham *et al*. (1992) experiment food intake increased from 41.8 g/bird d on the feed with the highest isoleucine content to 45.7 g/bird d on a marginally deficient feed, the intake finally decreasing to only 37.0 g/d on the lowest isoleucine feed. Similarly, in a trial reported by Clark *et al*. (1982) in which the response of broilers to a well balanced amino acid mixture was measured, food intakes increased from 33.9 g/d on the highest protein level to 37.2 on a marginally deficient feed, dropping to 28.0 on the lowest protein level used in the trial. Feed intake decreased over the phase III (23%) showing a higher capacity for thermal regulation of these birds at this phase, i.e., by adjusting the feed intake capacity

according to fat deposition and heat production. Clearly, the pullets in this trial were not capable of overconsuming energy in an attempt to consume sufficient dietary THR, with the consequence that body weight gains decreased to a greater extent than would have been the case had the birds been capable of consuming excess lipid. It is also important to remember that these birds need a minimum body fat content in order to lay eggs, as these reserves are important during production. Thus, the lowest level of fat should be carefully noted. Nevertheless, the advantage of measuring protein gain and using this to calculate efficiencies of utilisation rather than working with body weight gains is obvious.

Body and feather protein grow at different rates (Emmans, 1987; Hancock *et al*., 1995; Gous *et al*., 1996) but there is less evidence of the relative growth rates of these two proteins when the bird is subjected to feeds differing in protein content. The data in Table 3 are thus of interest, as they demonstrate that the ratio of feather to body protein does not remain constant at an age (or degree of maturity) when different dietary protein levels are fed. Irrespective of the age of the pullets, the ratio of body protein to feather protein increased at a rate of 0.0248 ± 0.0036 as the dietary THR content (mg/g) increased. Deficiencies in dietary protein therefore reduce the rate of feather protein growth relative to body protein growth

The responses in body and feather protein growth measured in this trial are of value in determining the optimum levels of THR to be included in feeds for growing pullets. The objective was not to define the optimum intake of THR in each phase, but to make use of the determined efficiencies of utilisation of THR for protein growth to define the amount of dietary THR that would be required by growing pullets on each day of the growing period, taking account of their daily potential rates of growth of body and feather protein. As the efficiency of utilisation remained the same in all three phases of growth it is not necessary to use different efficiencies to describe their mean daily THR requirements. By defining the rates of growth of body and feather protein, and calculating the amount of THR required each day to meet these requirements, taking account of the efficiency of utilisation of THR for protein growth, and including an amount to meet the requirement for THR to maintain body and feather protein, it is possible to determine the daily requirement for THR to enable a pullet to grow at its potential. These daily requirements need to be converted to dietary concentrations, from which a feeding programme can be devised that will minimize the periods of under- and over-feeding of THR whilst minimizing the number of feeds to be used in the feeding programme.

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CAPÍTULO 3 - DESCRIPTION OF A MODEL TO OPTIMISE THE FEEDING OF AMINO ACIDS TO GROWING PULLETS

Abstract

This study aimed to provide models to estimate threonine requirements for pullets. Two models were developed, the first dealing with the growth of body and feather protein only, whilst the second includes the additional amount required for the growth of the reproductive organs. The model considers the maintenance, feathers loss, protein depositions in body and feathers, threonine content in these parts, and threonine efficiency. The potential rates of body and feather protein growth are described by the Gompertz growth curve. The rates of maturing of the body, feather-free body and body protein were shown to be similar at 0.017/d (B). The rate of growth of the oviduct appears to be considerably faster than that of the ovary (0.139 *vs.* 0.084 /d) but the ovary attains a higher mature weight (78.4 *vs*. 58.7 g). The requirements predicted by the two models remain the same until the growth of the reproductive organs is initiated, the age at which this occurs being defined by the user. The model proposed in this paper seems to be reliable in estimating the requirements, since it considers the growth of the reproductive organs, and sexual maturity can be manipulated with the use of lighting programmes to change the age at which the reproductive organs start growing. The models predict realistic threonine requirements for pullets growing at their potential from hatching to sexual maturity.

Keywords: Gompertz curve, reproductive organs, desire food intake

1. Introduction

Models to predict amino acid requirements for pullets are scarce, and the development phase is very important, as it also involves the growth of reproductive organs, such as ovary and oviduct. Martin *et al.* (1994) published a model for calculating the amount of each amino acid that would be required by the average individual in a flock of growing pullets, considering separately the protein deposited in the feather-free body (BFF) and in the feathers, given that these components grow at different rates (Emmans, 1987; Hancock *et al*., 1995; Gous *et al*., 1996) and in most instances have different amino acid compositions (Emmans, 1987). Growth of these components was described by the Gompertz growth curve, and efficiencies of utilisation of amino acids for the growth of protein were regarded as being fixed, since the age of the pullets did not alter the regression coefficient reflecting the rate of protein deposition with increasing THR intake.

In a model proposed by Silva (2012) the requirement for amino acids needed to support the protein deposition in the ovary and oviduct that occurs prior to the attainment of sexual maturity was added to the model described by Martin *et al.* (1994). The growth of these two components has been described for broiler breeders by Bowmaker and Gous (1989). As sexual maturity may be manipulated with the use of lighting and feeding programmes (Lewis *et al.*, 2003) the age at which the reproductive organs start growing cannot be fixed in any model, and must remain variable. Because the requirement for amino acids increases when the bird begins to lay the model necessarily stops on that day, and a different approach needs to be taken to determine the optimum amount of each amino acid to include in the feed for laying hens.

The model described in this paper is designed to optimise the feeding of amino acids to growing pullets, and for this purpose, threonine is used as the reference amino acid given that the data used in the model are from experiments conducted by Bonato *et al*. (2013) involving the use of threonine limiting feeds. Maintenance threonine (THR) requirements was determined in previous studies (Bonato *et al*., 2011), and THR response trials were conducted over three periods during the growth of pullets to measure body and feather protein growth, food intake and the rate of body lipid deposition resulting from the feeding of THR-limiting feeds (Chapter 2 of this thesis). Thus, the objective of this study was to describe a model to predict THR requirements for pullets.

2. Material and methods

2.1. Models to estimate THR requirements

Two models are described, one dealing only with the growth of body and feather protein, whilst the second includes the additional amount required to support the growth of the reproductive organs prior to the attainment of sexual maturity. Both models are therefore the same up to the time when the reproductive organs begin to develop. The requirement for each essential amino acid is calculated for the average individual in the flock, whose rates of body and feather protein growth are described by the Gompertz growth curve. The requirement is partitioned into that for maintenance of both the BFF and the feathers, and for the deposition of protein in the BFF and feathers.

Model 1 (Martin *el al*., 1994) which excludes the growth of the reproductive organs, is described in Eq. 1 below:

$$AA = \left[(AAm_c \,.\, BP_m^{0.73} \,.\, u) + \left(FPL \,.\, FP \,.\, AA_f\right)\right] + \left[\left(\frac{AA_b .\ BPd}{k}\right) + \left(\frac{AA_f \,.\, FPd}{k}\right)\right] \quad [1]$$

Where: *AA* is the amino acid (THR in this case) requirement (mg/d) for the average bird in the population; AAm_c is the amino acid requirement for maintenance of BFF (mg.$BP_m^{0.73}$.*u*); $BP_m^{0.73}$ is the mature body protein weight (kg), *u* is the degree of maturity of body protein ($u=BP_t/BP_m$); *FPL* is the loss of feather protein (g); *FP* feather protein weight at time *t* (g); AA_b is the amino acid content in body protein (mg/g); AA_f is the amino acid content in feather protein (mg/g); *BPd* is the rate of body protein deposition (g/d); *FPd* is the rate of feather protein deposition (g/d), and *k* is the efficiency of utilisation of the amino acid for protein deposition. Bonato *et al*. (2011) estimated the maintenance requirement of THR as 75.6 mg/$BPm^{0.73}$u/d.

Model 2 (Martin *et al.*, 1994; Silva, 2012), which includes the requirement for the development of the reproductive organs, is described below:

$$AA = \left[(AAm_c \,.\, BP_m^{0.73} \,.\, u) + \left(FPL \,.\, FP \,.\, AA_f\right)\right] + \left[\left(\frac{AA_b . BPd}{k}\right) + \left(\frac{AA_f . FPd}{k}\right) + \left(\frac{AA_b . BPd_{ova}}{k}\right) + \left(\frac{AA_b . BPd_{ovi}}{k}\right)\right] \quad [2]$$

The first part of this equation is the same as Model 1, to which has been added $Bpd_{ova,}$ the rate of deposition of protein in the ovary (g/d); $BPd_{ovi,}$ the rate of protein deposition in the oviduct (g/d); and *k* which is the efficiency of utilisation of each amino acid for protein deposition.

The efficiency of utilisation of THR for the deposition of body and feather protein was measured in Chapter 2 of this thesis and this was shown to be constant over the three periods chosen, so the same value for *k* (0.855) may be applied throughout the growing period. The model 2 detects when the bird will lay its first egg by changing the day when the reproductive organs starts growing, and this will depending on lighting.

2.2. Growth parameters – description of Dekalb White pullets

In the trials reported in Chapter 2 of this thesis body weight (BW), feather-free body weight (BWFF), feather weight (FW), feather-free body protein content (BP) and feather protein content (FP) were measured in pullets at 0, 14, 31, 45, 59, 72, 96 and 108 d, and these measurements were used to describe the growth of these pullets and their components using the Gompertz (1825) growth curve. These measures were from references slaughterings in each age cited above and from treatments where none amino acid was limiting. The form of the equation used was that described by Emmans (1981) (Eq. 3). The estimates of the parameters of the fitted equations for each variable were estimated using the Gauss-Newton method, by means of the procedure "NLIN" SAS software (version 9.1), these being: P_i - initial weight (kg); P_m - weight at maturity (kg); and *B* – rate of maturing (/d); *ln* is the natural logarithm; P*t* is the weight of the bird at time *t*.

$$Pt = P_m . e^{-e\left(\ln\left(-\ln\left(\frac{Pi}{Pm}\right)\right)-(B.t)\right)} \quad [3]$$

The rate of deposition of protein in the body and feathers was obtained by differentiating Eq. 3, resulting in Eq. 4:

$$dP/_{dt} = B . P_m . \ln\left(\frac{P_m}{Pt}\right) \quad [4]$$

Silva (2012), based on Emmans (1989), found that the coefficient for protein loss via feathers was 0.04 g/d. The rate of THR deposition in each compartment was obtained by multiplying the rate of protein deposition (estimated using Eq. 4) by the THR content of protein in BFF and in feathers, analyzed in the laboratory.

The rates of growth of the ovary and oviduct were estimated by fitting the Gompertz equation to the weights of these two organs measured during their development. The chemical composition during the growth of these organs, making use of appropriate equations, these values were used to estimate their protein and lipid contents from which the daily gains in protein and lipid were calculated, according to Silva (2012).

