UNIVERSIDADE ESTADUAL PAULISTA-UNESP

CÂMPUS DE JABOTICABAL

OTIMIZAÇÃO DOS NÍVEIS DIETÉTICOS DE ISOLEUCINA, VALINA E TRIPTOFANO PARA MANTENÇA E PRODUÇÃO DE OVOS PARA POEDEIRAS COMERCIAIS

Vinícius Duarte de Oliveira

Zootecnista

2018

UNIVERSIDADE ESTADUAL PAULISTA-UNESP

CÂMPUS DE JABOTICABAL

OTIMIZAÇÃO DOS NÍVEIS DIETÉTICOS DE ISOLEUCINA, VALINA E TRIPTOFANO PARA MANTENÇA E PRODUÇÃO DE OVOS PARA POEDEIRAS COMERCIAIS

Vinícius Duarte de Oliveira

Orientador: Prof. Dr. Euclides Braga Malheiros

Co-orientadora: Profa. Dra. Nilva Kazue Sakomura

Co-orientador: Prof. Dr. Edney Pereira da Silva

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

2018

Oliveira, Vinícius Duarte
O48o Otimização dos níveis dietéticos de isoleucina, valina e triptofano para mantença e produção de ovos para poedeiras comerciais/ Vinícius Duarte de Oliveira. – – Jaboticabal, 2018
x, 50 p. : il. ; 29 cm

Dissertação (mestrado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2018
Orientador: Euclides Braga Malheiros
Banca examinadora: Nayara Tavares Ferreira , Matheus de Paula Reis
Bibliografia

1. Poedeiras-aminoácidos. 2. Poedeiras-exigências. 3. Poedeiras-modelagem. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 636.5

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

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: OTIMIZAÇÃO DOS NÍVEIS DIETÉTICOS DE ISOLEUCINA, VALINA E TRIPTOFANO PARA MANTENÇA E PRODUÇÃO DE OVOS PARA POEDEIRAS COMERCIAIS

AUTOR: VINÍCIUS DUARTE DE OLIVEIRA
ORIENTADOR: EUCLIDES BRAGA MALHEIROS
COORIENTADORA: NILVA KAZUE SAKOMURA
COORIENTADOR: EDNEY PEREIRA DA SILVA

Aprovado como parte das exigências para obtenção do Título de Mestre em ZOOTECNIA, pela Comissão Examinadora:

Prof. Dr. EUCLIDES BRAGA MALHEIROS
Departamento de Ciências Exatas / FCAV / UNESP - Jaboticabal

Pesquisadora Dra. NAYARA TAVARES FERREIRA
Nutrição Animal / Poli-Nutri / Osasco / SP

Pós-doutorando MATHEUS DE PAULA REIS
Departamento de Zootecnia / FCAV / UNESP - Jaboticabal

Jaboticabal, 09 de março de 2018

DADOS CURRICULARES DO AUTOR

VINÍCIUS DUARTE DE OLIVEIRA- filho de Sebastião Marcio de Oliveira e Silvana Aparecida Duarte Oliveira, nasceu no dia 04 de junho de 1993, na cidade de Viçosa, Minas Gerais. Em março de 2011 ingressou no curso de Zootecnia na Universidade de Federal de Viçosa, Minas Gerais, graduando-se em janeiro de 2016. Em março de 2016 ingressou no curso de mestrado em Zootecnia pela Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista – Campus de Jaboticabal, São Paulo, onde obteve bolsas pela CAPES e FAPESP, sob orientação do Prof. Dr. Euclides Braga Malheiros e defendendo está dissertação no dia 09 de março de 2018.

“*Olhar para trás após uma longa caminhada pode fazer perder a noção da distância que percorremos, mas se nos detivermos em nossa imagem, quando a iniciamos e ao término, certamente nos lembraremos o quanto nos custou chegar até o ponto final, e hoje temos a impressão de que tudo começou ontem. Não somos os mesmos, mas sabemos mais uns dos outros. E é por esse motivo que dizer adeus se torna complicado! Digamos então que nada se perderá. Pelo menos dentro da gente*...”

(João Guimarães Rosa)

“[...] Todo mundo ama um dia
Todo mundo chora
Um dia a gente chega
E no outro vai embora

Cada um de nós compõe
A sua própria história
E cada ser em si
Carrega o dom de ser capaz
De ser feliz [...]”

(Tocando em Frente – Renato Teixeira)

DEDICO...

Aos meus pais Marcio e Silvana, pelo amor incondicional e por sempre acreditarem em mim.

AGRADECIMENTOS

Ao Prof. Dr. Euclides Braga Malheiros pela orientação, aprendizado, amizade confiança e conselhos.

Aos Professores Dra. Nilva Kazue Sakomura e Dr. Edney Pereira da Silva pela co-orientação, apoio e oportunidade.

À Faculdade de Ciências Agrárias e Veterinárias - Unesp/Jaboticabal, pela estrutura e oportunidade de realizar o mestrado em Zootecnia.

À CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior e à FAPESP – Fundação de amparo à pesquisa do Estado de São Paulo (Processo FAPESP 2016/14754-5) pela concessão da bolsa de estudos viabilizando a execução do mestrado.

As empresas Evonik e Ajinomoto pelo apoio e parceria.

Aos membros da banca de qualificação Dr. Gabriel da Silva Viana, Dr. Juliano Cesar De Paula Dorigam e defesa Dra. Nayara Tavares Ferreira e Dr. Matheus de Paula Reis pela contribuição, críticas construtivas, disponibilidade e enriquecimento deste trabalho.

Aos meus pais, Marcio e Silvana que acreditaram e apoiaram a realização deste meu sonho, sempre com muito amor.

Ao meu irmão Vitor pelo companheirismo e amizade.

Aos meus avós pelos conselhos e apoio.

Aos meus familiares pelo carinho e confiança.

A equipe do LAVINESP: Matheus, Gabriel (Macaé), Nayara, Juliano, Marcos, Thiago, Leticia (Pomba), Mariana (Mari), Rafael (Kuki), Jefferson, Camila, Miryelle, Mirella, Leticia Pacheco, Karla, Fernando, Larissa (Pioia), Heloisa, Warley, Marllon, Raian, Palloma, Felipe (Torcido), Felipe (Celulari), Thaisa (Lorota), Bruno, Robson, Izildo e Vicente, pela colaboração e compreensão, todos foram muito importantes para o desenvolvimento do meu mestrado.

Aos Irmãos da Republica Antro do HV: Otávio (Tata), Murillo, Gilmar (Bartira), Kayo (Ky), Augusto (Buda), Cleber (Dido), Diego (Tofu), Marcelo (Balu), Thiago (Aladim), Alejandro (Alejo), Didimo, Felipe (Safadão), Igor, Fernando (Bahuam), Felipe (Nordestino) e Henrique pela amizade, ajuda e alegrias.

Aos meus amigos de Viçosa: Herbert, Marlon, Junior, Luís Felipe e Warlen e da UFV: Igor, Matheus (Cabelo), Valdir (Barão), Marcos (Gabiru), André, Leonardo e Lincoln por mesmo longe estarem sempre presente e participando de momentos importantes na minha vida.

Ao prof. Dr. Luiz Fernando Teixeira Albino e sua equipe de pesquisa da UFV pela amizade e aprendizado durante minha graduação.

Aos Funcionários da Unesp que sempre me ajudaram e tornaram possível a execução do mestrado.

A Deus por sempre me abençoar e capacitar na realização dos meus objetivos.

Muito obrigado!

SUMÁRIO

unesp UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 009999/14 do trabalho de pesquisa intitulado **"Modelagem da produção e das exigências nutricionais de aves e peixes – Resposta de produção das aves a ingestão de isoleucina, valina e triptofano"**, sob a responsabilidade da Prof.ª Dr.ª Nilva Kazue Sakomura está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 06 de junho de 2014.

Jaboticabal, 06 de junho de 2014.

Prof.ª Dr.ª Paola Castro Moraes
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209 2600 - fax 16 3202 4275 www.fcav.unesp.br

OTIMIZAÇÃO DOS NÍVEIS DIETÉTICOS DE ISOLEUCINA, VALINA E TRIPTOFANO PARA MANTENÇA E PRODUÇÃO DE OVOS PARA POEDEIRAS COMERCIAIS

RESUMO - A Determinação das ingestões ótimas de isoleucina (Ile), valina (val), triptofano (Trp) das galinhas poedeiras comercias pode permitir novas reduções no fornecimento da proteína bruta dietética. Para cumprir este objetivo as ingestões ótimas biológicas e econômicas de Ile, Val e Trp para poedeiras comercias foram avaliadas em três ensaios, sendo utilizadas 70 aves da linhagem Hisex White com 29 semanas de idade para cada ensaio. O delineamento experimental utilizado foi o inteiramente casualizado, com seis tratamentos e dez repetições de uma ave cada. As dietas experimentais foram formuladas pela técnica da diluição, obtendo-se assim níveis crescentes de Ile (2,80; 3,64; 5,11; 6,34; 6,95; 7,80 g/kg), Val (3,56; 4,58; 6,35; 7,83; 8,58; 9,60 g/kg) e Trp (0,81; 1,06; 1,50; 1,86; 2,05; 2,30 g/kg). Além disso, uma sétima dieta foi formulada em cada ensaio para verificar a limitância do aminoácido teste. Cada ensaio teve a duração de 10 semanas, sendo as seis primeiras de adaptação e as quatro últimas de coleta de dados. As variáveis de ingestão de aminoácidos, massa de ovo e conversão alimentar foram submetidas as análises de variância e regressão com nível de significância de α=5. Em seguida foram ajustados dois modelos matemáticos: linear platô (LP) e polinomial quadrático (PQ). Também foi estimada a ingestão ótima obtida pela 1º intersecção da PQ com o platô do LP. Para obtenção dos coeficientes para o "Reading Model", os dados foram analisados pelo programa EFG (2006). Os níveis de Ile, Val e Trp influenciaram significativamente todas a variáveis estudadas (p <0,01). Os níveis ótimos de Ile, Val e Trp estimados pelo LP foram 376, 679 e 150 mg/ave/dia, respectivamente. Considerando as ingestões estimadas pelo PQ foram obtidos 633 mg/ave/dia para Ile, 931 mg/ave/dia para Val e 218 mg/ave/dia para Trp. Com base na combinação dos dois modelos (LP+PQ), as ingestões de Ile, Val e Trp foram 531, 771 e 184 mg/ave/dia, respectivamente. Os coeficientes de produção (a) estimados foram 7,23; 9,83 e 2,28 mg/g de ovo para Ile, Val e Trp, respectivamente. A eficiência de utilização foi de 82% para Ile, 75% para Val e 85% para Trp. Os coeficientes de mantença (b) para Ile, Val e Trp foram 13,20; 0,03 e 7,48 mg/kg de peso corporal. Considerando o cenário econômico atual as ingestões ótimas econômicos de Val e Trp recomendados pelo "Reading Model" foram de 852 e 195 mg/ave/dia, para poedeiras comercias de 29 a 39 semanas.

Palavras-chaves: Aminoácidos, exigências e modelagem

OPTIMIZATION OF DIETARY LEVELS OF ISOLEUCINE, VALINE AND TRYPTOPHAN FOR THE MAINTENCE AND PRODUCTION OF EGGS FOR COMMERCIAL LAYING HENS.

ABSTRACT - Determination of optimal intakes of isoleucine (Ile), valine (val), tryptophan (Trp) of commercial laying hens may allow further reductions in the supply of dietary crude protein. To achieve this goal, the optimal economic intakes of isoleucine (Ile), valine (Val) and tryptophan (Trp) for commercial laying hens were evaluated in three trials, using 70 hens of Hisex White strain at 29 weeks of age for each assay. The experimental design was completely randomized, with six treatments and ten replications of one bird each. The experimental diets were formulated using the dilution technique, resulting in increasing dietary levels of Ile (2.80; 3.64; 5.11; 6.34; 6.95; 7.80 g.Kg-1), Val (3.56; 4.58; 6.35; 7.83; 8.58; 9.60 g.kg-1) and Trp (0.81; 1.06; 1.50; 1.86; 2.05; 2.30 g.kg-1). In addition, a seventh diet was formulated for each trial, in order to confirm if the amino acid test was the first-limiting. After a six-weeks adaptation period, the data collection were carried out for four weeks, thus each trial lasted ten weeks. The variables of amino acid intake, egg mass and feed conversion were submitted to analysis of variance and regression with significance level of α = 5. Then, two mathematical models were fitted: linear plateau (LP) and quadratic polynomial (PQ). It was also estimated the optimal intake obtained by the 1st intersection of the PQ with the LP plateau. To obtain the coefficients for the Reading Model, the data were analyzed by the EFG program (2006). Levels of Ile, Val and Trp significantly influenced all variables studied (p <0.01). The optimal levels of Ile, Val and Trp estimated by LP were 376, 679 and 150 mg / bird / day, respectively. Considering the intakes estimated by the PQ, 633 mg / bird / day were obtained for Ile, 931 mg / bird / day for Val and 218 mg / bird / day for Trp. Based on the combination of the two models (LP + PQ), Ile, Val and Trp intakes were 531, 771 and 184 mg / bird / day, respectively. The estimated coefficients of production (a) were 7.23; 9.83 and 2.28 mg / g egg for Ile, Val and Trp, respectively. The efficiency of use was 82% for Ile, 75% for Val and 85% for Trp. The maintenance coefficients (b) for Ile, Val and Trp were 13.20; 0.03 and 7.48 mg / kg body weight. Considering the current economic scenario, the optimal economic intakes of Val and Trp recommended by the Reading Model were 852 and 195 mg / bird / day, for commercial laying hens from 29 to 39 weeks

Keywords: amino acids, requirement and modeling

CAPITULO 1 – CONSIDERAÇÃO GERAIS

Introdução

A produção de ovos no Brasil vem crescendo ao longo dos anos e atualmente o pais aloja aproximadamente 96 milhões de pintainhas anualmente, produzindo cerca de 39 bilhões de ovos por ano (ABPA 2017). A perspectiva para os próximos anos é a continuidade deste crescimento devido a fatores como redução nos custos de produção, crescimento da população e popularização no valor do ovo.

