UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

EXIGÊNCIAS E OTIMIZAÇÃO DE ISOLEUCINA, VALINA, TRIPTOFANO E ARGININA PARA MATRIZES PESADAS

Michele Bernardino de Lima

Zootecnista

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Orientadora: Profa. Dra. Nilva Kazue Sakomura

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

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TÍTULO: EXIGÊNCIAS E OTIMIZAÇÃO DE ISOLEUCINA, VALINA, TRIPTOFANO E ARGININA PARA MATRIZES PESADAS

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DADOS CURRICULARES DO AUTOR

MICHELE BERNARDINO DE LIMA, filha de Julio Alves de Lima e Mariluce Bernardino de Lima, nasceu em Recife – PE, no dia treze de abril de 1985. Em agosto de 2005 iniciou o Curso de Zootecnia na Universidade Federal Rural de Pernambuco, Recife-PE. Iniciou atividades de pesquisa na área de avicultura no primeiro semestre e no segundo semestre foi contemplada com uma bolsa pelo Programa Institucional de Bolsas de Iniciação Científica do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) durante quatro anos. Em Agosto de 2010 iniciou as atividades no Programa de Pós-graduação na Escola Superior de Agricultura Luiz de Queiroz – ESAQ/USP onde foi bolsista da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Em julho de 2012 submeteu-se à defesa de dissertação para a obtenção do título de “Mestre em Ciências”. Em março de 2013, ingressou no curso de Doutorado em Zootecnia na Universidade Estadual Paulista “Júlio de Mesquita Filho” – Faculdade de Ciências Agrárias e Veterinárias, em Jaboticabal, São Paulo, onde foi bolsista do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), submetendo-se à defesa de tese no dia 22 de fevereiro de 2016 para a obtenção do título de “Doutor em Zootecnia”.

Se você não puder ser um pinheiro no topo da colina,
Seja um arbusto no vale - mas seja
O melhor arbusto à margem do regato:
Seja um ramo, se não puder ser uma árvore.
Se não puder ser um ramo, seja um pouco de relva,
E dê alegria a algum caminho:
Se não puder ser almíscar, seja então, apenas uma tília - Mas a tília mais viva do lago!
Não podemos ser todos capitães; temos de ser tripulação.
Há alguma coisa para todos nós aqui.
Há grandes obras e outras menores, a realizar,
E é a próxima a tarefa que devemos empreender.
Se você não puder ser uma estrada, seja apenas uma senda,
Se não puder ser Sol, seja uma estrela;
Não é pelo tamanho que terá êxito ou fracasso -
Mas seja o melhor do que quer que você seja!

(Douglas Malloch, 1877-1938)

À Deus, por sempre iluminar meu caminho e sempre estar comigo em todos os momentos de minha vida e por sempre me iluminar para escolher o caminho correto.

Dedico

Aos meus pais: Mariluce Bernardino de Lima e Julio Alves de Lima por tudo que fizeram por mim, pelo carinho, amor e dedicação.

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SUMÁRIO

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EXIGÊNCIAS E OTIMIZAÇÃO DE ISOLEUCINA, VALINA, TRIPTOFANO E ARGININA PARA MATRIZES PESADAS

RESUMO - Objetivou-se com esta pesquisa: 1) determinar as exigências de mantença de valina (Val), isoleucina (Ile) e triptofano (Trp) digestível utilizando diferentes sistemas de unidade; 2) avaliar as respostas de aves reprodutoras pesadas para diferentes ingestões de Val, Ile e Trp, determinar a eficiência de utilização e desenvolver um modelo fatorial; 3) calcular a ingestão ótima econômica de Val, Ile e Trp para aves reprodutoras pesadas utilizando o Modelo de Reading; 4) determinar as exigências de arginina digestível para manutenção utilizando diferentes sistemas de unidades; avaliar as respostas de aves reprodutoras pesadas para diferentes ingestões de arginina, estimar os parâmetros do Modelo de Reading pelo método da simulação e equação para aves reprodutoras pesadas e calcular a ingestão ótima econômica de arginina, considerando a relação entre custo-benefício e a variabilidade da população. Para o objetivo 1 foram realizados três ensaios utilizando 144 galos Cobb 500. A exigência de mantença foi obtida pela relação entre a ingestão do aminoácido e o nitrogênio retido. Os diferentes sistemas de unidade foram: mg/kg de peso corporal, mg/kg de peso metabólico e mg/kg de peso proteico. Para o objetivo 2 foram realizados três ensaios utilizando 192 aves reprodutoras pesadas. Os dados obtidos foram ingestão do aminoácido (IAA), peso corporal (PC) e massa de ovo (MO). O modelo modificado para calcular as exigências dos aminoácidos foi: $IAA=[AAm\times(PC\times0{,}196)^{0{,}73}]+[(N_{ovo}\times MO\times AA_{ovo})/k]$, onde AAm é o aminoácido para mantença, N_{ovo} é o nitrogênio do ovo, AA_{ovo} é o aminoácido do ovo e k é a eficiência de utilização. Para o objetivo 3 utilizou-se os dados de AAI, MO e PC do objetivo 2 que foram ajustados pelo modelo de Reading. Para determinar as exigências dos aminoácidos pelo método da simulação foram utilizados 10.000 aves. Para o objetivo 4 foram realizados dois ensaios, o primeiro utilizando 42 galos Ross e o segundo utilizando 64 aves reprodutoras pesadas Ross. Os procedimentos utilizados foram semelhantes aos objetivos 1, 2 e 3. As conclusões obtidas foram: A exigência de manutenção é mais adequadamente expressa como teor de proteína corporal. A predição do modelo foi melhorado utilizando os coeficientes estimados com unidades fisiologicamente relevantes. O modelo de Reading pode ser utilizado para estimar as ingestões ótimas de aminoácidos para galinhas sob diferentes cenários genéticos e econômico e, dependendo dos ingredientes disponíveis e seus preços, o custo de cada um dos aminoácidos pode variar.

Palavras-chave: aminoácidos, galos, matrizes de corte

REQUIREMENTS AND OPTIMIZATION OF ISOLEUCINE, VALINE, TRYPTOPHAN AND ARGININE FOR BROILER BREEDER HENS

ABSTRACT - The objective of this research were: 1) determine the requirements for maintenance of valine (Val), isoleucine (Ile) and tryptophan (Trp) digestible using different unit systems; 2) evaluate the responses of broiler breeder hens to different intakes of Val, Ile and Trp, determine the efficiency of utilization and develop a factorial model; 3) calculate the economic optimum intake of Val, Ile and Trp for broiler breeder hens using the Reading Model; 4) determine the digestible arginine requirements for maintenance using different unit systems; evaluate the responses of broiler breeder hens to different intakes of arginine, estimate the parameters of the Reading Model by the method of simulation and equation for broiler breeder hens and calculate the economic optimum intake of arginine, considering the relationship between cost-benefit and flock variability. For the objective 1 were conducted three trials using 144 Cobb 500 roosters. The requirement for maintenance was obtained by the relationship between amino acid intake and nitrogen retention. The different unit systems were: mg/kg of body weight, mg/kg of metabolic weight ($BW^{0.75}$) and metabolic protein weight at maturity ($BP_m^{0.73}\times u$).For the objective 2 were conducted three trials using 192 Cobb 500 broiler breeder hens. The data obtained were: amino acid intake (AAI), body weight (BW) and the egg output (EO). The modified model to calculate the requirements of amino acids was: $AAI=[AAm \times(BW\times0.196)^{0.73}]+[(N_{egg}\times EO\times AA_{egg})/k]$ where AAm is the amino acid for maintenance, N_{egg} is nitrogen egg, AA_{egg} amino acid in egg, k is efficiency of utilization. For the objective 3 was used the AAI, EO and BW data from objective 2 that were adjusted by Reading Model. To determine the requirements of amino acids by the simulation method were used 10,000 birds. For objective 4 were conducted two trials, the first using 42 Ross roosters and the second trial using 64 Ross broiler breeder hens. The procedures used were similar to the objectives 1, 2 and 3. The conclusions obtained were: The maintenance requirement is more appropriately expressed as body protein content. The prediction of the model was improved using the coefficients estimated here with physiologically relevant units. The Reading Model could be used to estimate the optimum amino acid intakes for hens under different genetic and economic scenarios and depending on the ingredients available and their prices, the cost of each amino acid will vary.

Keywords: amino acids, broiler breeder hens, roosters

LISTA DE TABELAS

CAPÍTULO 2 – Maintenance valine, isoleucine, and tryptophan requirements for poultry

CAPÍTULO 3 – The optimal digestible valine, isoleucine and tryptophan intakes of broiler breeder hens for rate of lay

CAPÍTULO 4 – Optimal economic intake of isoleucine, valine and tryptophan for broiler breeder hens

CAPÍTULO 5 – Requirements and optimization of arginine intakes for broiler breeder hens for egg production

LISTA DE FIGURAS

CAPÍTULO 2 – Maintenance valine, isoleucine, and tryptophan requirements for poultry

CAPÍTULO 3 – The optimal digestible valine, isoleucine and tryptophan intakes of broiler breeder hens for rate of lay

CAPÍTULO 5 – Requirements and optimization of arginine intakes for broiler breeder hens for egg production

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

INTRODUÇÃO

As melhorias no desempenho e rendimento de carcaça de frangos de corte modernos dão uma boa indicação dos atributos genéticos herdados (Peebles et al., 1998; Beer, 2009) e isto só foi possível devido à pressão de seleção empregada para melhorar o potencial produtivo e reprodutivo dos progenitores, sendo necessário também, uma boa gerência no manejo, nutrição e sanidade das aves.

Na prática, o pacote tecnológico empregado para gerir estes fatores (manejo, nutrição e sanidade) em cada granja, é específico para cada produtor, por razões econômicas e ambientais. O manejo nutricional é responsável pela maior parte dos custos de produção, portanto, minimizá-lo acaba sendo decisivo para sustentabilidade do sistema e competitividade no mercado globalizado.

Dentre os nutrientes de uma ração, os aminoácidos vêm assumindo grande importância, uma vez que determinam o nível proteico, afetam a produção de ovos e a viabilidade do produto final, o pintainho de corte e, consequentemente, o custo de produção. Devido à produção industrial dos aminoácidos, tornou-se prática comum incluí-los na dieta, como estratégia para reduzir custo, por meio da diminuição da inclusão do farelo de soja, reduzindo o nível proteico da dieta e a excreção nitrogenada para o ambiente.

Tendo em vista a sustentabilidade e competitividade do sistema, os níveis dos aminoácidos na dieta deveriam ser estabelecidos de acordo com a situação de cada granja, no entanto, nem sempre isto é possível, e na falta de soluções específicas, os nutricionistas utilizam margens de segurança para estabelecer os níveis de aminoácidos e garantir que o desempenho dos progenitores e da prole não sejam afetados por falta de nutrientes. Porém, adotando esta prática, a lucratividade pode ser afetada, uma vez que, a margem de segurança demanda custo financeiro, e é pouco provável uma resposta adicional na produção de ovos para pagar o excedente do aminoácido fornecido.

Do ponto de vista zootécnico, para definir os níveis de aminoácidos, a variabilidade do desempenho das aves, que é específica de cada granja e o custo benefício da suplementação do aminoácido devem ser levados em consideração. Ou seja, o ideal seria calcular o nível ótimo econômico de acordo com as condições de mercado, sobretudo, para os aminoácidos industriais que são *commodities*, portanto seus preços oscilam com frequência. Neste cenário, o produtor passaria a ter a opção de mudar ou não, os níveis de aminoácidos da dieta, de acordo com a variabilidade do lote e o cenário econômico, e assim ser competitivo e garantir sua inserção no mercado globalizado.

Entre os modelos matemáticos utilizados para definir os níveis de aminoácidos na ração, o Reading Model (Fisher et al., 1973) é capaz de se ajustar às condições de variabilidade de cada granja e estimar o nível ótimo econômico, concomitantemente. Este modelo também estima as exigências de aminoácidos considerando a variabilidade da população, além disso, os pequenos aumentos na ingestão de aminoácidos são embasados no ótimo econômico e são destinados às aves que ainda não atingiram sua capacidade máxima de produção, considera o custo benefício da suplementação em relação à taxa de produção e o valor marginal da produção de uma unidade de ovo.

Com o Reading Model, é possível estimar os coeficientes para mantença e produção de ovos, e estes coeficientes são obtidos em função da resposta da massa de ovo e do peso corporal em relação à ingestão do aminoácido. Na literatura, os estudos que definem os níveis dos aminoácidos na dieta de matrizes de corte são escassos, sobretudo para os aminoácidos isoleucina, valina, triptofano e arginina.

REFERENCIAL TEÓRICO

Isoleucina, valina, triptofano e arginina na dieta de matrizes pesadas

Os aminoácidos exercem importantes funções como componentes das proteínas, são essenciais para mantença e apresentam papel essencial em vários processos metabólicos e na produção (Sakomura e Rostango, 2007).

Valina e isoleucina são aminoácidos alifáticos, hidrofóbicos de cadeia ramificada que atuam em conjunto, desempenhando diversas funções no organismo (D'Mello e Lewis, 1970). Ambos apresentam estruturas e funções similares e isso pode levar a uma competição pelas mesmas enzimas de degradação (Harper, 1984), além de utilizarem as mesmas vias de absorção e de transporte, por isso são denominados antagonistas.

Aves de postura são mais susceptíveis ao antagonismo destes aminoácidos quando comparadas aos frangos de corte. Um estudo comparativo revelou que poedeiras reduziram não só a massa de ovo como também o peso corporal quando foi fornecido o dobro de isoleucina na dieta de 4 para 8 g/kg de ração (Peganova e Eder, 2002). O efeito depressor sobre a massa de ovo é atribuído ao antagonismo que existe entre esses aminoácidos, verificado não só em aves, mas também em outras espécies (Allen e Baker, 1972; Oestemer et al., 1973;.Smith e Austic, 1978; Taylor et al., 1984).

O NRC (1994) definiu como sendo 740 e 826 mg/dia, as exigências de isoleucina e valina para matrizes de corte, respectivamente, baseados em estudos de Bornstein et al. (1979) e Waldroup et al. (1976). Harms e Ivey (1992) determinaram ingestão de 778 mg de valina/dia e 625 mg de isoleucina/dia para matrizes de corte. Já Rostagno et al. (2011) recomendam a mesma ingestão de valina e isoleucina de 821 mg/ave dia para matrizes de corte.

O triptofano pertence à classe dos aminoácidos aromáticos e suas funções primárias estão relacionadas às sínteses de proteína, niacina e serotonina (Tackman et al., 1990; Mullen e Martin, 1992), portanto, tem sua essencialidade reconhecida para aves. Devido à sua baixa concentração nos ingredientes em relação a outros aminoácidos, pode ser um limitante na síntese de proteínas (Corzo et al., 2005) e, como limitante, afeta o comportamento das aves (Mench e Shea-Moore, 1995) como também pode deprimir o consumo de ração, comprometendo a homeostase da ave (Lacy et al., 1982).

Baseados em estudos realizados por Waldroup et al. (1976); Bornstein et al. (1979) e Wilson e Harms (1984), o NRC (1994) recomenda a ingestão de 165 mg de triptofano/ave por dia para matrizes de corte. As Tabelas Brasileiras para Suínos e Aves (Rostagno et al., 2011), recomendam a ingestão diária de 210 mg de triptofano/ave por dia. Apesar da atualização da exigência, o nível ideal para otimizar as respostas do lote dependerá da variabilidade do lote e de seu custo, portanto, fixar uma recomendação pode não ser a mais viável economicamente.

A arginina apresenta antagonismo com a lisina, isso porque ocorre a competição pelo mesmo sítio de absorção na borda escova intestinal entre esses aminoácidos, que possuem cadeias de estruturas semelhantes (D’Mello, 2003). O antagonismo ocorre quando um aminoácido está em excesso em relação ao outro, o que resulta na diminuição da síntese de creatinina, compromete as sínteses de prolina e de ornitina (Austic e Scott, 1975). O antagonismo entre estes aminoácidos pode ser induzido pelo desequilíbrio na relação entre eles, de modo que, o excesso de lisina promove aumento da atividade da arginase renal e excreção de ureia, aumentando o catabolismo da arginina no organismo e causando os sintomas de sua deficiência, devido às aves não possuírem ciclo da ureia funcional (D’Mello, 2003). Quando os níveis de lisina são excessivamente altos, também ocorre a excreção renal de arginina (Austic e Scott, 1975).

De modo geral, o antagonismo pode causar aumento e/ou redução da atividade de enzimas específicas do metabolismo dos aminoácidos. Além da maior atividade da arginase, o antagonismo lisina:arginina diminui a atividade da enzima glicina amidino-transferase no fígado e, possivelmente, limita a formação de creatina (Andriguetto et al., 1999), entretanto, o aumento do nível de arginina em dieta rica em lisina alivia o efeito depressivo causado pelo antagonismo (Gadelha et al., 2003).

Altos níveis de arginina também podem afetar a produção de ovos, devido à energia gasta para seu metabolismo e excreção, isto porque é o aminoácido com o número maior de moléculas de nitrogênio em sua estrutura, desta forma, ele depende de mais energia para sua degradação (Leeson e Summers, 2001).

O NRC (1994) recomenda a exigência de 966 mg de arginina/dia para matrizes de corte, baseados em estudos de Bornstein et al. (1979) e Waldroup et al. (1976).

Fisher (1998) recomendou 803 mg/ave dia, Rostagno et al. (2011) 1.049 mg de arginina/ave dia para matrizes de corte e Emkay et al. (2013) 1.026 mg/ave dia para maximizar a massa de ovos.

A realização de novos estudos pode contribuir na construção de um consenso, devido à variação dos resultados disponíveis na literatura, como também pode empregar novas abordagens para desafios recorrentes como, por exemplo, nutrir o lote de acordo com o ótimo econômico.

Métodos para determinação das exigências de aminoácidos

Os métodos utilizados para estudar as respostas das aves em função da ingestão do aminoácido são: dose-resposta e fatorial. O método dose-resposta consiste na determinação das exigências com base na resposta de desempenho dos animais submetidos a níveis crescentes de um determinado aminoácido na dieta (Sakomura e Rostagno, 2007) e por por ser prático e de fácil execução, tem sido a base para elaboração de Tabelas de exigências nutricionais como Nutrient Requirements of Poultry (NRC, 1994) e Tabelas Brasileiras para Aves e Suínos (Rostagno et al., 2000; Rostagno et al., 2005; Rostagno et al., 2011).

Este método caracteriza as interações traçando uma curva resposta em função de um nutriente limitante na dieta em um determinado período (Mercer et al., 1989) e tem sido a base dos estudos fatoriais. No entanto, alguns pesquisadores consideram uma limitação do método, por não predizer mudanças nas exigências ao longo do tempo (Hauschild et al., 2010).

O método fatorial, por sua vez, é baseado no princípio de que a exigência em energia ou nutrientes pelo animal, é a quantidade a ser fornecida para manutenção dos processos vitais, crescimento proteico, engorda e produção, fracionando a exigência total em proporções adequadas para cada uma dessas finalidades (Sakomura e Rostagno, 2007).

Este método representa uma ferramenta para compreender os processos envolvidos nos metabolismos energético e proteico dos animais (Sakomura e Rostagno, 2007) e é a base de estudos de modelagem, os quais visam a definição de um sistema adequado para a produção animal. A abordagem fatorial possibilita também a elaboração de modelos capazes de predizer as exigências nutricionais de aves de diferentes linhagens e idades, criadas sob diferentes condições, por contemplar diferenças de peso e composição corporal, potencial de crescimento e produção.

A aplicação do método fatorial depende da determinação dos parâmetros ou coeficientes que expressem a exigência de mantença e a eficiência de utilização do aminoácido dietético, informações estas obtidas a partir de estudos de dose-resposta.

Técnica da diluição como ferramenta para obter níveis experimentais para descrever a curva resposta das aves

O nível do aminoácido teste nas dietas experimentais em ensaio dose-resposta, pode ser formulado por duas técnicas: a suplementação gradativa (D'Mello, 1982) ou a diluição (Fisher e Morris, 1970). A técnica da suplementação consiste na formulação de uma dieta basal, para atender as exigências nutricionais de energia, minerais, vitaminas e aminoácidos essenciais, com exceção do aminoácido a ser estudado. Este é suplementado na dieta basal em níveis crescentes pela adição de uma fonte cristalina. Já a técnica da diluição consiste em diluir sequencialmente uma dieta com elevado teor de proteína e deficiente no aminoácido teste, com uma dieta livre de proteína, para obter os níveis intermediários do aminoácido a ser testado.

Com a técnica da diluição é possível manter a relação ideal dos aminoácidos, como também utilizar dietas cuja a constituição se aproxima de uma ração convencional (milho e farelo de soja) na qual a maior parte da proteína da dieta é proveniente daquela fornecida pelos ingredientes e não de aminoácidos sintéticos. Neste sentido, é desejável minimizar a inclusão de fontes sintéticas de aminoácido porque a velocidade dos processos digestivos, absortivos e metabólicos dos aminoácidos sintéticos é diferente dos aminoácidos provenientes de ingredientes convencionais.

Desta forma, Fisher e Morris (1970) preconizaram que é possível criar um aminoácido limitante na síntese proteica da ave, por meio da deficiência relativa do aminoácido, ou seja, independentemente da quantidade de proteína da dieta, a ave responderá ao primeiro limitante na síntese proteica. Assim, a técnica da diluição proporciona uma limitação na ingestão do aminoácido teste, que consequentemente limita decodificação de trinca de bases contida no mRNA, porque um dos aminoácidos estará em falta. Nesta técnica, faz-se necessário incluir um tratamento controle para comprovar se o aminoácido teste foi limitante.

Além desses aspectos, a redução da proteína é desejável para obtenção de uma curva resposta bem definida, da fase de mantença à estabilidade. Um aspecto importante na técnica da diluição é que os níveis dietéticos formulados para provocar respostas na fase de mantença são baixos, por volta 5% de proteína bruta e facilmente podem ser obtidos por essa técnica, que seria algo impraticável com a técnica da suplementação, pela alta quantidade de aminoácido sintético que demandaria, implicando em elevado custo.

Curva resposta e modelos matemáticos

As aves respondem à ingestão do aminoácido dietético e a sua resposta é dependente da amplitude dos níveis dietéticos testados, ou seja, do grau de deficiência que é imposto à ave. Em ensaios de exigência é desejável que os níveis testados dos aminoácidos atendam três fases da curva resposta, ou seja, a fase de mantença (ausência de resposta), a linear e a estabilidade de resposta.

Quando se tem uma curva resposta bem definida, diversos modelos podem ser aplicados para se estudar a resposta em função da ingestão *f*(I) e interpretar aspectos importantes como qualidade de proteína e variabilidade individual da resposta. De modo geral, dois procedimentos matemáticos têm grandes implicações sobre a interpretação da *f*(I), primeiro é o uso de retas, significando que a variabilidade individual sobre as respostas é mínima. Logo, a o efeito é atribuído à qualidade da proteína dietética, sendo necessário ter níveis na fase de mantença e linear. O segundo procedimento é o uso de curvas não lineares. Dos pontos de vista zootécnico e nutricional, a variação na taxa de incremento da resposta é causada pelo animal e em menor proporção pela qualidade da proteína dietética, e para ter um ajuste curvilíneo é necessário ter níveis na fase linear e estabilidade.