2.3. Estimating desire feed intake

In order to predict the amount of feed that the average individual in the population would need to consume each day in order to grow at its potential, the effective energy requirement (EERQ) (Emmans, 1989) of the pullet was calculated for each day of the growing period according to Eq. 5 below, and this was divided by the effective energy (EE) content of the given feed to calculate the desired feed intake (DFI) of the pullet (Emmans, 1981):

$$EEQR = MH + 50\frac{dP}{dt} + 56\,\frac{dL}{dt} \qquad [5]$$

Where: *EEQR* is the effective energy requirement (MJ/d); *MH* is the heat required for maintenance (MJ/d); *dP/dt* is the rate of deposition of body plus feather protein (g/d); *dL/dt* is the rate of deposition of body lipid (g/d). *MH* is given by Eq. 6:

$$MH = MM_E.P_m^{0.73}.u \qquad [6]$$

Where: M_E is 1.63 MJ, the EE needed per unit of maintenance each day; P_m body protein weight at maturity (kg); u is the degree of maturity of body protein $(u = BP_t / BP_m)$.

Desire food intake (DFI, g) (in a thermoneutral environment) were determined by Eq. 7:

$$DFI = EEQR/EEDiet$$

[7]

Where: *EEDiet* is the amount of efective energy in the diet, and is the average over 3 phases (10.6 MJ/kg), based on diets in Table 1.

Table 1. Composition (g/kg) of the basal diets used in each phase of the trial together with the analysed nutrient content of each diet

Ingredients	Diets					
	Phase I		Phase II		Phase III	
	Summit	N Free	Summit	N Free	Summit	N Free
Maize	555	-----	622	-----	669	-----
Soybean meal	391	-----	223	-----	157	-----
Starch	-----	543	-----	509	-----	540
Rice Husk	-----	120	-----	148	-----	150
Sugar	-----	120	-----	150	-----	120
Sand	-----	118	-----	100	-----	100
Wheat bran	-----	-----	87.0	-----	111	-----
Soybean oil	13.3	50.0	30.0	50.0	30.0	50.0
Dicalcium phosphate	17.4	23.6	15.0	21.2	10.6	16.8
Limestone	10.5	6.60	9.90	4.70	12.2	6.60
Salt	4.60	4.20	3.50	3.60	3.30	3.40
Potassium choride	-----	11.5	-----	11.3	-----	10.9
DL- Methionine (98 %)	3.50	-----	2.60	-----	2.00	-----
L-Lysine (78.5 %)	2.60	-----	2.40	-----	2.30	-----
L-Tryptophan (93.5 %)	-----	-----	0.40	-----	0.50	-----
L-Valine (98 %)	-----	-----	0.80	-----	0.50	-----
L-Isoleucine (98 %)	-----	-----	1.00	-----	0.80	-----
Vitamin/Mineral Premix[1]	1.50	1.50	1.50	1.50	1.50	1.50
Choline Chloride	0.70	0.70	0.70	0.70	0.70	0.70
Antioxidant	0.10	0.10	0.10	0.10	0.10	0.10
Nutrients						
Metabolizable energy[2] (MJ/kg)	12.1	12.1	12.1	12.1	12.1	12.1
Effective Energy[3]	9.87	NC[6]	10.8	NC	11.2	NC
Crude Protein[4]	223	6.70	180	6.70	153	6.70
Lysine[4,5]	14.0	-----	9.93	-----	8.32	-----
Methionine + cystine[4,5]	10.3	-----	8.05	-----	6.95	-----
Methionine[4,5]	6.70	-----	5.12	-----	4.27	-----
Tryptophan[4,5]	2.83	-----	2.57	-----	2.25	-----
Threonine[4,5]	8.15	-----	5.94	-----	5.10	-----
Arginine[4,5]	15.9	-----	11.2	-----	9.38	-----
Valine[4,5]	10.5	-----	8.53	-----	7.20	-----
Isoleucine[4,5]	9.89	-----	7.90	-----	6.55	-----
Leucine[4,5]	19.4	-----	15.2	-----	13.7	-----
Phenylalanine + tyrosine[4,5]	11.8	-----	8.59	-----	7.37	-----
Calcium	9.40	9.40	8.30	8.30	8.00	8.00
Sodium	1.80	1.80	1.60	1.60	1.50	1.50
Avaliable phosphorus	4.40	4.40	3.90	3.90	3.10	3.10
Potassium	8.70	8.70	6.60	6.60	5.70	5.70
Crude fibre	30.7	47.5	30.6	58.7	30.0	59.4

[1]Content/kg - vit. A = 8.000.000IU, vit. D3 = 2.300.000IU, vit. E = 12.350g, vit. B1 = 2.400mg, vit. B2 = 5.950mg, vit. B6 = 2.500mg, vit. B12 = 12.000mcg, Niacin = 38.000mg, pantothenic acid = 12.000mg, vit. K3 = 1.800mg, folic acid = 950 mg, selenium = 300mg, antioxidant = 250mg, Biotin = 60mg, manganese = 200.000mg, iron = 100.000mg, zinc = 160.000mg, copper = 16.000mg, iodine = 1.500mg.

[2]Predicted value calculated according to Rostagno *et al.* (2005).

[3]Effective energy of the diet according to Emmans (1987).

[4]Values analyzed by HPLC.

[5]Digestible amino acid composition determinated by trials with cecectomized roosters.

[6]Not calculated.

2.4. Estimating THR of diets

The THR content (%) in the given feed that would be required to supply the daily amount of THR required by the bird was then calculated as the daily THR requirement (mg/d) estimated using models 1 and 2 divided by the DFI (g/d). As it is not practical to change the composition of the feed daily, estimates were made of the optimum THR content to be included in the feed during defined feeding periods during the growing period (the user can define these periods or use the standard (0 to 6, 7 to 12 and 13 to 18 weeks).

3. Results

3.1. Describing the growth and composition of Dekalb White pullets

Estimates of the Gompertz model parameters describing the growth and composition of the Dekalb pullets, based on the data of Chapter 2 of this thesis are given in Table 2. These values were used in turn to describe the growth of BW, BWFF, FW, BP and FP, and the weekly weights of these variables are given in Table 3.

Table 2. Estimates of the Gompertz parameters that describe the growth of the body (BW), feather-free body (BWFF), feathers (FW) and body- (BP) and feather - (FP) protein

	Gompertz parameters[1]				
	BW	BWFF	FW	BP	FP
P_i (kg)	0.036 (±0.0012)	0.034 (±0.011)	0.002 (±0.0001)	0.005 (±0.0003)	0.001 (±0.0001)
P_m (kg)	1.595 (±0.191)	1.450 (±0.120)	0.145 (±0.0131)	0.261 (±0.0204)	0.128 (±0.0054)
B (/d)	0.017 (±0.0007)	0.017 (±0.0002)	0.021 (±0.0004)	0.017 (±0.0003)	0.020 (±0.0004)

[1] $Pt = P_m . e^{-e\left(\ln\left(-\ln\left(\frac{Pi}{Pm}\right)\right)-(B.t)\right)}$, where: P(t) - weight at time t; P_i – weight at day 1 (kg); P_m – maturity weight (kg); B – maturation rate (/d); t – age (days).

The rates of maturing of the body, the feather-free body and body protein were constant at 0.017/d (B), whereas that for feathers and feather protein was higher, indicating that feathers reach their mature weight earlier than do the components of the feather-free body. The mature weight of the feather-free body was estimated to be 1450 g and that of feathers, 145 g. The gains in protein (*dw/dt*) of the body and the feathers of Dekalb White birds to 20 weeks are illustrated in Figure 1 where the difference in the maximum growth rate, which

occurs at t^*, can be observed. These ages are, respectively, 80 and 68 days for body and feather protein.

Table 3. Mean weekly estimates of body weight (BW, kg), feather-free body weight (BWFF, kg), feather weight (FW, kg), feather-free body protein weight (BP, kg) and feather protein weight (FP, kg) of Dekalb White pullets estimated using the Gompertz model parameters given in Table 1

Week[1]	BW (kg)	BWFF (kg)	FW (g)	BP (g)	FP (g)
1	0.05	0.05	2.84	7.75	2.44
2	0.08	0.07	4.81	11.5	4.14
3	0.11	0.10	7.58	16.3	6.53
4	0.15	0.14	11.2	22.3	9.70
5	0.20	0.18	15.8	29.4	13.7
6	0.25	0.23	21.3	37.5	18.4
7	0.31	0.28	27.5	46.6	23.8
8	0.37	0.33	34.4	56.6	29.8
9	0.43	0.39	41.6	67.2	36.2
10	0.50	0.45	49.2	78.2	42.8
11	0.57	0.51	56.9	89.5	49.5
12	0.64	0.58	64.4	101	56.2
13	0.71	0.64	71.8	112	62.7
14	0.78	0.70	78.9	123	69.0
15	0.84	0.76	85.6	134	74.9
16	0.91	0.81	91.8	145	80.4
17	0.96	0.87	97.6	155	85.5
18	1.02	0.92	103	164	90.3
19	1.07	0.97	108	173	94.6
20[2]	1.12	1.01	112	181	98.4

[1]Calculated data per day and presented the average by week.

[2]Degree of maturity ($u = BP_t / BP_m$) of the bird at 20 weeks = 0.693.

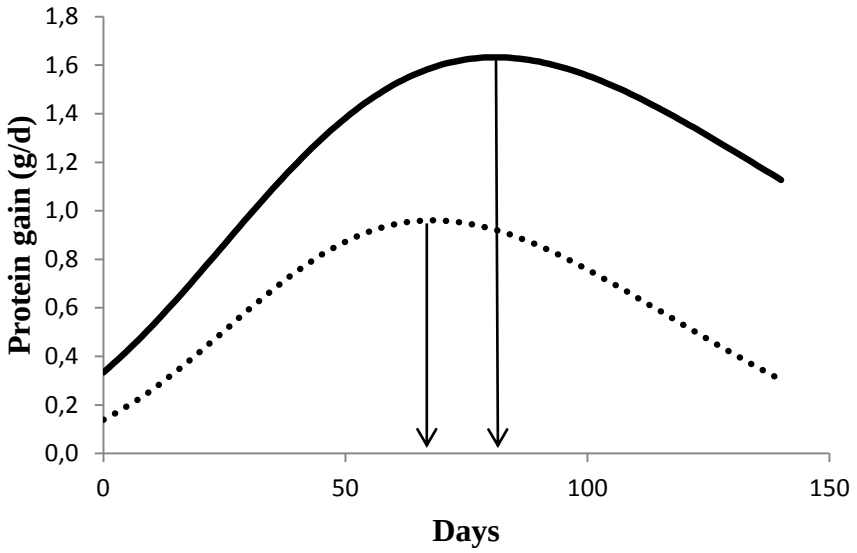

Figure 1. Body protein gain (solid line) and feathers gain (dotted line) of Dekalb White birds, estimated by Gompertz equation derived up to 18 weeks of age. Arrows indicate the ages at which protein growth is maximised (t^*).