A avicultura de postura passou por grandes avanços genéticos ao longo dos anos, melhorando a eficiência e aumentando a produtividade das aves. Devido essa modernização das linhagens de galinhas poedeiras, é necessária uma constante atualização das exigências nutricionais para o fornecimento adequado de energia e nutrientes, possibilitando a expressão máxima do potencial genético dessas aves.

O uso da modelagem na nutrição permite ao nutricionista, conhecer e corrigir a quantidade de nutrientes necessários para otimizar a produção avícola. Modelos determinísticos como o polinomial e o linear platô são amplamente utilizados e se caracterizam pela fácil interpretação da resposta das aves, estimando níveis de ingestão ótimas para um indivíduo referência, geralmente o indivíduo médio. Modelos estocásticos como o *Reading model* levam em consideração a variabilidade dos indivíduos e permitem a extrapolação dos resultados para a população e são ferramentas uteis nas tomadas de decisão, sobre a suplementação ou não de um determinado nutriente.

Assim, este estudo objetivou avaliar as respostas na produção de ovos à ingestão de isoleucina, valina e triptofano, determinar os coeficientes que definem as exigências dos aminoácidos testes para mantença e produção de ovos. Determinar a exigência para o indivíduo médio da população e do indivíduo superior da população para os aminoácidos estudados.

Referencial teórico

Isoleucina, valina e triptofano na dieta de poedeira comerciais

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, 2016). Em situações praticas, os aminoácidos sulfurados, lisina, treonina, arginina, isoleucina, valina e triptofano são os aminoácidos de maior importância na produção avícola (Baker & Han, 1994).

Ile e Val são aminoácidos alifáticos, hidrofóbicos de cadeia ramificada que atuam em conjunto, desempenhando diversas funções no organismo (D'Mello e Lewis, 1970). De acordo com Scottá (2014), Ile e Val são sintetizadas a partir do mesmo precursor, o piruvato, este numa reação dependente de tiamina pirofosfato (TTP) e catalisada pelas enzimas piruvato descarboxilase e por uma transcetolase, que dão origem ao hidroxietil-TTP. Deste ponto em diante, dependendo do composto com que o hidroxietil-TTP se condensar, haverá a formação da Ile ou Val. Ambos possuem antagonismo entre si, pois competem pelo mesmo sistema de transporte através das membranas celulares e utilizam os mesmos complexos enzimáticos para degradação (Peganova e Eder, 2003). As estimativas das exigências de Ile e Val determinadas em diversos trabalhos variam de 412 a 874 e de 567 a 773 mg/ave/dia, respectivamente (Harms e Russell, 2000; Peganova e Eder, 2002; Lelis et al., 2014; Rostagno et al., 2017). Essas variações podem ser causadas por fatores dietéticos, ambientais ou genéticos, mas a maioria resulta da variedade de métodos utilizados para calcular a exigência das aves (Huyghebaert et al., 1991).

O Trp pertence à classe dos aminoácidos aromáticos e suas funções primárias estão relacionadas às sínteses de proteína, niacina e serotonina (Tackman et al., 1990). É considerado o terceiro aminoácido limitante para aves alimentadas com dietas a base de milho e farelo de soja, precedida pela metionina e lisina (Peganova et al., 2003). Assim como para Ile e Val, há uma variação entre estimativas da exigência de Trp para galinhas poedeiras encontradas na literatura que variam de 142 a 198 mg/ave/dia (Russell e Harms, 1999; Peganova et al., 2003; Deponti et al., 2007; Calderano et al., 2012; e Rostagno et al, 2017).

Métodos para determinar as exigências de aminoácido

As exigências dos animais podem ser definidas como a quantidade de nutriente a ser fornecida para atender as necessidades fisiológicas de um indivíduo, e geralmente são apresentadas de duas formas: em termos absolutos ou em termos relativos, sendo que, na primeira forma o requerimento é apresentado em quantidades exigidas de cada nutriente por dia, e na segunda a exigência e expressa percentagem na dieta (Sakomura e Rostagno, 2016).

De acordo com estes autores os métodos utilizados para determinar as exigências dos animais são: o método dose resposta e o método fatorial. O método dose resposta é baseado na resposta do animal ao aumento na ingestão de um determinado nutriente, sendo este, o método mais utilizado para estabelecer as exigências dos monogástricos. Caracteriza-se por traçar uma curva resposta em função de um nutriente limitante na dieta em um determinado período (Mercer et al., 1989).

O método fatorial é baseado no princípio de que a exigência total do animal é a soma das quantidades a serem fornecida para mantença, crescimento e/ou produção. (Sakomura e Rostagno, 2016). A abordagem fatorial possibilita a elaboração de modelos capazes de predizer as exigências nutricionais de aves considerando diferentes condições fisiológicas e ambientais.

Uso da técnica da diluição na determinação das exigências de aminoácidos para aves

Para determinar as exigências nutricionais das aves utilizando-se do método dose resposta, é necessário que as dietas experimentais apresentem diferentes níveis do aminoácido a ser avaliado, e para atingir estes níveis são utilizadas duas técnicas: a técnica da suplementação que consiste na adição gradual do nutriente teste em uma dieta basal deficiente neste nutriente (D'Mello, 1982) e a técnica diluição onde os níveis do aminoácidos são obtidos pela diluição sequencial de uma dieta com alto teor de proteína deficiente no aminoácido teste, com outra dieta isenta de proteína, dietas estas, isoenergéticas e isonutritivas (Fisher e Morris, 1970).

As vantagens de se utilizar a técnica da diluição são: a facilidade de manter a relação dos aminoácidos em todos os níveis, permite reduzir a inclusão de fontes sintéticas de aminoácido, possibilitando a utilização de uma proteína cuja a constituição é próxima a de uma ração convencional, por exemplo, a base de milho e soja e apresenta uma maior facilidade de formular uma dieta suficientemente deficiente no aminoácido teste (Sakomura e Rostagno 2016).

Abordagens determinísticas e estocásticas

Na nutrição animal, o uso de modelos matemáticos é indispensável para determinação de níveis ótimos de ingestão dos nutrientes. Estes modelos podem ser divididos em modelos determinísticos e estocásticos.

Os modelos determinísticos fornecem explicações para as respostas das aves sem recorrer a elementos probabilísticos e desvios. Determina os resultados, aplicado a um indivíduo da população, normalmente, o indivíduo médio. Contribuindo assim, para a compreensão dos processos biológicos envolvidos na produção de ovos.

O modelo estocástico fornece explicações para as respostas das aves recorrendo a elementos probabilísticos e desvios. Os modelos estocásticos são menos utilizados que os modelos determinísticos, no entanto, por considerar a variabilidade dos indivíduos, podem operar a nível de população.

Uso de modelos matemáticos para determinar as exigências nutricionais das aves

Modelo determinístico: Dependendo da amplitude das respostas das aves aos níveis dietéticos testados, pode-se ajustar diversos modelos para determinar as suas exigências nutricionais. De acordo com Zhang e Coon (1994), diferentes exigências de aminoácidos podem ser derivadas dos mesmos conjuntos de dados dependendo da abordagem usada para interpretá-los .

Os modelos polinomial quadrático (PQ) e linear platô (LP) são exemplos de modelos bastante utilizados na interpretação de resultados de experimentos dose-resposta (Siqueira et al., 2009).

Os PQ, estimam a exigência em um único ponto máximo, os níveis ótimos de desempenho são estimados pela primeira derivada do modelo igual a 0. A porção ascendente é curvada, aumentando a uma taxa decrescente até atingir o ponto máximo. Existe um único ponto máximo, e outros aumentos na concentração de nutrientes resultam em um decréscimo no nível de desempenho.

De acordo com Pesti et al., (2009), Os nutricionistas às vezes optam por definir a exigência em algum valor diferente de 100% da resposta máxima, como podem ser com modelos assintóticos. Baker, (1986) e Robins et al., (1979) estimaram a exigência usando 95 ou 90% dos valores maximos obtidos pelo PQ.

Os modelos polinomiais são fáceis de se ajustar aos dados. Três níveis são suficientes para determinar os coeficientes de uma quadrática, mas são necessários mais pontos para estimar a forma da resposta ou uma exigência com qualquer confiança, sendo necessário para indicar a precisão dos coeficientes estimados (Pesti et al., 2009).

O LP, assume que a utilização de um nutriente limitante é constante até que sua exigência seja suprida e que não há respostas adicionais no desempenho acima deste ponto. Proporcionando assim, uma interpretação simplificada da curva-resposta (Robbins et al. 1979; Pack et al., 2003).

A medida que as concentrações de um nutriente aumentam na dieta, há um acréscimo na resposta até atingirem um ponto, a exigência, onde a resposta ótima é alcançada. Existe um platô após o nível da exigência em que o nutriente não é utilizado para deposição ou crescimento, nem é tóxico. A exigência é determinada por um valor x do ponto comum para ambas as linhas (Pesti et al, 2009), e segundo estes autores o LP e outros modelos não lineares são mais difíceis de se ajustar do que os polinômios simples, sendo, necessário um maior número de níveis do nutriente para obter boas estimativas dos segmentos de resposta ascendente e máxima do que com um PQ.

De acordo com Baker et al, (2002), o valor da primeira intersecção do LP e PQ seria uma alternativa ao uso destes modelos para recomendação, uma vez que a exigência determinada seria um valor intermediário obtido de forma objetiva.

Reading Model

Modelo estocástico: O *Reading Model* descrito por Fisher et al., (1973) ficou assim conhecido por ter sido desenvolvido na Universidade de Reading, Inglaterra. E se baseia na predição da ingestão ótima de aminoácidos a partir da partição da utilização dos aminoácidos em produção de massa de ovos e a mantença. Além disso, esse modelo permite a associação da ingestão ótima biológica com a variabilidade da população e um indicador econômico.

A equação abaixo é o *Reading Model* e descreve a resposta da população utilizando seis parâmetros, conforme Fisher et al. (1973):

$$\mathrm{Aopt}\frac{\mathrm{mg}}{\mathrm{dia}} = \mathrm{a} \times \mathrm{MOmax} + \mathrm{b} \times \mathrm{PC} + \sqrt{\mathrm{a}^2 \times \sigma^2\mathrm{MOmax} + \mathrm{b}^2 \times \sigma^2\mathrm{PC}} \times \mathrm{z}$$

Onde: Aopt é a ingestão ótima de aminoácidos (mg/d); MOmax é massa de ovo máxima média (g/dia); σ2MOmax é variância na massa de ovo máxima; PC é peso corporal médio (kg); σ2PC é variância de peso corporal, correlação e duas constantes que representam a quantidade de aminoácidos necessários por unidade de produção (a, mg/g de ovo) e para mantença (b, mg/kg PC). Nesta análise considerou-se como nula a correlação entre peso e massa de ovo, conforme Fisher et al. (1973).

A primeira parte da equação $a \times MOmax + b \times PC$ calcula a ingestão ótima para uma população considerando o indivíduo médio. E $\sqrt{\mathrm{a}^2 \times \sigma^2\mathrm{MOmax} + \mathrm{b}^2 \times \sigma^2\mathrm{PC}} \times \mathrm{z}$ descreve a variância populacional, sendo a exigência extra para os indivíduos mais produtivos. O *Reading Model* utiliza a relação custo benefício, que provém do k que corresponde a parte econômica da equação, pois z é a $\times$ k, sendo a o coeficiente de produção para 1 g de massa de ovo.

$$k = \frac{Custo\ por\ mg\ do\ aminoacido\ \$}{Valor\ da\ g\ d\ ovo\ \$}$$

O z indica o desvio padrão da curva normal que corta uma área a $\times$ k em uma cauda, é expresso em unidade padrão obtido a partir de tabelas da distribuição normal (é o valor de Z da Distribuição Normal Padrão, correspondente ao valor da expressão (1 - (a $\times$ k)). A multiplicação de Z pelo desvio dos requerimentos individuais tem-se o valor correspondente à distância y que deverá ser somado a exigência média (Pilbrow e Morris, 1974).

Referências bibliográficas

ABPA, 2017. Associação brasileira de Proteína Animal., Relatório Anual 2017., http://abpa-br.com.br/ (acesso e novembro de 2017).

Baker, D. H. 1986. Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. J. Nutr. 116:2339–2349.

Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. Poult. Sci. 73:1441– 1447.

Baker, D.H., Batal, a B., Parr, T.M., Augspurger, N.R., Parsons, C.M., 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81, 485–494. doi:10.1093/ps/81.4.485

Calderano, A., Gomes, P.C., Donzele, J.L., Barreto, S.L. de T., Vellasco, C.R., de Carvalho Mello, H.H., Lelis, G.R., da Rocha, T.C., 2012. Digestible tryptophan:digestible lysine ratio in diets for laying hens from 24 to 40 weeks of age. Rev. Bras. Zootec. 41, 2176–2182,2012. doi:http://dx.doi.org/10.1590/S1516-35982012001000006

Deponti, B.J., De Faria, D.E., De Faria Filho, D.E., Rombola, L.G., Araujo, L.F., Junqueira, O.M., 2007. Exigências de triptofano e padrão de recuperação do desempenho de poedeiras comerciais após alimentação com rações deficientes em triptofano. Rev. Bras. Zootec. 36, 1324–1330.

D'mello, J.P.F.; 1970. Lewis, D. Amino acid interactions in chick nutrition. 2. Interrelationships between leucine, isoleucine and valine. British Poultry Science, 11: 313 - 323.

D'mello, J.P.F., 1982 A Comparison of two Empirical Methods of determining Amino Acid Requirements. World's Poultry Science Journal 38:02 114-119.

Fisher, C., Morris, T.R., Jennings, R.C., 1973. A model for the description and prediction of the response of laying hens to amino acid intake. Br. Poult. Sci. 14, 469–484. doi:10.1080/00071667308416054

Fisher, C., Morris, T.R., 1970. The Determination Of The Methionine Requirement Of Laying Pullets By A Diet Dilution Technique. Br. Poult. Sci. 11, 67–82. doi:10.1080/00071667008415793

Harms, R.H., Russell, G.B., 2000. Evaluation of tryptophan requirement of the commercial layer by using a corn-soybean meal basal diet. Poult. Sci. 79, 740–742. doi:10.1093/ps/79.5.740

Huyghebaert, G., Groote, G. De, Butler, E.A., Morris, T.R., 1991. Optimum Isoleucine Requirement of Laying Hens and the Effect of Age. Br. Poult. Sci. 32, 471–481. doi:10.1080/00071669108417372

Lelis, G.R., Albino, L.F.T., Tavernari, F.C., Calderano, A.A., Rostagno, H.S., Barros, V.R.S.M., Maia, R.C., 2014. Digestible valine-to-digestible lysine ratios in brown commercial layer diets. Poult. Sci.

Mercer, L.P. et al., 1989 The Determination of nutritional requirements in rats: mathematical modeling of sigmoidal, inhibited nutrient-response curves. The Journal of Nutrition, 119: 10, p. 1465-1471,

Pack M, Hoehler D and Lemme A 2003. Economic assessment of amino acid response in growing poultry. In Amino acids in animal nutrition (ed. FJP D'Mello), pp. 459–483. CAB International, Wallingford, UK.

Peganova, S., Eder, K., 2003. Interactions of various supplies of isoleucine, valine, leucine and tryptophan on the performance of laying hens. Poult. Sci. 82, 100–105. doi:10.1093/ps/82.1.100

Peganova, S., Eder, K., 2002. Studies on requirement and excess of isoleucine in laying hens. Poult. Sci. 81, 1714–1721.

Peganova, S., Hirche, F., Eder, K., 2003. Requirement of tryptophan in relation to the supply of large neutral amino acids in laying hens. Poult. Sci. 82, 815–22.

Pesti, G.M., Vedenov, D., Cason, J.A., Billard, L., 2009. A comparison of methods to estimate nutritional requirements from experimental data. Br. Poult. Sci. 50, 16–32. doi:10.1080/00071660802530639

Pilbrow, P.J., Morris, T.R., 1974. Comparison of lysine requirements amongst eight stocks of laying fowl. Br. Poult. Sci. 15, 51–73. doi:10.1080/00071667408416081

Robbins, K. R., H. W. Norton, and D. H. Baker. 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109:1710–1714.

Rostagno, H.S., Albino, L.F.T., Hannas, M.I., Donzele, J.L., Sakomura, N.K., Perazzo, F.G., Saraiva, A., et al. (2017) Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. Viçosa: UFV.

Russell, G.B., Harms, R.H., 1999. Tryptophan requirement of the commercial hen. Poult. Sci. 78, 1283–5.

Sakomura, N.K. & Rostagno, H.S. (2016) Métodos de Pesquisa em Nutrição de Monogástricos. Jaboticabal: Funep.

Scottá, B.A., 2014. Valina, isoleucina e leucina para aves. J. Chem. Inf. Model. 8, 1689–1699. doi:10.1017/CBO9781107415324.004

Silva, E.P., Sakumura, N.K., Hauschild, L., Gous, R.M., 2015. Modelo de Reading para estimar a ingestão ótima econômica de aminoácidos para aves 450–457. doi:10.1590/0103-8478cr20120799

Siqueira, J.C., Sakomura, N.K., Nascimento, D.C.N., Fernandes, J.B.K., 2009. Modelos matemáticos para estimar as exigências de lisina digestível para aves de corte ISA Label. Rev. Bras. Zootec. 38, 1732–1737. doi:10.1590/S1516-35982009000900013

Tackman, J.M., Tews, J.K., Harper, a E., 1990. Dietary disproportions of amino acids in the rat: effects on food intake, plasma and brain amino acids and brain serotonin. J. Nutr. 120, 521–33.

Zhang, B., Coon, C.N., 1994. Nutrient Modelling for Laying Hens. J. Appl. Poult. Res. 3, 416–431. doi:10.1093/japr/3.4.416

CAPITULO 2 - Laying hen requirements for valine, isoleucine and tryptophan

Abstract. Determining laying hen requirements for valine (Val), isoleucine (Ile) and tryptophan (Trp) could allow further reductions on dietary CP supply. In order to fulfill this purpose, three simultaneous feeding trials (one for each amino acid) were conducted. In each trial, a total of seventy 29-week-old Hisex laying hens were randomly assigned to six levels the each amino acids studied, with ten individual replicates. Dietary levels of Ile ranged from 2.80 to 7.80 g/kg, Val from 3.56 to 9.60 g/kg and Trp from 0.81 to 2.30 g/kg. For each amino acid, an additional treatment was included to ensure that the amino acid studied in each trial was the first limiting in experimental diets. Such treatment was produced by adding L-Val, Ile, and Trp to the diets containing the lowest levels of these amino acids in order to achieve the second lowest level. All feeding trials lasted ten weeks, with the first six weeks for adaptation of birds to the experimental diets and the last four ones to collect data. Optimal SID Val, Ile, and Trp intake was estimated using polynomial quadratic and linear broken-line regression model, as well as by the association between both models. Except for egg weight in Ile trial, all the variables assessed were affected by increasing amino acid intake. The amount of SID Val, Ile, and Trp, which optimized egg output production according to polynomial quadratic model were 931, 633, and 218 mg/bird/day, respectively. According to linear broken-line model, these intakes were 679 mg SID Val, 376 mg SID Ile/kg, and 150 mg SID Trp/kg. The association between both models estimated daily SID Val, Ile, and Trp requirements considering egg output production in 771, 531, and 184 mg/bird/, respectively.

Keyword: Amino acid requirement, dilution technique, large neutral amino acids, models

Introduction

Increasingly world’s demand by high-quality and safe animal protein associated with pressures from consumer market on attenuating the noxious impacts of livestock on environment have been forcing poultry industry to reconsider some aspects in animal production in order to increase not only food supply, but also to improve the efficiency with which dietary protein is converted into final food products. Meeting bird requirements for amino acids more accurately through providing diets with a lower crude protein content supplemented with crystalline amino acids has shown to mitigate nitrogen excretion without compromising bird performance (Namroud et al., 2008, Ospina Rojas et al., 2012, Awad et al., 2014). Nevertheless, the knowledge of the ideal essential amino acid pattern in protein is a *sine qua non* condition to warrant how successful such nutritional strategy will be in bringing the aforementioned benefits for poultry production system.

Compared with other amino acids, few are the attempts made to establish laying hen requirements for valine (Val), isoleucine (Ile) and tryptophan (Trp). The NRC (1994) recommends a daily intake of 700 mg Val/bird, 650 mg Ile/bird, and 160 mg Trp/bird for white commercial layers. Despite frequently used as reference, one of the criticism of NRC is that amino acid requirements are expressed on the basis of total amino acid intake, whilst industry works with requirements expressed on digestible basis. Moreover, the data, which originated these estimates, are old and out of date and, therefore, may not represent the superior genotype of the current commercial layer strains. Bregendahl et al. (2008) reported an update of ideal protein for laying hens, in which Val, Ile, and Trp intake for maximum egg output of white egg-type layer are described as 501, 426, 120 mg/bird/day, respectively.

Years later, as an attempt of updating laying hen requirements for standardized ileal digestible essential amino acids using a meta-analysis approach, Van Krimpen et al. (2015) highlighted the lack of a reliable database regarding hen requirements for SID Trp, SID Val, and SID Ile. As a result, among these amino acids, only the amount of SID Trp required for optimum egg production (155 mg/bird), egg output (156 mg/bird), and feed conversion ratio (180 mg/bird) was determined, whilst optimum SID Val and Ile intake could not be established. Since then, few efforts have been put forward determining hen requirements for these amino acids. Given that hen requirements for some essential amino acids must be updated, and that such update could contribute for devising feeding tables for poultry, which in turn would guide nutritionist to optimize amino acid nutrition, we aimed to determine the requirements for SID Val, Ile, and Trp for laying hens in the egg-laying phase.

Material and methods

Ethics in animal care and use

All procedures described herein were adopted after the approval of institutional Animal Care and Use Committee of the Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, São Paulo, Brazil under protocol number n° 009999/14.

Bird husbandry and experimental design

Twenty seven-week-old Hysex White laying hens were selected form a local commercial flock and allotted in 50 x 45 x 40 cm (length x height x width) stainless steel cages in an environmental controlled facility. A total of 210 hens were used in three dose-response trials (70 hens/trial). At 29 weeks of age, all hens were weighed so that initial body weight and egg production (recorded for two weeks) were not different among treatment groups. In each trial, ten individual replicates were randomly assigned to each of seven experimental treatments. Throughout the entire experimental period, experimental diets and water were provided ad libitum to birds in trough feeders and nipple drinkers, respectively. Photoperiod was set at 16L:8D.

Experimental diets

Prior to mixing experimental diets, all feedstuffs were analyzed for total amino acid content and standardized ileal digestible amino acids were estimated using coefficient of digestibility described by AMINODat® 5.0, (2016). Crystalline amino acids were assumed to be 100% SID (Chung and Baker, 1992). Firstly, in order to produce the dietary treatments, in each trial a high protein 'summit' diet was formulated to exceed the amino acid studied (Val, Ile, and Trp) in 30% the level recommended by Rostagno et al. (2011), whilst the remaining essential amino acids were provided at 50% the recommended values by the same authors (Table 1).

[Insert Table 1]

Then, the aforementioned summit diets were graded diluted in a common isonutritive nitrogen-free diet (NFD) to produce serial dilutions (Fisher and Morris, 1970), which originated six increasing levels of SID Val, Ile, and Trp (Table 2). Except for crude protein and amino acids, all nutrients were supplied to meet or exceed Rostagno et al. (2011) recommendations for White commercial layers. Dietary levels of SID Val ranged from 3.56 to 9.60 g/kg, SID Ile levels ranged from 2.80 to 7.80 g/kg, whilst SID Trp ranged from 0.81 to 2.30 g/kg. In order to assure that studied amino acid was the first limiting in all dilution series, an additional treatment was produced from the diets containing the lowest levels of SID Val, Ile and Trp. These diets were supplemented with 1.02 g L-Val/kg diet, 0.84 g L-Ile/kg diet, or 0.25 g L-Trp/kg diet in order to achieve the second lowest level of the dilution series.

[Insert Table 2]

Data collection

All feeding trials lasted 10 weeks, but only the data collected at the final 4 weeks were used in the statistical analysis. Body weight was measured at the 1st, 6th, and the 10th week of the trials. Egg production was daily recorded. Feed intake and egg weight were weekly measured and egg output and feed conversion per egg output were calculated from these data.

Statistical analysis

All collected data were analyzed as one-way ANOVA using GLM procedure of SAS (SAS Institute Inc., Cary, NC). The broken-line and quadratic broken-line regression model was computed by the NLIN procedure (Robbins et al., 2006). Optimum SID Val, Ile, and Trp intake was estimated using polynomial quadratic regression model, linear broken-line regression model, and the association between both models. Statistical significance was considered at $p < 0.05$. As the other biological traits, amino acid intake can be described the normal distribution curve. In order to standardize amino acid intake, this variable was converted into the normal deviation Z (equation 1), which corresponds to the area under the standard normal curve, which represents different individuals in a population.

$$z = \frac{x - \mu}{\delta}$$

here x is the observed value for SID Val, Ile, and Trp intake, μ is the average amino acid intake (mg/day) and δ is the standardized deviation.