Modelos determinísticos (indivíduo) e estocásticos (população)

O modelo determinístico é definido por causas que se podem determinar, identificar e descrever adequadamente a *f*(I) sem recorrer a elementos probabilísticos e desvios. Se aplica a um indivíduo da população, normalmente, o indivíduo médio, porém as exigências do indivíduo médio não são suficientes para otimizar o desempenho do indivíduo superior da população.

O modelo estocástico recorre a elementos probabilísticos e desvios. Neste modelo, as variáveis respondem a uma distribuição específica, portanto, não há uma estimativa universal. Tais modelos não oferecem soluções únicas, mas apresentam distribuição de soluções associadas a probabilidade.

Na área de nutrição, a maioria dos modelos matemáticos disponíveis atualmente são os determinísticos. Os modelos estocásticos têm sua difusão limitada, porém mais complexos, pois consideram a variabilidade dos indivíduos e operam em nível de população.

Reading Model

A exigência do aminoácido pode ser influenciada pela escolha do modelo matemático para explicar a curva resposta das aves (Fisher et al., 1973). Dentre os modelos determinísticos (polinomial, exponencial e linear) é muito difícil distinguir qual deles é o mais adequado para explicar o comportamento da curva resposta das aves (Fisher et al., 1973). Estudos realizados na Unesp Jaboticabal (Silva, 2012) concluíram que as diferenças entre os modelos exponencial e linear pode ser de 884 mg.

Desta forma, o modelo matemático deve apresentar bom ajuste da resposta média da população à ingestão do aminoácido, como também explicar a variação em torno da média. Baseado nisto, pesquisadores da Universidade de Reading desenvolveram um modelo matemático próprio: o Reading Model. Este modelo fundamenta-se na predição das exigências de aminoácidos em função do peso corporal e da produção de ovos, ou seja, o modelo pressupõe que cada ave, tem um nível máximo de produção em massa de ovo.

Pelo Reading Model a estimativa da exigência é obtida usando populações, e os pequenos aumentos na ingestão do aminoácido estão relacionados com o ótimo econômico. Este modelo baseia-se na curva resposta de cada indivíduo da população assumindo uma relação entre o aminoácido limitante da dieta e a resposta em massa de ovo, fracionando as exigências em mantença e produção de acordo com método fatorial, conforme a seguinte fórmula:

$$\text{AAopt}_{\text{mg/dia}} = \boldsymbol{a}\text{Emax} + \boldsymbol{b}\text{PC} \ \sqrt{} \ [a^2 \times \sigma^2_{\text{Emax}} + b^2 \times \sigma^2_{\text{PC}}] + \boldsymbol{z}$$

Onde:

AAopt - ingestão ótima do aminoácido, mg/dia;

Emax - média da máxima produção de massa de ovos, g/dia;

σ^2Emax - desvio-padrão da máxima taxa de produção de massa de ovo;

PC - média de peso corporal (kg);

σ^2PC - desvio-padrão do peso corporal;

a e ***b*** **-** quantidades de aminoácidos associados a uma unidade de E (*a*) e uma unidade de PC (*b*), respectivamente;

y - desvio da exigência individual mg/dia;

z **-** probabilidade que representa o ótimo econômico.

Para determinar a máxima produção de massa de ovo (Emax), exigência para massa de ovo (*a*), mantença (*b*) e desvio da exigência **y**, são utilizados as respostas de massa de ovo (E), consumo do aminoácido (mg/dia), peso corporal (PC) e os desvios da massa de ovo e do peso corporal. Além de estimar a ingestão para média da população (μ) e o ótimo biológico (μ+y), permite, ainda, obter o ótimo econômico (μ+*z*×y) para cada granja e cenário econômico.

O nível ótimo econômico é o ponto em que a inclinação da curva resposta iguala a relação entre o custo de uma unidade extra do aminoácido para o retorno de uma unidade extra de massa de ovos. O desvio da exigência y é corrigido por fatores econômicos (*k*).

A exigência extra economicamente viável é dada pela seguinte expressão: *z*×y, onde *z* sugere quantas vezes y deve ser somado à exigência média da população μ.

O índice *z* corresponde ao desvio padrão da relação *a*×*k*, sendo o *k*, a relação custo benefício do fornecimento do aminoácido. *k*=custo por mg do aminoácido (R$)/valor do grama de ovo (R$).

Através da multiplicação de ***z*** pelo desvio das exigências (y) tem-se o valor economicamente viável que deverá ser somado à exigência média (Pilbrow e Morris, 1974). Este fornecimento destina-se, proporcionalmente, às aves que ainda não atingiram sua capacidade máxima de produção, corrigida pela taxa de produção e pelo valor marginal da produção de uma unidade de massa de ovo. Assim, a ingestão ótima ocorre quando o custo marginal do fornecimento de uma unidade de aminoácido for igual ao valor da resposta obtida por unidade do aminoácido fornecido.

Exigência de aminoácido para mantença

Conforme visto, o Reading Model estima a exigência do aminoácido para mantença, representada pelo coeficiente *b*. Na presente revisão, constatou-se que para aves de postura comercial o coeficiente *b,* estimado por este modelo, apresenta-se coerente com determinações em ensaio próprios de mantença (Fisher et al., 1973, Pilbrow e Morris, 1974). No entanto, para matrizes pesadas apenas alguns estudos foram realizados e não conseguiram estimativas coerentes (Bowmaker e Gous, 1991; Fisher et al., 2001; Silva et al., 2015, Lima et al., 2016). Os valores encontrados por estes autores foram próximos de zero após 10 semanas de experimentação, algo inesperado pelo grau de deficiência imposto pela dieta. Estatisticamente, a extrapolação com valor 0 mg/kg para mantença significa que as aves não reduziram suficientemente a produção de ovos quando foram submetidas ao maior grau de restrição do aminoácido. Bowmaker e Gous (1991) consideraram 9 semanas para coleta de dados (excluíram a última semana) e desta forma observaram que as aves reduziram drasticamente seu peso corporal nos níveis de maior grau de deficiência para manter a produção de 1 ovo de 70 g a cada 15 dias. Este sacrifício pode ser interpretado como mecanismo para manter a reprodução da espécie. Apesar do Reading Model ser um avanço frente aos demais modelos, algumas peculiaridades, de ordens biológicas podem afetar suas estimativas.

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CAPÍTULO 2 – MAINTENANCE VALINE, ISOLEUCINE, AND TRYPTOPHAN REQUIREMENTS FOR POULTRY

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ABSTRACT

Poultry maintenance requirements for valine, isoleucine, and tryptophan were measured by nitrogen balance using different unit systems. The nitrogen balance trial lasted five days with 48 hours of fasting (with roosters receiving only water+sucrose) and the last 72 hours for feeding and excreta collection. Forty grams of each diet first-limiting in valine, isoleucine, or tryptophan was fed by tube each day (three days) to give a range of intakes from 0 to 101, 0 to 119, and 0 to 34 mg/kg BW d of valine, isoleucine, and tryptophan, respectively. A nitrogen-free diet containing energy, vitamins, and minerals, meeting the rooster requirements, was offered ad libitum during these three days. To confirm that the amino acids studied were limiting, a treatment was added with a control diet formulated by adding 0.24 g/kg of L-valine, 0.21 g/kg of L-isoleucine, and 0.10 g/kg of L-tryptophan to the diets with lower amino acid level. Excreta were collected during the last 3 days of the balance period and the nitrogen content of the excreta was analyzed. For each amino acid, a linear regression between nitrogen retention (NR) and amino acid intake was performed. The equations from linear regression were: $NR = -98.6\ (\pm 10.1) + 2.4\ (\pm 0.2) \times Val$, $NR = -46.9\ (\pm 7.1) + 2.3\ (\pm 0.1) \times Ile$, $NR = -39.5\ (\pm 7.7) + 7.3\ (\pm 0.4) \times Trp$; where Val, Ile, and Trp are the intakes of valine, isoleucine, and tryptophan in mg/kg body weight per day, respectively. The valine, isoleucine, and tryptophan required to maintain the body at zero NR were calculated to be 41, 20, and 5 mg/kg body weight per day, respectively. For the system unit mg per kg of metabolic weight, the intake of valine, isoleucine, and tryptophan was 59, 32, and 9, respectively. Considering the degree of maturity of the animal and body protein content ($BP_m^{0.73} \times u$), the amounts of valine, isoleucine, and tryptophan required for maintenance were calculated to be 247, 134, and 37 mg per unit of maintenance protein ($BP_m^{0.73} \times u$) per day. Maintenance requirement is more adequately expressed as body protein content.

Key words: amino acids, breeder roosters, dilution technique, nitrogen retention, regression equation

INTRODUCTION

Determining the ideal amino acid intake in poultry husbandry, considering the specific requirements for maintenance and for egg production, has been suggested for more than seven decades (Heuser, 1941). Leveille and Fisher (1959) first tested and defined the maintenance concept as providing a minimal amount of amino acid to replace inevitable losses and maintain a constant nitrogen balance. Since then there has been little improvement in the methodologies to determine maintenance values although the concept of maintenance has been extensively studied (Fisher, 2013).

Several studies have established the maintenance requirements for the most limiting amino acids in practical diets for poultry, such as methionine+cysteine (Bonato et al., 2011), lysine (Bonato et al., 2011), and threonine (Nonis and Gous, 2008). For the amino acids valine, isoleucine, and tryptophan few studies have been conducted and the requirements obtained are outdated (Leveille and Fisher, 1959, 1960; Leveille et al., 1960; Ishibashi, 1973; Burnham and Gous, 1992). The studies published to date determining the amino acid requirements for maintenance in poultry have generated conflicting results (Leveille and Fisher, 1960; Ishibashi, 1973; Burnham and Gous, 1992, Baker et al., 1996). This divergence may reflect variations in the approaches these studies used, such as poultry of different physiological stages (growth and maturity) and different methodologies (nitrogen balance and comparative slaughter).

Most studies on the maintenance requirement express as mg of amino per kg of body weight or mg per kg of metabolic weight (Leveille and Fisher 1960; Baker et al., 1996; Edwards et al., 1997; Burnham and Gous, 1992; Sakomura and Coon, 2003). Nonetheless, because of the differences in body composition of birds especially regarding the fat content, the ideal would be to express amino acid maintenance requirement on the basis of protein weight of the bird, which would allow comparisons between studies (Burnham and Gous, 1992; Martin et al., 1994; Nonis and Gous, 2008; Bonato et al., 2011). Thus, there is still a need to investigate how the poultry respond to the intake of the amino acids valine, isoleucine, and tryptophan in maintenance, to generate values that adequately represent the requirements of these amino acids in this state.

Therefore, the aim of this study was to determine the specific requirements of digestible valine, isoleucine, and tryptophan for maintenance by different unit systems using Cobb 500 breeder roosters as an animal model. Knowledge about the specific requirements of these amino acids at maintenance will contribute to future studies aiming to improve the productivity of the poultry industry.

MATERIALS AND METHODS

The trials were conducted at the Poultry Science Laboratory of the São Paulo State University, UNESP Jaboticabal, São Paulo, Brazil. The Animal Ethics and Welfare

Committee of UNESP (CEUA) approved all experimental procedures used in the research under the Protocol number 9999/14.

***Birds and housing*.** Three nitrogen balance studies were performed and 144 Cobb 500 breeder roosters were housed individually. The roosters were housed in metabolic cages with individual feeder and nipple-type drinkers. During the experimental period, light was provided 24 hours per day.

Valine trial. In the valine trial 42 roosters were used, distributed into six treatments and six replicates, with an average weight of 4.15 ± 0.55 kg. To confirm that the amino acid studied was indeed limiting and that responses were due to the limitation of the test amino acid and not protein, we performed one more treatment consisting of a control diet. A summit diet was formulated containing 11.75 g/kg of digestible valine and a nitrogen-free diet was formulated based on corn starch and rice husk considering the same nutritional levels as in the summit diet, except for protein and amino acids (Table 1). The experimental levels of the amino acids were obtained from the dilution technique (Fisher and Morris, 1970) and had the following proportions in summit and nitrogen-free diets: 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0. The valine levels ranged from 0 to 1.175% in the diet. A total of 0.24 g/kg of L-valine was added in the diets with lower amino acid level (V2) up to the second amino acid level in the diet (V3).

Isoleucine trial. In the isoleucine trial 54 roosters were used, distributed into eight treatments and six replicates each, with an average weight of 5.69±0.48 kg, and one additional treatment was added (control diet), in the same way as described the valine trial. A summit diet was formulated containing 18.18 g/kg of digestible isoleucine and a nitrogen-free diet was also formulated (Table 1). The isoleucine levels ranged from 0 and 1.818% of the diet. The experimental levels were obtained with the dilution technique. For the control diet 0.21 g/kg of L-isoleucine was added to the diets with lower amino acid level (I2) up to the second amino acid level in the diet (I3).

Table 1. Composition (g/kg) of the summit and N-free diet.

Ingredients	Summit valine	N-free valine	Summit isoleucine	Summit tryptophan	N-Free
Corn starch		431.9			673.3
Soybean meal	224.9		342.6	364.1	
Corn	675.9		370.0	275.6	
Corn gluten meal			150.2	202.9	
Sugar		350.0			150.0
Rice husk		100.0			108.9
Soybean oil	21.5	30.0	34.4	32.2	24.7
Dicalcium Phosphate	11.4	16.2	10.1	10.0	16.2
Potassium chloride	2.7	14.3			14.3
Sodium chloride	5.3	5.5	5.4	5.4	5.5
Limestone	7.9	4.3	8.1	8.0	4.1
Premix[1]	3.0	3.0	3.0	3.0	3.0
L-Arginine (980 g/kg)	6.3		12.4	15.9	
L-Valine (970 g/kg)	5.2		13.0	15.9	
DL-Methionine (980	7.3		12.1	14.5	
L-Lysine HCl (780	5.2		10.8	14.0	
L-Isoleucine (985	6.2		7.1	12.5	
L- Threonine (980	5.6		9.7	11.9	
L- Phenylalanine	5.0		4.5	5.9	
L-Glycine (985 g/kg)	1.0		3.6	5.3	
L-Leucine (980 g/kg)	4.1				
L-Tryptophan (980	1.6		3.1	2.9	
Inert (Sand)		44.8			
Total	1000.	1000.0	1000.0	1000.0	1000.
	Calculated nutritional composition and amino acids (g/kg) [2]				
Metabolizable	13.40	13.40	13.40	13.40	13.40
Crude protein [3]	196/2	5.69/5.94	352/374	407/394	0.14/0
Lysine	11.14		19.49	22.87	
Methionine+cystine	11.70		20.50	24.05	
Methionine	9.51		16.35	19.42	
Tryptophan	3.23		5.67	5.70	
Threonine	10.76		18.86	22.13	
Arginine	15.63		27.34	32.08	
Valine	11.75		24.70	28.98	
Phenylalanine	12.14		19.04	22.49	
Phenylalanine	17.25		30.20	35.43	
Glycine+Serine	13.92		24.36	28.58	
Histidine	3.94		6.55	7.19	
Isoleucine	12.11		18.18	24.89	
Leucine	17.25		30.20	35.43	

[1] Premix provided per mg/kg diet: retinyl acetate, 3.60; cholecalciferol, 0.055; all-rac-α-tocopherol acetate, 30; thiamine, 2.2; riboflavin, 6; pyridoxine.HCl, 3.3; cobalamin, 0.016; nicotinamide, 53; pantothenic acid, 13; menadione, 2.5; folic acid, 1; Se (as Na_2SeO_3), 0.25; Mn (as $MnSO_4.H_2O$), 75; Fe(as $FeSO_4.H_2O$), 50; Zn (as ZnO), 70; Cu (as $CuSO_4.5H_2O$), 6.5; I (as KIO_3), 1.5.
[2] Digestible amino acid.
[3] Calculated and Analyzed crude protein (N*6.25), respectively.

Tryptophan trial. In the tryptophan trial 48 birds were used, distributed into seven treatments and six replicates each with an average weight of 6.11±0.38 kg. In this study, a control diet was also added. A summit diet was formulated containing 5.70 g/kg of digestible tryptophan and a nitrogen-free diet was also formulated (Table 1). The experimental levels of the amino acids were obtained from the dilution technique (Fisher and Morris, 1970) and had the following proportions in summit and nitrogen-free diets: 0:100, 22:78, 33:67, 44:56, 56:44, 67:33, 78:22, and 100:0. The tryptophan levels ranged from 0 to 0.570% of the diet. For the control diet 0.10 g/kg of L-tryptophan was added to the diets with lower amino acid level (T2) up to the second amino acid level in the diet (T3).

***Experimental diets*.** Based on the recommendations of Rostagno et al. (2011) the requirement for the amino acid tested was multiplied by 0.4 and 0.8 for other amino acids, creating a minimum relative deficiency of 40% between the test amino acid and other amino acids, as suggested by Siqueira et al. (2011) and Bonato et al. (2013). Based on the ideal profile of amino acid/lysine, summit diets with high protein content were formulated (Table 1), meeting the requirements for energy, minerals, and other nutrients proposed by Rostagno et al. (2011). In all diets, the minimum contents of potassium, calcium, available phosphorus, and sodium were 7.50, 6.50, 3.00, and 2.30 g/kg, respectively.

***Experimental procedure and data collection*.** The experiment lasted 120 hours and in the first 48 hours the birds went through a period of fasting to empty the gastrointestinal tract. In this period they received 60 ml of water with sucrose (50% each) once a day. For the next 72 hours, the birds were fed 40 grams of the experimental diets daily. At the same time, nitrogen-free diet was provided ad libitum to maintain energy homeostasis and minimize metabolic and endogenous losses of birds. As a marker for excreta, 1% of iron oxide was used at the beginning and end of the collection period. Trays covered with plastic were placed under the metabolic cages to collect the excreta. The excreta were collected twice a day (at 8h and 16h) and placed in plastic containers that were kept at -20 °C. Feed intake was measured for the 72 hours of the feeding phase, determined by feed consumption provided by forced feeding plus the nitrogen-free diet provided ad libitum in the feeder. At the end of each trial, two birds were killed and feathers were separated from the carcass for further analysis.

Analytical procedures. The amount of excreta from each animal was weighed after the three days of collection. The excreta were homogenized in a blender, and samples were weighed into Petri dishes. After, the samples were lyophilized for 72 hours. Next, samples were ground in a ball mill for 2 minutes. These dried samples of the experimental diets, excreta, and carcass (free of feathers) were analyzed for crude protein by Kjeldahl (method 2001.11) and ether extract (method 920.39) for carcass (free of feathers), according to AOAC (1995) procedures.

Statistical analysis. The nitrogen retention (NR) was determined by the difference between nitrogen intake and excretion. The nitrogen retention responses to the amino acid intakes were expressed in three unit systems: 1) mg per kg of body weight (BW), mg/BW per day; 2) mg per kg of metabolic weight ($BW^{0.75}$), mg/$BW^{0.75}$ per day; and 3) mg per unit of metabolic protein weight at maturity ($BP_m^{0.73} \times u$), mg/ $BP_m^{0.73} \times u$ per day, where u is the maturity rate, given by the relation between protein weight at time (BP_t) and protein weight at maturity (BP_m) where $u = BP_t/BP_m$), according to Burnham and Gous (1992). In these trials, *u* was considered equal to 1, because the birds were adults and had reached maturity. The metabolic protein weight at maturity was obtained by multiplying the percentage of protein and the weight of carcass free of feathers, raised to power 0.73. A linear regression between amino acid intake and NR was carried out. The requirement for maintenance was considered as the amount of amino acid to maintain NR equal to zero (NR = 0), as described by Sakomura and Rostagno (2007). Statistical analyses were performed using the PROC REG from SAS 9.2 software.

RESULTS

The assumptions of normality of errors and homogeneity of variance were tested and satisfied. The responses of the roosters to the control diets showed that the amino acids studied were limiting in the diet, because they provided retentions between treatments V2, I2, T2 and V3, I3, T3, validating this study.

Valine trial

The results of feed intake, amino acid intake, and nitrogen retention for different systems of units in the valine trial are presented in Table 2. The feed intake observed in the valine trial was 118±16 g/day. The experimental diets provided ranges from negative to positive NR values. As the amino acid intake increased, the NR values also increased. In the valine trial, three levels showed negative NR.

Table 2. Average values for feed intake (FI), valine intake (ValI), and nitrogen retention (NR) in different systems of units.

Diet	Levels (%)	FI	ValI			NR		
		g/d	mg/kg BW d	mg/kg BW$^{0.75}$ d	mg/kg BP$_m^{0.73}$ d	mg/kg BW d	mg/kg BW$^{0.75}$ d	mg/kg BP$_m^{0.73}$ d
V1	0.000	111.0	0.0	0.0	0.0	-96.3	-137.5	-577.0
V2	0.235	124.5	21.8	30.5	129.8	-37.8	-53.0	-222.5
V3	0.470	108.6	43.4	61.2	256.8	-10.4	-15.8	-66.0
V4	0.705	110.8	60.0	86.2	362.4	55.6	79.0	332.0
V5	0.940	126.3	81.8	116.0	488.3	102.8	145.5	611.0
V6	1.175	125.8	100.8	143.2	601.6	141.6	199.8	839.0
	Root MSE	16.1	18.3	13.9	42.6	40.1	54.9	230.4

V = valine diets; BW = Body weight; BP$_m^{0.73}$ = Body protein at maturity; Root MSE = root mean square error; Val = Valine.

Isoleucine trial

The results of feed intake, amino acid intake, and nitrogen retention for the different systems of units in the isoleucine trial are shown in Table 3. The feed intake observed in the isoleucine trial was 143±29.86 g/day. In the isoleucine trial, there were only negative responses on NR values in the nitrogen-free diet.

Table 3. Average values for feed intake (FI), isoleucine intake (IleI), and nitrogen retention (NR) in different systems of units.

Diet	Levels (%)	FI	IleI			NR		
		g/d	mg/kg BW d	mg/kg $BW^{0.75}$ d	mg/kg $BP_m^{0.73}$ d	mg/kg BW d	mg/kg BW $^{0.75}$ d	mg/kg $BP_m^{0.73}$ d
I1	0.000	112.3	0.0	0.0	0.0	-58.2	-89.7	-379.5
I2	0.404	142.8	27.0	41.5	175.0	20.5	31.2	131.3
I3	0.606	148.6	39.6	60.6	256.6	67.2	103.6	438.0
I4	0.808	133.5	50.8	79.17	335.8	68.8	106.7	451.5
I4	1.010	153.8	64.7	100.5	424.7	94.2	145.5	615.8
I6	1.212	144.8	78.2	120.6	510.6	140.6	216.8	916.6
I7	1.414	155.5	94.5	144.0	608.3	189.0	286.8	1211.5
I8	1.818	156.2	118.6	183.2	774.4	221.4	342.0	1448.2
	Root MSE	29.86	6.04	6.96	28.74	27.39	40.54	30.83

I = Isoleucine diets; BW = Body weight; $BP_m^{0.73}$ = Body protein at maturity; Root MSE = root mean square error.

Tryptophan trial

The results of feed intake, amino acid intake, and nitrogen retention for the different systems of units in the tryptophan trial are shown in Table 4. The feed intake observed in the tryptophan trial was 167±29.86 g/day. In the tryptophan trial, only negative responses were observed in regard to NR values in the nitrogen-free diet.

Table 4. Average values for feed intake (FI), tryptophan intake (TrpI), and nitrogen retention (NR) in different systems of units.