3.2. Describing the growth and composition of reproductive organs

In the trial conducted by Silva (2012) growth of the reproductive organs began around the fifteenth week of age, with the ovary reaching a maximum rate of growth between 130 and 135 d and the oviduct between 121 and 122 d. However, because the age at maturity may be manipulated with the use of lighting and feeding programmes, the growth of these organs for modelling purposes was assumed to start at day 0 rather than at the ages measured in the trial. In this way, growth of these organs may be initiated at any age in the model, thereby making the model more universally applicable. Estimates of the Gompertz model parameters describing the growth of the ovary and oviduct of Dekalb pullets, based on the data of Silva (2012) and starting at day 0 are given in Table 4. The rate of growth of the oviduct appears to be considerably faster than that of the ovary (0.139 *vs.* 0.084 /d) but the ovary attains a higher mature weight than the oviduct (78.4 *vs.* 58.7 g). The gain in protein in the ovary and oviduct are illustrated in Figure 2.

Table 4. Gompertz parameters of growth of ovary and oviduct of Dekalb White strain, with growth starting at day 0

Compartment	Parameters[1]		
	P_m (g)[2]	B (/d)[3]	t* (d)[4]
Ovary	78.4 (±9.25)	0.084 (±0.0016)	52 (±2.66)
Oviduct	58.7 (±7.22)	0.139 (±0.0345)	46 (±2.18)

[1]Data from Silva (2012). [2]P_m – maturity weight (g). [3]B – maturation rate (/d). [4]t*- age or time that organ is growing at maxima rate or curve inflection (d) from day 0, day which the reproductive organs begin to develop.

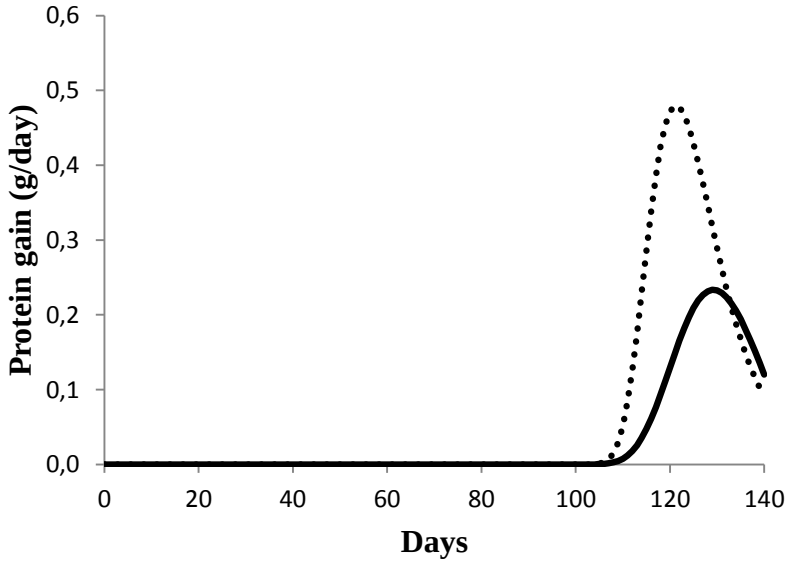

Figure 2. The rate of gain in protein in the ovary (continuous line) and the oviduct (dotted line) of Dekalb White pullets estimated using the Gompertz equation derived.

The most appropriate equations for describing changes in the protein and lipid contents during the development of the reproductive organs differ from each other (Table 5). These equations were adjusted order to explain how changes occur in the composition of protein and fat during growth of the reproductive organs. And were based on data from Silva (2012).

Table 5. Fitted regressions for protein and fat contents (g/kg) during the development of ovary and oviduct growth

	Ovary	R^2
Protein	133 (±4.30) + 0.50 (±0.50) age - 0.020 (±0.010) age^2	33.6
Fat	79.4 (±0.40)	---
	Oviduct	
Protein	160 + 31.1 * (2.10^{age})	88.2
Fat	113 (±14.0) - 0.30 (±1.60) age - 0.0450 (±0.0350) age^2	80.4

3.3. Estimating DFI, EERQ and THR requirements

The mean EE content of the experimental diets, averaged over the 3 phases of the trial conducted in Chapter 2 of this thesis was 10.6 MJ/kg. This value was used to calculate the DFI of the average pullet in the population, based on her *EERQ*. The estimated daily EE and THR requirements (based on Models 1 and 2), DFI and the required dietary THR content to ensure the desired THR intake are given in Table 6.

Table 6. Daily threonine requirements based on Model 1 ($THRr_1$) and Model 2 ($THRr_2$), effective energy requirement ($EERQ_1$ and $EERQ_2$), desired feed intake (DFI_1 and DFI_2), required dietary threonine content based on model (THR_1 and THR_2), per week, for Dekalb-type pullets

Week[a]	$THRr_1$[b] (mg/d)	$THRr_2$[c] (mg/d)	$EERQ_1$[d] MJ/d	$EERQ_2$ MJ/d	DFI_1[e] g	DFI_2 g	THR_1[f] (g/kg)	THR_2 (g/kg)
1	36.8		57.9		5.46		6.75	
2	51.8		80.8		7.63		6.80	
3	69.5		108		10.2		6.82	
4	89.0		138		13.1		6.81	
5	110		172		16.2		6.78	
6	131		206		19.4		6.72	
7	151		241		22.7		6.65	
8	170		275		26.0		6.55	
9	188		309		29.1		6.44	
10	203		341		32.1		6.32	
11	216		370		34.9		6.19	
12	227		398		37.5		6.05	
13	236		423		39.9		5.92	
14	243		446		42.0		5.78	
15	248	248	466	466	44.0	44.0	5.65	5.65
16	252	258	485	497	45.7	46.9	5.51	5.51
17	255	280	501	548	47.3	51.7	5.39	5.41
18	256	284	515	564	48.6	53.2	5.27	5.33
19	257	276	528	558	49.8	52.6	5.16	5.24
20	257	266	539	550	50.9	51.9	5.05	5.13

[a]Calculated data per day and presented the average by week.

[b]$AA = \left[(AAm_c \,.\, BP_m^{0.73} \,.\, u) + \left(FPL \,.\, FP \,.\, AA_f\right)\right] + \left[\left(\frac{AA_b \,.\, BPd}{k}\right) + \left(\frac{AA_f \,.FPd}{k}\right)\right]$ (md/d).

[c]$AA = model\ 1 + \left(\frac{AA_b.BPd_{ova}}{k}\right) + \left(\frac{AA_b.BPd_{ovi}}{k}\right)$] (md/d).

[d]$EEQR = MH + 50\frac{dP}{dt} + 56\ \frac{dL}{dt}$ (MJ/d).

[e]$DFI = EEQR/EEDiet$ (g).

[f]THR (g/kg) = [(daily THR requirement estimated by Models 1 and 2/DFI)] based on an EEDiet of 10.6 MJ/kg.

The desired feed intake (Table 6) compared with food intake predicted by pulltes guide manage (Dekalb White Strain) are presented in Figure 3.

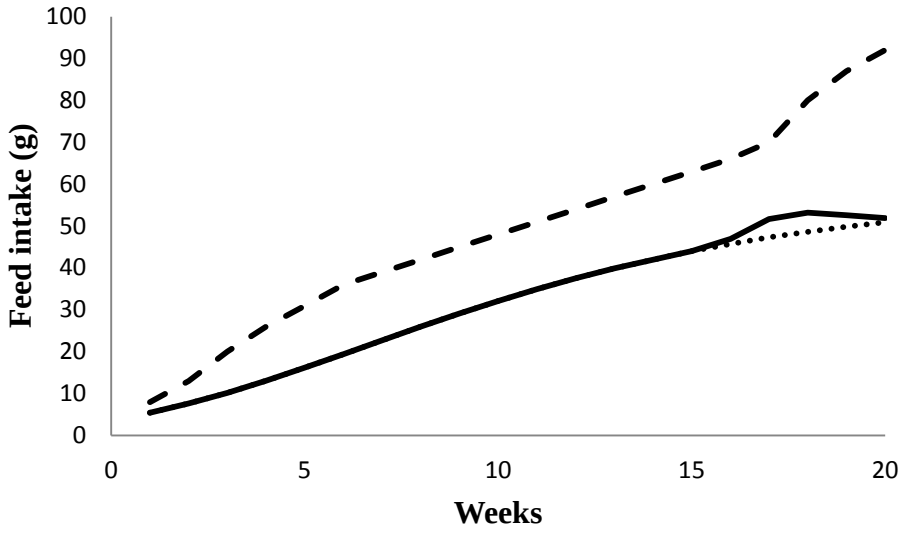

Figure 3. Feed intake according to pullets guide manage (Dekalb White strain) (dashed line), desired food intake according to model 1 (dotted line) and model 2 (continuous line).

The THR requirement per d and per kg feed for Dekalb-type pullets based on the two models proposed are illustrated in Figure 4. The requirements predicted by the two models remain the same until the growth of the reproductive organs is initiated (Table 4, Figure 4), the age at which this occurs being defined by the user. The relatively small increase in the daily requirement to meet the needs for ovary and oviduct growth results in a small increase in the required THR content of the feed during this time. Four feeding phases are suggested according to the requirements and pullets age. A summary of THR requirements for each phase, whereas diferente dietary effective energy contentes are presente in Table 7.

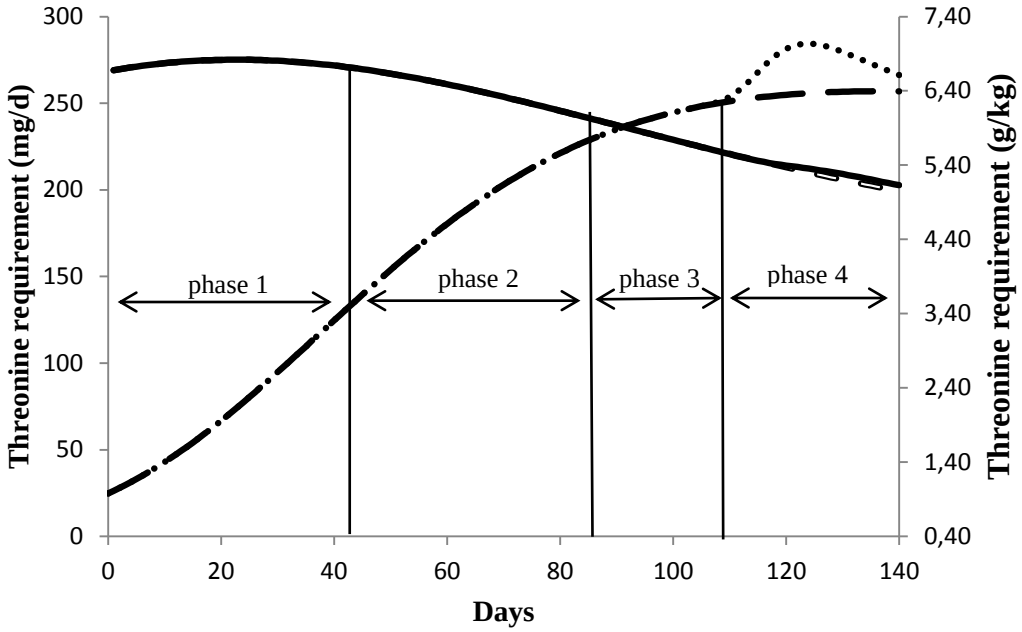

Figure 4. Estimated threonine requirement by model 1 (mg/d - dotted and dashed line, and % - solid line) and model 2 (mg/d - small dotted line, and % - dashed line) as a function of age (from 1 to 20 weeks).