Results

Regardless of the feeding trial (Val, Ile, and Trp) hens fed counter-proof diets had the performed traits improved compared with those fed the lowest level of the amino acid studied, indicating that all the amino acids under study were indeed the first limiting in experimental diets. All performance responses are detailed in Table 3.

[Insert Table 3 and 4]

Valine Trial

Performance responses of hens fed increasing SID Val levels are detailed in Table 3. Graded SID Val intake elicited a quadratic response in egg production, egg weight, egg output, and feed conversion, which were optimized at 829, 1,155, 931, and 771 mg/bird/day, respectively. Fitting performance data to broken-line model, the breakpoint estimated for maximum egg production, egg weight, egg output, and optimum feed conversion were 562, 740, 679, and 570 mg SID Val/bird/day, respectively. Associating both models the optimum egg production, egg weight, egg output, and feed conversion were achieved at 704, 815, 771, and 674 mg SID Val/bird/day, respectively. The SID Val intake estimated by polynomial quadratic, broken-line model, and the association of both models, which optimizes egg output, fed approximately 93, 66, 80% of the individuals, respectively (Figure 1A and 1B).

[Insert Figure 1A and 1B]

Isoleucine trial

Performance responses of hens fed increasing SID Ile levels are detailed in Table 3. Increasing SID Ile intake elicited a quadratic response in all the performance traits assessed, optimizing egg production, egg weight, egg output, and feed conversion at 599, 775, 633, and 534 mg/bird/day, respectively. The breakpoint estimated by the broken-line model was for egg production, egg weight, egg output, and feed conversion was 331, 610, 376, and 177 mg SID Ile/bird/day, respectively. The combination of both models provided the optimum egg production, egg weight, egg output, and feed conversion at 488, 692, 531, and 71 mg/bird/day, respectively. The estimate of SID Ile intake provided by polynomial quadratic, broken-line model, and the association of both models, optimized egg output of approximately 80, 32, and 63% of the individuals, respectively (Figure 1A and 1B).

[Insert Figure 2A and 2B]

.

Tryptophan trial

Performance responses of hens fed increasing SID Trp levels are detailed in Table 3. Contrary to the other amino acids studied here, egg weight data did not fit to any regression model. Egg production, egg output, and feed conversion were quadratically influenced by graded SID Trp intake being optimized at 208, 218, and 182 mg/bird/day. Broken-line model estimated the breakpoint for egg production, egg output, and feed conversion at 147, 150, and 102 mg SID Trp/bird/day, respectively. Considering the estimates produced by the association of the aforementioned models optimum SID Trp intake for egg production, egg output, and feed conversion was 179, 184, and 146 mg/bird/day, respectively. The estimates of SID Trp intake generated using polynomial quadratic, broken-line, and the association between these models, optimized egg output of 93, 66, and 83% of the hens, respectively (Figure 3A and 3B).

[Insert Figure 3A and 3B]

Discussion

We conducted the current research in order to determine the SID Val, Ile, and Trp requirements of laying hens in the egg-laying phase. Analyzing the performance responses, the collected data support the conclusion for the afore raised question. Although the laying hen performance is a set of several variables (feed intake, egg weight, egg output and feed conversion ratio), we consider daily egg output production as the variable with the most important biological and economic meaning, and thereby optimum intake of the amino acids studied herein was considered as that, which optimized such variable (Table 4). In the current research, optimum egg output reached the greater values at daily intake of 931 mg SID Val/bird, 633 mg Ile/bird, and 208 mg SID Trp/bird according to polynomial quadratic regression. According to linear broken-line model, such variable was optimized at 679 mg SID Val/bird, 376 mg SID Ile/bird, and 134 mg

SID Trp/bird (Table 3). The association between both models estimated a daily SID Val, Ile, and Trp intake for optimum egg output in 771, 531, and 184 mg/bird, respectively.

As expected, the estimates from polynomial quadratic were higher compared to those estimated by linear-broken line model. With regard to such models, both are assumed to biases estimates in some points, which becomes the truth about their estimates still a matter of controversy. Such controversies are centred on the fact that polynomial quadratic model is said to overestimate requirements by assuming a bilateral symmetry in bird response, whilst linear broken-line model ignores the minimum diminishing return law by assuming an interruption on performance improvements from the breakpoint and thereby is assumed to underestimate requirements (Lamberson and Firman, 2002). We believe that what prevails as truth from such statement is the description of animal responses provided by each model, which, however, in our understanding, does not imply in over or underestimating nutritional requirements. Protein accretion rates in modern broiler strains have improved considerably due to intense genetic evolution, yet genetic variability among individuals remains existing. A population is represented by approximately 25% of individuals with a low genetic potential, whose nutritional requirements are lower compared with 50% of average individuals and the remaining 25% with the higher requirements (Kaps and Lamberson, 2004). Based on such assumption, we randomized amino acid intake data using a Z table, and regressed the values of Z in function of egg output. As result, we obtained the inflexion point and a breakpoint, which corresponded to optimum Z values according to polynomial quadratic and linear broken-line model, respectively. From these values, using the normal curve distribution we found that the estimates of optimum SID Val, Ile, and Trp intake originated from polynomial quadratic regression fed approximately 93, 80, and 93% of the population of hens used in each respective trial. According to the estimates of linear broken-line model, hens, which have their requirements met for SID Val, Ile, and Trp were equivalent to 66, 32, and 66%, respectively. The association of both models has been used as a tool to avoid the occurrence of the so-called “under and overestimates” (Baker et al., 2002). By associating both models, the estimates produced for SID Val, Ile, and Trp satisfied the requirements of 82, 63, and 82% of the hens, respectively. With these outcomes, models do not under or overestimates, but rather produces estimates, which feed different fractions of a population.

Adopting lower requirements, i.e. choosing to feed hens with lower genetic potential, as reference to devise a feeding program implicates on the fact that birds whose potential is higher will inevitably eat more as an attempt of meeting their requirement for amino acids (Emmans, 1981). Despite the deficiency of the first limiting nutrient may trigger off such increase in food consumption, it will not necessarily ensure that birds will consume to have their requirements fully met, since bulk capacity may be a constraining factor. In this case, hens would unsuccessfully eat more but not express their higher genetic potential, which hence would lead to the extra-intake of nutrients without the expected return. In overall terms, such framework could lead to an inefficiency in nutrition management and economic losses. Obviously, that choosing to feed the individuals with the highest genetic potential does not mean optimum economic return. Such decision is an economic one and is correlated with the factors such as the cost of the additional amino acids and the number of birds that will benefit from the higher level.

Contrasting our outcomes with published literature, we can notice discrepancies regarding hen responses to increasing amino acid supply. In the current study, all the variables assessed exhibited responses to the dietary increment in the dose of the amino acid under study, except for egg weight in Ile trial. Contrary to our findings, Lelis et al. (2014) and Azzam et al. (2015) found no effects of increasing SID Val in egg output concluding that the minimum level in study (502 and 783 mg/bird/day, respectively) were sufficient to meet hen requirements. Likewise, Mello et al. (2012) did not found effects of SID Ile intake on hen performance concluding that 532 mg SID Ile/bird/day or less ensure proper egg output production. Regarding references about SID Trp requirements, Calderano et al, (2012) using linear broken-line model pointed out that the optimum SID Trp intake for maximum egg output should be 144 mg Trp/bird/day. An overall analysis of these literatures either demonstrates a lack of hen responses to amino acid intake, or indicate that our estimates are higher than those already published. Since the aforementioned authors used the same models that we used to estimate requirements, the most marked difference between our study and the others above is that the levels of the amino acids studied here were obtained using the dilution technique, whilst the remaining authors used the graded supplementation technique.

Since the sixties of the last century, providing graded dietary concentrations of the amino acid under study via supplementing its crystalline form in diets deficient in that amino acid has been the most traditional method to estimate poultry requirements for essential amino acids (Dean and Scott, 1965; D'Mello, 1982). Graded supplementation technique, although still most commonly used in dose-response trials, present limitations that arise doubts about the reliability of the estimates produced from such method. Firstly, diets formulated using the so-called supplementation technique do not provide the same balance between essential amino acids, since the concentration of the amino acid under study varies, whilst the other amino acids remain equal. According to D'Mello, (1982), how this fact would affect results were never amplified beyond the criticisms. However, published reports provide clear evidences, which refute such statement. Wiltafsky et al. (2009a) noticed a linear decrease in the plasmatic concentration of leucine, glycine, lysine, tryptophan, and tyrosine of piglets fed diets in which SID Ile were graded supplemented in crystalline form. In another subsequent trial, Wiltafsky et al. (2009b) observed the same response that those noticed in Ile trial, when L-Val supplementation increased, except for plasmatic Leu and Gly concentration. Such behaviour may be explained by the fact that the branched chain amino acids (BCAA) Val, Ile, and Leu, which as well as the other large neutral amino acids (LNAA) such as histidine, phenylalanine, tyrosine, methionine, and tryptophan share the same absorptive system in the intestine and hence may compete for the intestinal transporters (D'Mello, 2003).

Moreover, excessive Val, Ile, and Leu dietary supply may reduce brain Trp uptake due to competition for hemato-cephalic barrier transport (Peganova and Eder, 2003). Pig requirements for Trp has shown to be affected by the dietary supply of LNAA (Boomgaardt and Baker, 1973; Henry et al., 1992). Since, Trp is a precursor of the neurotransmitter serotonin, which modulates feeding behaviour (Tackman et al., 1990; Mullen and Martin, 1992), low Trp concentration in the brain due to low intestinal absorption or excessive plasmatic concentration of BCAAs may decrease food intake and impair performance. These outcomes may support Azzam et al. (2015) findings who observed a depression on hen food consumption in response to increase in L-Val supplementation. It is worth recalling that the antagonism among amino acids also takes place in the liver, where the amino acids after absorbed are split into C-skeletons and ammonia. Leucine C-skeleton (□-ketoisocaproate) has shown to stimulate the branched-chain a-keto acid dehydrogenase complex (BCKDH) activity, resulting in Val and Ile catabolism in pig and rat liver (Langer and Fuller, 2000). Wiltafsky, (2009) noticed that increasing dietary SID Leu supply elicited an increase in BCKDH activity in pig liver.

Given the background above, the deleterious effects from the interactions among the amino acids under study with other essential amino acids are clearly proved, which reinforces the idea that adding crystalline amino acids gradually to diets seems not to be the more proper way to produce a variation in dietary amino acid concentration. Another claim to refute supplementation technique as the most proper technique to formulate diets is that at some point the amino acid studied will be not the first limiting one in diets and that additional responses will be limited by the deficit in other amino acids. It sounds like animal responses to supplementation technique is restricted to establish the ideal dietary amino acid profile, whilst in the dilution technique, where the balance among essential amino acids remains equal, the performance would be determined by the amount of amino acid that the bird can eat to meet their requirements. Another advantage of dilution technique is that the amino acids are supplied bound to peptides, which minimize the competition for intestinal transporters. The highest the inclusion of free amino acids in diets, the highest is the probability of imbalanced amino acid profile in plasma, which has shown to depress food consumption and decrease efficiency of nitrogen utilization (Austic et al., 2000; Fatufe et al., 2005).

As detailed above, all the experimental treatments tested in the current trials were produced from the dilution technique (Fisher and Morris, 1970). Irrespective of the feeding trial, the lowest amino acid supply, the highest was the loss in body weight. At the beginning of the feeding trials, hen average initial body weight was approximately 1.4 kg, but at the end, such values ranged from 1.2 to 1.5 kg depending on the trial and the amino acid. Although the balance among essential amino acids is equal among dilution series studied, the amount of amino acids in the more diluted diets are not sufficient to support egg protein synthesis. As an attempt of maintaining egg output, these birds increase bodily protein catabolism rates so that the amino acids of the muscle tissue can warrant egg production. Therefore, body weight loss observed in hens fed the lowest levels of amino acids may be presumably attributed to bodily protein catabolism. Such responses were already expected and are in accordance with previous findings originated from studies in which dilution technique was used to produce experimental treatments (Bowmaker and Gous, 1991; Burnham et al., 1992). As experimental diets became more diluted, poorer was the dietary amino acid content, but equal was the AMEn content. Since such dietary amino acid pattern does not support the optimum expression of egg output, the amount of energy provided by diets is far in excess of the amount required to sustain egg production and egg weight. Therefore, in order to avoid imbalances in energy intake triggered by an excessive and unnecessary intake of energy, hens tend to eat less (Bowmaker and Gous, 1991). It helps us to explain our outcomes of food intake, which increased by 35, 40, and 35% when SID Val, Ile, and Trp dietary supply increased from the lowest to highest level.

The experiment design adopted in the current trials allowed us estimating SID Val, Ile, and Trp intake for optimum egg output in laying hens; and the models used to obtain such estimates, as expected, produced different values, which as demonstrated, fed different fractions of a population. The decision of which individuals will receive diets to meet their requirements is economic and depends on the dynamic between amino acid and egg prices. The main advantage of our study is the fact that we worked with individuals. Such fact allowed us obtaining the variance in egg output and body weight, which in turn in a future may applied in stochastic models to predict amino requirements. Although literature provide a detailed description about the antagonism among LNAA, little is known about from which level the amino acids under study affect the metabolism of others, and to what extent hen performance is impaired by an imbalance among these amino acids. Such knowledge gap suggests that research efforts must still have done in this sense.