Diet	Levels (%)	FI	TrpI,			NR		
		g/d	mg/kg BW d	mg/kg $BW^{0.75}$ d	mg/kg $BP_m^{0.73}$ d	mg/kg BW d	mg/kg $BW^{0.75}$ d	mg/kg $BP_m^{0.73}$ d
T1	0.000	158.0	0.0	0.0	0.0	-48.8	-76.2	-322.0
T2	0.095	154.0	6.0	9.8	40.5	13.8	21.3	89.5
T3	0.190	162.8	12.0	18.8	79.8	61.2	95.7	405.5
T4	0.285	162.7	17.5	27.7	118.0	82.8	129.8	550.0
T5	0.380	166.2	23.2	36.8	155.8	121.2	191.2	810.2
T6	0.475	172.3	29.7	46.3	196.5	182.8	287.0	1215.2
T7	0.570	194.7	34.0	53.7	227.2	207.0	327.5	1388.3
Root MSE		33.5	1.5	1.7	7.0	29.2	44.4	187.7

T= Tryptophan diets; BW = Body weight; $BP_m^{0.73}$ = Body protein at maturity; Root MSE = root mean square error.

The regression equations obtained for all the different systems of units regarding the valine, isoleucine, and tryptophan trials are shown in Table 5. The coefficients of determination (R^2) in the studies were 90%, 92%, and 92% for valine, isoleucine, and tryptophan trials, respectively, indicating that there was a good fit of the data.

Table 5. Maintenance requirements for digestible valine, isoleucine, and tryptophan estimated from linear regression equation based on three units.

Amino acid	Regression equation[1]	R^2	Requirement
Valine			
mg/kg BW d	NR = -98.6 (±10.1) + 2.4 (±0.2) × Val	0.90	41
mg/kg $BW^{0.75}$ d	NR = -140.6 (±14.7) + 2.4 (± 0.2) × Val	0.90	59
mg/kg $BP_m^{0.73}$ d	NR = -591.7 (±62.2) + 2.4 (±0.2) × Val	0.90	247
Isoleucine			
mg/kg BW d	NR = -46.9 (±7.1) + 2.3 (±0.1) × Ile	0.92	20
mg/kg $BW^{0.75}$ d	NR = -72.6 (±11.1) + 2.3 (±0.1) × Ile	0.92	32
mg/kg $BP_m^{0.73}$ d	NR = -307.9 (±46.7) + 2.3 (± 0.1) × Ile	0.92	134
Tryptophan			
mg/kg BW d	NR = -39.5 (±7.7) + 7.3 (±0.4) × Trp	0.92	5
mg/kg $BW^{0.75}$ d	NR = -62.9 (±12.5) + 7.3 (±0.4) × Trp	0.92	9
mg/kg $BP_m^{0.73}$ d	NR = -266.7 (±52.4) + 7.3 (±0.4) × Trp	0.92	37

[1] The data of the control diet were not utilized in the statistical analyses. BW = Body weight; BPm = Body protein at maturity; NR = nitrogen retention; Val = valine; Ile = isoleucine; Trp = tryptophan.

To cancel the unit effect, the observed response (Y) and amino acid intake (X) studied were calculated as a percentage of the maximum value observed in X and Y axis for each amino acid and plotted in a standardized scale of 0 to 100%. The data are shown in Figures 1, 2, and 3 for the valine, isoleucine, and tryptophan trials, respectively. The different unit systems showed overlapping results and similar emerging slope for each of the amino acid trials tested. The systems mg/kg $BW^{0.75}$ per day and mg/kg $BP_m^{0.73}$ per day were remarkably similar in the case of the valine and tryptophan trials.

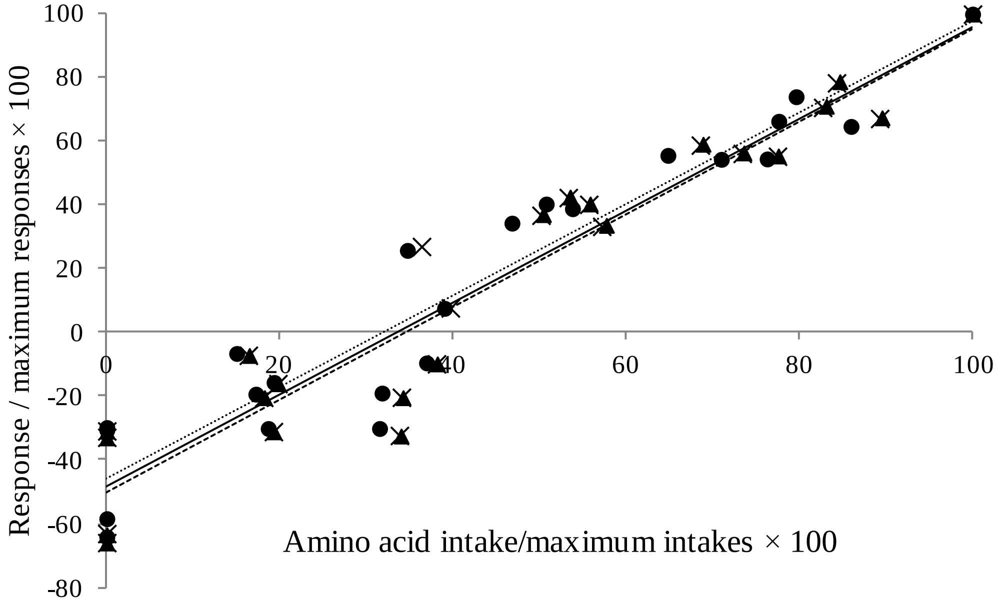

Figure 1. Graph representing a linear regression between nitrogen retention and valine intake, expressed in relation to a maximum response (%). (....., ·) mg/kg BW per day; (—, ×) mg/kg BW$^{0.75}$ per day; (– –, ▲) mg/BPm$^{0.73}$ per day.

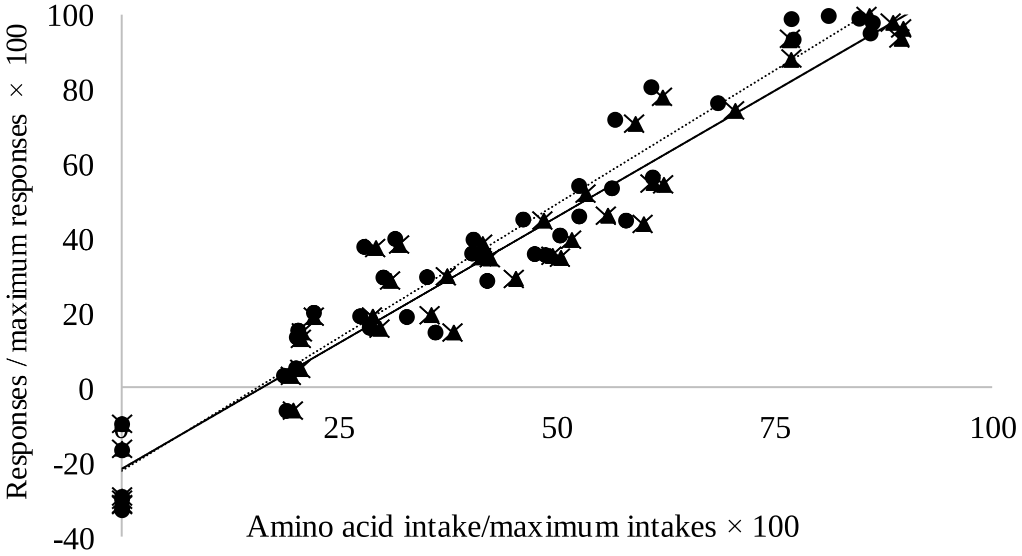

Figure 2. Graph representing a linear regression between nitrogen retention and isoleucine intake, expressed in relation to a maximum response (%). (..... ,•) mg/kg BW per day; (___, ×) mg/kg BW$^{0.75}$ per day; (- -, ▲) mg/BPm$^{0.73}$ per day.

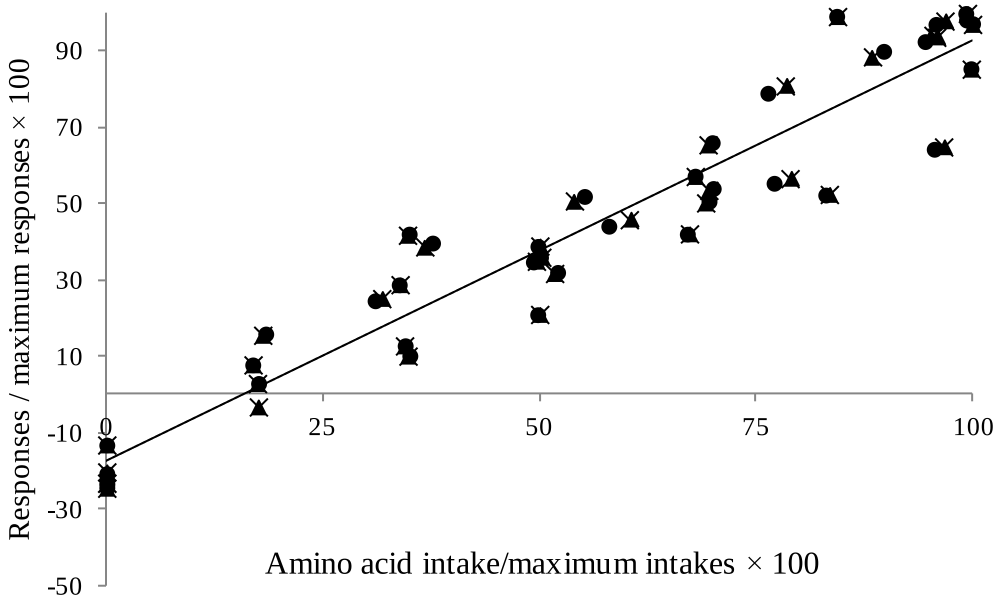

Figure 3. Graph representing a linear regression between nitrogen retention and tryptophan intake, expressed in relation to a maximum response (%). (.....,·) mg/kg BW per day; (——, ×) mg/kg $BW^{0.75}$ per day; (– –, ▲) mg/kg $BPm^{0.73}$ per day.

DISCUSSION

The specific maintenance requirements for valine, isoleucine, and tryptophan for poultry were estimated with different unit systems in order to search for values that represent the actual need for these amino acids in this physiologic state.

The maintenance requirements determined for valine in the present study (41 mg/kg BW d) differed from those reported in other studies, which were 55 mg/kg BW d (Leveille and Fisher, 1960), 15 mg/kg BW d (Ishibashi, 1973), and 60 mg/kg BW d (Fisher, 1983). The lower value reported by Ishibashi (1973) can be explained by the sigmoidal function used by this author because, as explained by Baker (2005), any mathematical function affects the interpretation of the results that, in turn, can influence the estimated value. The difference between the values found herein and the studies by Leveille and Fisher (1960) and Fisher (1983) may be due to the feeding adjustments that were performed to improve the energy homeostasis of these birds.

The amino acids necessary to maintain the integrity of body proteins in birds include those that are inevitably lost through the digestive tract, peeling, and feather loss (Baker, 2005; Sakomura and Rostagno 2007; Kebreab et al., 2008; Wu, 2014). Some researchers argue that the maintenance requirements are positively correlated with the amino acid composition of feathers (Leveille et al., 1960; Silva et al., 2014). Valine is found in high concentration in feather's protein (69.2 mg/g) (Leveille et al., 1960), therefore, it is possible that birds have an increased requirement for valine for the keratin synthesis in order to replace feather losses.

The isoleucine requirement for maintenance of the breeder roosters was calculated to be 20 mg/kg BW d. The daily values published in the literature vary from 15 to 60 mg/kg BW d (Burnham and Gous, 1992; Leveille and Fisher, 1960; Huyghebaert et al., 1991; Ishibashi, 1973). However, the diet and strain of birds used in this study differed from the studies of these authors, which may account for the differences in the results. The effect of strain can be found in the intercept of the equation, because the linear coefficient of the adjusted models $NR = -46.9 + 2.3 \times Ile$ versus $NR = -135 + 2.21 \times Ile$ (Burnham and Gous, 1992) were close, i.e., the strains have similar responses. However, the intercept of the equations cannot be explained in a simple way. According to Kebreab et al. (2008), there are several factors such as the dietary energy:protein ratio that can affect the metabolic and endogenous losses and consequently the value of the maintenance requirement. The diets employed in this study had higher energy:protein ratio, higher concentration of isoleucine and dietary protein, and lower leucine in dietary protein than those described in Burnham and Gous (1992). High energy:protein ratio and the inclusion of lipid sources in the diet possibly reduce the oxidation of leucine and isoleucine (Adibi, 1976). Moreover, addition of fatty acids reduces the decarboxylation of branched chain amino acids and this may be related to the nitrogen-sparing effect described by some authors (Adibi, 1976), which consequently reduces endogenous losses. The isoleucine:leucine ratio in our diet was 1:1.4, while Burnham and Gous (1992) used 1: 2.8. Higher concentration of isoleucine in relation to leucine can improve the use of isoleucine. Indeed, the increase in dietary leucine has been reported as a factor that reduces the efficiency of utilization of isoleucine (Allen and Baker, 1972). This may have contributed to the reduced endogenous and metabolic losses, resulting in the value of -46.9 mg/kg BW per day in this study and -135mg/kg BW per day found by Burnham and Gous (1992). Thus, the lower values of isoleucine requirement found may be due to reduced oxidation of this amino acid and reduced endogenous and metabolic losses caused by the inclusion of lipid sources and the ratio of isoleucine:leucine in the diet.

The maintenance requirement for tryptophan determined in this study was 5 mg/kg BW d and was similar to the values obtained by Ishibashi (1973) and Leveille and Fisher (1960) of 5 and 7 mg/BW for roosters, respectively. Our results were also close to the values obtained in studies on different species using different methodologies published by Baker et al. (1966), Wethli and Morris (1978), and Kim (1997), who determined values of 5, 6, and 5 mg/kg BW d for swine, laying hens, and broilers, respectively. For pullets, the values obtained were 10 mg/kg BW d (Morris and Wethli, 1978) and 11 mg/kg BW d (McDonald and Morris, 1985). The value obtained for laying hens was 6 mg/kg BW d (Wethli and Morris, 1978).

Many researchers recommend requirements for maintenance using the system mg/kg BW per day or mg/kg $BW^{0.75}$ per day (Leveille and Fisher, 1960; Leveille et al., 1960). However, these systems do not consider the differences in body composition. According to Taylor (1980) and Emmans (1989), the requirement for maintenance should be related to protein weight of the bird and maturity of body protein (*u*), which is the ratio of the weight of protein of the present state divided by the weight of the mature protein. The scale used by these authors does not consider the fat content, which may eliminate the imprecision in determining the amino acid requirements due to variation of the fat content, which vary widely in birds of the same genotype and body weight (Gous et al., 1984; Burnham and Gous, 1992; Nonis and Gous, 2008; Siqueira et al., 2011; Bonato et al., 2011; Bonato et al., 2013).

The effect of the unit systems in defining valine, isoleucine, and tryptophan maintenance requirements was also evaluated. The values were calculated as a percentage of the maximum observed value in the X and Y axes and plotted on a standardized scale from 0 to 100% (Figures 1, 2, and 3). The lines representing the different unit systems showed great overlapping, especially for the systems mg/kg $BW^{0.75}$ per day and mg/kg $BP_m^{0.73}$ per day. This similarity probably reflects the fact that the same samples were used to perform all calculations.

The system mg/kg $BP_m^{0.73} \times u$ per day allows the proportion of the maintenance requirement for any age to be extrapolated, as long as the degree of maturity of the protein is considered. To illustrate this, the data from Bae et al. (1999) for valine and isoleucine and from Kim et al. (1997) for tryptophan were used. These studies determined requirements of valine, isoleucine, and tryptophan for maintenance in growing birds (average weight of 0.269 kg). Based on their maintenance requirements the valine, isoleucine, and tryptophan intakes for maintenance were calculated to be 1.1, 1.0, and 0.6 mg/bird, respectively.

Considering again the data from Bae et al. (1999) and Kim et al. (1997), we calculated body weight, metabolic weight, and maturity of body protein and applied the maintenance requirements found in this study for valine, isoleucine, and tryptophan. For valine the intakes calculated with systems mg/kg BW d, mg/$BW^{0.75}$, and mg/kg $BP_m^{0.73}$ d × u were 11.2, 22.3, and 11.6 mg/bird, respectively. For isoleucine the values found for the systems mg/kg BW d, mg/kg $BW^{0.75}$ d, and mg/kg $BP_m^{0.73}$ d × u, were 5.7, 12.5, and 6.4 mg/bird, respectively. For tryptophan the intakes calculated using the systems mg/kg BW d, mg/kg $BW^{0.75}$ d, and mg/kg $BP_m^{0.73}$ d × u, were 1.2, 3.2, and 1.4 mg/bird, respectively. Based on these comparisons, it is clear to see an underestimation of amino acid intake for maintenance when the maintenance requirements recommended by Bae et al. (1999) and Kim et al. (1997) are used. The values found in the present study can be attributed to the methodological characteristics adopted including the use of breeder roosters, the feeding system, and the mathematical function employed.

Burnham and Gous (1992) suggested that the best way to express the amino acid requirement for maintenance in growing and adult birds is according to protein weight, because there is no demand for amino acid to maintain the adipose tissue. In addition, the body lipid content is influenced by environmental factors and diet (Emmans, 1989; Gous et al., 1999), mainly after maturity. However, the protein weight of the animal remains constant (Gous et al., 1999; Marcato et al., 2008; Silva et al., 2013) and the increase in weight occurs due to increase in body fat (Gous et al., 1999; Marcato et al., 2008). Thus, the maintenance unit based on protein weight and degree of maturity is the best way to express the requirements. Based on this unit, the determined requirements are: 24, 134, and 37 mg/kg $BP_m^{0.73}$ per day for valine, isoleucine, and tryptophan, respectively.

This study shows that determination of amino acid requirements at maintenance can vary according to the type of animal and methodology used. It is believed that the feeding and the calculations based on protein weight and degree of maturity employed herein express more precisely the amino acid requirements at maintenance for poultry than do other systems. The data concerning calculated requirements for the amino acids valine, isoleucine and tryptophan may contribute to future studies aiming to improve poultry production, through use in factorial models to increase precision in diet formulation.

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CAPÍTULO 3 – THE OPTIMAL DIGESTIBLE VALINE, ISOLEUCINE AND TRYPTOPHAN INTAKES OF BROILER BREEDER HENS FOR RATE OF LAY

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CAPÍTULO 3 – THE OPTIMAL DIGESTIBLE VALINE, ISOLEUCINE AND TRYPTOPHAN INTAKES OF BROILER BREEDER HENS FOR RATE OF LAY

ABSTRACT

Three concurrent assays were conducted with objective of to evaluate the response of broiler breeder hens to valine (Val), isoleucine (Ile), and tryptophan (Trp) intake, determine amino acid utilization efficiency (k), and develop a factorial model. One hundred ninety-two hens were used in each amino acid (AA) assay. A completely random design was used, which consisted of eight treatments, eight replicates, and one hen per cage. The diets were formulated by dilution technique using one summit diet and one nitrogen (N)-free diet, resulting in AA levels that ranged from 1.97 to 9.85, 1.97 to 9.85, and 5 to 2.5 g/kg of Val, Ile, and Trp, respectively. A validating diet was included for each amino acid studied to confirm that the response of the birds was a function of the limiting amino acid. Each experiment lasted nine weeks (five weeks of adaptation and four weeks for data collection). The data obtained were AA intake (AAI), body weight (BW), and egg output (EO). Broken line model was used to evaluate the responses. The model design used was $AAI=[AA_m\times(BW\times0.196)^{0.73}]+[(N_{egg}\times EO\times AA_{egg})/k]$, where AA_m is AA for maintenance (247, 134, or 37 mg/$BP_m^{0.73}$ for Val, Ile, and Trp respectively); $BP_m^{0.73}$ is mature body protein or $(BW\times0.196)^{0.73}$; k is 0.66 for Val, 0.60 for Ile, or 0.56 for Trp; N_{egg} is the N content in the egg (1.89g/100g); and AA_{egg} is the AA content in the egg (413, 338, or 108 mg/g for Val, Ile, and Trp respectively). The additional response seen with the supplementation of the crystalline amino acid confirmed that Val, Ile, and Trp were the first limiting amino acid. The values estimated by the model for utilization efficiency were: 66, 60, and 56% for Val, Ile, and Trp, respectively. The AAI estimated by the model at 30 weeks was 853, 692, and 228 mg/day for Val, Ile, and Trp, respectively. The prediction of the model was improved using the coefficients estimated here with physiologically relevant units.

Keywords: amino acid; broiler breeder hen; efficiency of utilization; egg output; mathematical model; rate of lay

Abbreviations: AA, amino acids; AAI, amino acid intake, BW, body weight, BWG, body weight gain, E, efficiency, EO, egg output, Ile, Isoleucine; RL, rate of lay; Trp, Triptofano; Val, valine

INTRODUCTION

Constant genetic improvements have enhanced poultry industry productivity in recent decades. In parallel with these advances, feed producers must frequently redefine nutritional plans considering new breed requirements, scientific developments, and local or global economic situation of the productive chain. Despite existing recommendations (NRC, 1994; Rostagno et al., 2011), industry professionals often attempt to improve rate of lay, egg weight, and hatchling weight by manipulating protein daily intake with no attention to specific amino acid requirements. Nevertheless, a number of reports suggest that high protein intake negatively affects fertility and hatchability (Pearson and Herron, 1982; Shafey, 2002; Ekmay et al., 2013). In addition, increasing the intake of amino acids such as lysine and isoleucine adversely affects hen fertility and egg hatchability (Ekmay et al., 2013).

Mathematical models represent an important tool that incorporates productivity variables to update nutritional requirements. These models also allow for greater flexibility than experimental methods in determining what variables might be more relevant to specific regions or even individual producers (Gous, 2014). However, to have good predictive value models must incorporate the correct factors and coefficients. Furthermore, these factors and coefficients must often be adjusted to new breeds and conditions.

Specifically regarding broiler breeder hens, existing factorial models mostly rely on coefficients and data from commercial laying hens. This is clearly not an optimal situation. For example, laying hens have an amino acid utilization efficiency of 85% whereas for broiler breeders this number is closer to 49% (Bowmaker and Gous, 1991; and Silva et al. 2015a). These differences arise from the egg-laying potential of the two types of hens, and demonstrate how poorly data from one breed, inserted into a model, would predict nutritional needs of the other.

Therefore, more studies are necessary to define the relevant variables and correct coefficients to be used in mathematical models for the nutrition of broiler breeders. Thus, in this study we evaluated the response of broiler breeder hens to different concentrations of valine, isoleucine, and tryptophan, three amino acids that are rarely studied in this context. We determined amino acid utilization efficiency and adapted the mathematical model originally developed by Bornstein et al. (1979).

MATERIALS AND METHODS

Birds and experimental design

Three dose response studies were performed using broiler breeder hens. The Animal Ethics and Welfare Committee of Universidade Estadual Paulista approved all experimental procedures used in this study under the Protocol number 9999/14.

One hundred ninety-two broiler breeder hens of Cobb 500® genotype with 48 weeks of age were used, with sixty-four birds per trial housed individually in metabolic cages. The cages were equipped with individual feeders and nipple drinkers. The experimental design was a completely randomized design with eight treatments (seven increasing levels of the amino acid and a control diet) and eight replicates.