Table 7. Threonine requirements (THR, g/kg) estimated for phases 1 (1 to 6 weeks), 2 (7 to 12 weeks), and 3 (13 to 20 weeks) according to dietary effective energy (EE, MJ/kg) content

EE	THR (g/kg)		
MJ/kg	Phase 1	Phase 2	Phase 3
9.5	6.09	5.75	4.97
10.0	6.41	6.05	5.23
10.5	6.73	6.35	5.49
10.6	6.78	6.37	5.59
11.0	7.05	6.66	5.75
11.5	7.37	6.96	6.01
12.0	7.69	7.26	6.28

4. Discussion

Any method of determining the optimum amino acid content to be used in a feeding programme for growing pullets should be based on the potential growth rate of the pullets, which would define the amount of each essential nutrient that is required by the bird on each day of growth. This is assuming that the pullet producer wishes to grow pullets at their

potential; if not, the desired growth rate would need to be defined after which the same procedure would follow in both cases. The potential growth rates of laying-type pullets have been studied previously by Martin *et al.* (1994) and the values of the Gompertz parameters of the three strains measured for body feather free are Pm (kg) 1.748 (BW), 0.312 (BP), and B (/d) 0.020 (BP); and for feathers are Pm (kg) 0.171 (FW), 0.112 (FP), and B (/d) 0.026 (FP). The mature weights and rates are above those find in this paper, and means that Dekalb White pullets reach maturity body and proteic weight slowly than the strains studied by Martin *et al*. (1994) (Hisex, Ross Brown and Amber-Link), and also has a low weight at maturity. As for the feathers, the strain studied in this paper, has a slower feathering, but with a greater maturity protein weight. During the period studied (growth phases), the birds have not reached maturity (maturity (u) = 0.693 at 20 weeks), meaning that they began lay eggs without reaching maturity.

Regarding the growth of the reproductive organs, our maturity weights and rate of maturing are lower than found by Bowmaker and Gous (1989) with broiler breeders, except for oviduct rate of maturing. The oviduct matures before the ovary (46 *vs* 52 days), and this is due to pulltes oviduct needs to be ready before the ovulation starts. The age at occours the maximum gain are for ovary and oviduct, respectively, 124 and 120 days, the authors citated above, found at 168 days for both organs. Already Kwakkel *et al*. (1995) studing Leghorn White pullets estimated an age at maximum gain at 140 days. The differences from literature are due the diferente strains used. The way that protein and fat are deposited in each of these organs are different (Table 5) and this is directly related to the growth and maturation of each organ. In this study we assume that the birds will begin to lay eggs at 20 weeks, but this can be modified according to the light stimulation, since this factor will determine if the reproductive organs will begin to develop.

Because the age at sexual maturity in a flock of laying pullets can be manipulated with the use of lighting and feeding programmes (Lewis *et al*., 2003) the age at which the reproductive organs begin to develop should not be fixed in a model to predict the nutrient requirements of a flock of pullets during growth. Once the majority of the flock has started laying, nutrient requirements change and need to be dealt with using a different approach to that used here. It is likely that 0.5 of a flock of pullets would reach sexual maturity by 20 weeks of age, hence the period of growth described in Table 3 ceases at 20 weeks. In the model developed here, the age at which the ovary and oviduct begin to develop is user-

controlled; hence the model is sufficiently versatile to account for changes in age at sexual maturity in the flock. But the beginning of organ development will never be before 9 or 10 weeks old, by bird/strain growth capacity, the user must take this into consideration and be cautious when defining when these organs will begin to develop. The same kind of approach was used by Martin *et al*. (1994) in order to predict lysine, methionine+cystine and tryptophan for pullets, but without considers reproductive organs. Anyway, this method (model) has never been used to calculate THR requirements.

The calculation of a DFI based on the EE content of the feed and the EE requirement each day is a way of estimating the food intake of pullets during the growing period, from which it is possible to determine the necessary amino acid concentration in the feed on each day that would be needed to support the potential growth of the pullet. Any EE content can be entered into the program, and because the resultant THR contents are related to the EE content chosen, the EE content used would need to be specified when formulating feeds for the pullets. In Figure 3 is the comparison between feed intake predicted by pullet management guide and desired food intake by the models 1 and 2. There is an average difference of 37% more consumption recommended by the pullet management guide. It must be considered that the desired food intake takes into account the effective energy content of the diet, and this can change according to the ingredients used in the feed. Anyway, the estimated feed intake is still a major challenge, as many factors may affect, such as temperature, ingredients used, limiting nutrients, etc.

Any feeding programme could be chosen to be used during the growing period but a sensible approach would be to divide the 18 or 20 week period into three phases of about six weeks each. If the mean THR requirement during each phase is calculated from the values in Table 6 for a feed containing 10.6 MJ EE/kg, the period of under and over-supply of THR during each period would be minimised. The resultant values are given in Table 7 together with the ‘optimum’ THR contents when a range of EEC’s are used to predict DFI and hence THR content.

When the birds are given less THR in the feed than they need, i.e. at the start of each phase of feeding, the birds will attempt to consume sufficient of the THR by overconsuming energy, and they will get fatter than the desired level of fatness (Emmans, 1981). There is evidence that this happens (Gous, *et al.*, 1990), the extent to which energy can be overconsumed depends on the amount of lipid that can be stored in the body: if the genotype

is such that a large amount of lipid can be stored, then the bird will be capable of consuming relatively more of an imbalanced feed than one whose ability to fatten is limited genetically (Emmans, 1981; 1987). In the period when excess THR is fed the bird is able to reduce its intake of food because some of its energy requirements can be met by utilising body lipid reserves, and there is evidence for this also (Gous, *et al*., 2012).

THR content of the feed chosen should not stay very far from the required amount because these pullets do not seem to be capable of overconsuming energy, which means that if the THR level in the feed is too low, food intake will not compensate for this and the pullet growth will be compromised (Chapter 2 of this thesis). The difference between the THR requirement predicted by model 1 and model 2, from 15 weeks, is 6% on average, and this difference is due the inclusion of the growth of reproductive organs, and this allows estimating amino acid requirements with higher precision in the pre-laying phase. Thus, our results therefore evidence the need to determine the daily amount of Thr to be given to pullets by using a factorial model that considers body and feathers growth and the development of reproductive organs. The observed increase in Thr requirements in model 2 coincides with the age of maximum growth of the reproductive organs, indicating that this period is critical for the uniform development of these organs. Thus, ensure an adequate supply of Thr at this phase should contribute to the uniformity in the age pullets reach sexual maturity and begin laying eggs.

Based on this assumptions, as demonstrated in Figure 4, four feeding phases are suggested according to the requirements and pullets age, phase 1 from 1 to 6 weeks, phase 2 from 7 to 12 weeks, phase 3 from 13 to 15 weeks, and phase 4 from 16 to 20 weeks. It would be important to have more phases as much as possible, so the birds would not receive nutrients in excess or deficiency, but in practical situations are used 3 or 4 phases. The last phase is important because it is where is the maturation of the reproductive organs occur. However, these phases are in accordance with the predicted demands in this study, and can be changed according to the light stimulation. Simulation modelling is an accurate method to predict the requirements, however, these predictions are based on *ad libitum* food intake, and many pullets are reared under food restriction programme. So, the problem of determining an optimal feeding programme for pullets has still to be solved (Martin *et al.*, 1994).

The model proposed in this paper seems to be reliable in estimating the requirements, since it considers the growth of the reproductive organs, and sexual maturity can be

manipulated with the use of lighting programmes to change the age at which the reproductive organs start growing. Because the requirement for amino acids increases when the bird begins to lay the model necessarily stops on that day, and another model needs to be used to, taking into account the requirements for laying eggs.

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CAPÍTULO 4 - AMINO ACID REQUIREMENTS FOR PULLETS BASED ON POTENTIAL PROTEIN DEPOSITION AND THE EFFICIENCY OF AMINO ACID UTILIZATION

Abstract

The aim of this study was to estimate lysine (lys), methionine+cystine (met+cys) and threonine (thr) requirements for pullets based on protein deposition and efficiency of amino acid dietary, based on Goettingen University approach. Three assays of nitrogen balance were conducted with White Dekalb pullets, in periods I (14 to 28 d), II (56 to 70 d) and III (96 to 112 d). In each experiment, 56 pullets were distributed in a completely randomized design, with 5 treatments and 6 replications. The treatments were 5 diets with different nitrogen levels keeping constant the ideal ratio (amino acid/lys), except the amino acid test. A diet consisting at the first level which was added a small amount of each amino acid for the confirmation of this amino acid was limiting. The data of nitrogen intake (NI) and nitrogen excretion (NEX) were colleted and fitted by exponential equation, for each period to determine the NMR (nitrogen maintenance requirement). The maximum theoretical potential for nitrogen retention (NRmaxT) was determined by the exponencial relation between NI and nitrogen deposited (ND) for each period. Based on diets limiting in ly, met+cys and thr, the efficiency ($b.c^{-1}$) was obtained. The quality of dietary protein (b) is dependent on concentration of the limiting amino acid in the protein of the diet (c). Intake limiting amino acid (LAAI) was calculated.. All fitted equations were significant ($P<0.01$). The NMR determined were 270, 303 and 348 $mg/kg^{0.67}$/day, and NRmaxT were 3208, 2353 and 1739 $mg/kg^{0.67}$/day, for each period. The $b.c^{-1}$ values were: 50, 90 and 100 for lys, 170, 230 and 350 for met+cys and 100, 160 and 180 for thr, for periods I, II and III, respetively. These parameters were used in the formula and LAAI was determined considering 40, 50 and 60% of NRmaxT for the periods I, II and III, respectively. In general, the based on 50% of the NRmaxT values are lower than the recommended values, which is due the methodology applied in this study (factorial model, type of approach), although the requirements are consistent for pullets.