Acknowledgments

The authors thank the São Paulo Research Foundation – FAPESP (BRAZIL) for financial support grant number n° 2016/14754-5 and 2013/25761-4 and Ajinomoto Biolatina and Evonik Industries for donating the amino acids used in the current research and for the analysis of amino acid content in diets.

References

Austic, R.E., Keene, J.C., Yuan, J.H., 2000 Effect of dietary protein level on amino acid imbalanceand toxicity. Proc. Cornell Nutrition Conference for Feed Manufacturers (Rochester, NY) October 24-26.

Awad, E.A., Fadlullah, M., Zulkifli, I., Farjam, A.S., Chwen, L.T., 2014. Amino acids fortification of low-protein diet for broilers under tropical climate: Ideal essential amino acids profile. Ital. J. Anim. Sci. 13, 270–274. doi:10.4081/ijas.2014.3166

Azzam, M.M.M., Dong, X.Y., Dai, L., Zou, X.T., 2015. Effect of excess dietary L-valine on laying hen performance, egg quality, serum free amino acids, immune function and antioxidant enzyme activity. Br. Poult. Sci. 56, 72–78. doi:10.1080/00071668.2014.989487

Baker, D.H., Batal, a B., Parr, T.M., Augspurger, N.R., Parsons, C.M., 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81, 485–494. doi:10.1093/ps/81.4.485

Blundell, J.E., Latham C.J., 1978. Pharmacologicalmanip- ulation of feeding behavior: Possible influences of serotonin and dopamine on food intake. Pages 83–109 in Central Mechanism of Anorectic Drugs. S. Garattini and R. Sa- manin, ed. Raven Press, New York.

Boomgaardt, J., Baker, D.H., 1973. The lysine requirement of growing chicks fed sesame meal-gelatin diets at three protein levels. Poult. Sci.. 52, 586-591.

Bowmaker, J.E., & Gous, R.M., 1991 The response of broiler breeder hens to dietary lysine and methionine. Br. Poult. Sci. 32, 1069-1088.doi: 10.1080/00071669108417430.

Bregendahl, K., Roberts, S.A., Kerr, B., Hoehler, D., 2008. Ideal Ratios of Isoleucine, Methionine, Methionine Plus Cystine, Threonine, Tryptophan, and Valine Relative to Lysine for White Leghorn-Type Laying Hens of Twenty-Eight to Thirty-Four Weeks of Age. Poult. Sci. 87, 744–758. doi:10.3382/ps.2007-00412

Burnham, D., Gous, R.M., Emmans, G.C., 1992. Isoleucine requirements of the chicken: The effect of excess leucine and valine on the response to isoleucine. Br. Poult. Sci. 33, 71–87. Doi:10.1080/00071669208417445

Calderano, A., Gomes, P.C., Donzele, J.L., Barreto, S.L. de T., Vellasco, C.R., de Carvalho Mello, H.H., Lelis, G.R., da Rocha, T.C., 2012. Digestible tryptophan:digestible lysine ratio in diets for laying hens from 24 to 40 weeks of age. Rev. Bras. Zootec. 41, 2176–2182. doi: 10.1590/S1516-35982012001000006

Chung, T.K., Baker, D.H., 1992. Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. J. Anim. Sci. 70, 3781–3790. doi:/1992.70123781x

Dean, W.F., Scott, H.M., 1964. The Development of an Amino Acid Reference Diet for the Early Growth of Chicks. Poult. Sci. 44, 803–808.

Degussa AG. 2006. AminoDat 3.0. Hanau, Germany

D’mello, J. P. F., 2003. Amino acids in animal nutrition. 2 ed. CABI Publishing, Edinburgh. pp. 187–202

D'mello, J.P.F., 1982 A Comparison of two Empirical Methods of determining Amino Acid Requirements. World's Poult. Sci. J. 38, 114-119.

Emmans, G. C., 1981. A model of the growth and feed intake of ad libitum fed animals, particularly poultry. Pages 103–110 in: Computers in Animal Production. Animal Production, Occasional Publication No 5. G. M. Hillyer, C. T. Whittemore, and R. G. Gunn, ed. British Society of Animal Production, London, U.K.

Fatufe, A.A., Rodehutscord, M., 2005. Growth, body composition, and marginal efficiency of methionine utilization are affected by nonessential amino acid nitrogen supplementation in male broiler chicken. Poult. Sci. 84, 1584–1592. doi:10.1093/ps/84.10.1584

Fisher, C., Morris, T.R., 1970. The Determination Of The Methionine Requirement Of Laying Pullets By A Diet Dilution Technique. Br. Poult. Sci. 11, 67–82. doi:10.1080/00071667008415793

Henry, Y., Sève, B., Colléaux, Y., Ganier, P., Saligaut, C., Jégo, P., 1992. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs, in relation to plasma free amino acids and hypothalamic serotonin. J. Anim. Sci. 70, 1873–1887. doi:10.2527/1992.7061873x

Kaps M., Lamberson, W.R., 2004. 'Biostatistics for Animal Science.' (CABI Publishing: Wallingford, UK)

Krimpen, M.M. Van, Veldkamp, T., Riel, J.W. Van, Khaksar, V., Hashemipour, H., Blok, M.C., Spek, W., n.d. Estimating requirements for apparent faecal and standardised ileal digestible amino acids in laying hens by a meta-analysis approach. Wageningen, Wageningen UR (University & Research centre) Livestock Research, Livestock Research Report 848

Lamberson, W.R., Firman, J.D., 2002. A comparison of quadratic versus segmented regression procedures for estimating nutrient requirements. Poult. Sci. 81, 481–484.

Langer, S., Fuller, M.F., 2000. Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on nitrogen retention and amino acid utilization. Br. J. Nutr. 83, 49–58.

Lelis, G.R., Albino, L.F.T., Tavernari, F.C., Calderano, A.A., Rostagno, H.S., Barros, V.R.S.M., Maia, R.C., 2014. Digestible valine-to-digestible lysine ratios in brown commercial layer diets. J. Appl. Poult. 23, 683–690. doi:10.3382/japr.2014-00984.

Mello, H.H.C., Gomes, P.C., da Rocha, T.C., Donzele, J.L., de Almeida, R.L., Troni, A.R., de Carvalho, B.R., Viana, G. S., 2012. Determination of digestible isoleucine: Lysine ratio in diets for laying hens aged 42-58 weeks. Rev. Bras. Zootec. 41, 1313–1317. doi:10.1590/S1516-35982012000500032

Mullen, B. J., and Martin, R. J. 1992. The effect of dietary fat on diet selection may involve central serotonin. Ani. J. Physiol. 263, 559-563.

Namroud, N.F., Shivazad, M., Zaghari, M., 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poult. Sci. 87, 2250–2258. doi:10.3382/ps.2007-00499

NRC. 1994. Nutrient requirements of poultry. Washington DC.

Peganova, S., Eder, K., 2003. Interactions of various supplies of isoleucine, valine, leucine and tryptophan on the performance of laying hens. Poult. Sci. 82, 100–105. doi:10.1093/ps/82.1.100

Peisker, M., P. H. Simmins, H. Monge, and F. Liebert. 1998. Tryptophan as a feed intake stimulant. Feed Mix 6, 8–12.

Robbins, K.R., Saxton, a M., Southern, L.L., 2006. Estimation of nutrient requirements using broken-line regression analysis J. Anim. Sci. 84E, E155-E165.

Rojas, I.C.O., Murakami, A.E., Fanhani, J.C., Picoli, K.P., Barbosa, M.J.B., 2015. Tryptophan, threonine and isoleucine supplementation in low-protein diets for commercial laying hens. Semin. Ciências Agrárias 36, 1735-1744. doi:10.5433/1679-0359.2015v36n3p1735

Rostagno, H.S., Albino, L.F.T., Donzele, J.L., Gomes, P.C., Oliveira, R.F., Lopes, D.C., Ferreira, A.S., et al., 2011 Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. Viçosa: UFV.

SAS Institute. 2010. SAS/STAT User's Guide: Statistics. Version 9.2. SAS Inst. Inc., Cary, NC.

Tackman, J.M., Tews, J.K., Harper, a E., 1990. Dietary disproportions of amino acids in the rat: effects on food intake, plasma and brain amino acids and brain serotonin. J. Nutr. 120, 521–33.

Wiltafsky, M.K., Bartelt, J., Relandeau, C., Roth, F.X., 2009a. Estimation of the optimum ratio of standardized ileal digestible isoleucine to lysine for eight- to twenty-five-kilogram pigs in diets containing spray-dried blood cells or corn gluten feed as a protein source. J. Anim. Sci. 87, 2554–2564. doi:10.2527/jas.2008-1320

Wiltafsky, M.K., Schmidtlein, B., Roth, F.X., 2009b. Estimates of the optimum dietary ratio of standardized ileal digestible valine to lysine for eight to twenty-five kilograms of body weight pigs. J. Anim. Sci. 87, 2544–2553. doi:10.2527/jas.2008-1221

Wiltafsky, M.K. 2009. Isoleucine and valine requirements of piglets and activity and gene expression of key enzymes of the branched-chain amino acid metabolism in response to dietary leucine excess. Ph.D thesis, 140p., Technische Universität München, München, Germany.

Table 1- Composition and nutritional content (%) of experimental diets

Ingredients	Summit			N-free
	Isoleucine	Valine	Tryptophan	
Corn (8.5%)	429	429	493	-
Wheat bran	126	126	-	-
Soybean meal (45%)	254	254	278	-
Maize gluten meal 60%	-	-	80.0	-
Sugar	-	-	-	150
Limestone	92.1	92.1	91.3	87.5
Maize starch	-	-	-	556
Soybean oil	60.0	60.0	25.3	30.0
Dicalcium phosphate	14.5	14.4	16.2	19.5
Rice husk	-	-	-	139
Salt	5.10	5.10	5.10	5.10
L-Lysine- HCl (78%)	3.90	3.90	2.70	-
DL-Methionine (99%)	5.10	5.10	3.50	-
L-Threonine (98,5%)	2.90	2.90	1.50	-
L-Valine (98%)	3.10	1.70	1.20	-
L-Tryptophan (98%)	0.60	0.60	0.20	-
L-Arginine (98%)	0.40	0.40	-	-
L-Isoleucine (99%)	0.60	1.70	-	-
Premix[1]	1.00	1.00	1.00	1.00
Potassium chloride	-	-	-	10.7
Choline chloride (60%)	1.00	1.00	1.00	1.00
Antioxidant	0.10	0.10	0.10	0.10
Calculated composition				
Crude Protein	182	188	221	0.85
Metabolizable energy (kcal/kg)	2.85	2.85	2.85	2.85
Dig. Met + Cys	9.90	9.90	10.2	0.02
Dig. Methionine	7.00	7.10	6.60	0.01
Dig. Lysine	11.4	11.5	11.8	0.02
Dig. Threonine	8.80	8.70	9.20	0.19
Dig. Tryptophan	2.50	2.60	2.30	-
Dig. Isoleucine	7.90	8.50	9.00	0.01
Dig. Valine	11.2	9.60	11.3	0.03
Dig. Leucine	14.3	14.1	21.7	0.03
Calcium	39.0	39.0	39.0	39.0
Available phosphorus	3.50	3.50	3.50	3.50
Sodium	2.20	2.20	2.20	2.20
Potassium	7.20	7.20	6.00	5.60

[1]Supplied per kg total diet: folic acid 2400 mg; pantothenic acid 30 g; biotin 160 mg; butylated hydroxytoluene 100 mg; niacin 84 g; Se 600 mg; Vit A 200000000 UI; Vit B1 5000 mg; Vit B12 36000 mcg; Vit B2 1300 g; Vit B6 7000 mg; Vit D3 5000000 UI; Vit E 375000 UI; Vit K3 4000 mg; Mn 150 g; Zn 140 g; Fe 90; Cu 15g; I 15 mg.

Table 2 – Graded levels of isoleucine, valine and tryptophan (g/kg) dilution series (%).

Levels	Isoleucine (g/kg)	Valine (g/kg)	Tryptophan (g/kg)	Summit (%)	N-free (%)
L1	2.80	3.56	0.81	35	65
L2	3.64	4.58	1.06	46	54
L3	5.11	6.35	1.50	65	35
L4	6.34	7.83	1.86	81	19
L5	6.95	8.58	2.05	89	11
L6	7.80	9.60	2.30	100	0
L7*	3.64[1]	4.58[2]	1.06[3]	35	65

*Counter-proofs diets (L7). [1]L1 + 0.84 $g.kg^{-1}$ of L-isoleucine; [2]L1 + 1.02 $g.kg^{-1}$ of L-valine. [3]L1 + 0.25 $g.kg^{-1}$ of L-tryptophan.

Table 3 – Means and standard deviation of feed intake (FI), amino acid intake (AAi), egg production (EP), egg weight (EW), egg output (EO) and body weight (BW) of laying hens fed diets with increasing levels of isoleucine, valine and tryptophan.