Bird management

Two weeks before the beginning of the experiment, all birds were fed 150 g per day of a diet designed to meet their nutritional requirements during this period, according to recommendation of Rostagno et al. (2011). The rate of lay was monitored to provide a baseline for the experimental period. The experiment lasted nine weeks, with the first five weeks of adaptation and the last four weeks for data collection.

The lighting program adopted during the experiment was 17 hours of light. The hens were raised in a poultry house with a negative-pressure system with controlled temperature of 21 °C. The daily management was performed according to the Cobb 500 guidelines.

Experimental diets

For each amino acid assay a high protein summit diet, based on corn and soybean meal, was formulated, containing 9.85 g/kg of Val, 9.85 g/kg of Ile, and 2.50 g/kg of Trp (Table 1).

Table 1. Composition (g/kg) of the summit (high protein) and nitrogen-free (N-free) diets used in the valine, isoleucine, and tryptophan response trials.

Ingredients	Valine summit	Isoleucine summit	Tryptophan summit	N-free
Corn	442.6	414.4	494.8	-
Corn gluten meal 60%	-	-	10.0	-
Soybean meal	428.7	453.6	372.6	-
Corn starch	-	-	-	516.9
Rice husk	-	-	-	150.0
Sugar	-	-	-	150.0
Soybean oil	27.1	32.9	14.1	40.0
Limestone	66.7	66.7	66.7	61.9
Dicalcium phosphate	15.1	14.9	15.6	21.6
Salt	5.9	5.9	5.9	5.9
Potassium chloride	-	-	-	18.1
DL-methionine (99%)	5.0	4.8	5.3	-
L-Lysine HCl (78%)	1.0	0.2	2.7	-
L-Threonine	2.6	2.3	3.2	-
L-Tryptophan	0.2	0.1	-	-
L-Arginine	-	-	1.0	-
L-Valine	-	1.3	2.4	-
L- Isoleucine	2.1	-	2.8	-
Choline chloride 60%	1.0	1.0	1.0	1.0
Vitamin premix[a]	1.0	1.0	1.0	1.0
Trace premix[b]	1.0	1.0	1.0	1.0
BHT[c]	0.1	0.1	0.1	0.1
Inert (Sand)	-	-	-	32.4
Total	1000.0	1000.0	1000.0	1000.0
Nutrient content[d] (Digestible amino acid composition)				
Crude protein, g/kg	241.5	249.09	231.99	0.14
Methionine	7.92	7.82	8.07	-
Methionine+cysteine	11.11	11.11	11.11	-
Lysine	12.77	12.77	12.77	-
Threonine	10.34	10.34	10.34	-
Tryptophan	2.94	2.94	2.5	-
Arginine	15.18	15.89	14.68	-
Valine	9.85	11.49	11.49	-
Isoleucine	11.49	9.85	11.49	-
Leucine	17.96	18.51	17.67	-

[a]Content per kg diet: vitamin A, 9.000 IU; vitamin D3, 2.600 IU; vitamin E, 14 IU; vitamin K3, 16 mg; vitamin B1, 22 mg; vitamin B2, 6 mg; vitamin B6, 3 mg; vitamin B12, 10 mcg; nicotinic acid, 0.03 g; pantothenic acid, 0.15 g; folic acid, 0.6 mg; biotin, 1 mg.

[b] Content per kg diet: Cu, 8 mg; Fe, 0.05 g; Mn, 0.07 g; Zn, 0.05 g; I, 1.2 mg; Se, 0.2 mg. [c] Antioxidant butylated hydroxytoluene. [d] All diets contained: AMEn, 2,750kcal/kg; Calcium, 30 g/kg; Non-phytate phosphorus, 4 g/kg; and Sodium, 2.50 g/kg.

For each trial a nitrogen-free diet was formulated to meet the same nutritional levels as the summit diets, except for protein and amino acids. The nitrogen-free diets were used to dilute the summit diets, in appropriate proportions, to obtain the range of Val, Ile, and Trp (Table 2) contents required for each dilution series (Fisher and Morris, 1970).

To confirm that the response of the birds to each dilution series was in function of the respective limiting amino acid, a control diet was included for each amino acid assay. A small quantity of the respective crystalline amino acid was added to the diet with the lowest level of the amino acid tested sufficient to meet the level of the amino acid in the second-lowest level in the dilution series (Table 2).

Table 2. Proportions of the summit diet diluted with the corresponding nitrogen-free diet in the valine (Val), isoleucine (Ile), and tryptophan (Trp) trials and the resulting concentrations of the limiting amino acids in the diets.

Valine			Isoleucine			Tryptophan		
Summit	N-free	Val	Summit	N-free	Ile	Summit	N-free	Trp
%	%	g/kg	%	%	g/kg	%	%	g/kg
20	80	1.97	20	80	1.97	20	80	0.50
30	70	2.95	30	70	2.95	30	70	0.75
40	60	3.94	40	60	3.94	40	60	1.00
50	50	4.92	50	50	4.92	50	50	1.25
60	40	5.91	60	40	5.91	60	40	1.50
70	30	6.89	70	30	6.89	70	30	1.75
100	0	9.85	100	0	9.85	100	0	2.50
20	80	2.95[a]	20	80	2.95[b]	20	80	0.75[c]

[a] 1.016 g L-Val 96.5 % /kg added to $D1_{Val}$; [b] 0.995 g L-Ile 98.5 % /kg added to $D1_{Ile}$; [c] 0.026 g L-Trp 98 % /kg added to $D1_{Trp}$

Allocation of diets and measurements

The birds were fed 150 g per day of feed, at the same time each morning, and at the end of the week the leftovers were weighed to quantify the weekly consumption of the feed. The body weight of the hens was measured on the first, sixth, and tenth weeks of the assay. Egg production was recorded daily and egg weight was measured on three consecutive days each week.

Modelling of responses

The responses (Y) on efficiency of utilization and rate of lay (%) were regressed as a function of the amino acid intake (X) in mg/hen per day using the broken line model with one slope for rate of lay ($Y=L+U\times(R-X)$) and two slopes for efficiency of utilization ($Y=L+U\times(R-X)\times V+(X-R)$), where X is the input (amino acid intake) and Y is the output (rate of laying or efficiency of utilization) of the models, L is the maximum response of the model, R is the amino acid intake for maximum response, and the parameter U and V represents the slope in the models.

Estimating amino acid intake using a factorial model

Two models were compared, one from the literature (M1) and the other a model developed in this study (M2). The model M1 ($AAI = [(AA_m/0.85) \times BW] + [BWG \times (0.21 \times AA_t)] + [EO \times (63 \times AA_y + 158 \times AA_t)]$) was proposed by Bornstein et al. (1979), where: AAI is the amino acid intake (mg/day); AA_m is the amino acid for maintenance; 0.85 is the protein absorption rate; BW is the body weight; BWG is the body weight gain; 0.21 is the amount of protein in gain; AA_t is the amino acid in the protein fraction of the tissue; EO is the egg output; 63 is the nitrogen concentration in the egg; AA_y is the fraction of amino acid in egg yolk protein; and 158 is the amount of nitrogen in the tissue. The coefficient used for weight gain (0.21) was obtained from Bornstein et al. (1979). A second model was proposed (M2) AAI = [AA_m × (BW × 0.196) $^{0.73}$] + [((N_{egg} × EO) × AA_{egg}) / k]); where AAI is the amino acid intake; AA_m is the amount of amino acid for maintenance (mg/$BP_m^{0.73}$×u); *u* is the maturity rate obtained dividing body protein weight at age t (BPt) by the body protein weight at maturity (BP_m), i.e. u= BPt / BPm. In this study, *u* was considered as 1 because the broiler breeders were mature; BW is the body weight of the hens; EO is the egg output; k is the efficiency of utilization from the present data; 0.196 is the amount of nitrogen contained in the body of the birds without feathers analysed in the laboratory; N_{egg} is the amount of nitrogen in the egg (1.89 g N/100g according to Fisher, 1998); and AA_{egg} is the amount of amino acids in the egg (mg/g N) obtained from composition presented by Lunven et al. (1973).

To predict valine, isoleucine, and tryptophan intake, the data of body weight and egg mass of 60 broiler breeder hens of Cobb (500) strain were used. These data were obtained from individual monitoring of the hens during the period from 25 to 60 weeks of age.

Statistical analysis

The broken line model with one slope and with two slopes utilized for rate of lay and efficiency of utilization was estimated using the PROC NLIN procedure. The linear plateau models were adjusted according to the procedures described by Robbins et al. (2006). The average values (x_i) obtained were standardized (z=x_i-μ)/σ) considering μ (average) and the σ (deviation) of the egg output and body weight. Afterwards, we calculated the corresponding values in the cumulative distribution (NORM.DIST.S (*z*;true)) in Excel®. The statistical analyses were performed using SAS software, 2010 (Statistical Analysis System, version 9.2).

RESULTS

Responses of broiler breeder hens to dietary valine, isoleucine, and tryptophan

Bird responses to different levels of dietary Val, Ile, and Trp are shown in Table 3. The additional response (control diet) seen with the supplementation of the synthetic amino acid confirmed that Val, Ile, and Trp were the first limiting amino acid.

Table 3. Average responses to treatments and standard deviation (±SD) for daily feed intake (g/hen), daily amino acid intake (mg/hen), daily rate of lay (%), egg weight (g), daily egg output (g/bird), body weight (kg), and efficiency of broiler breeder hens from 53 to 57 weeks of age.

Levels g/kg	Feed Intake	Amino acid intake	Rate of lay	Egg Weight	Egg Output	Body Weight	Efficiency
				Valine			
1.97	119.8±20.3	236.1±39.9	38.9±18.5	62.4±3.2	18.6±13.4	4.1±0.3	139.9±68.9
2.95	136.3±18.3	401.9±53.9	53.1±17.3	62.2±5.6	32.1±14.1	4.2±0.4	111.1±41.0
3.94	144.9±4.9	570.9±19.2	65.2±4.2	66.3±5.6	44.3±4.3	4.5±0.6	98.0±17.7
4.92	141.9±8.6	698.3±42.5	63.9 ±7.4	69.5±7.2	45.7±5.5	4.4±0.3	76.1±8.0
5.91	143.9±7.9	850.9±46.9	62.6±9.4	68.9±6.8	44.8±5.8	4.5±0.4	54.7±11.0
6.89	147.5±1.5	1016.0±10.4	68.3±3.7	70.7±4.3	47.2±3.2	4.7±0.2	46.8±2.2
9.85	146.9±3.1	1446.6±30.6	69.3±6.9	69.3±7.8	45.1±7.6	4.7±0.2	28.6±5.1
2.95[a]	132.9±15.7	392.3±46.3	50.2±3.9	67.7±6.5	28.8±3.5	4.0±0.5	92.2±9.7
n	61	61	61	56	57	61	53
				Isoleucine			
1.97	131.4±14.8	258.9±29.2	41.8±14.7	65.1±6.3	27.9±11.1	4.0±0.4	120.5±4.3
2.95	141.8±6.9	418.4±20.3	56.4±8.2	68.9±6.3	39.8±4.16	4.3±0.3	80.0±9.3
3.94	144.5±2.9	569.3±11.7	65.2±8.5	67.0±5.6	43.6±4.9	4.3±0.3	59.0±6.8
4.92	141.3±6.2	695.0±30.4	60.2±9.8	70.8±2.1	42.8±6.5	4.6±0.5	48.5±4.6
5.91	146.2±1.8	863.8±10.9	60.7±9.2	72.4±4.9	43.9±8.7	4.7±0.4	37.1±7.1
6.89	146.1±2.6	1007.2±19.4	59.5±13.1	67.1±3.5	41.1±6.5	4.6±0.3	29.0±4.3
9.85	147.3±2.3	1451.3±22.9	69.2±6.0	70.6±4.9	49.0±6.6	4.6±0.3	23.2±2.9
2.95[b]	140.1±8.9	413.2±26.5	45.9±12.4	67.1±6.8	30.9±7.6	4.2±0.3	73.3±3.3
n	62	61	59	62	59	62	48
				Tryptophan			
0.50	135.1±10.9	67.6±5.5	38.8±22.1	65.7±7.1	26.0±13.1	3.9±0.5	118.3±33.9
0.75	139.8±11.1	104.8±8.3	52.0±12.2	65.9±6.9	31.9±14.9	4.4±0.3	109.8±17.5
1.00	139.9±16.9	139.9±16.9	57.6±9.2	70.9±2.6	40.6±5.5	4.3±0.5	77.7±8.5
1.25	145.0±2.4	181.3±3.0	52.7±16.7	72.2±5.9	43.2±4.5	4.4±0.3	62.3±4.6
1.50	143.6±6.3	215.5±9.4	53.1±16.3	71.7±5.2	43.3±7.4	4.6±0.2	49.8±5.9
1.75	147.1±2.1	257.4±3.7	66.5±7.9	70.1±5.9	44.9±6.4	4.6±0.3	43.8±6.1
2.50	148.1±1.2	370.7±2.9	63.8±8.4	72.9±4.3	44.9±2.4	4.9±0.2	29.1±4.5
0.75[c]	132.6±3.3	94.9±8.9	40.2±13.9	66.3±3.8	27.9±7.4	3.9±0.4	85.6±14.0
n	64	63	62	62	58	64	50

[a, b, c] Control diet. [a]Added 1.016 g/kg of L-valine; [b]Added 0.995 g/kg of L-isoleucine;[c]Added 0.026 g/kg L-Tryptophan. n: number of observations.

Feed intake

Each hen received 150 g of feed per day, however birds fed with lowest level and second-lowest level of the amino acid diets had lower feed intake in comparison to other treatments for the three amino acids assessed (Table 3).

Rate of lay, egg weight, and egg output

The highest rates of lay were observed for birds fed the 0.985 g/kg of the valine diet, reaching 69% for Val and 69% for Ile. Regarding Trp, birds on the 1.75 g/kg diet had the best rate of lay (67%). On the other hand, hens feeding on the lowest amino-acid diet produced 44%, 40%, and 42% fewer eggs than the maximally performing diets for Val, Ile, and Trp, respectively. Birds fed lowest level or second-lowest level of the amino acid tended to lay eggs that were approximately 10% lighter than those from birds feeding on sixth or seventh level of the amino acid for the three amino acids evaluated. Egg output was also reduced by 61%, 43%, and 42% for birds feeding on lowest level when compared to the maximally performing diets for Val, Ile, and Trp, respectively. Output was especially affected with the Val-lowest level diet (18.6 g/hen per day).

Modelling the responses: *amino acid intake vs. rate of lay and efficiency of utilization*

Adjusted models for rate of lay in function of dietary amino acid intake were:

$\text{Rate of lay}_{Val} = 67.69-0.07\times(654-X)$; if $X>654$ then $(654-X)=0$ Eq.1

$\text{Rate of lay}_{Ile} = 65.71-0.11\times(495-X)$; if $X>495$ then $(495-X)=0$ Eq.2

$\text{Rate of lay}_{Trp} = 63.82-0.23\times(172-X)$; if $X>172$ then $(172-X)=0$ Eq.3

Errors associated with L, U, and R were, respectively: 3%, 7%, and 9% for Val; 2%, 9%, and 9% for Iso; and 3%, 17%, and 23% for Trp.

Adjusted models for efficiency of utilization in function of dietary amino acid intake were:

$\text{Efficiency}_{Val} = 65.50-0.05\times(X-654)+0.21\times(654-X)$,

if $X<654$ then $(X-654)=0$ and if $X>654$ then $(654-X)=0$ Eq.4

$\text{Efficiency}_{Ile} = 59.65-0.05\times(X-495)+0.27\times(495-X)$, if $X<495$, then $(X-495)=0$ and if $X>495$, then $(495-X)=0$ Eq.5

$\text{Efficiency}_{\text{Trp}} = 55.69-0.14\times(X-172)+0.67\times(172-X)$,

if $X<172$, then $(X-172)=0$ and if $X>172$, then $(172-X)=0$ Eq.6

Errors associated with L, U, R, and V were, respectively: 18%, 40%, 15%, and 20% for Val; 5%, 20%, 5%, and 11% for Ile; and 11%, 28%, 9%, and 13% for Trp.

Rate of lay increased with increasing amino acid intake up to the response plateau and efficiency of utilization decreased with increasing amino acid intake (Figure 1). The adjusted model for utilization efficiency includes two slopes (Eqs 4, 5, and 6). The amino acid concentration at which the second slope starts is the same at which the rate of lay reaches the response plateau (Eqs 1, 2 and 3).

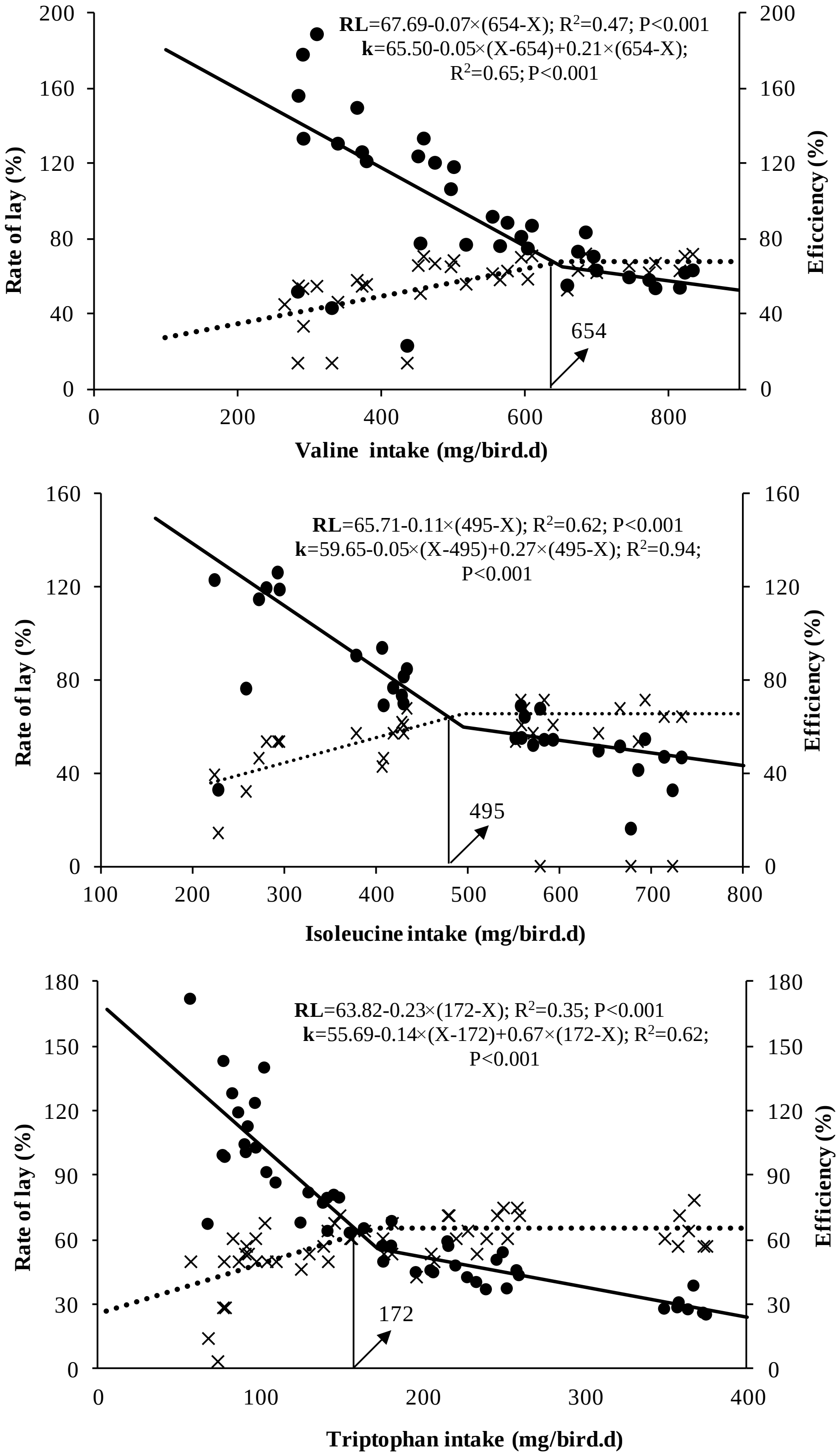

Figure 1. Relation between amino acid intakes and Rate of lay, RL (**........**, ×), and efficiency, k (**____**, •).

Factorial model for the estimation of ideal valine, isoleucine, and tryptophan intake

Ideal amino acid intake is given by the factorial model:

$$AAI=[AA_m\times(BW\times0.196)^{0.73}]+[((N_{egg}\times EO)\times AA_{egg})/k] \quad (M2)$$

Where: AAI = amino acid intake; AA_m = maintenance amino acid intake; BW = body weight measured as $mg/BP_m^{0.73}\times u$ where u= proteic weight in time/proteic weight at maturity, a ratio that equals 1 for mature animals; 0.196 = amount of nitrogen contained in the body of the birds without feathers; $N_{egg,}$ = amount of nitrogen in the egg (1.89 g/100g) (Fisher, 1998); EO = egg output (g); AA_{egg} = amount of amino acids in the egg (mg/g N) using values for each amino acid reported by Lunven et al. (1973); and k is the efficiency of utilization determined in the present study. Equations obtained for the three amino acids were:

$$AAI_{Val}=[247\times(BW\times0.196)^{0.73}]+[((1.89\times EO)\times413)/0.66]) \quad \text{Eq.7}$$

$$AAI_{Ile} = [134\times(BW\times0.196)^{0.73}]+[((1.89\times EO)\times338)/0.60]) \quad \text{Eq.8}$$

$$AAI_{Trp} = [37\times(BW\times0.196)^{0.73}]+[((1.89\times EO)\times108)/0.56]) \quad \text{Eq.9}$$

Model simulation and evaluation

Factorial models M1 and M2 were simulated with body weight and egg output from 60 individually monitored hens. Simulation results are shown in Table 4. The M1 model compared to M2 predicted 20% and 15% higher intakes for Val and Ile, respectively. The predicted intake for Trp was 50% lower in M1 than in M2. The observed differences between the models remained during the whole period assessed.

Table 4. Prediction of valine, isoleucine, and tryptophan intake for 60 broiler breeder hens from 25 to 60 weeks of age using two mathematic models with body weight (BW), body weight gain (BWG), and egg output (EO) data.

Age weeks	BW Kg	BWG g/day	EO g/day	Valine intake		Isoleucine intake mg/day		Tryptophan intake	
				M1[a]	M2[b]	M1[a]	M2[b]	M1[a]	M2[b]
25	3.89	6	37.1	790	645	601	507	111	166
30	4.10	6	53.9	1057	853	793	692	149	228
35	4.32	6	55.7	1098	883	824	716	155	236
40	4.54	4	55.6	1108	889	834	719	156	237
45	4.68	4	50.0	1032	828	781	662	145	217
50	4.82	0	48.7	1020	817	774	650	143	213
55	4.83	0	47.7	1005	805	764	640	141	209
60	4.83	0	45.6	974	780	741	617	137	202

[a] M1. $AAI=[(AA_m/0.85)\times BW]+[BWG\times(0.21\times AA_t)]+[EO\times(63\times AAy+158\times AA_t)]$ from Bornstein et al. (1979).

[b] M2. $AAI=[AA_m\times(BW\times0.196)^{0.73}]+[((N_{egg}\times EO)\times AA_{egg})/k]$ developed in this study.

Application of the factorial model to estimate amino acid intakes for a population

Average egg output and body weight values for 60 hens at 25, 30, 35, 40, 45, 50, 55, and 60 weeks of age were applied in the M2 model to predict Val, Ile, and Trp intake (Table 5).