Keywords: nitrogen balance, nitrogen retention, nitrogen maintenance requirement, quality of dietary protein

1. Introduction

Poultry is one of the most important providers of animal protein to human diets (Embrapa, 2011). The egg is the main product of laying hens in commercial enterprises and contributes to the food protein supply. To ensure optimal development of layers (including their reproductive organs), the pullets need an adequate supply of nutrients during growth, particularly the supply of amino acids for body protein synthesis is very important.

To achieve the optimal content and dietary ratio of amino acids, it is necessary to determine both the efficiency of amino acid utilization and the rate of protein deposition (Baker et al, 2002). Several methodologies have been proposed to determine these parameters (Emmans, 1989; Martin el al., 1994; Edwards and Baker 1999; Sakomura and Rostagno, 2007). However, these procedures utilize large numbers of animals and require slaughtering of a relatively large number of birds to measure their responses. However, researchers at Georg-August-University in Goettingen, Germany, have proposed another approach. They have published studies based on factorial models using dose-response parameters obtained in nitrogen balance trials (Samadi and Liebert. 2006 a,b; Samadi and Liebert. 2007a,b; Samadi and Liebert, 2008, Liebert, 2008, 2013; Wecke and Liebert, 2013).

This approach makes use of a nonlinear mathematical model to estimate the efficiency of amino acid utilization (bc-1), taking into account the theoretical maximum for daily nitrogen deposition (NDmaxT) and the nitrogen maintenance requirement (NMR) for determination of amino acid requirements.

Most studies have been conducted using broiler chickens (Samadi and Liebert, 2006b; 2007a,b; 2008; Wecke and Liebert, 2013), swine (Thong and Liebert, 2004; Wecke and Liebert, 2009, 2010) or fish (Liebert, 2009). However, within the review undertaken, up to now no studies have been performed using pullets. The current study was designed to determine the amino acid requirements of pullets based on the Goettingen approach.

2. Materials and methods

Four nitrogen balance trials were conducted in the Laboratory of Poultry Sciences of the Faculty of Agricultural and Veterinary Sciences, on the Jaboticabal campus in São Paulo, Brazil.

The model parameters as determined in the four nitrogen balance (NB) trials were the nitrogen maintenance requirement (NMR) and the theoretical maximum for daily nitrogen

retention ($NR_{max}T$). The last three NB trials were performed both to determine the efficiency of lysine (Lys), methionine (Met) and threonine (Thr) utilization (bc^{-1}) and to estimate the requirements for these amino acids of Dekalb White pullets in various growth phases (**I**: 14 to 28 d, **II**: 56 to 70 d and **III**: 96 to 112 d). The procedures applied in this study are in accordance with earlier reports (Thong and Liebert, 2004; Samadi and Liebert, 2006a,b; 2007a,b; 2008; Wecke and Liebert, 2009, 2013; Liebert, 2008; 2009) performed to estimate the NMR, $NR_{max}T$, efficiency of amino acid utilization and amino acid requirements making use of nitrogen balance trials.

2.1. Animals, housing and experimental design

A total of 360 Dekalb White birds were used in the trials. The management of the pullets and the lighting program followed the recommendations of the pullet management guide. The experimental design was completely randomized, with five treatments and six replicates of one bird per cage. At the beginning of each phase, the body weight of the birds in the experimental units was standardized. The pullets were housed in metabolic cages with a floor space of 0.25 m^2/bird.

2.2. Dietary treatments

Prior to the start of each experimental period, the birds received diets formulated according to the recommendations of the Brazilian Tables for Poultry and Swine (Rostagno et al., 2005).

The first trial (T1) was conducted using graded dietary protein supply to determine the NMR and NRmaxT of the Dekalb White genotype (Table 1). Three trials (T2-T4) were conducted to estimate the model parameters (NMR, NRmaxT) and the efficiency of utilization of Lys, Met and Thr, respectively. The dietary treatments consisted of five levels of graded amino acid(s) supply from equal protein quality (e.g., L1, L2, L3, L4 and L5). In trial two (T2), Lys was the limiting amino acid in the diets; in trial three (T3), Met was the limiting amino acids in the diets; and in trial four (T4), Thr was the limiting amino acid in the diets (Tables 1 and 2). Each trial included a counter-proof treatment in which a small amount of the amino acid under investigation, Lys (T2), Met (T3) or Thr (T4), was added to a diet with the same composition of the L1 diet, to verify that the amino acid studied was in the first limiting position in each trial.

Diets were formulated using principles of the diet dilution technique (Fisher and Morris, 1970), according to the requirements of Rostagno et al. (2005). Table 2 presents the amino acid contents relative to the crude protein content and the ratio to the lysine content for each series of diets containing the various limiting amino acids. The treatments were the same in each study period.

Table 1. Nutritional composition of the experimental diets (g/kg) of four trials

Nutritional composition	First trial (T1)					Lysine trial (T2)					Methionine trial (T3)					Threonine trial (T4)				
	L1	L2	L3	L4	L5	$L_{Lys}1$	$L_{Lys}2$	$L_{Lys}3$	$L_{Lys}4$	$L_{Lys}5$	L_M1	L_M2	L_M3	L_M4	L_M5	$L_{Thr}1$	$L_{Thr}2$	$L_{Thr}3$	$L_{Thr}4$	$L_{Thr}5$
ME (MJ/kg)[1]	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.4	12.1	12.1	12.1	12.1	12.1
Crude protein[2]	**62.4**	**125**	**187**	**250**	**312**	**75.3**	**150**	**220**	**293**	**365**	**75.3**	**150**	**220**	**293**	**365**	**69.3**	**128**	**190**	**254**	**312**
Crude fiber	58.3	54.0	49.8	45.4	41.0	55.3	52.6	41.0	47.3	44.6	58.3	54.0	49.8	45.4	41.0	44.3	42.2	38.0	34.7	31.6
Calcium	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40	9.40
Available phosphorus	4.38	4.38	4.38	4.38	4.38	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.37	4.37	4.37	4.37	4.37
Potassium	5.22	6.49	7.75	9.05	10.3	5.20	5.20	5.20	5.20	5.20	5.22	6.49	7.75	9.05	10.32	8.59	8.59	8.59	8.59	8.59
Sodium	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Lysine[3]	3.14	6.43	9.72	13.0	16.3	**3.63**	**7.37**	**11.1**	**14.7**	**18.3**	4.27	8.73	12.87	17.20	21.46	3.48	6.46	10.24	13.71	16.99
Methionine+cystine	2.29	4.69	7.10	9.50	11.9	2.97	6.03	9.04	12.0	14.9	**2.58**	5.28	7.78	10.40	12.97	2.54	4.94	7.48	10.02	12.42
Methionine	1.54	3.16	4.78	6.39	8.01	1.87	3.80	5.71	7.58	9.42	1.49	**3.06**	**4.51**	**6.02**	**7.51**	1.74	3.38	5.12	6.86	8.50
Threonine	2.10	4.31	6.51	8.72	10.9	2.78	5.66	8.49	11.3	14.0	2.78	5.69	8.38	11.20	13.97	**1.91**	**3.72**	**5.63**	**7.54**	**9.35**
Tryptophan	2.10	4.31	6.51	8.72	10.9	0.77	1.55	2.33	3.10	3.85	0.76	1.56	2.30	3.08	3.84	0.63	1.23	1.87	2.50	3.10
Valine	2.54	5.21	7.87	10.5	13.2	4.60	6.87	10.3	13.7	17.0	3.36	6.88	10.13	13.54	16.89	2.68	5.20	7.87	10.55	13.07
Arginine	3.60	7.39	11.2	15.0	18.7	3.38	9.35	14.0	18.6	23.2	4.60	9.40	13.86	18.51	23.10	3.72	7.23	10.95	14.67	18.18
Leucine	5.58	11.4	17.3	23.1	29.0	7.34	14.9	22.4	29.7	36.9	7.24	14.81	21.82	29.16	36.38	6.88	13.35	20.23	27.11	33.59
Isoleucine	2.45	50.2	75.9	10.2	12.7	3.18	6.46	9.70	12.9	16.0	3.17	6.48	9.54	12.75	15.91	2.44	4.73	7.17	9.61	11.90

[1]Metabolizable energy, calculated according to WPSA. [2, 3]The values presented for the crude protein and amino acid contents were determined; the values for all of the other nutrients were calculated.

Table 2. Amino acid content of dietary protein (gAA/100 g CP) and ratio to lysine (%) in the diets limiting in different amino acids

Amino acid[1]	Lysine Trial (T2)		Methionine Trial (T3)		Threonine Trial (T4)	
	L1 to L5 (g/100 g CP)	AA ratio to[2] Lys (%)	L1 to L5 (g/100 g CP)	AA ratio to[3] Lys (%)	L1 to L5 (g/100 g CP)	AA ratio to[4] Lys (%)
Lysine	**4.95**	**100**	5.82	100	5.37	100
Methionine+ Cystine	4.05	82	3.52	60	3.92	73
Methionine	2.56	52	**2.04**	**35**	2.69	50
Threonine	3.80	77	3.79	65	**2.95**	**55**
Tryptophan	1.04	21	1.04	18	0.98	18
Arginine	6.28	127	6.26	108	5.74	107
Valine	4.62	93	4.58	79	4.13	77
Isoleucine	4.34	88	4.31	74	3.76	70
Leucine	10.0	202	9.86	170	10.6	198

[1]Amino acid content determined in the feed. [2,3,4] Ratio of the amino acid relative to lysine (100%).

2.3. Experimental Procedures

Each experimental period was divided into an adaptation period (5 days) and a period with total excreta collection (10 days). For the first two days of the adaptation period, the feed was available ad libitum to determine the optimal feed intake under the metabolic cage conditions. Based on their ad libitum feed intake, the birds received a controlled quantity of feed for the next three days. This procedure was followed for the collection period. In the collection period, the excreta were collected once a day (in the afternoon) and immediately frozen at -20°C for later analysis. The amounts of feed intake (FI, g) and total excreta collected were quantified.

The excreta were freeze-dried for 72 hours under controlled conditions (-80°C; -80 kPa; SuperModulyo; Thermo Fisher). The dry matter was determined using a forced air oven at 105ºC for 24 hours, and the nitrogen contents of the diets and excreta were quantified using the Kjeldahl method (AOAC, 1990: method 2001.11, Kjeltec 8400; Foss). To determine the amino acid composition of the diets, the samples were hydrolyzed with 6 M hydrochloric acid under nitrogen for 24 hours. The amino acids released by acid hydrolysis were separated using reversed-phase HPLC and detected in the UV range at 254 nm.

The nitrogen intake (NI, mg/BWkg$^{0.67}$/d) and nitrogen excretion (NEX, mg/BWkg$^{0.67}$/d) were determined. The nitrogen balance (ND, mg/BWkg$^{0.67}$/d) was calculated from the difference between the NI and NEX, respectively.