	Levels ($g.kg^{-1}$)	FI ($g.dia^{-1}$)	AAi ($g.dia^{-1}$)	EP (%)	EW (g)	EO (g)	BW (g)
				Valine			
L1	3.56	59.6±9.3	182.3±28.4	38.9±9.7	47.2±3.6	18.3±4.7	1128±237
L2	4.58	67.4±27.8	275.7±113.6	56.3±12.5	51.5±3.0	29.2±7.8	1198±195
L3	6.35	101.5±18.5	587.9±107.0	91.1±9.3	56.7±2.6	51.6±5.2	1422±177
L4	7.83	102.9±8.3	736.0±59.1	96.8±3.1	61.3±2.9	59.3±3.0	1480±122
L5	8.58	103.8±5.2	815.1±41.2	96.8±4.6	61.0±3.1	59.0±3.2	1475±148
L6	9.60	96.7±9.1	855.5±80.3	94.6±7.2	60.7±2.6	57.4±4.6	1426±115
L7*	4.58*	69.2±17.7	296.9±74.9	36.6±14.2	52.5±3.1	19.1±7.4	1153±148
Mean		88.7±19.8	575.6±285.0	79.1±25.1	56.4±5.9	45.8±17.6	1354±159.0
P>F		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
				Isoleucine			
L1	2.80	61.0±12.1	149.4±29.7	30.9±15.6	49.6±2.8	15.2±7.5	1124±81
L2	3.64	76.5±13.3	250.3±43.4	71.1±14.7	51.7±2.9	36.7±7.2	1250±156
L3	5.11	95.3±22.0	441.3±102.1	89.3±19.3	58.4±4.1	52.6±12.9	1402±253
L4	6.34	103.4±9.6	591.2±55.1	96.4±3.6	60.4±3.1	58.2±3.0	1511±152
L5	6.95	100.6±5.9	633.0±37.4	97.9±2.5	60.9±3.0	59.5±2.7	1500±110
L6	7.80	101.7±8.3	720.9±58.6	97.5±4.8	61.1±3.3	59.5±3.1	1472±90
L7*	3.64*	71.6±12.4	234.0±40.5	35.7±12.7	50.8±2.6	18.3±6.9	1239±178
Mean		89.7±17.2	464.4±226.2	80.5±26.3	57.0±5.1	46.9±17.8	1376±156.7
P>F		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
				Tryptophan			
L1	0.81	67.8±10.5	50.3±7.8	30.3±8.4	55.5±2.3	16.8±4.7	1208±106
L2	1.06	72.9±11.5	72.2±11.4	49.1±12.9	59.2±2.6	29.1±7.9	1220±78
L3	1.50	105.1±12.7	147.3±17.9	95.8±4.2	58.2±3.9	55.7±4.0	1483±157
L4	1.86	103.0±8.4	178.4±14.5	98.8±1.8	60.9±4.3	60.2±4.8	1500±88
L5	2.05	101.3±10.2	192.7±19.4	98.8±2.5	59.2±3.9	58.5±4.0	1436±80
L6	2.30	104.7±9.8	224.6±21.1	97.9±2.5	61.1±3.3	59.7±3.5	1479±153
L7*	1.06*	68.3±8.8	67.6±8.7	30.6±8.8	57.3±3.1	17.6±5.3	1221±103
Mean		89.0±17.2	133.3±69.2	71.6±30.6	58.7±2.1	42.5±18.8	1364±136.4
P>F		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

P= value associated with probability; F= f-test value;; *counter-proof diet

Table 4 – Equations for egg output (EM) according to isoleucine, valine and tryptophan intake of commercial laying hens.

Model	Equation	Intake	R^2
	Valine		
BL	$Y = 58.86 - 0.0775\ (679.3 - Val)$	679	0.96
PQ	$Y = -2.592 + 0.136\ Val – 0.00007304Val^2$	931	0.96
BL + PQ	$58.86 = -2.592 + 0.136\ Val – 0.00007304Val^2$	771	-
	Isoleucine		
BL	$Y = 58.83 - 0.1895\ (376.4 - Ile)$	376	0.96
PQ	$Y = -13.589 + 0.2348\ Ile – 0.00018535\ Ile^2$	633	0.94
BL + PQ	$58.83 = -13.589 + 0.2348\ Ile – 0.00018535\ Ile^2$	531	-
	Tryptophan		
BL	$Y = 59.41 - 0.408\ (150.6 - Trp)$	150	0.92
PQ	$Y = -11.5223 + 0.6663\ Trp – 0.0015256\ Typ^2$	218	0.91
BL + PQ	$59.41 = -11.5223 + 0.6663\ Trp – 0.0015256\ Typ^2$	184	-

R^2- coefficient of determination; BL – broken-line; PQ - polynomial quadratic; LP+PQ- first intercept of the PQ with the BL.

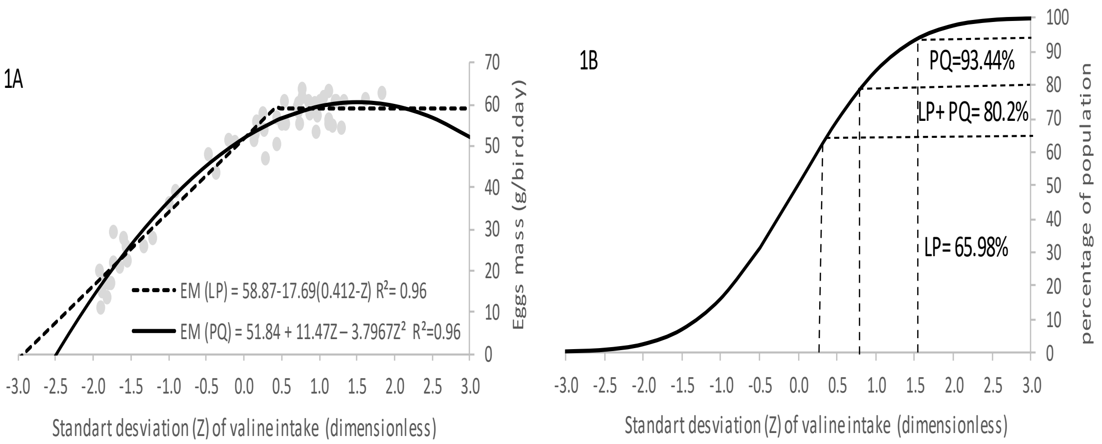

Figure 1A relationship between egg output (g/bird/day) and standard normal deviation of SID Val intake of commercial laying hens. Figure 1B relationship between standard normal deviation of SID Val intake and percentage of the population served by each model.

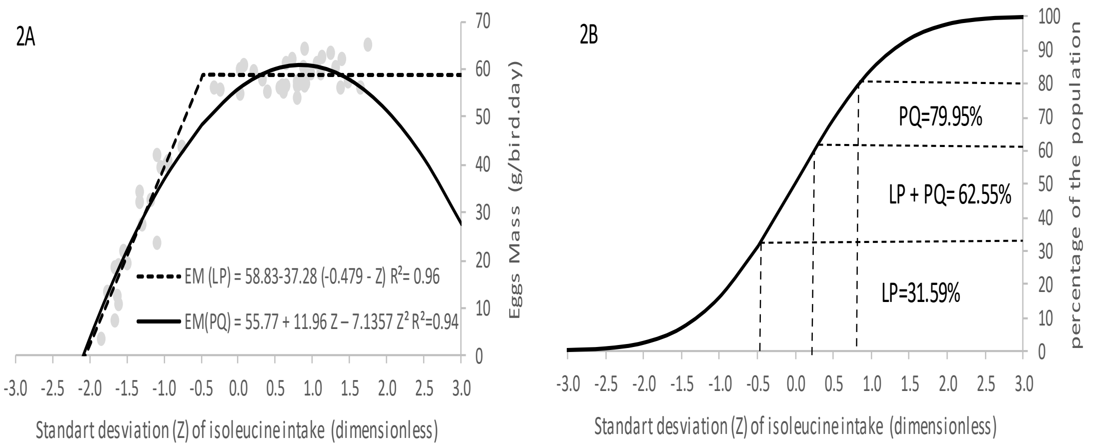

Figure 2A relationship between egg output (g/bird/day) and standard normal deviation of SID Ile intake of commercial laying hens. Figure 2B relationship between standard normal deviation of SID Ile intake and percentage of the population served by each model.

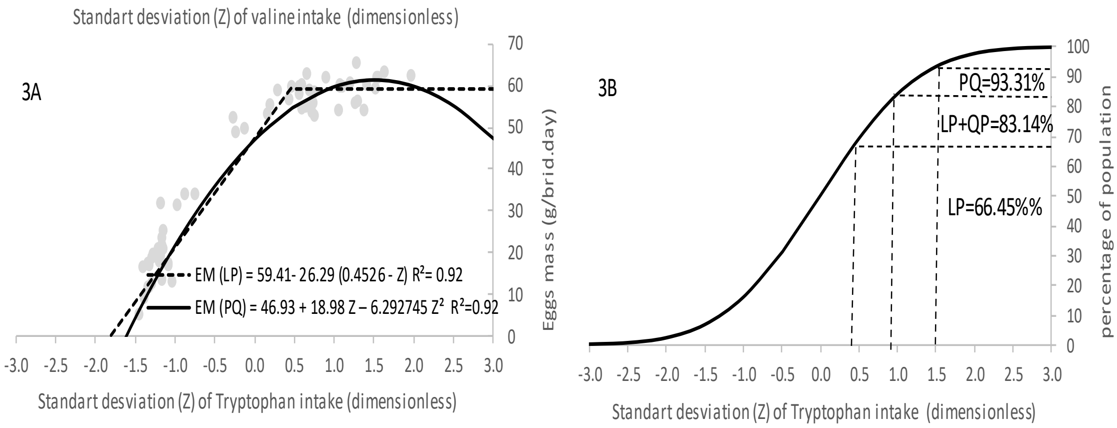

Figure 3A relationship between egg output (g /bird/day) and standard normal deviation of SID Trp intake of commercial laying hens. Figure 3B relationship between standard normal deviation of SID Trp intake and percentage of the population served by each model.

CAPITULO 3 - The optimal isoleucine, valine and tryptophan intake for laying hens using Reading model

Abstract

1. The optimal biological and/or economic intakes of isoleucine (Ile), valine (Val) and tryptophan (Trp) were determined using two hundred ten 29-week-old Hisex White hens in three fedding trials (70 hens/trials).
2. In each trial, ten individual replicates were randomly assigned to six dietary treatments obtained using the dilution technique to produce a range in dietary levels of Ile (2.80 to 7.80 g/kg), Val (3.56 to 9.60 g/kg) and Trp (0.81 to 2.30 g/kg). A seventh diet was formulated for each 10-week feeding trial, in order to confirm if the amino acid test was the first-limiting. After a six-week adaptation period, the data collection was carried out for four weeks.
3. Egg output, amino acid intake and body weight data were analyzed as one-way ANOVA. The results were also analyzed according to the Reading model, thereby taking into account the mean performance and the variation of individuals about this mean.
4. The estimated coefficients for egg output production (*a*) were 7.23; 9.83 and 2.28 mg/g egg for Ile, Val and Trp, respectively. The efficiency of utilization was 82% for Ile, 75% for Val and 85% for Trp. The maintenance coefficients (*b*) for Ile, Val and Trp were 13.20; 0.03 and 7.48 mg/kg of BW.
5. Considering the current economic scenario, the optimal economic levels of Val and Trp recommended by the Reading Model were 852 and 195 mg/hen/day for 29-to-39-week-old commercial laying hens, respectively.

Keywords: amino acid, dilution technique, egg output, optimal economic intake, maintenance, requirement

Introduction

From the sixties of last century onward, increasing emphasis has been placed on the establishment of the minimum levels of essential amino acids in poultry diet formulation. Linear programming techniques have shown that either dietary protein level or the cost of practical diets are very affected by the assumptions made about poultry requirement for some essential amino acids (Fisher and Morris, 1970). In this context, adjusting the level of each nutrient so that profit margins can maximized instead of aiming to achieve maximum productivity can be an interesting approach for modern egg production industry. This implies abandoning the idea of a " fixed " requirement for a nutrient and replacing it by data relating rates of output to levels of input (Fisher and Morris, 1970; Fisher *et al.,* 1973; McDonald and Morris, 1985; Gous *et al.,* 1987; Huyghebaert *et al.,* 1991).

The Reading Model (Fisher *et al.,* 1973) is a good starting point to understand the dynamic among nutrition, individual, population, cost and income (Silva *et al.,* 2015a,b,c; Gous, 2016). However, firstly it is necessary to describe the response curves of birds to Ile, Val and Trp intakes to estimate the Reading Model coefficients and only after that, it would be possible to estimate the optimal economic intakes of such amino acids according to the animal production, population variability and the desired cost-benefit. Thereby, this model is presented as a valuable tool to reduce feed costs and hence increase profitability considering several production and economic scenarios, specifically, for Val, Ile and Trp that can be limiting in layer low-protein diets.

Thus, prior to decreasing protein content in layer diets with a view to mitigate environmental nitrogen excretion, it is crucial to understand to how what extent varies the requirements of the individuals with the lower to the higher genetic potential (Huyghebaert *et al.,* 1991). Based on the price of feed and the final product, in this case eggs, the Reading model could estimate the most economic amino acid intake. Given such background, the objectives of the present study were to estimate the optimal economic intakes of Ile, Val and Trp for commercial laying hens and to simulate optimal economic levels using the Reading Model taking into account different production and market conditions.