Table 5. Population data for 60 broiler breeder hens and prediction of the minimum (min), average (μ), and maximum (max) values for valine, isoleucine, and tryptophan intake based on the application of the factorial models.

Age weeks	Population data					
	Egg outptut (g)			Body weight (kg)		
	Min	μ±SD	max	min	μ±SD	max
25	13	37±14	53	3.3	3.9±0.3	4.4
30	20	54±10	70	3.5	4.1±0.3	4.6
35	35	56±7	70	3.7	4.3±0.4	5.0
40	29	56±10	80	3.6	4.5±0.4	5.3
45	19	50±10	65	3.6	4.7±0.4	5.5
50	15	49±10	68	3.4	4.8±0.4	5.5
55	24	48±9	68	3.4	4.8±0.4	5.6
60	22	46±10	63	3.1	4.8±0.5	5.7

	Amino acid intakes predicted								
	Valine			Isoleucine			Tryptophan		
	Min	μ±SD	max	min	μ±SD	max	min	μ±SD	max
	mg/bird per day								
25	349	645±171	833	244	507±15	676	76	166±52	223
30	454	853±121	1051	332	692±10	869	105	228±37	288
35	641	883±87	1054	498	716±78	870	162	236±26	288
40	582	889±117	1186	439	719±10	982	141	237±36	326
45	458	828±122	1022	328	662±10	832	103	217±37	274
50	423	817±127	1063	292	650±11	865	91	213±38	285
55	533	805±113	1061	392	640±10	866	125	209±34	286
60	485	780±127	1011	358	617±11	816	114	202±38	268

SD = standard deviation.

For egg output, the coefficient of variation (CV) at 25 weeks was high (35%) because of the low rate of lay, but after this time point it stabilized at the average value of 19%. For body weight, the CV ranged between 7 and 10% of the average value of 4.5 kg.

The average egg output values and standard deviation accounted for 46.55% of the population, whereas maximum egg output values accounted for 90.07% of the population. For body weight, average values corresponded to 54.55% of the population, and maximum values to 94.66%.

Predicted values for Val, Ile, and Trp intake varied by 544, 487, and 165 mg/hen per day, respectively (Table 5). Differences between the maximum and average intakes were 223 (Val), 197 (Ile), and 66 (Trp) mg/hen per day, yielding maximum values approximately 30% higher than average intake values. Differences between the average and minimum intakes were 322 (Val), 290 (Ile), and 99 (Trp) mg/hen per day, corresponding to a difference of approximately 43%.

DISCUSSION

We evaluated the responses of broiler breeder hens to different intakes of Val, Ile, and Trp intake. Furthermore, we determined amino acid efficiency of utilization, and adapted the mathematical model originally developed by Bornstein et al. (1979). Our results include the dose-responses obtained by progressive dilution of the three amino acids in breeder diets. However, more importantly, we provide a revised mathematical model that can be used to define amino acid levels depending on producer goals and needs.

Responses of broiler breeder hens to dietary valine, isoleucine, and tryptophan

We applied the concept of relative deficiency by dilution technique (Fisher and Morris, 1970) to limit the amount of Val, Ile, and Trp amino acids in dietary protein. Compared to lowest level, the control diet for each amino acid increased rate of lay, egg weight, and egg output, demonstrating that the amino acids were in fact limiting (Table 3). The control diet for Val provided the highest improvement in rate of lay compared to lowest level, followed by Ile and Trp, indicating the limiting potential of these amino acids. Previous studies using the same methodology also based their conclusions about limiting potential on the relative responses to their highest dilution and control diets (Wethli and Morris, 1978; Bowmaker and Gous, 1991).

Dietary amino acid concentration had little influence on feed intake (Table 3). Only the lower amino acid intake resulted in decreased intake. Another study reported a similar effect (Gous et al., 1987). This counter-intuitive effect may result from the larger volumes as well as higher relative concentration of other nutrients in the more diluted diets. Among amino acids, feed intake only stabilized with diet 1.25 g/kg for Trp and third level of the amino acid for the other two amino acids. The Trp has been shown to increase feed intake in other farm animals (Sève, 1999). Thus, the observed difference may result from a depressant effect on feed intake of very low Trp concentrations. Similar Trp effects on hens have been previously reported (Morris and Wethli, 1978).

Egg weight and rate of lay decreased with lower amino acid concentrations (Table 3). Previous studies evaluating other amino acids have reported two-fold greater reductions (Morris and Gous, 1988; Bowmaker and Gous, 1991). This discrepancy may result from the age of hens. The older hens used in the current study have larger body reserves and more easily mobilize tissue mass for egg formation, even at very low amino acid concentrations (Bowmaker and Gous, 1991).

Utilization efficiency and rate of lay

At lower amino acids concentrations, hens reduced protein intake to near-maintenance requirements, when they would be expected to stop laying eggs. However, in parallel with reducing body weight (data not shown), broiler breeders kept producing more than 2 eggs per week, totalling 18 to 27 eggs during the entire experimental period. These results agree with those previously obtained by Bowmaker and Gous (1991) who reported the production of one egg per week in parallel with body weight loss. Thus, broiler breeders react differently to dietary restriction when compared to laying hens. The former probably prioritize egg laying even at the expense of body energy stores.

Efficiency of utilization increased in lower amino acid concentrations, probably because, with the mobilization of body mass towards egg laying, the apparent feed conversion improved. The amino acid concentration at which the second slope of the efficiency of utilization curve starts is the same at which the rate of lay reaches the response plateau for the three amino acids tested (Figures 1, 2, and 3). These results agree with the hypothesis that broiler breeder efficiency is a complex variable related to rate of lay (Fisher, 1994; 1998). Fisher et al. (2001) used a methodology similar to ours to calculate efficiency of utilization of broiler breeder hens of four different ages. The authors found that lysine efficiency of utilization was 82%, 76%, 59%, and stabilized at 57% for hens aged 26, 37, 48, and 60 weeks, respectively. Similar lysine efficiencies were reported in other studies (Bowmaker and Gous, 1991; Silva et al., 2015a). We report here a compatible average efficiency for the three amino acids (Val, Ile, and Trp) of 66%.

Factorial model for the estimation of ideal valine, isoleucine, and tryptophan intake

Based on maintenance and efficiency of utilization coefficients, as well as physiological aspects, we revised the amino acid requirement model originally proposed by Bornstein et al (1979). The main alterations we propose to the model involve metric, units, and maintenance percentage values. The maintenance requirement coefficient we propose includes protein weight at maturity expressed as

$\text{mg}/_{\text{kg}}\text{BP}_{\text{m}}^{0.73}\times u$ as previously recommended by Emmnas (1989) in substitution for body weight. This change accounts for widely different body fat percentages, which may distort results because lipid reserve maintenance does not require amino acids. We also considered 100% efficiency for maintenance as opposed to the 85% proposed by Bornstein et al. (1979). Other models also employ 100% efficiency for maintenance (Martin et al., 1994; Samadi and Liebert, 2007a, 2007b, 2008). This change is justified by the very low amino acid concentrations required for maintenance. The efficiency of utilization value introduced in the model was 60% for all ages, as opposed to the 85% previously used (Hurwitz and Bornstein, 1973). We propose this change based on the fact that broiler breeders require higher intake than laying hens to deposit the same amount of egg amino acids (Silva et al. 2015a,b). However, because this input value is greatly related to rate of lay (Fisher, 1994, 1998), further studies can improve on it by modelling efficiency of utilization in a dynamic form.

After the changes introduced, the M2 model predicted lower required intake of Val and Ile. This results mostly from the correction in maintenance efficiency. On the other hand, our M2 model yielded higher requirements of Trp because of new amino acid deposition coefficients that reflect ideal egg amino acid concentrations in the egg (Lunven et al., 1973).

We measured the egg output and body weight of 60 individual hens to evaluate the predictive power of the model at minimum, average, and high performances (Table 5). This was done according to previous suggestions that limiting amino acid intakes should be calculated based on population variations (Fisher et al., 1973) Depending on the distribution of high and low-productivity birds, the average population will not represent 50% of individuals. Here, we found that recommended intake would be optimal for 47% of the population instead of 50%, because some individuals were producing with long pauses between laying sequences, also justifying the high CV for the minimum predicted intake of Val, Trp, and Ile.

The difference between the maximum and average intake corresponded to 30%. This value can be used as a correlation between population average and the average maximum value. Thus, the average value times 1.3 approximates the maximum intake for 90% of this population of individuals. However, this value varies depending on the distribution and uniformity of body weight and egg output. It is appropriate to consider economic aspects of the determination of the limiting amino acid intake (Fisher et al., 1973).

The M2 predicted intake was 25% and 29% higher for Val and Trp, respectively, and 12% lower for Ile than recommended by the NRC (1994), considering the period of 25 to 60 weeks of age. A lower intake of Ile has also been recommended by Fisher (1998). Emkay et al. (2013) found that rates of lay for Val, Ile, and Trp reach a plateau at 829, 794, and 234 mg/d, respectively. These authors do not provide intake variation in the estimates, but based on the values provided, the M2 model differed by +46, -85, and -0.3 mg/d of Val, Ile, and Trp, respectively, in the same experimental period.

The M2 model yielded lower Ile intakes than recommended by Emkay et al. (2013) at all ages. However, our model only takes into account of body protein weight and egg amino acids to predict intake, with no fertility variable. However, the best fertility rates observed by Emkay et al. (2013) were at Ile intakes lower than 625 mg/d. Their average optimal intakes for rate of lay and fertility would be 709.5 mg/d, only 0.5 mg/d above the value predicted by our model.

Altogether, our results suggest that the M2 model incorporates the most physiologically relevant units, and values to provide accurate estimates of amino acid requirements by broiler breeder hens. Future work should refine M2 taking into account the dynamics of utilization efficiency, and considering an association between efficiency of utilization and fertility.

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CAPÍTULO 4 – OPTIMAL ECONOMIC INTAKE OF ISOLEUCINE, VALINE AND TRYPTOPHAN FOR BROILER BREEDER HENS

ABSTRACT

Considering that the variation within a population and the cost-benefit ratio of amino acid supplementation has not been a procedure often used by nutritionists in the definition of dietary levels, the objective of this study was to calculate the optimal economic intake of valine (Val), isoleucine (Ile) and tryptophan (Trp) for broiler breeder hens by the simulation method and using an equation while considering the relationship between cost-benefit and the flock variability, using the Reading Model. One hundred ninety-two hens were used in each amino acid (AA) feeding trial. A completely randomized design was used, which consisted of eight treatments, eight replicates, and one hen per cage. The diets were formulated by dilution technique using one summit diet and one nitrogen (N)-free diet, resulting in AA levels that ranged from 1.97 to 9.85, 1.97 to 9.85, and 5 to 2.5 g/kg of Val, Ile, and Trp, respectively. Each experiment lasted nine weeks (five weeks of adaptation and four weeks of data collection). The amino acid intake, egg output (EO) and body weight (BW) were adjusted using a Reading model. For the simulation method was used 10,000 birds and for each bird, was calculated the value of amino acid intake (AAI). The EO was calculated by the equation: EO=AAI-[b×BW]/a, where "*a*" is requirement of the amino acid per gram of egg (mg/g) and "*b*" is amino acid requirement per kilogram of BW (mg/kg). The models generated by predicting Val, Ile and Trp intake were the following: Val=12.5×EO+41×BW, Ile=9.9×EO+20×BW and Trp=3.1×EO+5×BW, where EO=egg output, g/bird per day, and BW=body weight, kg/bird. Based on the above models, birds with 3,5 kg of body weight and an egg output of 50 g/d, the flock require 768 mg of Val/bird per day, 565 mg of Ile/bird per day and 172 mg of Trp/bird per day. The optimum economic intake for flock uniformity (5% coefficient of variation) was calculated at 837, 604 and 192 mg of Val, Ile and Trp/bird per day, respectively by equation and 824, 565 and 187 mg of Val, Ile and Trp/bird per day was calculated while using the simulation method. The Reading Model could be used to estimate the optimum amino acid intakes for hens under different genetic and economic scenarios and depending on the ingredients available and their prices, the cost of each amino acid will vary.

Keywords: amino acid, body weight, dilution technique, egg output, population response, Reading model

INTRODUCTION

Broiler breeder hens at reproduction phase are reared under controlled feeding conditions. The feed is provided and it is assumed that all birds are fed equally. However, the composition and nutritional levels established by the companies are the dietary factors that depend on the physical capacity for intake by the bird. On the other hand, if these factors are uncontrolled, they can contribute to the non-uniformity of the intake and can affect the egg production, because broiler breeder hens doesn’t regulate the limiting nutrient by increasing their intake, as occur in other birds when reared under ad libitum conditions (Gous, 2015). In addition, there are some other factors related to the environment, handling and dominance affecting the uniformity of the flock.

One possible solution would be the use of methodologies that take into account the uniformity or variation in the performance of the population, considering the nutritional levels of the nutrients in the diets provided to the broiler breeders. The first mathematical model parameterized for calculating the amino acid intake for broiler breeders was developed by Bornstein et al. (1979) and recently modified by Lima et al. (2016). In both studies, the amino acid intake calculated are valid for uniform flocks and do not consider the uniformity in egg output and body weight. Moreover, these studies do not mention an economic indicator, which relates the cost of amino acid supply and the additional revenue predicted with the number of birds feeding the extra amino acid provided beyond the average, especially for flocks with high variation in egg output and body weight.

Considering that variation within a population and the cost-benefit ratio of amino acid supplementation has not been a procedure often used by nutritionists in the definition of dietary levels, the flock of birds reared under any restrictions would be producing below its genetic potential (Fisher, 2015). Thus, the economic index of the flocks can be improved by using some mathematical procedures. According to Fisher et al. (1973), three methods can be applied for describing the experimental data and for predicting amino acid requirements: a computer simulation method; exact equation for the curve and an approximate method derived of the first method while using in practical situations. An alternative is to apply stochastic models to define the nutritional levels of the diets (Fisher, 2015; Silva et al, 2015a).

The “Reading Model” developed by Fisher et al. (1973), applied to a simulated population, can provide an approximation of the number of birds meeting their nutritional requirements according to the level of the amino acid. For amino acid levels provided beyond the average, it is possible to calculate the cost and the additional revenue obtained by the extra egg output produced, indicating an optimal economic relationship with the simulated levels (Fisher et al., 1973). Despite the application of the Reading Model, fewer studies with broiler breeder hens were conducted to determine the parameters for the essential amino acids, which currently has been found coefficients for only lysine and threonine (Bowmaker and Gous, 1991; Silva et al., 2015b) and using the method of the equation, that not considers the flock variability.

The aim of the present research study was to calculate the optimal economic intake of valine (Val), isoleucine (Ile) and tryptophan (Trp) for broiler breeder hens by simulation and equation considering the cost-benefit ratio and the flock variability while using the Reading Model.

MATERIALS AND METHODS

Birds and experimental design

Three dose response feeding trials were performed utilizing broiler breeder hens. The studies were conducted at the Faculdade de Ciências Agrárias e Veterinárias, UNESP Univ Estadual Paulista, Campus Jaboticabal, Departament of Animal Science, Poultry Science Laboratory, SP, Brazil with the approval of the Animal Ethics and Welfare Committee of UNESP (Protocol number 9999/14).

One hundred ninety-two Cobb 500 broiler breeder hens at 48 weeks of age were used, with sixty-four birds per trial housed individually in metabolic cages, equipped with individual feeders and nipple drinkers. A completely randomized design was used for all the three feeding trials; each consisted of eight treatments (seven increasing levels of the amino acid and a control diet) and eight replicates.

Bird management

Two weeks before the beginning of the experiment, all birds received 150 g per day of a diet designed to meet their nutritional requirements during this period, according to the method described by Rostagno et al. (2011). The rate of lay was monitored to provide a baseline for the experimental period. The experiment lasted nine weeks, with five weeks of adaptation and four weeks of data collection.

The lighting program adopted was 17 hours of light. The hens were raised in a poultry house with a negative-pressure system and controlled temperature of 21 °C. The daily management was performed according to the Cobb 500 guidelines (Cobb-Vantress Inc 2012).

Experimental diets

For each amino acid a high protein summit diet was formulated, based on corn and soybean meal, containing 9.85 g/kg of Val, 9.85 g/kg of Ile, and 2.50 g/kg of Trp (Table 1).

Table 1. Composition (g/kg) and nutrient content (digestible amino acid composition) of the summit and nitrogen-free diets.

Ingredients	Summit Valine	Isoleucine	Tryptophan	Nitrogen-free
Corn	442.6	414.4	494.8	-
Corn gluten meal 60%	-	-	10.0	-
Soybean meal	428.7	453.6	372.6	-
Corn starch	-	-	-	516.9
Rice husk	-	-	-	150.0
Sugar	-	-	-	150.0
Soybean oil	27.1	32.9	14.1	40.0
Limestone	66.7	66.7	66.7	61.9
Dicalcium phosphate	15.1	14.9	15.6	21.6
Salt	5.9	5.9	5.9	5.9
Potassium chloride	-	-	-	18.1
DL-methionine (99%)	5.0	4.8	5.3	-
L-Lysine HCl (78%)	1.0	0.2	2.7	-
L-Threonine	2.6	2.3	3.2	-
L-Tryptophan	0.2	0.1	-	-
L-Arginine	-	-	1.0	-
L-Valine	-	1.3	2.4	-
L- Isoleucine	2.1	-	2.8	-
Choline chloride 60%	1.0	1.0	1.0	1.0
Vitamin premix[a]	1.0	1.0	1.0	1.0
Mineral premix[b]	1.0	1.0	1.0	1.0
BHT[c]	0.1	0.1	0.1	0.1
Inert (Sand)	-	-	-	32.4
Total	1000.0	1000.0	1000.0	1000.0
Nutrient content[d] (Digestible amino acid composition)				
Crude protein, g/kg	241.5	249.09	231.99	0.14
Methionine	7.92	7.82	8.07	-
Methionine+cysteine	11.11	11.11	11.11	-
Lysine	12.77	12.77	12.77	-
Threonine	10.34	10.34	10.34	-
Tryptophan	2.94	2.94	2.5	-
Arginine	15.18	15.89	14.68	-
Valine	9.85	11.49	11.49	-
Isoleucine	11.49	9.85	11.49	-
Leucine	17.96	18.51	17.67	-

[a] Content per kg premix: vitamin A, 9.000.000 IU; vitamin D3, 2.600.000 IU; vitamin E, 14.000 IU; vitamin K3, 1.600 mg; vitamin B1, 2.200 mg; vitamin B2, 6.000 mg; vitamin B6, 3.000 mg; vitamin B12, 10.000 mcg; nicotinic acid, 30 g; pantothenic acid, 15 g; folic acid, 600 mg; biotin, 100 mg; [b] Content per kg premix: Cu, 8.000 mg; Fe, 50 g; Mn, 70 g; Zn, 50 g; I, 1.200 mg; Se, 200 mg

[c] Antioxidant - butylated hydroxytoluene; [d] All diets was formulated to contain: AMEn, 2,750kcal/kg; Calcium, 3%; Available phosphorus, 0.400%; and Sodium, 0.250%.

For each trial a nitrogen-free diet was formulated to meet the same nutritional levels as the summit diets (mineral, vitamin and energy), except for protein and amino acids. The nitrogen-free diets were used to dilute the summit diets, in suitable proportions, to obtain the range of Val, Ile, and Trp (Table 2) contents required for each dilution series (Fisher and Morris, 1970).

To confirm that the response of the birds to each dilution series was in function of the respective limiting amino acid, a control diet was included for each amino acid feeding trial (control diet). A small quantity of the respective crystalline amino acid was added to the diet with the lowest level of the amino acid tested (First level) sufficient to meet the level of the amino acid in the second-lowest level in the dilution series (Table 2).

Table 2. Proportions of the summit diet diluted with the corresponding nitrogen-free diet in the valine (Val), isoleucine (Ile), and tryptophan (Trp) trials and the resulting concentrations of the limiting amino acids in the diets.

Valine			Isoleucine			Tryptophan		
Summit	N-free	Val	Summit	N-free	Ile	Summit	N-free	Trp
%		g/kg	%		g/kg	%		g/kg
20	80	1.97	20	80	1.97	20	80	0.50
30	70	2.95	30	70	2.95	30	70	0.75
40	60	3.94	40	60	3.94	40	60	1.00
50	50	4.92	50	50	4.92	50	50	1.25
60	40	5.91	60	40	5.91	60	40	1.50
70	30	6.89	70	30	6.89	70	30	1.75
100	0	9.85	100	0	9.85	100	0	2.50
20	80	2.95[a]	20	80	2.95[b]	20	80	0.75[c]

[a] Added 1.016 g of L-valine/kg of diet; [b] Added 0.995 g of L-isoleucine/kg of diet; [c] Added 0.026 g L-Tryptophan/kg of diet. [a, b, c] Control diet.

Allocation of diets and measurements

The birds were fed 150 g per day of feed, at the same time, and at the end of the week the leftovers were weighed to quantify the weekly consumption of the feed. The body weight of the hens was measured on the first, sixth, and tenth weeks of each feeding trial. Egg production was recorded on daily basis and egg weight was measured on three consecutive days each week.

Statistical analyses and mathematical models

For statistical analysis, the control treatment data was not utilized, because it was used only to validate if the amino acids were limiting in the diets. At the end of each trial, were analysed the average and standard deviations of the feed intake, amino acid intake, egg output and body weight. Before regression analysis, treatments were analysed using the PROC GLM procedure of SAS (2010) for each trial.

Reading model

The responses of egg output and body weight were applied Reading model (Fisher et al., 1973), according to the following general expression: $AAI_{opt}=\mu+y\times z$, where AAI_{opt} is the optimum economic intake; μ is the mean intake of the population which is calculated by equation $AAI=a\times Emax+b\times BW$; *y* is the standard deviation of the amino acid requirements; and *z* is the economic index that suggests how many times the deviation of the requirement (*y*) must be added to the average population requirement.

The parameters are follows: amino acid intake in mg/bird day (AAI); maximal egg output, g/day (Emax); standard deviation of the Emax, g/day (σ^2_{Emax}); body weight (BW), standard deviation of BW (σ^2_{BW}); requirement of the amino acid per gram of egg, mg/g (*a*) and for amino acid requirement per kilogram of BW, mg/kg (***b***). The standard deviation of the requirements was calculated as follows: $y = \sqrt{(a^2\times\sigma^2_{Emax}+b^2\times\sigma^2_{BW})}$.

According to Morris and Wethli (1978), the variance of a×Emax+b×BW, assuming zero correlation between Emax and BW, is $a^2 \times \sigma^2_{Emax} + b^2 \times \sigma^2_{BW}$.

The variables analyzed were amino acid intake, average and standard deviations of the EO and BW. For this study, the amino acid optimization module of EFG software (1995) was used to estimate the coefficients of the model.

Application of the model to calculate the optimum economic intake

The optimum economic intake AAI_{opt} was calculated using the simulation and the equation described for Reading Model (Fisher et al., 1973). Using the Reading Model, the AAI_{opt} represents the extra amount of amino acid (mg/bird per day) that can be economically supplied above the mean requirement of the population. The extra amino acid that is economically viable is given by the expression: y×z, where z is the index that suggests how many times the deviation (*y*) should be added to the mean requirement of the population. The AAI_{opt} was set using the equation as described by Pilbrow and Morris (1974), where *z* corrects the deviation *y* by economic factors (k): $AAI_{opt} = a \times Emax + b \times BW + \sqrt{(a^2 \times \sigma^2_{Emax} + b^2 \times \sigma^2_{BW})} \times z$.