2.4. Nitrogen maintenance requirement (NMR) and maximum potential of nitrogen deposition (NDmaxT)

The NMR (mg/$BW_{kg}^{0.67}$/d) was estimated by fitting an exponential function of NI and NEX (NEX = NMR·$e^{b \cdot NI}$) (Thong and Liebert, 2004). The NMR is the result of an extrapolation when the NI is equal to zero; e is the basic number of the natural logarithm; and *b* is the equation parameter that represents the slope of the exponential function. Nitrogen retention (NR, mg/$BW_{kg}^{0.67}$/d) is the ND plus the NMR, and the theoretical maximum for daily nitrogen retention ($NR_{max}T$, mg/$BW_{kg}^{0.67}$/d) is the threshold value of the exponential function between the NI and ND (Thong and Liebert, 2004), i.e.:

$$NR = NR_{max}T \times (1 - e^{-b \times NI})$$

or

$$ND = NR_{max}T \times (1 - e^{-b \times NI}) - NMR.$$

The data obtained from the four trials were used to determine the NMR and the $NR_{max}T$. The $ND_{max}T$ was obtained from the difference between the $NR_{max}T$ and the NMR. The $PD_{max}T$ was obtained from the $ND_{max}T \times 6.25$.

Because these parameters express the theoretical potential for protein deposition of the genotype studied, the data from the four trials were analyzed together.

2.5. Amino acid efficiency

The efficiencies of utilization of the amino acids (bc^{-1}) were calculated using the data from T2, T3 and T4 that involved individual limiting amino acids, according to (Thong and Liebert, 2004) the following equation:

$$b = [ln NR_{max}T - ln(NR_{max}T - NR)] / NI$$

where *b* is the slope of the exponential function resulting from graded amino acid or protein supply and indicates the dietary protein quality independent on NI.

The amino acid intake which is needed for an aimed NR is determined using the following equation (Thong and Liebert, 2004), as derived by logarithmization and transformation of the basic function when NI is replaced by intake of the LAA:

$$LAAI = [ln NR_{max}T - ln(NR_{max}T - NR)] / 16 \cdot bc^{-1}$$

where LAAI is the daily intake of the limiting amino acid [mg/$BW_{kg}^{0.67}$] which is needed for the aimed response level (NR); and bc^{-1} is the linear slope between concentration of the LAA (*c*=g AA/100 g CP) in the feed protein and protein quality *b*. The bc^{-1} that was considered was in the linear area, where in each trial there was an amino acid limiting. The conversion factor for the nitrogen intake based on the amino acid is given by the following equation: NI = 16 LAAI / *c*.

2.6. Amino acid requirements

The $NR_{max}T$ is the theoretical maximum of the potential for nitrogen retention. In practice, it is impossible for the birds to achieve this theoretical threshold value by real performance level. Consequently, graded levels of making use of the potential (p.e. 40, 50 and 60% of the $ND_{max}T$) were used to calculate the Lys, Met and Thr requirements for pullets.

2.7. Statistical analysis

The values for the parameters of equations were estimated by applying the Gauss method to the NLIN procedure using SAS software (version 9.2). This method considers the sum of the least squares of the distances between the model and each observed point.

3. Results

3.1. Nitrogen balance

We studied chickens of the Dekalb White strain throughout the same growth periods in all of the trials. Therefore, the results for all of the nitrogen balance periods within equal age periods are summarized in Table 3. As the content of the limiting nutrient increased, the values for the NI, NEX and nitrogen balance (ND) also increased. The birds fed the L1 diet had a lower FI and BW compared to the birds fed the diets with higher protein contents, and the values of these variables were almost constant for the latter birds.

Table 3. Body weight (BW, kg), feed intake (FI, g/d) nitrogen intake (NI, mg/$BW_{kg}^{0.67}$/d), nitrogen excretion (NEX, mg/$BW_{kg}^{0.67}$/d) and nitrogen balance (ND, mg/$BW_{kg}^{0.67}$/d) of the Dekalb White pullets as summarized for the individual age periods (I: 14 to 28 d, II: 56 to 70 d and III: 96 to 112 d)

Parameters	L1	L2	L3	L4	L5
			14 to 28 days		
BW	159	205	216	226	173
FI	18	24	25	23	17
NI	654	1221	1743	2176	2568
NEX	330	516	747	1029	1087
ND	324	705	996	1147	1481
			56 to 70 days		
BW	492	545	571	585	586
FI	32	34	34	35	34
NI	526	954	1315	1775	2357
NEX	295	431	642	971	1286
ND	231	524	673	803	1072
			96 to 112 days		
BW[1]	805	904	872	940	905
FI	43	51	44	43	40
NI	455	936	1385	1668	2066
NEX	289	477	689	997	1365
ND	166	459	696	671	701

[1]BW: average of the body weights at the beginning and end of each collection period.

3.2. NMR and NRmaxT

The NMR, $NR_{max}T$ and $ND_{max}T$ values were calculated from the data in Table 3. The nitrogen maintenance requirements increased with age until the birds' growth began to stabilize, and the retention and deposition of nitrogen decreased with the increasing age.

Table 4. Nitrogen maintenance requirements (NMR, mg/$BW_{kg}^{0.67}$/d), theoretical maximum for daily nitrogen retention ($NR_{max}T$, mg/$BW_{kg}^{0.67}$/d) and theoretical maximum for daily nitrogen deposition ($ND_{max}T$, mg/$BW_{kg}^{0.67}$/d) during each growth period

Period	NMR[1]	$ND_{max}T$[3]	$NR_{max}T$[2]
14 to 28 d	270	2938	3208
56 to 70 d	303	2050	2353
96 to 112 d	348	1391	1739

[1]NEX = NMR ($e^{b \cdot NI}$). [2]ND = $NR_{max}T$ (1 - $e^{-b \cdot NI}$) – NMR. [3]$ND_{max}T$ – theoretical maximum for daily nitrogen deposition, calculated using the equation $NR_{max}T = ND_{max}T$ – NMR.

Figure 1 shows the exponential function between the NI and NEX values, which provides the NMR values. Because there are no observations in which the NI is equal to zero, the NMR values are extrapolations of the exponential function.

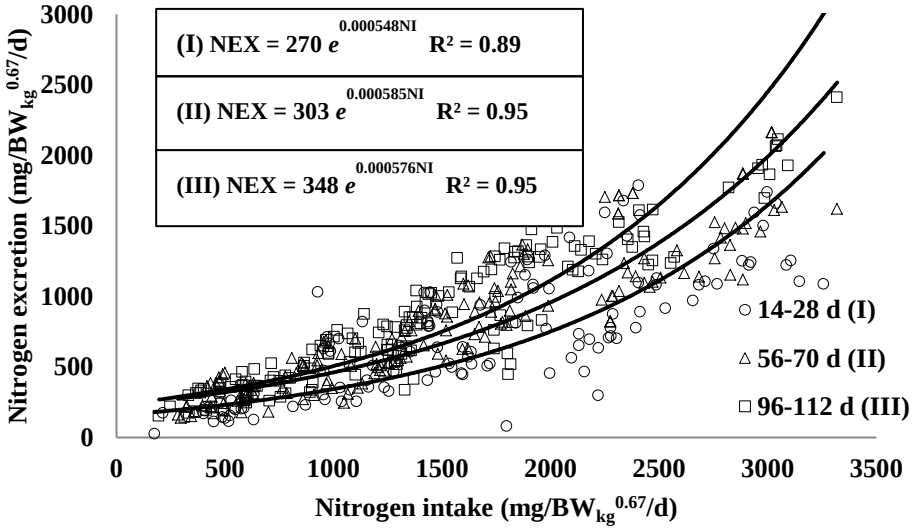

Figure 1. Nitrogen maintenance requirements (NMRs) determined from the exponential function of nitrogen intake and nitrogen excretion values for the pullets in growth periods I (14 to 28 days), II (56 to 70 days) and III (96 to 112 days).

Figure 2 shows the $ND_{max}T$ values obtained from the exponential function between the NI and ND values. This figure reveals that the ND data from approximation were higher than the observed values. This result was expected due to the theoretical basis of this methodology, which considers the maximum theoretical potential that characterizes the genotype, whereas in practice, the birds cannot achieve this daily deposition rate.

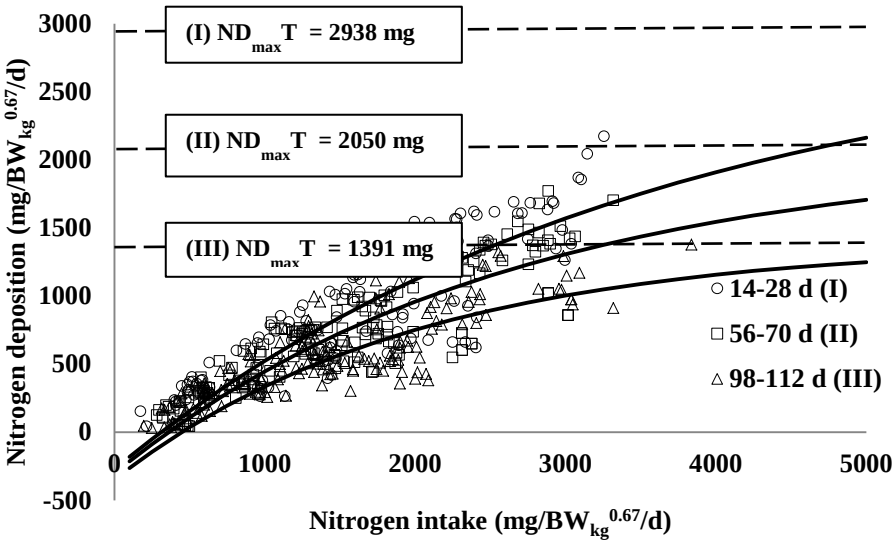

Figure 2. Theoretical maximum for the daily nitrogen deposition ($ND_{max}T$) of the pullets during periods I (14 to 28 days), II (56 to 70 days) and III (96 to 112 days), based on the relationship of their nitrogen intake and their nitrogen deposition.

The Figures 3, 4 and 5 show the responses of nitrogen deposition of the pullets with different amino acids intake (Lys, Met and Thr) at the periods studied.