Materials and methods

Bird husbandry

The trials were performed in the Poultry Science Laboratory at São Paulo State University, UNESP-Jaboticabal, São Paulo, Brazil. All experimental proceedings were approved by the Ethics Committee on the use of animals of UNESP (CEUA) under protocol n° 009999/14.

Three simultaneous feeding trials (one for each amino acid) were performed environmentally controlled facility equipped with negative pressure system to maintain the environmental temperature in thermoneutral zone. A total of 70 Hisex White hens weighing about 1.4 kg, from 29 to 39 weeks of old, were used in each trial. The birds were weighed individually, and the egg production was recorded prior to the beginning of the experiment, so that initial body weight and egg production rate were similar among treatment groups. The birds were housed in stainless steel cages (50 × 45 × 40 cm), equipped with individual trough feeders and nipple drinkers. The management and light program (16L:8D) were adopted according to the Hisex White strain guideline recommendations.

Experimental design

It was used a completely randomized design with six treatments and ten individual replicates. Treatments consisted of six increasing levels of the test amino acids (Ile, Val and Trp). The seventh diet of each amino acid (counter-proof diet) was formulated to confirm whether the test amino acid was the first limiting amino acid in diets and as a way of validating that the responses occurred as a function of the test amino acid, regardless of the crude protein content of diets (Fisher and Morris, 1970; Gous and Morris, 1985).

Experimental Diets

The experimental diets were formulated using the dilution technique (Fisher and Morris, 1970). Two diets were formulated, a high-protein diet (summit diet) and a nitrogen-free diet. These diets were formulated to be isoenergetic and isonutritives, except for protein and amino acids contents (Table 1).

The nutritional requirements used to formulate both diets were those described by Rostagno *et al.* (2011). The summit diets were formulated to exceed in 30% higher the test amino acid recommendation and 50% the other amino acids, resulting in a minimum relative deficiency of the amino acid test of 20%. Each summit diet was diluted sequentially with nitrogen-free diet to produce diets containing graded levels of Ile, Val and Trp (Table 2).

Data collection

Each trial lasted 10 weeks. The birds were submitted to a six-week adaptation period followed by a data collection period of four weeks (Fisher and Morris, 1970). The feed leftovers were weighed weekly to calculate the food intake. Eggs were collected and weighed daily. The body weight was measured at the first, sixth and tenth week of the experimental period. Food intake was calculated by the difference between the amount of feed provided and its leftovers. The egg output was obtained by multiplying the egg production by egg weight.

Statistical analysis

Collected performance data were analyzed as one-way ANOVA. The software program EFG (2006) was used in the amino acid optimize module in order to generate the coefficients for the adjustment of the Reading Model for each amino acid. The equation below represents the Reading Model and estimates the optimal economic intake for different production scenarios using six parameters (Fisher *et al.,* 1973):

$$\text{Aopt} = a\,\bar{E}0_{\text{max}} + b\,\overline{BW} + Z\sqrt{a^2\,\sigma^2\overline{EO}_{\text{max}} + b^2\,\sigma^2\overline{BW}}$$

where:

Aopt is the optimal economic intake estimated (g/day);

a = mg amino acid per g egg output (mg/g);

b = maintenance requirement or mg amino acid per unit body weight (mg/kg);

$\overline{EO}_{max}$ = estimated maximum potential egg output of the flock (g/day);

$\overline{BW}$ = average body weight (kg),

$\sigma^2 EO_{max}$ = variance of maximum egg output,

$\sigma^2 BW$ = variance of body weight;

rEW = correlation between maximum egg output and body weight was considered as null in this analysis, as proposed by Fisher *et al.* (1973).

Z = deviation of a standard normal curve which cuts off an area equal to *ak* in one tail, where *k* = marginal cost of 1 mg amino acid input/marginal value of 1 g egg output. Where *Z* indicates the standard deviation of the normal curve that determines an area *ak* in a tail and it is expressed as the standard unit obtained from normal distribution tables (it is the *Z* value of the standard normal distribution, corresponding to the value of the expression (1-*ak*) The product of *Z* by the deviation of the individual requirements correspond to the value that represents the distance *y* that should be added to the average requirement (Pilbrow and Morris, 1974).

Results

Effect of amino acid supply on rate of laying, egg weight and body weight

Laying hen performance responses to Ile, Val, and trp intake are detailed in table 2. Hens fed counter-proof diets exhibited improvements in performance traits compared with those fed the lowest levels of the amino acids under study, which confirms that indeed all the amino acids were the first limiting in their respective trials. Regardless of the feeding trial, all the performance traits assessed herein were impaired as the dietary level of the amino acid under study decreased ($p<0.05$). As dietary amino acid content decreased, the rate of laying was impaired in greater extent compared with egg weight. The average initial body weight of hens at the beginning of the trials was 1.4 kg. By the end of the period, body loss in hens fed the lowest level of Ile, Val, and trp was 20% in both Ile and Val trials, and 14% in Trp trial.

Egg output response to isoleucine, valine and tryptophan

The Reading Model was adjusted using amino acid intake, body weight and egg output data. Parameters used to fit the curves were: for both amino acids: EO_{max} = 58.5, 58.8, and 59.4 g/bird/day for Ile, Val, and Trp; σEOmax = 15 g/bird/day for both Ile and Val, and 12 g/bird/day for Trp; BW = 1.36 kg and σBW = 0.13 kg for all amino acids; and rEW = 0.0. The equations for an individual hen derived from this experiment were:

Isoleucine intake (mg/hen d) = 7.23 EO_{max} + 13.2 B*W*,

Valine intake (mg/hen d) = 9.83 EO_{max} + 0.03 B*W*, and

Tryptophan intake (mg/hen d) = 2.28 EO_{max} + 7.48 B*W*,

where EO_{max} = maximum egg output, g/hen d, and BW = body mass, kg/hen.

Optimum economic intake of amino acids

These calculations have been repeated using values for *a* and *b* obtained in the present analyses, assuming the standard deviations of 15 g/hen d for egg output and 0.13 kg for body weight per hen. Considering the current scenario, Val and Trp are sold for 7.30 and 12.00 dollars per kg, respectively, whilst the average selling price of a 60 g egg, in December 2017, was about $ 0.062 in the State of São Paulo (Agrolink, 2017). The optimal economic intake of Val and Trp were calculated using the prices specified above and coefficient values resulted from this study, except for the maintenance coefficients *b,* which did not result in coherent values, and thus, were replaced by the values determined by Lima et al. (2016). If rEW is assumed to be zero, this standard value is converted to real units. The standard deviation (**SD**) of individual bird requirements is obtained extracting the square root of the variance of ($a^2\ \sigma^2 EO_{max} + b^2\ \sigma^2 BW$). The SD obtained for Ile, Val and Trp was 108, 147 and 34 mg, respectively. The numerical value of *Z* was obtained from normal curve table, by finding the value *Z* such that an area (1-*a*k), where a = 9.83 Val and 2.28 Trp and cost-benefit ratios: k = 0.0071 for Val and k = 0.0116 for Trp. The *Z* obtained for Val and Trp were 1.48 and 1.93, respectively. Considering the coefficients and variances given above, the equation 1 was reduced to:

Isoleucine intake (mg/hen d) = 7.23 EO_{max} + 20 B*W* + 108 *Z*

Valine intake (mg/hen d) = 9.83 EO_{max} + 41 B*W* + 147 *Z*

Tryptophan intake (mg/hen d) = 2.28 EO_{max} + 5 B*W* +34 *Z*

For Val, the optimum intake calculated was 852 mg/hen d for laying hens with B*W* of 1.36 kg, EO_{max} of 58.8 g/hen d and k of 0.0071. Finally, the estimated Trp requirement for laying hens with B*W* of 1.36 kg, EO_{max} 59.4 g/hen d and k of 0.0116 was 195 mg/hen d.

The simulations, which estimate the optimum economic intake for hen flocks considering the potential rate of egg output, mean body weight and the cost ratio of amino acid input and egg output are demonstrated in Table 4. In order to simulate different conditions, the body weight of the birds varied from 1.3 to 2.0 kg and EO from 50 to 60 g/hen d and k from 0.005 to 0.009. According to the simulation results, it is possible to observe that as body weight increases, the estimated optimal intake also increases due to the influence of maintenance requirement, and that as amino acid price decreases there is an increment in the recommendation of optimal economic dose.

Discussion

Laying hen responses to amino acid intake

The key prerequisite of any dose-response trial is to confirm weather the nutrient under study is indeed limiting in feed (Bowmaker and Gous, 1991). In the current research, in each feeding trial, an additional diet, herein named counter-proof treatment, was formulated to test and validate such assumption. Based on the improvements in hen performance traits due to the supplementation of L-Isoleucine, L-Valine, and L-Tryptophan in the diets where these amino acids were supplied at the lowest level, we could conclude that Ile, Val, and Trp were indeed the first-limiting ones. We noticed that laying hens fed diets marginally deficient in amino acids exhibited a depression in voluntary food consumption compared with birds fed diets containing the highest amino acid supplies. Such outcomes support previous findings that as diets become poorer in nitrogen; hens tend to decrease food consumption (Bowmaker and Gous, 1991; Bendezu *et al.,* 2015; Silva *et al.,* 2015a; Silva *et al.,* 2015b). According Bowmaker and Gous (1991), these more diluted diets have lower amino acid concentrations but similar energy concentrations to the diets containing higher amino acid content, so the amount of energy consumed by the birds fed these diets is excessive to support a low egg output possible on diets with such low amino acid concentrations. In this case, the dietary energy: protein ratio provides a higher energy intake to support an egg output production, which does not exist. Thereby, biological mechanisms to reduce food consumption are activated as way to equilibrate the amount of energy consumed. A progressive increasing loss in body weight was observed as dietary amino acid concentration decreased. The observed reduction in body weight (Table 3) can be attributed to the mobilization of bodily protein reserves, which may have occurred to meet the maintenance requirement and support a minimum rate of laying and egg weight that the diets whose protein and amino acid content were not capable of supporting. Such outcomes may be also supported by previous studies (Bowmaker and Gous, 1991; Bendezu et a., 2015; Silva *et al.,* 2015a; Silva *et al.,* 2015b). The egg weight decreased at lower concentration of Ile, Val and Trp, but in smaller proportions compared to that observed for rate of laying. According to Bowmaker and Gous, (1991), the smaller reduction in egg weight has an ecological basis and may be attributed to the need to ensure minimum conditions for embryo survival.

Maintenance and egg output coefficients to estimate hen Ile, Val, and Trp intake

Reading model is a flexible approach used to estimate either the amount of amino acid required by the average individual based on biological aspects or the amount required for optimum economic return. In so doing, the model encompasses in its structure a part in which the coefficients for maintenance (*b*) and egg output (*a*) estimate the requirements for amino acids based on body weight and egg output values; and a second part in which body weight and egg output variation, associated with the an economic factor (k) are taken into account to estimate the optimum economic intake. The *a* coefficient estimates the amount of amino acid required to produce 1 g of egg output, whilst the *b* coefficient is an estimate of the daily maintenance requirement of the hen in mg amino acid per kg body weight. In the current research the *a* coefficients estimated for Ile, Val, and Trp models were 7.23, 9.83, and 2.28 mg/g EO/day, respectively. Comparing these values with those reported by Huyghebaert et al. (1991) for Ile (8.4 mg/g EO/day), McDonald and Morris, (1985) for Val (8.9 mg/g EO/day), and Wethli and Morris, (1978) for Trp (2.18 mg/g EO/day) we notice narrow differences in the coefficients, which may confirm the reliability of our estimates.

Conversely, our responses did not provide realistic coefficients of maintenance (*b*), whose values for Ile, Val, and Trp were 13.2, 0.03, and 7.48 mg/kg BW, respectively. Pilbrow and Morris, (1974) highlighted the high correlation between the coefficients *a* and *b* of the Reading model, which indicates that *b* values must be judged considering the values of *a* (Wethli and Morris, 1978). In our research, we found acceptable values for *a* coefficients, which indicates that theoretically *b* coefficient should have the same biological meaning. However, we found *b* values that cannot be biologically condoned when a comparison is made with published literature, except for Trp whose estimate of 7.48 mg/kg BW are close to the 8.13 mg/kg BW described by Wethli and Morris, (1978). The *b* coefficient obtained here for Ile (13.2 mg/kg) was aproximetly 2 times greater than the 7.1 mg/kg BW reported by Huyghebaert *et al.* (1991), whilst our *b* for Val (0.03 mg/kg) beyond exhibiting values approximately 7 times lower the 7.6 mg/kg BW reported by McDonald and Morris, (1985), was almost equivalent to zero. In a review, Sakomura et al. (2015) highlithed that the Reading model is not the ideal approach for determining maintenance requirement for amino acids, whose estimates, according to Burham and Gous, (1992) must be measured independently of egg production. Thus, in order to perform the simulations below described, the *b* coefficients estimated in this study were replaced to those determined by Lima et al. (2016) using adult cockrels. Such authors described the coefficients for maintenance of Ile, Val, and Trp in 20, 41, and 5 mg/kg BW, respectively.