The *z* value is the standard deviation that represented by the product between the cost-benefit and amino acid requirement per gram of egg (a×k). Where, *k* is the cost-benefit ratio, given by US$ mg of industrial amino acid/US$ g of egg.

The AAI_{opt} is reached when the EO response is equal to the relationship between the costs of an extra unit of the amino acid that returns an extra unit of EO. Extra amino acid may be supplied to hens that do not reach their Emax; this amount is corrected to their production rate and the value of producing a unit of EO. Thus, the AAI_{opt} should be given only when the cost of the amino acid supply is equal to the EO response value obtained by a unit of amino acid supplied.

The data applied to the model was of a given population with an average Emax of 50 g/day and 3.5 kg of BW. The uniformity of the flock was obtained considering 100% minus the coefficient of variation (CV). For body weight, was admitted a fixed value for uniformity of 90% or CV of 10%, corresponding to σ^2_{BW} of 0.350 kg.

For the variable EO were considered scenarios for five-uniformity within a flock of 95%, 90%, 85%, 80% and 75%. Based on CV were calculated σ^2_{Emax} for each scenario of 2.5, 5.0, 7.5, 10.0, and 12.5 g/day, given as a source of variation in the model to calculate the AAI_{opt}.

The method described by Lima et al (2016) was used for obtaining the coefficients of the amino acid requirements for maintenance (*b*) (valine (41 mg/kg), isoleucine (20 mg/kg) and tryptophan (5 mg/kg)). Those maintenance coefficients were determined in nitrogen balance experiments using Cobb roosters.

The prices considered were 12 US$/kg for L-valine 96.5%, 18 US$/kg for L-tryptophan 98%, 48 US$/kg for L-isoleucine 99%, and US$ 0.45/unit of 65g for fertile egg.

Procedure based on *simulation using 10,000 birds*

A simulation method based on Monte Carlo random sampling techniques was used to draw samples of birds from two normal, non-correlated distributions for Emax and BW, according to Fisher et al. (1973). The values for *a* and *b* are determined from Reading model. For each bird, was calculated value of AAI. The EO was calculated by the equation: EO=AAI-[b×BW]/a. This procedure is repeated for all 10,000 birds in the selected sample, leading to an estimate EO for an average flock considering AAI and was repeated for 10 AAI increased levels, that was established the level of stability of the response. All 10 amino acids intake levels were obtained by adding fractional intakes calculated for the average population. For valine intakes, each increasing of 56 mg resulted in values varied from 768 to 2,000 mg/bird per day.

For isoleucine intakes each increasing of 45 mg resulted in varied between 567 and 1,557 mg/bird per day. For tryptophan intakes each increasing of 15 mg resulted in varied between 170 and 500 mg/bird per day. By this procedure is possible to make small increases in amino acid intake and to calculate the optimum economic intake based on the input cost and revenue of output using large populations. In this simulation method was considered the same scenarios of uniformity for EO and BW, and the same prices for industrial amino acids and egg and the maintenance coefficients as used by Lima et al. (2016).

RESULTS

Responses of broiler breeder hens to dietary valine, isoleucine, and tryptophan

Bird responses to different dietary levels of Val, Ile, and Trp are shown in Table 3. The additional response (treatment control) seen with the supplementation of the L-amino acid confirmed that Val, Ile, and Trp were the first limiting amino acid.

Table 3. Mean and standard deviation (SD) of the feed intake, amino acid intake, egg output and body weight (kg), of broiler breeder hens (53 until 57 weeks).

Levels g/kg	Feed intake (g/bird d)		AA intake (mg/bird d)		Egg output (g/bird d)		Body weight (kg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Valine								
1.97	119.8	20.3	236.1	39.9	18.6	13.4	4.1	0.3
2.95	136.3	18.3	401.9	53.9	32.1	14.1	4.2	0.4
3.94	144.9	4.9	570.9	19.2	44.3	4.3	4.5	0.6
4.92	141.9	8.6	698.3	42.5	45.7	5.5	4.4	0.3
5.91	143.9	7.9	850.9	46.9	44.8	5.8	4.5	0.4
6.89	147.5	1.5	1016.0	10.4	47.2	3.2	4.7	0.2
9.85	146.9	3.1	1446.6	30.6	45.1	7.6	4.7	0.2
2.95[a]	132.9	15.7	392.3	46.3	28.8	3.5	4.0	0.5
P-value	0,0006		<.0001		<.0001		0.0024	
CV[d]	8.6		5.59		22.09		8.49	
Isoleucine								
1.97	131.4	14.8	258.9	29.2	27.9	11.1	4.0	0.4
2.95	141.8	6.9	418.4	20.3	39.8	4.16	4.3	0.3
3.94	144.5	2.9	569.3	11.7	43.6	4.9	4.3	0.3
4.92	141.3	6.2	695.0	30.4	42.8	6.5	4.6	0.5
5.91	146.2	1.8	863.8	10.9	43.9	8.7	4.7	0.4
6.89	146.1	2.6	1007.2	19.4	41.1	6.5	4.6	0.3
9.85	147.3	2.3	1451.3	22.9	49.0	6.6	4.6	0.3
2.95[b]	140.1	8.9	413.2	26.5	30.9	7.6	4.2	0.3
P-value	0.0017		<.0001		<.0001		0.0017	
CV[d]	4.9		3.2		18.2		7.84	
Tryptophan								
0.50	135.1	10.9	67.6	5.5	26.0	13.1	3.9	0.5
0.75	139.8	11.1	104.8	8.3	31.9	14.9	4.4	0.3
1.00	139.9	16.9	139.9	16.9	40.6	5.5	4.3	0.5
1.25	145.0	2.4	181.3	3.0	43.2	4.5	4.4	0.3
1.50	143.6	6.3	215.5	9.4	43.3	7.4	4.6	0.2
1.75	147.1	2.1	257.4	3.7	44.9	6.4	4.6	0.3
2.50	148.1	1.2	370.7	2.9	44.9	2.4	4.9	0.2
0.75[c]	132.6	3.3	94.9	8.9	27.9	7.4	3.9	0.4
P-value	0.0005		<.0001		<.0001		<.0001	
CV[d]	6.8		4.9		24.2		8.5	

[a] Added 1.016 g of L-valine/kg of diet; [b] Added 0.995 g of L-isoleucine/kg of diet; [c] Added 0.026 g L-Tryptophan/kg of diet. [d] Coefficient of variation; [a, b, c] Control diet.

Fitting the Reading Model

The Reading model was fitted using the data of amino acid intake, EO and BW. The BW and σ^2_{BW} were 4.39kg and 0.439kg, respectively, used for the three amino acids. The estimated parameters by model for Emax were: 45.79, 44.41 and 43.77 g/day for for Val, Ile and Trp, respectively, for σ^2_{Emax} were 4, 4 and 4.5 g/day for Val, Ile and Trp, respectively; for *a* were 12.48, 9.91 and 3.1 mg/g; and for *b* were 2.52, 0.02 and 0.01 mg/kg BW for Val, Ile and Trp, respectively.

Application of the models to calculate optimum economic intake for a flock

While using practical situations to calculate amino acid intake for a flock, was considered five scenarios of uniformity. The equations for each scenario are listed as follows:

$Val_{opt} = 12\times50+41\times3.5\sqrt{}(12^2\times\sigma^2_{Emax}+41^2\times0.350^2)\times2.0212$ Eq. 1

$Ile_{opt} = 9.91\times50+20\times3.5\sqrt{}(9.91^2\times\sigma^2_{Emax}+20^2\times0.350^2)\times1.485$ Eq. 2

$Trp_{opt} = 3.1\times50+5\times3.5\sqrt{}(3.1^2\times\sigma^2_{Emax}+5^2\times0.350^2)\times2.406$ Eq. 3

The *y* values considering σ^2_{Emax} for 95%, 90%, 85%, 80% and 75% of uniformity were 34, 64, 95, 126 and 157 mg of valine. The *y* values were multiplied by the *z* value and added to the average population, resulting in Val_{opt} of 837, 896, 958, 1,021, 1,083 mg/bird per day, respectively (Eq 1).

The *y* values considering σ^2_{Emax} for 95%, 90%, 85%, 80% and 75% of uniformity were 26, 50, 75, 99 and 124 mg of isoleucine. The *y* values were multiplied by the *z* value and added to the average population, resulting in Ile_{opt} of 604, 639, 676, 712, 750 mg/bird per day, respectively (Eq 2).

The *y* values considering σ^2_{Emax} for 95%, 90%, 85%, 80% and 75% of uniformity were 8, 16, 23, 31 and 39 mg of tryptophan. The *y* values were multiplied by the *z* and added value to the average population, resulting in trp_{opt} of 192, 210, 229, 247, 265 mg/bird per day, respectively (Eq 3).

Application of the model to calculate optimum economic intake based on simulation of a flock

Tables 4, 5 and 6 show the results of applying the models to a flock of 10.000 birds simulated according to the flock described in the material and methods section of the chapter. This procedure considers the responses of birds to each amino acid intakes. Although 10 amino acids intake levels were generated, it was shown in the respective tables only the levels that promoted the bird’s responses. The economic optimal intake by simulation method was established when the income was higher or equal to the cost.

Table 4. Number of birds responding in intake valine according to coefficient of variation of population [a].

Valine		Coefficient of variation, %														
Intake	Cost	5			10			15			20			25		
mg/bird d	US$	nº birds	EO	Income[b]	nº birds	EO	Income[b]	nº birds	EO	Income[b]	nº birds	EO	Income[b]	nº birds	EO	Income[b]
768	92	5006	49	1693	5004	48	1660	4981	47	1616	4953	46	1580	4965	45	1552
824	99	503	50	174	1920	49	657	2867	49	965	3239	48	1075	3570	47	1164
880	106	5	50	2	399	50	138	1190	49	408	1852	49	629	2356	48	790
936	112	-	-	-	32	50	11	362	50	125	924	50	318	1439	49	491
992	119	-	-	-	2	50	1	67	50	23	346	50	120	763	50	263
1048	126	-	-	-	-	-	-	11	50	4	129	50	45	359	50	124
1104	132	-	-	-	-	-	-	-	-	-	37	50	13	164	50	57
1160	139	-	-	-	-	-	-	-	-	-	6	50	2	64	50	22
1216	146	-	-	-	-	-	-	-	-	-	-	-	-	18	50	6
1272	153	-	-	-	-	-	-	-	-	-	-	-	-	6	50	2
Intake predicted of valine, mg/day																
Population average				768			768			768			768			768
Economic optimal intake[c]																
Equation				837			898			959			1022			1085
Simulation[c]				824			880			936			992			1048

[a] Simulation of flock using 10,000 birds; [b] Cost in US$; [c] Cost US$/kg of egg: 6.923; US$/kg of L-valine: 12.0.

Table 5. Number of birds responding in intake isoleucine according to coefficient of variation of population [a].

Isoleucine		Coefficient of variation, CV														
Intake	Cost	5%			10%			15%			20%			25%		
mg/bird d	US$	nº birds	EO	Income[b]	nº birds	EO	Income[b]	nº birds	EO	Income[b]	nº birds	EO	Income[b]	nº birds	EO	Income[b]
565	271	4954	49	1679	4985	48	1658	4963	47	1616	5017	46	1592	5051	45	1561
610	293	397	50	137	1876	50	644	2711	49	915	3272	48	1081	3660	47	1181
655	315	3	50	1	346	50	120	1174	50	403	1865	49	631	2393	48	795
700	336	-	-	-	27	50	9	356	50	123	893	49	306	1418	49	479
745	358	-	-	-	-	-	-	75	50	26	336	50	116	742	49	253
790	379	-	-	-	-	-	-	12	50	4	115	50	40	336	50	115
835	401	-	-	-	-	-	-	-	-	-	33	50	11	143	50	49
880	423	-	-	-	-	-	-	-	-	-	5	50	2	44	50	15
925	444	-	-	-	-	-	-	-	-	-	-	-	-	11	50	4
970	466	-	-	-	-	-	-	-	-	-	-	-	-	2	50	1
Intake predicted of isoleucine, mg/day																
Population average				565			565			565			565			565
Economic optimal intake[c]																
Equation				604			639			676			712			750
Simulation[c]				565			610			655			655			700

[a] Simulation of flock using 10,000 birds; [b] Cost in US$; [c] Cost US$/kg of egg: 6.923; US$/kg of L-isoleucine: 48.0.

Table 6. Number of birds responding in intake tryptophan according to coefficient of variation of population [a].

Tryptophan		Coefficient of variation, %														
Intake	Cost	5			10			15			20			25		
mg/dird d	US$	nº birds	EO	Income[b]	nº birds	EO	Income[b]	nº birds	EO	Income[b]	nº birds	EO	Income[b]	nº birds	EO	Income[b]
172	31	4972	49	1684	4970	48	1650	5023	47	1638	4995	46	1591	4975	45	1543
187	34	306	50	106	1701	50	583	2619	49	887	3212	48	1067	3513	47	1139
202	36	-	-	-	273	50	94	962	50	331	1708	49	581	2184	48	729
217	39	-	-	-	18	50	6	245	50	85	734	50	253	1269	49	431
232	42	-	-	-				54	50	19	255	50	88	634	49	217
247	45	-	-	-	-	-	-	7	50	2	70	50	24	272	50	94
262	47	-	-	-	-	-	-	-	-	-	11	50	4	100	50	34
277	50	-	-	-	-	-	-	-	-	-				28	50	10
292	53	-	-	-	-	-	-	-	-	-				10	50	3
307	55	-	-	-	-	-	-	-	-	-				4	50	1
Intake predicted of tryptophan, mg/day																
Population average				172			172			172			172			172
Economic optimal intake[c]																
Equation				192			210			229			247			265
Simulation[c]				187			202			217			232			247

[a] Simulation of flock using 10,000 birds; [b] Cost in US$; [c] Cost US$/kg of egg: 6.923; US$/kg of L-tryptofan: 18.

At the increase of amino acid intake, the feeding cost for 10,000 birds was superior. As the flock increased, the CV also improved the number of birds that accounted for each intake. Regardless of the amino acid, the first level above the average amino acid intake calculated for a flock with 5% of CV was sufficient to satisfy most of the birds in the flock and to obtain a higher revenue.

One exception from this rule was observed for the amino acid isoleucine due to the high cost of the industrial purified source (Table 5). The income obtained allowed to meet approximately 86% of individuals in the flock, i.e. 14% of the birds did not produced the Emax desired, which remained limited because of the Ile intake.

DISCUSSION

Responses of broiler breeders and data adjusted by Reading Model

We estimated the parameters of the Reading model to estimate valine, isoleucine and tryptophan intakes based on responses of broiler breeders hens. Furthermore, the optimal economic intake of valine, isoleucine and tryptophan, was calculated by taking consideration the cost of the amino acid and the revenue for eggs and the coefficient of variation for egg output. In addition, we considered the flock variability for egg output and the cost-benefits according to the economic scenario.

We applied the concept of the relative deficiency by using the dilution technique (Fisher and Morris, 1970) to limit the amount of Val, Ile, and Trp in the dietary protein. Compared to first level, the control diet diet for each amino acid increased the egg output, demonstrating that the amino acids were limiting (Table 3). The control diet for Val provided the highest improvement in rate of lay compared to first level, followed by Ile and Trp, indicating the limiting potential of these amino acids. Previous studies using the same methodology also based their conclusions about limiting potential on the relative responses to their highest dilution and control diets (Wethli and Morris, 1978; Bowmaker and Gous, 1991, Silva et al., 2015a,b).

The Reading Model has coefficients with biological meaning and the optimum economic intake of the limiting amino acid may be determined by considering the cost of the amino acid and the eggs revenue (Morris and Blackburn, 1982; McDonald and Morris, 1985; Fisher, 2015; Bendezu et al., 2015).

In the Reading model, the maintenance requirement was obtained by the extrapolation for egg output (E) equal to zero, thus, it is necessary for the responses to be close to zero for production. However, the reduced production and egg weight were insufficient to accurately estimate the coefficient of maintenance. According to Fisher et al. (2001), it is difficult to describe any precise biological meaning to the concept of amino acid requirements for maintenance when these are estimated by extrapolation of the response curves. Thus, in case of the research studies on the broiler breeder (Fisher et al., 2001, Silva et al., 2015b) and laying hens (McDonald and Morris, 1985; Silva et al., 2015a) the Reading model did not estimate good results of maintenance.

Optimum economic intake of the flock based on equation and simulation

The optimum amino acid intake was calculated by two procedure. The first solve an equation with coefficients that describe the bird response to amino acid intake for egg output (*a*) and body weight (*b*) considering the model input variables as the means and standard deviations of the egg output and body weight in a flock of birds. The second, procedure i.e., the simulation method consisted of the random simulation of 10,000 birds and uses Linear-plateau to describe the response of an individual in the population. The Emax of each individual is determined by increasing in the limiting amino acid intake above the maintenance resulting in a linear response up to the individual genetic potential. The average value is obtained by considering the 10,000 individual responses. Equation and simulation method are based on the Reading model to determine the optimum economic intake of the limiting amino acid of the population, considering the marginal cost of the amino acid and the marginal income from egg output (Fisher et al., 1973; Fisher, 2015).

These results showed a difference of 20%, 6% and 5% for Val (960 mg/bird d for equation and 802 mg/bird d for simulation), Ile (676 mg/bird d for equation and 637 mg/bird d for simulation) and Trp (229 mg/bird d for equation and 217 mg/bird d for simulation), respectively. Was observed a higher cost:benefit ratio as the difference between the two procedures evaluated become greater. By applying the equation for the optimum economic intake, we observed differences of 12, 39, 159 mg/bird d for Trp, Ile and Val respectively. These intakes can provide, for each flock with 10,000 birds, an increase of 0.1, 0.4, and 1.6 kg per day of L-Trp, L-Ile and L-Val, which represents an additional cost of 2.0, 20.0 and 19.0 US$ per day, respectively.

The equation method assumed that all birds above of average respond similarly with the extra amino acid supplied. Therefore, the calculated intake for the average population wasn´t changed by increasing the CV of the flock in all simulations (Tables 4, 5 and 6). At each level of simulated amino acid intake, the birds of high and low productive potential presented differences between the distributions of BW and Emax in the population, but the mean response was curvilinear. This is attributed to the non-linearity of the responses when considering the average of 10,000 birds simulated for each level of amino acid intake and thus simulation explains the difference between them.

The optimum intakes of the valine, isoleucine and tryptophan are expressed in mg/bird d, but to formulate the feed it is necessary to know the feed intake of the hen. Based on the intake of 150 g, body weight of 3.5 kg and egg output of 50 g, the concentrations of Val, Ile and Trp required in the feed (for 5, 10, 15, 20 and 25% of CV) would be 5.49, 5.87, 6.24, 6.61 and 6.99 g/kg for Val, 3.77, 4.07, 4.37, 4.37, 4.67 g/kg for Ile and 1.25, 1.35, 1.45, 1.55 and 1.65 g/kg for Trp. Here we demonstrated that the optimum economic amino acid intake varies according to body weight, potential egg output and also with changes on cost:benefit ratio, hence it is necessary to the need to recalculate this optimum amino acid intake (Bendezu et al., 2015; Silva et al., 2015 a,b).

The proposed procedures in this study complements the models developed by Bornstein et al. (1979) and recently modified by Lima et al. (2016), which provide only one intake for a uniform population. In practical situations, it is common to feed the birds that are producing above the average with the aim to optimize the production for different economic scenarios. The proposed methods allows to reproduce the observed variation in egg output and body weight, making possible to predict the number of birds that have potential to respond in egg production. Consequently, it is possible to relate the cost of the amino acid supply to feed birds that have potential to respond with the extra amino acid provided above the average for an additional revenue, especially for flocks with high-variation in egg output and body weight (Tables 4, 5 and 6).

However, both procedures preconize that the increase in the amino acid supply of a population will result in conditions that promote the expression of the productive potential of each individual, thus, improving the flock uniformity. This context has been rarely studied in the poultry science area, and so far, no sufficient studies exist that show the amino acid supply limitation for the population, since the lower amino acid price in relation to the price of egg generally provides greater amino acid intake recommendation by the model independent of the procedure.

The flocks require were: 768 mg of Val/bird per day, 565 mg of Ile/bird per day and 172 mg of Trp/bird per day. The optimum economic intake for flock uniformity (5% coefficient of variation) was calculated at 837, 604 and 192 mg of Val, Ile and Trp/bird per day, respectively by the equation and 824, 565 and 187 mg of Val, Ile and Trp/bird per day by simulation method. The Reading Model could be used to estimate the optimum amino acid intakes for hens under different genetic and economic scenarios and depending on the ingredients available and their prices, the marginal cost of each amino acid will vary. Therefore, further studies can be performed to confirm if the increase in the supply of amino acids leads to uniformity of the population, since the supply for 100% of the population would allow that all individuals manifest differences in their genetic potential.

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CAPÍTULO 5 – REQUIREMENTS AND OPTIMIZATION OF ARGININE INTAKES FOR BROILER BREEDER HENS FOR EGG PRODUCTION

ABSTRACT

The objective this study were: to determine the specific requirements of arginine for maintenance using different unit systems; evaluate the response of broiler breeder hens to different intakes of arginine, estimate the parameters of the Reading Model for broiler breeder hens by a simulation and equation method, and calculate the optimal economic intake of arginine, considering the relationship between cost-benefit and the flock variability. Two studies were conducted: One nitrogen balance trial (Experiment 1) was performed using roosters and another dose response trial was performed using broiler breeder hens (Experiment 2). The nitrogen balance trial lasted five days with 48 hours of fasting (with roosters receiving only water+sucrose) and the last 72 hours for feeding and excreta collection. Forty grams of each diet first-limiting in arginine was fed by tube each day (three days) to give a range of intakes from 0 to 18.5 g/kg BW d of arginine. Excreta were collected during the last 3 days of the balance period and the nitrogen content of the excreta was analyzed. A linear regression between nitrogen retention (NR) and arginine intake was performed. Sixty four hens were used. A completely random design was used, which consisted of eight treatments, eight replicates, and one hen per cage. The diets were formulated by dilution technique using one summit diet and one nitrogen (N)-free diet, resulting in AA levels that ranged from 2.37 to 10.27 g of arginine/kg. The data obtained were AA intake (AAI), body weight (BW), and egg output (EO). Broken line model was used to evaluate the responses. The amino acid intake, egg output (EO) and body weight (BW) were adjusted using a Reading model.

For the simulation method was used 10,000 birds and for each bird, was calculated the value of amino acid intake (AAI). The EO was calculated by the equation: $EO=AAI-[b\times BW]/a$, where “a” is requirement of the amino acid per gram of egg (mg/g) and “b” is mg/kg of BW (mg/kg). The equation from linear regression was: $NR = -39.9 (\pm 4.5) + 1.8 (\pm 0.1) \times Arg$. The arginine required to maintain the body at zero NR were calculated to be 23 mg/kg body weight per day, respectively. For the system unit mg per kg of metabolic weight, the intake of arginine was 36. Considering the degree of maturity of the animal and body protein content the amounts of arginine required for maintenance was calculated to be 151 mg per unit of maintenance protein ($BP_m^{0.73} \times u$) per day. The values estimated by the model for utilization efficiency was 62%. The AAI estimated by the model at 30 weeks was 753 mg/day. The prediction of the model was improved using the coefficients estimated here with physiologically relevant units. The model generated by predicting Arg intake were the following: $Arg=13.6\times EO+23\times BW$. The flock require was 835 mg of Arg/bird per day. The optimum economic intake for flock uniformity (5% coefficient of variation) was calculated at 900 of Arg/bird d by equation and 891 of Arg/bird per day was calculated while using the simulation method.