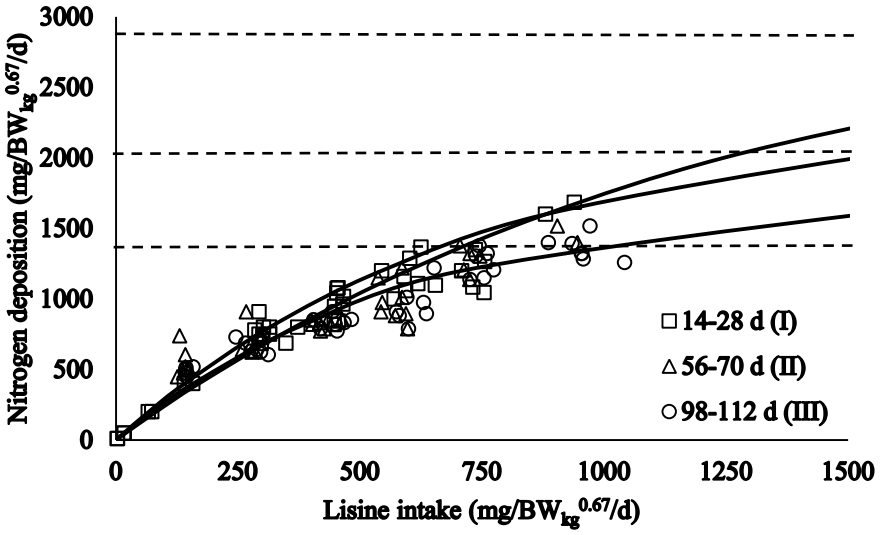

Figure 3. Responses of nitrogen deposition of the pullets during periods I (14 to 28 days), II (56 to 70 days) and III (96 to 112 days) with different lysine intakes.

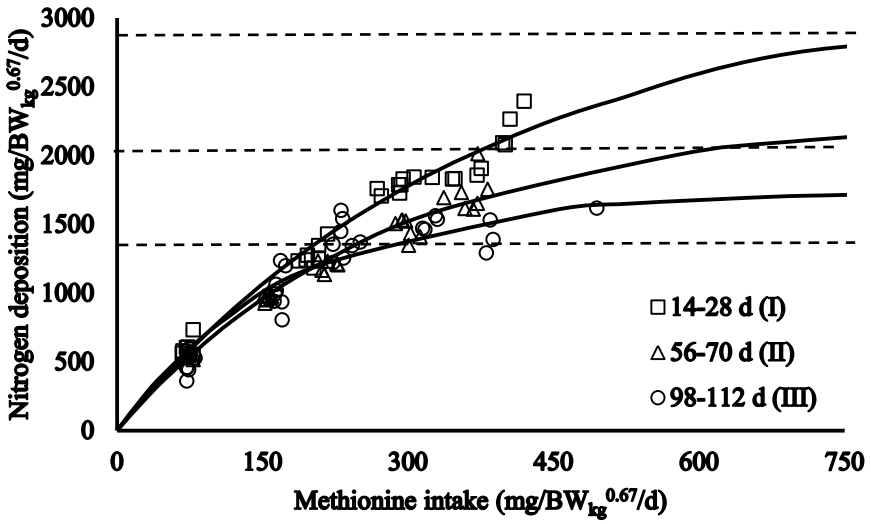

Figure 4. Responses of nitrogen deposition of the pullets during periods I (14 to 28 days), II (56 to 70 days) and III (96 to 112 days) with different methionine intakes.

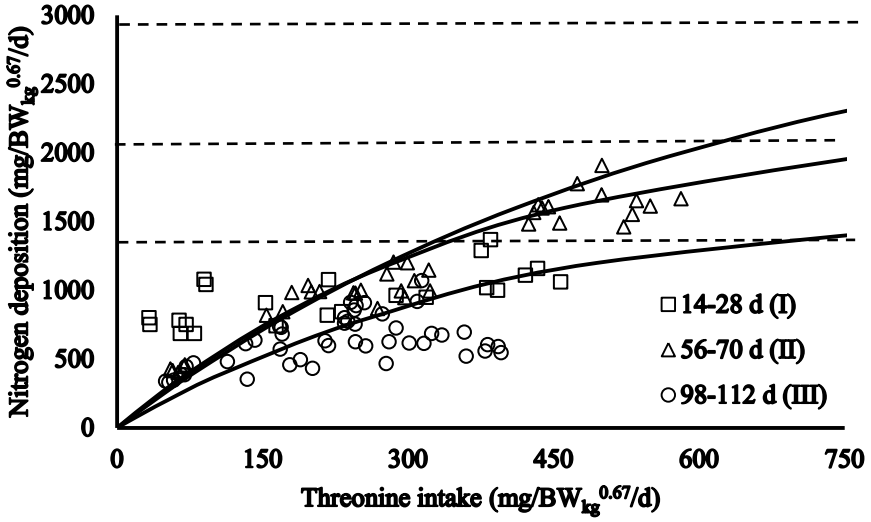

Figure 5. Responses of nitrogen deposition of the pullets during periods I (14 to 28 days), II (56 to 70 days) and III (96 to 112 days) with different threonine intakes.

3.3. Amino acid efficiency and requirements

The daily protein deposition can be considered as a percentage of the theoretical potential $ND_{max}T$. Subsequent calculations are based on 40, 50 and 60% of the potential to be near to real performance data of these birds. However, other percentages to make use of the potential can be applied, depending on the situation.

Based on the efficiencies of amino acid utilization and the protein deposition, the amino acid requirements were calculated, and then the optimal amino acid contents in the diet were estimated by considering the FI (Tables 5, 6 and 7).

Table 5. Model calculation of individual AA requirements and optimal dietary contents of Lys, Met and Thr for pullets during growth period I (14 to 28 d, mean BW=222g), depending on protein deposition (PD) and observed AA efficiency

	Lys			Met			Thr		
PD (g/d)[1]	2.9	3.7	4.4	2.9	3.7	4.4	2.9	3.7	4.4
Efficiency of AA (bc^{-1})[2]		50			170			100	
AA requirement[3]									
mg/$BW_{kg}^{0.67}$/d	651	884	1168	190	257	340	304	413	546
mg/d (at mean BW)	238	322	426	69	94	124	111	151	199
Daily feed intake (g)[4]	Optimal content in the diet (g/kg)								
25	9.50	12.9	17.1	2.77	3.75	4.96	4.44	6.03	7.96

[1]Protein deposition values at 40, 50 and 60% of the theoretical maximum for the daily nitrogen deposition ($ND_{max}T$). [2]Efficiency of amino acid utilization (bc^{-1}) considering that b = [ln$NR_{max}T$ - ln($NR_{max}T$ – NR)] / NI and c = protein content of the feed). [3]Amino acid requirements were calculated using the equation LAAI = [ln$NR_{max}T$ - ln ($NR_{max}T$ - NR)] / 16bc^{-1}. [4]Daily feed intake at 25 g according to the pullet management guide.

Table 6. Model calculation of individual AA requirements and optimal dietary contents of Lys, Met and Thr for pullets during growth period II (56 to 70 d, mean BW=582g), depending on protein deposition (PD) and observed AA efficiency

	Lys			Met			Thr		
PD (g/d)[1]	4.1	5.1	6.1	4.1	5.1	6.1	4.1	5.1	6.1
Efficiency of AA (bc^{-1})[2]		90			230			160	
AA requirement[3]									
mg/$BW_{kg}^{0.67}$/d	374	507	671	141	192	253	196	266	351
mg/d (at mean BW)	260	353	467	98	133	176	136	185	245
Daily feed intake (g)[4]	Optimal content in the diet (g/kg)								
45	5.78	7.85	10.4	2.18	2.96	3.92	3.03	4.11	5.43

[1]Protein deposition values at 40, 50 and 60% of the theoretical maximum for the daily nitrogen deposition ($ND_{max}T$). [2]Efficiency of amino acid utilization (bc^{-1}) considering that b = [ln$NR_{max}T$ - ln($NR_{max}T$ – NR)] / NI and c = protein content of the feed). [3]Amino acid requirements were calculated using the equation LAAI = [ln$NR_{max}T$ - ln ($NR_{max}T$ - NR)] / 16bc^{-1}. [4]Daily feed intake at 45 g according to the pullet management guide.

Table 7. Model calculation of individual AA requirements and optimal dietary contents of Lys, Met and Thr for pullets during growth period II (96 to 112 d, mean BW=920g), depending on protein deposition (PD) and observed AA efficiency

	Lys			Met			Thr		
PD (g/d)[1]	4.1	5.1	6.2	4.1	5.1	6.2	4.1	5.1	6.2
Efficiency of AA (bc^{-1})[2]		100			350			180	
AA requirement[3]									
mg/$BW_{kg}^{0.67}$/d	309	419	554	91	123	162	177	240	318
mg/d (at mean BW)	292	397	524	86	116	154	167	227	300
Daily feed intake (g)[4]	Optimal content in the diet (g/kg)								
65	4.00	5.43	7.18	1.51	2.05	2.71	2.10	2.85	3.76

[1]Protein deposition values at 40, 50 and 60% of the theoretical maximum for the daily nitrogen deposition ($ND_{max}T$). [2]Efficiency of amino acid utilization (bc^{-1}) considering that b = [ln$NR_{max}T$ - ln($NR_{max}T$ – NR)] / NI and c = protein content of the feed). [3]Amino acid requirements were calculated using the equation LAAI = [ln$NR_{max}T$ - ln ($NR_{max}T$ - NR)] / 16bc^{-1}. [4]Daily feed intake at 65 g according to the pullet management guide.

4. Discussion

The aims of this study were to model the amino acid requirements and to estimate the optimal amino acid contents in the diet according to the aimed performance of the pullets and their nutritional plan, based on the Goettingen approach. This approach considers the NMR, $NR_{max}T$, NR, $ND_{max}T$ and the efficiency of amino acid utilization as obtained from nitrogen balance trials.

This method considers that the amino acid requirements are specific related the aimed percentage to make use of the theoretical potential for daily protein deposition according to the genotype. Therefore, a description of the potential for N deposition of various strains is indispensable. As in other approaches for growth studies, the increased deposition of nitrogen or protein with age was considered here. Unlike other approaches, the description of nitrogen retention was fractionated into two parts, the first being the NMR, which is independent of the nitrogen intake and appears to be specific for each genotype. The second part is the physiological response boundary for certain nitrogen deposition rates with high nitrogen intakes.

The average NMR value that was calculated based on the three growth phases studied was 307 mg/$BW_{kg}^{0.67}$/d, considering that the NMR was extrapolated from NI = 0. The NMR value for laying hens that was determined by Filardi *et al.* (2000) was 178 mg/$BW_{kg}^{0.75}$/d. The NMR values found in this study were higher than the values of 153 mg/$BW_{kg}^{0.75}$/d for Passeriformes (Allen and Hume, 2001) and 171 mg/$BW_{kg}^{0.67}$/d for slow-growing broilers

(Samadi and Liebert, 2007b); however, they were 18% higher than the values determined for broilers (Samadi and Liebert, 2006a).

According to Reeds and Lobley (1980), when nitrogen in absent in the diet (NI = 0), catabolism or degradation of the body's proteins occurs to maintain the pool of free amino acids for protein synthesis according to the metabolic priorities; the result of this degradation process is quantified in the NMR, so that the differences found in the NMR values can be associated with the protein synthesis and degradation rates of different genotypes.