Keywords: breeder roosters, dilution technique, egg output, nitrogen retention, Reading model

INTRODUCTION

The definition of nutritional levels for broiler breeder hens must consider feeding management, which is based on controlled feed intake and this makes it impossible for a bird to increase the feed intake to meet the first limiting nutrient.

In short periods, broiler breeder hens can use fat stores to maintain egg production (Nonis and Gous, 2012). Differently the body lipid, the protein has limited storage capacity, and therefore, the amino acids are most limiting nutrient resources for broiler breeder hens (Nonis and Gous, 2012). Among the amino acids, the arginine has some essential functions in the physiology of the bird, such as: constitution of proteins; precursor of nitrogenous compounds, creatine, action in muscle metabolism (Fernandes et al., 2011); precursor of nitric oxide, action on the immune system; stimulates the release of luteinizing hormone and action on ovulation (Basiouni et al., 2009); constitutes the arginine vasotocin involved in uterine contraction and oviposition (Basiouni et al., 2009). In addition, the antagonism with lysine reduces their availability with the excess of lysine. Even being an important amino acid, studies of the response of broiler breeder hens to different intakes of arginine are scarce (Ball et al., 2007), especially, using factorial approach.

Some researchers consider that the factorial calculation of nutrient intakes to broiler breeder hens is more accurate (Bornstein et al., 1979; Emmans and Fisher, 1986; Fisher, 1998; Lima et al., 2016b). This approach considers the partition intake to maintain body weight, growth and egg production (Sakomura et al., 2011). However, studies with broiler breeder hens in the production phase doesn’t have good estimate of maintenance coefficients for body weight (Lima et al., 2016a) and the authors have suggested the use of adult animals because they represent the physiological state of maintenance, because growth is practically null in this phase (Lima et al., 2016a; Dorigam et al., 2016).

Another important factor is the efficiency of utilization of amino acids for egg production (Fisher, 1998), which are inferior compared with laying hens (Silva et al., 2015a) due to the greater length of the pauses between the posture sequences (Gous and Nonis, 2010; Ferreira et al., 2014). This highlights that the amino acid intake definition must be precise because it involves additional cost with the excess of nutrients (Ekmay et al., 2013) and biological response of the birds (Nonis and Gous, 2012).

Some mathematical models can be applied for broiler breeders, one of them was recently reparametrized by Lima et al. (2016b), considering maintenance of body protein weight and utilization efficiency for amino acid deposition in the egg, applied to individual and classified as deterministic model. Another option is the Reading Model, which uses the factorial calculation and allows you to simulate responses of populations and analyze the economic optimum intake based on the most productive individuals in the population (Silva et al., 2015a,b).

To apply these models, it is necessary to determine the maintenance coefficients and efficiency of utilization of arginine for broiler breeder hens and with these coefficients, it is possible to compare the difference between deterministic and stochastic models and analyzing the difference between the estimates of arginine intake, considering the individual and economic response of the population.

Therefore, the aim of this study were: to determine the specific requirements of arginine for maintenance using different unit systems determined with Ross breeder roosters as an animal model; evaluate the response of broiler breeder hens to different intakes of arginine, estimate the parameters of the Reading Model for broiler breeder hens by a simulation and equation method, and calculate the optimal economic intake of arginine, considering the relationship between cost-benefit and the flock variability.

MATERIALS AND METHODS

Two studies were conducted at the Poultry Science Laboratory of the São Paulo State University, UNESP Jaboticabal, São Paulo, Brazil. One nitrogen balance trial was performed using roosters and another dose response trial was performed using broiler breeder hens.

The Ethics Committee approval

The Animal Ethics and Welfare Committee of Universidade Estadual Paulista approved all experimental procedures used in this study under the Protocol number 9999/14.

Experiment 1

***Birds and housing*.** One nitrogen balance study was performed using 42 Ross adult roosters. The roosters were housed individually in metabolic cages with individual feeder and nipple-type drinkers. During the experimental period, light was provided 24 hours per day. The roosters were distributed into six treatments and six replicates, with an average weight of 6.60 ± 0.57 kg. To confirm that the amino acid studied was indeed limiting and that the responses were due to the limitation of the test amino acid and not protein, we added one more treatment consisting of a control diet.

***Experimental diets*.** The experimental diets were formulated by dilution technique (Fisher and Morris, 1970). The experimental levels of amino acids were obtained by the following proportions of the summit and nitrogen-free diets, respectively: 0:100, 17:83, 33:67, 50:50, 67:33, and 100:0 (Table 1). A summit diet was formulated containing 18.55 g of digestible arginine/kg, according to recommendations of (Rostagno et al., 2011) for the amino acid tested, which was multiplied by 0.4 and 0.8 for other amino acids, based on the ideal profile of amino acid/lysine; creating a minimum relative deficiency of 40% between the test amino acid and other amino acids, as suggested by Siqueira et al. (2011) and Bonato et al. (2011). Moreover, the summit diets were formulated and meeting the requirements for energy, minerals, and other nutrients proposed by Rostagno et al. (2011). In all diets, the minimum contents of potassium, calcium, available phosphorus, and sodium were 7.50, 6.50, 3.00, and 2.30 g/kg, respectively. A nitrogen-free diet was also formulated, which was based on corn starch, sugar and rice husk and considering the same nutritional levels as in the summit diet, except for protein and amino acids (Table 2), The arginine levels ranged from 0 to 18.55 g/kg in the diet. To formulate the control diet, a total of 0.312 g/kg of L-arginine was added in the diets with lower amino acid level up to the second amino acid level in the diet.

Table 1. Proportions of the summit diet diluted with the corresponding nitrogen-free diet in the arginine (Arg) trial and the resulting concentrations of the limiting amino acids in the diets.

Breeder roosters			Broiler Breeder hens		
Arg	Summit	N-free	Arg	Summit	N-free
g/kg	%	%	g/kg	%	%
0	0	100	2.37	23	77
3.10	17	83	3.16	31	69
6.19	33	67	4.74	46	54
9.28	50	50	6.32	62	38
12.37	67	33	7.11	69	31
18.55	100	0	7.90	77	23
6.19[a]	17	83	10.27	100	0
-	-	-	3.16 [b]	23	77

[a] Added 0.312 g of L-Arg 99 % /kg in third level

[b] Added 0.080 g L-Arg 99 % /kg in second level

Table 2. Composition (g/kg) of the summit (high protein) and nitrogen-free (N-free) diets used in the arginine response trials.

Ingredients	Broiler Breeder hens		Breeder roosters	
	Summit	N-Free	Summit	N-Free
Corn	631.51	-	463.32	-
Corn starch	-	567.38	-	673.18
Soybean meal	240.00	-	329.46	-
Rice husk	-	159.40	-	109.98
Sugar	-	150.00	-	150.00
Corn gluten meal 60%	21.96	-	97.88	-
Limestone	68.53	63.48	7.89	4.08
Soybean oil	-	20.00	34.31	24.73
Dicalcium phosphate	13.79	18.92	10.48	16.22
Wheat bran	10.00	-	-	-
Potassium chloride	-	15.49	-	14.33
Salt	3.33	3.34	5.37	5.49
DL-Methionine (99%)	3.39	-	9.75	-
L-Lysine.HCl (78%)	1.78	-	7.40	-
L-Isoleucine	1.28	-	7.76	-
L-Threonine	1.14	-	7.33	-
L-Valine	0.98	-	10.00	-
L-Tryptophan	0.32	-	2.28	-
L-Arginine	-	-	4.79	-
Vitamim premix[a]	1.00	1.00	1.00	1.00
Mineral premix[b]	1.00	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00
AMEn	2.800	2.800	3.200	3.200
Crude protein	17.973	0.014	28.722	0.014
Methionine	0.589	-	1.336	-
Methionine+cysteine	0.840	-	1.684	-
Lysine	0.900	-	1.601	-
Threonine	0.705	-	1.549	-
Tryptophan	0.210	-	0.466	-
Valine	0.840	-	2.029	-
Isoleucine	0.795	-	1.742	-
Arginine	1.027	-	1.855	-
Sodium	0.150	0.150	0.230	0.230
Calcium	3.000	3.000	0.650	0.650
Available phosphorus	0.350	0.350	0.300	0.300
Chlorine	0.284	0.753	0.513	1.010
Potassium	0.634	0.600	0.750	0.750

[a]Content per kg product: Nicotidic acid: 30g, Pantothenic acid: 15g, Folic acid: 600 mg, Biotin: 100 mg, Vit A: 9.000.000 UI, Vit D3: 2.600.000 UI, Vit E: 14.000 UI, Vit K3: 1.600 mg; Vit B1: 2.200 mg, Vit B2: 6.000 mg, Vit B6:3.000 mg, Vit B12: 10.000 mcg. [b]Content per kg product: Cu 8.000,00 mg, Fe 50.00 g, I: 1.200,00 mg; Mn 70.00 g; Se: 200,00 mg, Zn 50.00g.

***Experimental procedure*.** The experiment lasted 120 hours. In the first 48 hours, the birds went through a fasting period to empty the gastrointestinal tract. In this period they received 60 ml of water with sucrose (50% each) once a day. For the next 72 hours, the birds were fed according to body weight (approximately 40 grams of the experimental diets daily). At the same time, nitrogen-free diet was provided ad libitum to maintain energy homeostasis and minimize metabolic and endogenous losses of birds. As a marker for excreta, 1% of iron oxide was used at the beginning and end of the collection period. Trays covered with plastic were placed under the metabolic cages to collect the excreta. The excreta were collected twice a day (at 8h and 16h) and placed in plastic pots that were kept at -20 °C. Feed intake was measured for the 72 hours of the feeding phase, determined by feed consumption provided by precise feeding plus the nitrogen-free diet provided ad libitum in the feeder. At the end of each trial, two birds were sacrificed and feathers were separated from the carcass for further analysis.

***Laboratory analysis*.** The amount of excreta from each rooster was weighed after three days of collection. The excreta were homogenized in a blender, and samples were weighed in Petri dishes. Afterward, the samples were lyophilized for 72 hours. Subsequently, samples were grounded in a ball mill for 2 minutes. These dried samples of the experimental diets, excreta, and carcass (free of feathers) were analyzed for crude protein by Kjeldahl (method 2001.11) and ether extract (method 920.39) for carcass (free of feathers), according to AOAC (1995) procedures.

***Statistical analysis*.** The nitrogen retention (NR) was determined by the difference between nitrogen intake and excretion. The nitrogen retention responses to the amino acid intakes were expressed in three unit systems: 1) mg per kg of body weight (BW), mg/BW per day; 2) mg per kg of metabolic weight ($BW^{0.75}$), mg/$BW^{0.75}$ per day; and 3) mg per unit of metabolic protein weight at maturity ($BP_m^{0.73} \times u$), mg/ $BP_m^{0.73} \times u$ per day, where u is the maturity rate, given by the relation between protein weight at time t (BP_t) and protein weight at maturity (BP_m) where $u = BP_t/BP_m$, according to Burnham and Gous (1992). In these trials, *u* was considered equal to 1, because the birds were adults and had reached maturity. The metabolic protein weight at maturity was obtained by multiplying the percentage of protein and the weight of carcass free of feathers, raised to power 0.73. A linear regression between amino acid intake and NR was carried out. The requirement for maintenance was considered as the amount of amino acid to maintain NR equal to zero (NR=0), as described by Sakomura and Rostagno (2007). Statistical analyses were performed using the PROC REG from SAS 9.2 software.

Experiment 2

Birds and housing. Sixty-four broiler breeder hens of Ross genotype with 35 weeks of age were used, housed individually in metabolic cages. The cages were equipped with individual feeders and nipple drinkers. The experimental design was a completely randomized design with eight treatments (seven increasing levels of the amino acid and a control diet) and eight replicates. Two weeks before the beginning of the experiment, all birds were fed 168 g per day of a diet designed to meet their nutritional requirements during this period, according to recommendation of Rostagno et al. (2011). The egg production was monitored to provide a baseline for the experimental period. The experiment lasted ten weeks, with the first six weeks of adaptation and the last four weeks for data collection. The lighting program adopted during the experiment was 14 hours of light. The hens were raised in a poultry house with a negative-pressure system with controlled temperature of 21 °C. The daily management was performed according to the Ross guidelines (Ross, 2013).

Experimental diets. A summit diet was formulated containing 10.27 g of digestible arginine/kg, according to recommendations of (Ekmay et al., 2014) and meeting the requirements for energy, minerals, and other nutrients proposed by Rostagno et al. (2011). A nitrogen-free diet was formulated to meet the same nutritional levels as the summit diets, except for protein and amino acids. The nitrogen-free diets were used to dilute the summit diets, in appropriate proportions, to obtain the range of Arginine (Table 1) contents required for each dilution series (Fisher and Morris, 1970). To confirm that the response of the birds was in function of the respective limiting arginine, a control diet was included. A small quantity of the respective crystalline amino acid was added (0.080 g/kg) to the diet with the lowest level of the amino acid tested sufficient to meet the level of the amino acid in the second-lowest level in the dilution series (Table 2).

Experimental procedure. The birds were fed 168 g per day of feed, at the same time each morning, and at the end of the week the leftovers were weighed to quantify the weekly consumption of the feed. The body weight of the hens was measured on the first, sixth, and tenth weeks of the assay. Egg production was recorded daily and egg weight was measured on three consecutive days each week.

Amino acid intake by factorial model. Two models were compared, one from the literature (M1) and the other a model adapted by Lima et al. (2016b) (M2). The model M1 ($AAI=[(AA_m/0.85) \times BW] + [BWG \times (0.21 \times AA_t)] + [EO \times (63 \times AA_y + 158 \times AA_t)]$) was proposed by Bornstein et al. (1979), where: AAI is the amino acid intake (mg/day); AA_m is the amino acid for maintenance; 0.85 is the protein absorption rate; BW is the body weight; BWG is the body weight gain; 0.21 is the amount of protein in gain; AA_t is the amino acid in the protein fraction of the tissue; EO is the egg output; 63 is the nitrogen concentration in the egg; AA_y is the fraction of amino acid in egg yolk protein; and 158 is the amount of nitrogen in the tissue. The coefficient used for weight gain (0.21) was obtained from Bornstein et al. (1979). A second model (M2) $AAI = [AA_m \times (BW \times 0.196)^{0.73}] + [((N_{egg} \times EO) \times AA_{egg})/k]$; where AAI is the amino acid intake; AA_m is the amount of amino acid for maintenance ($mg/BP_m^{0.73} \times u$); *u* is the maturity rate obtained dividing body protein weight at age t (BPt) by the body protein weight at maturity (BP_m), i.e. u=BPt/BPm. In this study, *u* was considered as 1 because the broiler breeders were mature; BW is the body weight of the hens; EO is the egg output; k is the efficiency of utilization; 0.196 is the amount of nitrogen analysed in the body without feathers; N_{egg} is the amount of nitrogen in the egg (1.89 g N/100g according to Fisher, 1998); and AA_{egg} is the amount of amino acids in the egg (mg/g N) obtained from composition presented by Lunven et al. (1973).

To predict arginine intake, the data of body weight and egg output of 60 broiler breeder hens of Cobb (500) strain were used. These data were obtained from individual monitoring of the hens during the period from 25 to 60 weeks of age.

Reading model. The responses of egg output and body weight were applied to Reading model (Fisher et al., 1973), according to the following general expression: $AAI_{opt}=\mu+y\times z$, where AAI_{opt} is the optimum economic intake; µ is the mean intake of the population ($AAI=a\times Emax+b\times BW$); y is the standard deviation of the amino acid requirements; and z is the economic index that suggests how many times the deviation of the requirement (y) must be added to the requirement of average population.

The parameters are follows: amino acid intake in mg/bird per day (AAI); maximal egg output, g/day (Emax); standard deviation of the Emax, g/day (σ^2Emax); body weight (BW), standard deviation of BW (σ^2BW); requirement of the amino acid per gram of egg, mg/g (a) and for amino acid requirement per kilogram of BW, mg/kg ($\boldsymbol{b}$). The standard deviation of the requirements was calculated as follows: $y = \sqrt{(a^2\times\sigma^2_{Emax}+b^2\times\sigma^2_{BW})}$.

The variables fitted to model were amino acid intake, average and standard deviations of the EO and BW. Were used to estimate the coefficients of the model the amino acid using optimization module of EFG software (2012).

Application of the model to calculate the optimum economic intake. We calculated the AAI_{opt} by simulation procedure and using the equation described for Reading Model (Fisher et al., 1973). The data applied to model were obtained from a population with an average Emax of 54 g/day and 4.4 kg of BW. The uniformity of the flock was obtained considering 100% minus the coefficient of variation (CV). For body weight, it was considered 90% for uniformity, corresponding to 0.440 kg of σ^2BW. For EO were considered five uniformity within a flock of 95%, 90%, 85%, 80% and 75%. Based on CV, the σ^2_{Emax} were calculated for each scenario of 2.5, 5.0, 7.5, 10.0, and 12.5 g/day, given as a source of variation in the model to calculate the AAI_{opt}.

The maintenance (b) and egg production (a) coefficients were previously determined in this study. The price for L-arginine 99% was 24 US$/kg and for fertile egg US$ 0.50/unit of 65g.

Simulation of population of 10,000 birds. A simulation method based on Monte Carlo random sampling technique was used to generate a population of birds from two normal, non-correlated distributions for Emax and BW, according to the procedure described by Fisher et al. (1973). It was calculated the AAI and EO by the equation: EO=AAI-[b×BW]/a for 10,000 birds and estimated an average flock. This procedure was used for 10 arginine increased levels, that was established the level of stability of the response. All 10 amino acids intake levels were obtained by adding fractional intakes calculated for the average population. For arginine intakes, each increasing of 56 mg resulted in values varied from 8,35 to 2,067 mg/bird per day. By this procedure is possible provide small increases in amino acid intake and calculate the optimum economic intake based on input cost and output revenue considering large populations. In this simulation, the same scenarios of uniformity for EO and BW were considered, and the same prices for industrial amino acids and egg and the requirement coefficients determined in this study as described before.

Statistical analysis. The responses (Y) of efficiency of utilization and egg production (%) were regressed as a function of the arginine intake (X) in mg/hen per day using the broken line model with one slope (Y=L+U×(R-X)), where X is the input (arginine intake) and Y is the output (egg production or efficiency of utilization), L is the maximum response of the model, R is the amino acid intake for maximum response, and the parameter U represents the slope of the models. Statistical analyses were performed only in the seven treatments, because the eighth treatment was used only to validate the amino acids limiting in the diets. We analysed the average and standard deviations of the feed intake, amino acid intake, egg output and body weight. Before regression analysis, treatments were analysed using the PROC GLM procedure of SAS. The broken line model with one slope utilized for egg production and efficiency of utilization was estimated using the PROC NLIN procedure. The linear plateau models were adjusted according to the procedures described by Robbins et al. (2006). The statistical analyses were performed using SAS 2010 (Statistical Analysis System, version 9.2).

RESULTS

Experiment 1

The assumptions of normality of errors and homogeneity of variance were tested and satisfied. The responses of the roosters to the control diets showed that the amino acids studied was limiting in the diet, because provided retentions in-between the first and the second treatments, validating this study.

The results of amino acid intake and nitrogen retention for the different systems of units are shown in Table 3. The experimental diets provided ranges from negative to positive NR values. As the amino acid intake increased, the NR values also increased. There were negative responses on NR values for nitrogen-free diet and for the second level of arginine (3.10 g/kg).

Table 3. Arginine levels (g/kg), average (±standard deviation) daily arginine intake (ArgI), and daily nitrogen retention (NR) in different systems of units.

Levels Arginine g/kg	ArgI			NR		
	mg/kg BW	mg/kg $BW^{0.75}$	mg/kg $BP_m^{0.73}$	mg/kg BW	mg/kg $BW^{0.75}$	mg/kg $BP_m^{0.73}$
0.00	0.0±0.0	0.0±0.0	0.0±0.0	-45.9±19.5	-70.7±29.0	-299.1±122.5
3.10	16.5±0.1	26.3±0.5	111.4±2.2	-5.3±9.7	-8.5±15.4	-36.2±65.4
6.19	33.7±0.6	53.7±1.6	227.9±7.2	45.7±8.2	73.6±13.0	312.4±55.1
9.28	50.5±1.1	80.1±2.0	339.6±8.5	61.9±35.6	82.4±63.5	349.3±269.2
12.37	66.4±0.4	105.0±0.8	445.2±3.4	64.2±20.7	11.7±38.3	473.2±162.6
18.55	99.9±0.6	162.6±1.3	690.9±5.7	128.9±16.5	209.9±26.1	891.8±110.8

BW = Body weight; $BP_m^{0.73}$ = Body protein at maturity.

The regression equations obtained for all the different systems of units regarding the arginine are shown in Table 4. The coefficients of determination (R^2) in the studies were 88% indicating that there was a good fit of the data. The maintenance requirements for digestible arginine estimated from linear regression equation based on three units were 23 mg/kg BW per day, 36 mg/kg $BW^{0.75}$ per day and 151 mg/kg $BP_m^{0.73}$ per day.

Table 4. Maintenance requirements for digestible arginine estimated from linear regression equation based on three units.

Unit systems	Regression equation[1]	R^2	Requirement
mg/kg BW per day	NR = -39.9 (±4.5) + 1.8 (±0.1) × Arg	0.88	23
mg/kg $BW^{0.75}$ per day	NR = -62.2 (±7.2) + 1.7 (± 0.1) × Arg	0.88	36
mg/kg $BP_m^{0.73}$ per day	NR = -262.9 (±30.4) + 1.7 (±0.1) × Arg	0.88	151

[1] The data of the control diet were not utilized in the statistical analyses. BW = Body weight; $BW^{0.75}$= metabolic weight; $BP_m^{0.73}$ = Body protein at maturity; NR = nitrogen retention.

Experiment 2

Responses of broiler breeder hens to dietary arginine

Bird responses to different levels of dietary arginine are shown in Table 5. The additional response (control diet) seen with the supplementation of the synthetic amino acid confirmed that arginine was the first limiting amino acid.

Table 5. Average responses to the dietary levels and standard deviation (±SD) for daily feed intake (g/hen), daily amino acid intake (mg/hen), daily egg production (%), egg weight (g), daily egg output (g/bird), body weight (g), and efficiency of broiler breeder hens from 32 to 42 weeks of age.

Levels g/kg	Feed Intake	Amino acid intake	Egg production	Egg Weight	Egg Output	Body Weight	Efficiency
2.37	133.22±20.79	315.73±49.28	42.11±25.60	56.29±3.89	23.72±14.16	3733.38±377.36	59.73±10.99
3.16	153.52±13.84	485.11±43.74	58.85±9.96	63.53±3.34	37.79±5.12	3884.00±213.26	61.17±7.58
4.74	159.57±9.45	756.36±44.78	80.29±8.73	62.32±2.60	49.91±4.31	4062.38±162.96	59.95±4.21
6.32	163.57±3.14	1033.74±19.85	75.60±4.99	68.42±2.78	51.73±3.87	4309.86±114.08	44.23±2.32
7.11	163.60±4.56	1163.17±32.39	82.81±8.29	65.16±3.07	54.65±4.28	4187.88±96.20	39.86±3.36
7.90	166.11±2.18	1312.26±17.26	78.13±6.16	66.77±4.10	54.35±3.87	4228.00±194.50	35.86±1.94
10.27	167.40±0.92	1719.20±9.44	79.09±6.33	69.08±3.26	55.39±5.15	4422.13±215.79	26.80±2.17
3.16	146.95±22.39	464.34±70.76	55.66±7.28	58.94±2.85	32.79±4.51	3849.13±280.15	52.97±6.26
n	63	63	63	62	63	63	56

[a] Control diet supplied with 0.080 g/kg of L-arginine. n: number of observations. The data from control diet was not used in the calculation of the efficiency variable.

Feed intake

Each hen received 168 g of feed per day, however birds fed with lowest level and second-lowest level of the amino acid diets had lower feed intake in comparison to other treatments (Table 5).

Egg production, egg weight, and egg output

The highest rates of lay were observed for birds fed 7.11 g/kg of the arginine diet, reaching 83%. On the other hand, hens feeding on the lowest amino acid level produced only 49% fewer eggs than the maximally performing diets (7,11 g/kg). Birds fed lowest level or the second level of the amino acid tended to lay eggs with approximately 19% or 13 g lighter than those birds feeding on sixth or seventh level of the amino acid. For egg output, there was a reduction of 43% for birds feeding on lowest level when compared to the maximally performing diets (10.27 g/kg).

Modelling the responses: *amino acid intake vs. egg production and efficiency of utilization*

The model fitted for egg production was:

Egg production = 79.85(±1,33)-0.09(±0.02)×(723.7(±53,26)-X); if X>723.7 then (723.7-X)=0.

The model fitted for efficiency of utilization was:

Efficiency = 61.75(±1.96)-0.04(±0.003)×(525.5(±75.16)-X); if X>525.5 then (525.5-X)=0.

There was an increase in egg production by increasing the amino acid intake up to the response plateau. For efficiency of utilization, it was observed a response plateau for the first three levels of the amino acids and, subsequently, decreasing with increased amino acid intake (Figure 1). The estimated values, errors for each parameters, and the interval of confidence of the model parameters (superior and inferior limit), are shown in Table 6.

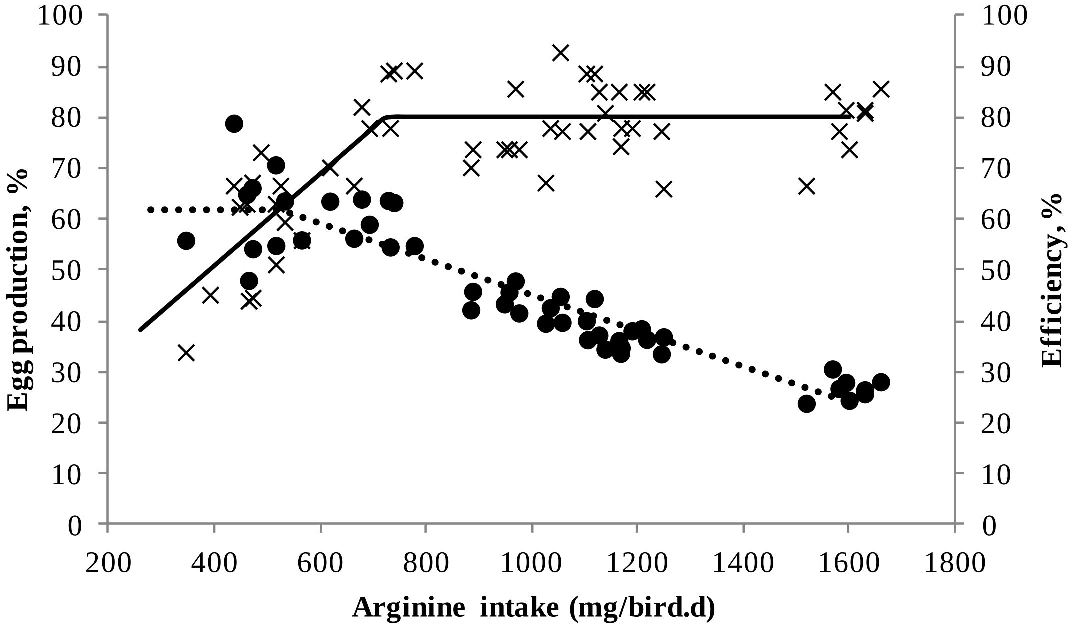

Figure 1. Relation between arginine intake and egg production (______, ×) and efficiency (............, ●)

Table 6. Model parameters and their respective standard error and confidence interval.

Parameters	Egg production			
	Mean	SE	Confidence interval	
			Superior	Inferior
L	79.8474	1.3288	82.5178	77.1770
U	-0.0901	0.0209	-0.0482	-0.1321
R	723.7	53.26	830.8	616.7
	Efficiency			
L	61.7475	1.9607	65.6942	57.8008
U	-0.035	0.00272	-0.0295	-0.0405
R	525.5	75.16	676.8	374.2

SE=standard error

Factorial model for estimating arginine intake

The equation determined was:

$$AAI=[151\times(BW\times0.196)^{0.73}]+[((1.89\times EO)\times380)/0.62 \qquad (M2)$$

Evaluation of the model

To evaluate the model developed in this study (M2) was compared to model of Bornstein et al. (1979). Factorial models M1 and M2 were simulated using body weight and egg output from 60 hens individually monitored. The results of simulation are shown in Table 7. The M1 model predicted 30% higher intakes compared to M2.

Table 7. Prediction of arginine intake (mg/day) for 60 broiler breeder hens from 25 to 60 weeks of age using two mathematic models with body weight (BW), body weight gain (BWG), and egg output (EO) data and prediction of the minimum (min), average (μ), and maximum (max) values for arginine intake based on the application of the factorial models.

Population data[1]										Arginine intakes		
Age weeks	Egg outptut			Body weight			BWG g/day	M1[a]	M2[b]	mg/bird d		
	Min	μ	Max	Min	μ	Max				Min	μ	Max
25	13	37.1	53	3.3	3.89	4.4	6	790	553	268	553	735
30	20	53.9	70	3.5	4.10	4.6	6	1057	753	364	753	944
35	35	55.7	70	3.7	4.32	5.0	6	1098	779	544	779	945
40	29	55.6	80	3.6	4.54	5.3	4	1108	782	480	782	1067
45	19	50.0	65	3.6	4.68	5.5	4	1032	721	361	721	905
50	15	48.7	68	3.4	4.82	5.5	0	1020	709	322	709	941
55	24	47.7	68	3.4	4.83	5.6	0	1005	697	430	697	942
60	22	45.6	63	3.1	4.83	5.7	0	974	673	268	673	735

[a] M1. $AAI=[(AA_m/0.85)\times BW]+[BWG\times(0.21\times AA_t)]+[EO\times(63\times AAy+158\times AA_t)]$ from Hurwitz and Bornstein (1973). [b] M2. $AAI=[AA_m\times(BW\times0.196)^{0.73}]+[((N_{egg}\times EO)\times AA_{egg})/k]$ developed in this study. [1] Data from Ferrreira et al. (2015).

Application of the factorial model to estimate amino acid intakes for a population

Average egg output and body weight values for 60 hens at 25, 30, 35, 40, 45, 50, 55, and 60 weeks of age were applied in the M2 model to predict arginine intake (Table 7).

For egg output, the coefficient of variation (CV) at 25 weeks was high (35%) because of the low egg production, but after this point, the response stabilized at the average value of 19%. For body weight, the CV ranged between 7% and 10% of the average value of 4.5 kg.

The average egg output values and standard deviation accounted for 46.55% of the population, whereas maximum egg output values accounted for

90.07% of the population. For body weight, average values corresponded to 54.55% of the population, and maximum values to 94.66%.

Predicted values for arginine intake varied by 526 mg/hen per day, respectively (Table 7). Differences between the maximum and average intakes was 213 mg/hen per day, yielding maximum values approximately 30% higher than average intake values. Differences between the average and minimum intakes were 313 mg/hen per day, corresponding to a difference of 44%.

Fitting the Reading Model

The Reading model was fitted according to the data of amino acid intake, EO and BW. The estimated coefficients for Emax was 53.85 g/day; σ^2_{Emax} were 7g/day; BW was 4.40 kg; σ^2_{BW} was 0.440kg; *a* were 13.57 mg/g; and for *b* were 0.00 mg/kg BW. Based on the parameters, the equation for predicting the optimum intake of arginine was: $Arg_{opt}=13.57\times53.85+0.00\times4.40+\sqrt{(13.57^2\times4.0^2+0^2\times0.44^2)}$.

Application of model to calculate optimum economic intake for a breeder flock

To simulate practical situations, five scenarios of uniformity were considered to calculate amino acid intake for a flock of hens. The model is: $Arg_{opt} =13.57\times54+23\times4.4\sqrt{(13.57^2\times\sigma\ \sigma^2_{Emax}\ 23^2\times0.440^2)}\times1.7242$.

The *y* values considering σ^2_{Emax} for 95%, 90%, 85%, 80% and 75% of uniformity were 38, 74, 110, 147 and 183 mg of arginine. The *y* values were multiplied by the *z* value and added to the average population, resulting in Arg_{opt} of 900, 962, 1025, 1,088, 1,152 mg/bird per day, respectively.

Application of the model to calculate optimum economic intake based on simulation of a flock

Table 8 show the results of applying the models to a flock of 10,000 birds simulated according to the flock described in material and methods. This procedure consider the responses of birds to each amino acid intakes. Although 10 arginine intake levels were generated, it was shown in the respective tables only the levels that promoted the bird’s responses. The other levels were suppressed due to the repetitive information.

Table 8. Number of birds responding in intake arginine accordance with the coefficient of variation of population[a].

Arginine		Coefficient of variation, %														
Intake	Cos		5			10			15			20			25	
mg/bird	US$	nº	E	Income	nº	E	Income	nº	E	Income	nº	E	Income	nº	E	Income
835	200	5024	53	2046	5014	52	2001	5031	51	1966	5038	50	1927	4990	49	1872
891	214	690	54	286	2305	53	946	3031	52	1223	3557	51	1409	3732	51	1452
947	227	18	54	7	637	54	264	1585	53	651	2246	53	910	2663	52	1063
1003	241	-	-	-	99	54	41	670	54	277	1306	53	536	1805	53	733
1059	254	-	-	-	3	54	1	201	54	83	620	54	257	1105	53	454
1115	268	-	-	-	-	-	-	41	54	17	283	54	117	630	54	260
1171	281	-	-	-	-	-	-	6	54	2	108	54	45	338	54	140
1227	294	-	-	-	-	-	-	-	-	-	29	54	12	170	54	71
1283	308	-	-	-	-	-	-	-	-	-	5	54	2	68	54	28
1339	321	-	-	-	-	-	-	-	-	-	-	-	-	21	54	9
1395	335	-	-	-	-	-	-	-	-	-	-	-	-	5	54	2
1451	348	-	-	-	-	-	-	-	-	-	-	-	-	2	54	1
Intake predicted of arginine, mg/day																
Population average				835			835			835			835			835
Economic Optimal intake[c]																
Equation				900			962			1025			1088			1152
Simulation[c]				891			947			1003			1059			1115

[a] Simulation of flock using 10,000 birds.

[b] Cost in US$.

[c] Cost US$/kg of egg: 7.692; US$/kg of L-arginine 24.

An increase of amino acid intake increased the feeding cost for 10,000 birds. As the flock increased, the CV also increased the number of birds that accounted for each intake.

The first level above the average amino acid intake calculated for a flock with 5% of CV was sufficient to satisfy almost all of the birds in the flock and to obtain higher revenue. The cost for feeding a flock increased as the CV increased (Table 8).

DISCUSSION

Experiment 1 – Arginine maintenance requirements

The requirements of arginine for maintenance of poultry were estimated in function of body weight, metabolic body weight and body protein weight. The present study do not have the same limitations as other studies, e.g., using growing birds, that are likely to cause a great variation in the experimental results, because we utilized adult birds which growth is minimal. The requirement of arginine for maintenance was determined in this study as 23 mg/kg BW day and was close to the values obtained by Ishibashi (1973) (25 mg/kg BW) and lower than those reported in other studies, e.g. 32 mg/kg BW d obtained by Dorigam et al. (2015), 54 mg/kg BW d by Leveille and Fisher (1959) and 60 mg/kg BW d by Fisher (1983). Unlike other species, poultry has high arginine requirements (Ball et al., 2007) and varies widely. In rooster, this explanation is supported by the composition of feathers which contain large amounts of arginine (8.0g Arg /16 g N) according to Leville and Fisher (1959). Therefore, not only the growing birds have a large requirement for this amino acid, but also, it is one of the most important essential amino acids in the diet of mature rooster (Leveille and Fisher (1959).

Many authors suggested that the best way to express the amino acid requirement for maintenance is according to body protein weight, because there is no demand for amino acid to maintain the adipose tissue (Emmans and Oldham, 1988; Burnham and Gous, 1992; Gous, 2007; Bonato et al., 2011; Lima et al, 2016a) minerals or water. On the other hand, reporting maintenance requirements based on body weight or metabolic body weight does not take into account differences in body composition. The body lipid content is influenced by environmental and dietary factors, mainly after maturity, but the protein weight of the animal remains constant (Gous et al., 1999; Marcato et al., 2008; Silva et al., 2013) and the increase in weight occurs due to increase in body fat (Gous et al., 1999; Marcato et al., 2008). Based on maintenance unit ($BPm^{0.73} \times u$), the determined requirement was 151 mg/kg $BPm^{0.73}$ per day.

According to Sakomura et al. (2015), the genetic selection for greater amounts of lean mass, determine the necessity of reassessing maintenance requirements. These authors suggested that the maintenance requirements for arginine of broiler breeder hens is 174 mg/$kg^{0.75}$ per day or 651 mg/kg CP per day. The authors explain that the amino acid profile of the endogenous losses of the breeder hen is unknown, the losses are likely greater as compared with the broiler chick and the rooster, resulting in the greater maintenance requirements observed.

This study shows that the determination of the amino acid requirements for maintenance can vary according to the type of animal and methodology used. It is believed that the feeding and the calculations based on protein weight and degree of maturity employed herein express more precisely the amino acid requirements for maintenance for poultry than do other systems. The data concerning the calculation of the requirements for the amino acids arginine may contribute to future studies aiming to improve poultry production, through use in factorial models to increase precision in diet formulation.

Experiment 2 - Reponses to arginine intakes

We evaluated the responses of broiler breeder hens to different intakes of arginine and determined amino acid efficiency of utilization. Based on factorial approach we developed mathematical model to estimate of the arginine intake. Fitting the Reading model, we estimate the parameters and based on simulation procedure we determined optimal economic intake of arginine considering the marginal cost of the arginine and the marginal revenue for eggs and the coefficient of variation for egg output. In addition, we considered the flock variability for egg output and the cost-benefits according to economic scenario.

We applied the dilution technique (Fisher and Morris, 1970) to limit the amount of arginine in dietary protein. Compared to lowest level, the control diet increased egg production, egg weight, and egg output, demonstrating that the arginine was limiting (Table 6). Previous studies using the same methodology also observed a limitation in the responses to their lowest level and control diets (Bowmaker and Gous, 1991; Lima et al, 2016b).

The first and second level of the amino acid intake resulted in a decreased intake (Table 6). Others studies reported a similar effect (Gous et al., 1987; Lima et al. 2016b). This counter-intuitive effect may result from the larger volumes as well as higher relative concentration of other nutrients in the more diluted diets. The feed intake is stabilized with diet 7.11 g/kg.

Egg weight and egg production decreased with lower arginine concentrations (Table 6). Previous studies evaluating other amino acids have reported greater reductions (Morris and Gous, 1988; Bowmaker and Gous, 1991) which can be related to the age of the hens.

Lima et al (2016b) observed lowest differences between low and high levels of the studied amino acids for egg production and egg weight because used older hens. According to Bowmaker and Gous (1991), the older hens have larger body reserves and mobilize more easily the tissue mass for egg formation, even at very low amino acid intakes.

Efficiency of utilization and rate of lay

The hens reduced protein intake to a near-maintenance requirements, but kept producing more than 3 eggs per week, totalling 3 to 18 eggs during the experimental period. These results agree with those previously obtained by Bowmaker and Gous (1991) who reported the production of one egg per week and also body weight loss, because these hens prioritize egg laying even at the expense of body energy stores.

In other study, broiler breeder hens kept producing more than 2 eggs per week, totalling 18 to 27 eggs during the experimental period (Lima et al., 2016b), as the hens were older (48 to 58 weeks) and have more body reserves, therefore they produced more eggs during the experimental period.

The efficiency of utilization increased in lower arginine intakes, probably because the mobilization of body mass. The egg production increased with the increased arginine intakes until reaches the response plateau (723.7 mg/bird d). Fisher (1994; 1998) explain that broiler breeder efficiency is a complex variable related to egg production. Fisher et al. (2001) showed that efficiency of utilization was stabilized at 57% of broiler breeders hens from 26 to 60 weeks.

We report here a compatible average efficiency for arginine of 62%.Similar lysine efficiencies were reported in other studies (Bowmaker and Gous, 1991; Silva et al., 2015b; Lima et al. 2016).

Modeling of arginine intake for broiler breeders

We presented two procedures to calculate arginine intake, one based on the performance of the birds and another considering performance, the cost benefit of the limiting amino acid supplementation. The information presented here aim to proportionate for producers and nutritionists, differences and limitations of the available methods, according to our evaluation.

The factorial model consist in assigning precision in the biological meaning of the model coefficients published by Bornstein et al. (1979). The consequence of the modification is to reduce the estimated intake of arginine, when calculated considering the changes in Lima et al. (2016b) and the coefficients determined in this study. A comparative analysis between estimates of Bornstein et al. (1979) model and the estimated maximum intake presented in Table 7 shows that the model of Bornstein et al. (1979) is, on average, 90% of the intake of the most productive individuals in the population.

However, the lower is the EO (45.6 g/day - Table 7) the greater the difference, that based on the evaluated population, can be interpreted as an overestimated value (974/735=1.33). The justification for the use Bornstein et al. (1979) model during four decades is attributed to the estimate of the model that provide a consistent intake with the nutritional requirement of the superior individual of the population. This avoid a nutritional deficiency in the population, being a positive aspect, which may have been the main reason for not having developed options for new models. We believe that the calculation of intake to meet the superior individual of the population should be an option of the producer or nutritionist and not an intrinsic feature/implicit of the model. This evaluation is only possible because we study the model to estimate the intake of the amino acid separately in the characterization of the variability of the population. The results made it clear that the variation of the population should be considered in establishing the amino acid intake. Table 7 shows that the arginine intake for 40-week-old breeder hens can vary from 480 to 1.067 mg/bird d, and the greater the distance is between the individuals, superior and inferior, and the number of subjects in the respective tracks, the decision on the intake needs an economic criterion.

Even this discussion on the population is important, the factorial model proposed in this research have a deterministic nature, i.e., there is no parameter that includes information on the population variability, and therefore requires a previous characterization of the population to be applied. The information necessary to simulate a population are EO and BW and should represent the population or characteristic of the population in which is desirable to meet the requirement with the calculated intake, the average of EO and BW of the population, or EO and BW of the upper or lower individual of the population. Based on this information, meeting the superior, inferior or the average individual becomes a user option.

The Reading Model represents an advance in this discussion. This model takes into account variability of EO and BW and economic criteria. The parameters used here has undergone some changes from that presented by

Fisher et al. (1973) to allow a calculation with biological interpretation. The first modification was the maintenance requirement determined in adult roosters (Experiment 1) because the coefficient is set equal to zero by Reading Model. The lack of fit for the maintenance has been observed in previous studies (Silva et al., 2015b; Lima et al., 2016b) and can be considered a limitation of this model in the studies for broiler breeder hens. In contrast to the deterministic factorial model, the Reading Model show the intake for average population and can be applied considering the exact equation and by computer simulation, the latter being so essentially stochastic, allow both economic inferences. A second modification in relation to Fisher et al. (1973) procedure was performed in the method by computer simulation. Here the conclusion about the economic optimum intake was based on number of birds met and additional margin of profit from birds, which now have their requirements met. An advantage of this procedure compared to the exact equation method is the possibility to view the number of birds in the flock which their requirements are met with the level of the amino acid intake, even the exact equation of Reading Model providing major recommendations by up to 3% for the largest CV when compared to the method by computer simulation. According to the results shown in Table 8, to produce 53 g/day of EO in a flock of 10,000 birds with 75% uniformity (CV=25%) it is required an intake of 1,059 mg/bird.day of arginine, that meet approximately 8,895 birds in relation to the flock with 95% uniformity (CV=5%), this intake is an additional cost of US$ 54.00.

The model of Bornstein et al. (1979) applied to the same EO (53 g/day) estimates the intake of 1,056 mg/bird.day of arginine. Comparing this value with the intake obtained for the simulated flock with 95% of uniformity, the estimate was 19% (1.056/891) greater than that obtained by computer simulation method. According to this simulation, the recommendation of Bornstein et al. (1979) when applied to a uniform flock, can increase the cost at about US$ 54.00. When compared to a flock with 75% of uniformity, there is no difference between the recommendations (0.003% = 1,056/1,059) and becomes appropriate.

The factorial model proposed by Lima et al. (2016b) is a theoretical advance in calculating the utilization efficiency and maintenance coefficient for arginine based on protein weight. On the other hand, these coefficients are determined using a straight line in previous research (Pomar et al., 2003; Silva et al., 2014) linear relations are procedures that fit the average population. When comparing the recommendation of the factorial model and the Reading model for a uniform population (CV=5%) there is a difference on the recommendation of 11.5% (835/749). This difference is attributed to the utilization efficiency (54.4%=7,387/13:57) of Reading Model be 12% (54.4/62) lower. When applied a correction in the utilization efficiency of the Reading Model to 62%, the coefficient *a* would be 11.91 mg/g and the arginine intake for the average population becomes 744 mg/bird d, annulling the differences between models. However, this exercise is useful only to illustrate the difference between the models, since the coefficient *a* of the Reading Model was estimated by the method of least squares, considering the fit of the response curve of the arginine levels to a simulated population.

Therefore, it is necessary further studies to better understand the stochasticity in the factorial model for broiler breeder hens, as well as to advance in the mechanistic calculating in the utilization efficiency, since that, there is no standardization in calculating the utilization efficiency for breeder hens and it is known that there are reports that the body mobilization would be an artifice used by these birds (Ekmay, 2013). The models presented here consider the utilization efficiency in different forms, with the factorial model, the only one that fixes the mobilization of the arginine from body for egg production and the Reading Model, more simply, assuming values for the coefficients that enabled the highest approach between the observed and predicted responses. Despite being a useful tool, it needs to improve the biological interpretation of parameters specifically for broiler breeder hens, since these limiting factors have not been verified in studies with laying hens (Silva et al., 2015b).

Regarding literature, the factorial model was able to estimate the arginine intake with proximity to recent studies. Ekmay et al. (2013) conducted two trials and determined the arginine intakes based on the performance of the birds, using the quadratic polynomial model and found 1,026 mg/ave.day as recommended level. When compared with the estimated factorial model for the most productive individual in a similar period (35-40 weeks), the recommendation becomes similar with Ekmay et al. (2013). However, it is not possible to compare estimates of Reading Model because it is considered the genetic potential variables, economic scenario and, depending on available ingredients and their prices, the recommendation will vary.

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