According to the traditional method described by Sakomura and Rostagno (2007), the NMR is determined by the positive and negative NB responses. The negative balance is limited by the intensity of protein degradation, which tends to increase the values of the endogenous losses and affects the NMR value. Thus, with this approach, the positive NB is used to determine the NMR, and hence, the result is closer to the observed value.

The $NR_{max}T$ is dependent on the genotype and changes with age, as shown by our results. The $ND_{max}T$ value decreased from period I (14 to 28 d) to period III (96 to 112 d) and tended to reach zero when growth is finished in adult birds, when fat is deposited but no additional protein is deposited (Samadi and Liebert, 2006b; 2007a; Marcato *et al*., 2010).

Traditionally, the potential for growth has been described by the Gompertz growth model, based on the body weight (BP) *versus* time (t). By dividing the first derivative of this equation by the metabolic weight of the bird (dBP/dt ÷ $BW_{kg}^{0.67}$), it is possible to determine the nitrogen deposition rate (BP÷6,25) in mg/$BW_{kg}^{0.67}$/d. Considering the results of Martin *et al.* (1994), which were obtained on the same basis used in this study (mg/$BW_{kg}^{0.67}$/d), the $ND_{max}T$ values were 860, 865 and 791 mg/$BW_{kg}^{0.67}$/d for the Hisex, Ross Brown and Amber-Link strains, respectively. The differences between their values and those of the present study are related to methodological aspects. Martin *et al.* (1994) used the comparative slaughter technique to determine protein deposition, and the dietary nitrogen intake levels were in agreement with the practical conditions, in contrast to the conditions applied in this study.

Several of the differences between the above-mentioned approaches and the approach applied in this study are that the $ND_{max}T$ values were determined using nondestructive methods and the birds were subjected to high levels of nitrogen intake to characterize the nitrogen use limit of this modern strain, which provided knowledge that can be used to understand and explore their full deposition potential, thus limiting dietary amino acid levels to minimize nitrogen excretion and to avoid the use of excessive levels of nutrients.

The maximum potential of nitrogen deposition is the point where the protein deposition rate begins to decrease, which represents the theoretical potential. The theoretical maximum protein deposition ($PD_{max}T$) obtained from the $ND_{max}T$ characterizes the genetic potential of the strain, and it is not possible to attain it by improving the diet; therefore, this theoretical threshold value can be expressed as a percentage of the $PD_{max}T$ values (Samadi and Liebert, 2006a).

The different amino acids intake (Figures 3, 4 and 5) provided different depositions of nitrogen. This is due to the fact that each amino acid has a different importance in the body. However, as this method does not use the comparative slaughter is not possible to know the deposition of each amino acid. However, it can be observed the importance of them in nitrogen deposition.

To estimate dietary amino acid requirements, it is necessary to know the efficiency of amino acid utilization. In the approach taken in this study, the efficiency is obtained from the relationship between *b* (protein quality of the feed) and *c* (concentration of the limiting amino acid in 100 g of dietary protein). To compare the efficiencies of amino acid utilization found in this study with those in the literature, a calculation must be performed. Considering a protein deposition rate of 60% for each growth phase, based on the maximum potential for protein deposition and the Lys, Met and Thr contents of the body plus the feathers, according to Emmans (1989) and considering the estimated amino acid intake (Tables 5, 6 and 7), the calculated efficiencies for body deposition of the individual AAs in the three growth phases are86, 73 and 75% for Lys, 75, 73 and 65% for Met and 94, 86 and 78% for Thr. The values obtained in this study are within the range of those reported in the literature (Edwards *et al*., 1999; Fatufe *et al*., 2004; Hurwitz and Bornstein, 1973; Martin el al., 1994; Edwards and Baker, 1999; Edwards *et al*., 1997). The great variation among the reported values is due to the use of different approaches, and moreover, these results are mostly for broilers because no studies using pullets were found in the literature. However, the efficiency of amino acid utilization is an important dietary parameter that must be thoroughly studied because it represents how the birds use an amino acid for growth.

All of the information regarding the Dekalb White pullets that was obtained in this study using the methods of the ”Goettingen approach” was applied in the LAAI equations to provide the limiting amino acid intake as a percentage of the $ND_{max}T$. The applied 40, 50 and 60% of the theoretical potential were in the range as recommended by Samadi and Liebert

(2006b) near to practical deposition values. These results were transformed to obtain the optimal content in the diet (g/kg) according to the feed intake (g) and considering the recommendations for the strain, although accurate prediction of the feed intake which may occur under practical housing conditions is difficult (Samadi and Liebert, 2006b). However, this modeling methodology allows adjustment of the requirements according to the situation becauseit is possible to adapt the aimed daily protein deposition and observed feed intake.

The values found in this study based on 50% of the maximum protein deposition ($ND_{max}T$), which was the mean value assessed in this study, and the feed intake observed in growth period I are higher than the predicted values presented in the literature, whereas the values for period II and III are lower, except for lysine which were higher (NRC, 1994; Rostagno *et al*., 2011). This result is due to the pullets depositing more protein in their bodies plus their feathers during the first period of growth (6 to 12 weeks) and thereafter beginning to decrease protein deposition. In general, the values are lower than the recommended values, which is due the methodology applied in this study (factorial model, type of approach), although the requirements are consistent for pullets.

While characterizing threshold protein deposition, Sakomura *et al*. (2012) obtained the standard deposition rate (protein deposition rate under normal conditions) and the $PD_{max}T$ and found that pullets have a maximum relative potential of 11% from 1 to 63 d, meaning that they have a capacity for growth that could be exploited by nutritionists. According to Sakomura *et al*. (2012), another application of the knowledge of physiological limits is the identification of the more demanding individuals in the population, which can facilitate the decision about the ideal profile of amino acids in the dietary protein and suggest an alternative nutritional management strategy to standardize plotting sexual maturation and, consequently, egg production.

In conclusion, the “Goettingen approach” as applied in this study allows estimating of both daily amino acid requirements and optimal in-feed contents, considering the genetical growth potential of the birds, aimed protein deposition and expected feed intake. Among the advantages of this method are the simplicity of the trials, the use of a small number of animals, the lack of the necessity to slaughter them so that they may be re-used and its low cost. In addition, it is also possible to estimate the nitrogen maintenance requirement, the nitrogen deposition, and efficiency of utilization of amino acids with high accuracy using this method. However, further studies should be conducted to estimate the amino acid

requirements of pullets during their growth period, duo to the importance of this period in the development of hens.

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CAPÍTULO 5 – IMPLICAÇÕES

A Treonina é um aminoácido essencial e está envolvido em diversos processos metabólicos, e não pode ser sintetizado a partir de outros aminoácidos. É o aminoácido em maior concentração na mucina e nos anticorpos, sendo que sua deficiência pode comprometer o funcionamento do sistema digestivo e imunológico e reduzir sua disponibilidade para síntese de proteína muscular. Assim, este aminoácido foi escolhido como referência para realização dos estudos desta tese.

No capítulo 2 foi abordado o método dose-resposta para estudar as respostas das aves a níveis crescentes de treonina na dieta, pela técnica da diluição, onde a relação entre os aminoácidos é mantida. Os resultados nos permitiram conhecer melhor como é a deposição de proteína e treonina no corpo e penas, deposição de gordura no corpo, relação de deposição do aminoácido no corpo e penas com o avançar da idade e eficiência de utilização da treonina. Todas estas informações são fundamentais para que um modelo de precição das exigências possa ser elaborado.

Já no capítulo 3, as informações do capítulo 2 puderam ser aplicadas na elaboração de dois modelos para predizer as exigências de treonina para aves de postura em fase de crescimento, um considerando a exigência de mantença, perda de penas, deposição do aminoácido no corpo e nas penas, e também a eficiência de utilização destas aves. No outro modelo, além destes fatores, também foi considerada a deposição de treonina nos órgãos reprodutivos (ovário e oviduto) e a eficiência. As deposições diárias foram estimadas por meio dos parâmetros da derivada da curva de Gompertz aplicada aos dados do capítulo 2. Os dados de crescimento dos órgãos reprodutivos são de extrema importância, já que nos permite conhecer e também manipular a idade quando estes começarão a se desenvolver. Como os resultados deste capítulo é possível entender que é imprescindível considerar o crescimento do ovário e oviduto nas exigências destas aves (que naturalmente é em torno de 15 semanas), e também é possível manipular o modelo para que estes se desenvolvam mais precocemente ou tardiamente, por meio do estímulo luminoso. Isto é extremamente importante na maturação sexual destas aves, e na uniformidade do lote.

Ainda no capítulo 3, também foi estimado o consumo desejado de ração por meio do cálculo da exigência de energia efetiva para mantença e deposição de proteína e gordura das aves e da energia enfetiva da dieta. Com este consumo foi possível estimar a quantidade de treonina da dieta das aves por dia, semana ou fase. E com base nas exigências diárias, pode-se

observar que o ideal é dividir em 4 fases de crescimento, sendo a inicial, cria, recria e pré-postura, melhorando o desempenho e uniformidade do lote e garantindo uma uniformidade no início da postura de ovos. Vale ressaltar também que os estudos para estimar as exigências de aminoácidos para aves de postura em fase de crescimento são escassos, e um modelo fatorial onde se conhece a mantença, crescimento e deposição no corpo, penas e órgãos reprodutivos e eficiência utilização do aminoácido destas aves, é de fundamental importância na nutrição. O modelo pode ser utilizado para estimar a exigência de outros aminoácidos, bastando conhecer o conteúdo do aminoácido no corpo e penas e sua eficiência de utilização.

O Capítulo 4 traz uma abordagem diferente para estimativa das exigências de aminoácidos (metodologia de Goettigen). Foram feitos ensaios de balanço de nitrogênio e os resultados foram utilizados para calcular o máximo potencial teórico de retenção e deposição de nitrogênio, exigência de nitrogênio para mantença, e eficiência dos aminoácidos (lisina, metionina e treonina). Com estes dados foi possível estimar as exigências diárias para cada fase do crescimento, e com base no consumo de ração preconizado pelo manual da linhagem, foi estimado o conteúdo do aminoácido na dieta. Esta metodologia se baseia na utilização de uma percentagem do máximo potencial de deposição (40, 50, 60, 70%) para que possa ser aplicado em diferentes situações práticas. Este método é de fácil execução e baixo custo (poucas aves utilizadas e curto período experimental).

Assim, é importante ressaltar que os modelos de predição das exigências constituem a base para o desenvolvimento de softwares capazes de simular as situações resultantes das modificações nos programas nutricionais das aves. Espera-se com estes resultados contribuir para o enriquecimento do conhecimento e aprendizado dos estudos sobre a modelagem, aplicação prática dos modelos (ajudando no planejamento nutricional) e desenvolvimento e aperfeiçoamento de softwares de predição das exigências dos aminoácidos.