Requirements based on biological and economic aspects

A simulation introducing the information regarding body weight and egg mass described in Hisex guideline as inputs in our models was used to compare how narrow the our estimates were from those published in literature. In this case, the feeding table chosen as reference was the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017). Our estimates are contrasted with the requirements in the Table 3. We noticed that regardless of the age, our estimates for optimum Ile, Val, and Trp intake for 22-to-56-week-old laying hens were approximately 30, 17, and 27% lower than those described by Rostagno et al. (2017). Such discrepancies may be attributed to the way that the estimates were obtained. The same inputs used in our models were introduced in the following factorial model for lysine described by Rostagno et al. (2017): Lys (g/bird/day) = 0.07 x $BW^{0.75}$ (kg) + 0.020 x BWG (g/bird/day) + 0.0124 EO (g/bird/day).

Since the latter authors do not describe factorial models for the amino acids studied herein, we used the ratios of Ile, Val, and Trp relative to Lys recommended in Brazilian Tables for Poultry and Swine in order to estimate the optimum intake the these amino acids (Table 3). Although Lys intake for laying hens has been originated from a factorial model that as ours encompass body weight and egg output, the final estimates for Ile, Val, and Trp were produced from optimum amino acid to Lys ratios, which were obtained from models, which estimate doses that optimize the performance traits assessed such as polynomial quadratic model. Hauschild et al (2010) have demonstrated that this model produces estimates that feed individuals with a higher genetic potential than the average ones, i.e. with higher requirements, whilst factorial models estimate requirements for the average individuals.

The second part of Reading model takes into account the cost-benefit ratio between the price of input (L-amino acid price) and output (egg price) and the inherent variability which exist among individuals, being classified as an stochastic model due to the latter characteristic. The SD of individual bird requirements is obtained by extracting the square root of the variance of (a^2 $\sigma^2 EO_{max}$ + b^2 σ^2BW), and depends on parameters such as σEO_{max} and σBW, whose values were affected by genetic selection due to the increase of EO_{max} and the decrease in *BW*. In the past, EO_{max} was approximately 50 g/bird/day and the CV was assumed to be 30%, which therefore resulted in a σE_{max} equivalent to 15 g (Morris and Wethli, 1978; Wethli and Morris, 1978; McDonald and Morris, 1985; Huyghebaert *et al.,* 1991). The current genetic potential of hens determined in our trials for EO_{max} were 58.5, 58.8, and 59.4 g/bird/day in Ile, Val, and Trp trials, respectively, whilst B*W* was determined as 1.36 kg. The SD of observed for EO_{max} and *BW* were 15 g/bird/day and 0.13 kg for all amino acids, respectively.

In the current study, the amino acids under study have a higher cost than the conventional ones such as lysine, methionine, and threonine. The simulations of different economic scenarios exhibited the same trend for all the amino acids, regardless of the BW and EO values used: the estimated optimum economic amino acid intake decreased as the ratio of marginal cost to marginal revenue increased (Table 4). It is worth mentioning that due to Ile is not sold as feed-grade crystalline amino acid, estimating its optimum economic intake could not represent a practical reality, and for such reason only for Val and Trp are taken into account to perform the simulations described in Table 4. Optimum economic amino acid intake is dynamic and varies with the body weight, potential egg output and also with of the cost-benefit ratio changes, which hence becomes necessary the need to recalculate this optimum (Bendezu *et al.,* 2013). McDonald and Morris (1985) emphasized that the optimum economic has been calculated assuming that the concentration of the relevant amino acid is limiting for egg output. If this condition is not fulfilled, maximum profit will not necessarily be obtained.

Under a biological point of view, optimum performance will be obtained by supplying a dietary amino acid pattern, which ensure optimal protein synthesis rates. Usually, the optimum dose of such amino acids are determined using empirical deterministic models, whose focus is to determine the dose, which will optimize performance trait assessed. Although widely used, such approach ignores that in population there are individuals, whose genetic potential are superior, and therefore may respond to an extra intake of amino acids. Rostagno *et al.* (2017) recommend a daily intake of Val and Trp of 773 and 191 mg/bird/day of Val and Trp respectively, for hens weighing 1.35 kg and with egg output production of 59 g/day. Taking into account the same genetic potential and based on the current market situation (k = 0.007), i.e. the current price of L-amino acids and the price of the eggs, we estimated a higher optimal Val intake (852 mg/bird/day) compared with the aforementioned authors. The Trp requirements estimated by Rostagno et al., (2017) were close to the estimated in this study, and it were close to the optimal economic intakes. Based on the values of the normal standard deviation Z (1- ak), we observed that the optimal economic intake of Val and Trp meet the requirements of 93 and 97% of the population.

There is some important point in increasing dietary amino acid supply to meet the requirement of birds whose potential is greater than the mean. By choosing feed birds, whose genetic potential are lower than those with the highest one, the nutritionist will make the superior hen eat more in order to achieve its genetic potential. The main implication from this, it is that such hen will not perhaps be able to consume such additional amount of feed. A solution for such dilema is deciding which bird to feed based on economic aspects. Such decision depends on the cost of the extra dose of the amino acid, the number of birds that will benefit from the higher level and the reduction in food intake that would result. Among such three factors that should be considered, the Reading model takes into account the first two, indicating therefore its applicability in an economic scenario. As demonstrated by our study, which in turn support years of other many researches, Reading model has proved to be a useful to fulfill such purpose. Although in a very recent future, our estimates of the optimal economic intake of Val and Trp will be not representative due to a different market scenario. However, using the coefficients determined herein and introducing the price of eggs and amino acid, as well as the variability in egg output and body weight, which represent better its reality, a nutritionist can easily use Reading model to optimize hen egg production.

Acknowledgements

The authors thank the São Paulo Research Foundation – FAPESP (BRAZIL) for financial support grant number n° 2016/14754-5 and 2013/25761-4 and Ajinomoto Biolatina and Evonik Industries for donating the amino acids used in the current research and for the analysis of amino acid content in diets.

References

Bendezu, H.C.P., Sakomura, N.K., Veturine, K.S., Sato, J., Hauschild, L., Malheiros, E.B. & Gous, R.M. (2013). Response of laying hens to amino acid intake. In: International Symposium: Modelling In Pig And Poultry Production, Jaboticabal- SP..Compact Disc.

Bendezu, H.C.P., Sakomura, N.K., Hauschild, L., Silva, E.P., Dorigam, J.C.P., Malheiros, E.B. & Fernandes, J. B. K. (2015) Response of laying hens to methionine + cystine intake by dilution technique. *Revista Brasileira de Zootecnia,* **44:** 15-21, DOI:10.1590/S1806-92902015000100003

Bowmaker, J.E., & Gous, R.M. (1991) The response of broiler breeder hens to dietary lysine and methionine. *British Poultry Science,* **32:** 1069-1088, DOI: 10.1080/00071669108417430.

Burnham, D., Gous, R.M., Emmans, G.C. (1992) Isoleucine requirements of the chicken: The effect of excess leucine and valine on the response to isoleucine. *British Poultry Science,* **33**: 71–87. Doi:10.1080/00071669208417445

EFG Software, (2006) www.efgsoftware.net (accessed September 2017)

Fisher, C., Morris. T.R. & Jennings, R.C. (1973) A model for the description and prediction of the response of laying hens to amino acid intake. *British Poultry Science*, **14:**469–484. DOI: 10.1080/00071667308416054.

Fisher, C., & Morris, T.R. (1970) The determination of the methionine requirement of laying pullets by a diet dilution technique. *British Poultry Science*, **11**: 67–82. DOI: 10.1080/00071667008415793.

Gous, R. M. (2016) The rewards of using a modelling approach in directing poultry research. *British Poultry Science*, **57:** 1- 11. DOI:10.1080/00071668.2015.1119244.

Gous, R. M., Griessel, M. & Morris, T.R. (1987) Effect of dietary energy concentration on the response of laying hens to amino acids, *British Poultry Science,* **28**: 427-436. DOI: 10.1080/00071668708416977.

Gous, R.M. & Morris, T.R. (1985) Evaluation of a diet dilution technique for measuring the response of broiler chickens to increasing concentrations of lysine. *British Poultry Science*, 26:2, 147-161. DOI 10.1080/00071668508416799.

Hauschild, L., Pomar, C. & Lovatto, P.A. (2010) Systematic comparison of the empirical and factorial methods used to estimate the nutrient requirements of growing pigs. *Animal: an international journal of animal bioscience*, 4: 714-723 DOI: 10.1017/S1751731109991546.

Huyghebaert, G., De Groote, G., Butler, E.A. & Morris. T.R. (1991) Optimum isoleucine requirement of laying hens and the efect of Age. *British Poultry Science*, **32:** 471–481. DOI: 10.1080/00071669108417372.

Lima, M.B., Sakomura, N.K., Dorigam, J.C.P., Silva, E.P., Ferreira. N.T. & Fernandes. J.B.K., (2016) Maintenance valine, isoleucine, and tryptophan requirements for poultry. *Poultry Science*, **95**: 842–850. DOI: 10.3382/ps/pev380.

McDonald, M.W. & Morris, T. R. (1985) Quantitative review of optimum amino acid intakes for young laying pullets. *British Poultry Science*, **26**: 253–264. DOI: 10.1080/00071668508416811.

Morris, T.R. & Wethli, E. (1978) The tryptophan requirements of young laying pullets, *British Poultry Science*, **19:** 455-466, DOI: 10.1080/00071667808416501.

Pilbrow, P.J. & Morris, T.R. (1974) Comparison of lysine requirements amongst eight stocks of laying fowl. *British Poultry Science*, **15**: 51–73. DOI: 10.1080/00071667408416081.

Rostagno, H.S., Albino, L.F.T., Hannas, M.I., Donzele, J.L., Sakomura, N.K., Perazzo, F.G., Saraiva, A., et al. (2017) Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. Viçosa: UFV.

Rostagno, H.S., Albino, L.F.T., Donzele, J.L., Gomes, P.C., Oliveira, R.F., Lopes, D.C., Ferreira, A.S., et al. (2011) Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. Viçosa: UFV.

Sakomura, N.K., Silva, E.P., Dorigam, J.C.J., Gous, R.M. & St-Pierre, N. (2015) Modeling amino acid requirements of poultry. *Journal of Applied Poultry Research,* **24**: 267–282. DOI: 10.3382/japr/pfv024

Silva, E.P., Malheiros, E.B., Sakomura, N.K., Venturini, K.S., Hauschild, L., Dorigam, J.C.P. & Fernandes, J.B.K. (2015) Lysine requirements of laying hens. *Livestock Science*, **173**: 69 –77. DOI: 10.1016/j.livsci.2015.01.005.

Silva, E.P., Sakomura, N.K., Hauschild, L. & Gous, R.M. (2015) Reading model to estimate optimum economic intakes of amino acids for poultry. *Ciência Rural*, **45:** 450 –457. DOI: 10.1590/0103-8478cr20120799.

Silva, E.P., Sakomura, N.K., Oliveira, C.F.S., Costa, F.G.P., Dorigam, J. C. P., Malheiros, E.B. (2015) The optimal lysine and threonine intake for Cobb broiler breeder hens using Reading model. *Livestock Science*, **174**: 59 –65. DOI: 10.1016/j.livsci.2015.01.009.

Wethli, E. & Morris, T.R. (1978) Effects of age on the tryptophan requirement of laying hens, *British Poultry Science*, **19**, 559-565, DOI: 10.1080/00071667808416513.

Table 1- Composition and nutritional content (%) of experimental diets*

Ingredients	Summit			N-free
	Isoleucine	Valine	Tryptophan	
Corn (8.5%)	429.2	429.3	492.6	-
Wheat bran	126.0	126.3	-	-
Soybean meal (45%)	254.4	254.3	278.3	-
Maize gluten meal 60%	-	-	80.0	-
Sugar	-	-	-	150.0
Limestone	92.1	92.1	91.3	87.5
Maize starch	-	-	-	556.1
Soybean oil	60.0	60.0	25.3	30.0
Dicalcium phosphate	14.5	14.4	16.2	19.5
Rice husk	-	-	-	139.0
Salt	5.1	5.1	5.1	5.1
L-Lysine- HCl (78%)	3.9	3.9	2.7	-
DL-Methionine (99%)	5.1	5.1	3.5	-
L-Threonine (98,5%)	2.9	2.9	1.5	-
L-Valine (98%)	3.1	1.7	1.2	-
L-Tryptophan (98%)	0.6	0.6	0.2	-
L-Arginine (98%)	0.4	0.4	-	-
L-Isoleucine (99%)	0.6	1.7	-	-
Vit. and Min. Supplement [1]	1.0	1.0	1.0	1.0
Potassium chloride	-	-	-	10.7
Choline chloride (60%)	1.0	1.0	1.0	1.0
B HT	0.1	0.1	0.1	0.1
Calculated compostion				
Crude Protein, g/kg	181.9	187.6	221.2	08.5
AMEn, kcal/kg	2.85	2.85	2.85	2.85
Digestible amino acids, g/kg				
Met + Cys	9.9	9.9	10.2	00.2
Methionine	7.0	7.1	6.6	00.1
Lysine	11.4	11.5	11.8	00.2
Calcium	39.0	39.0	39.0	39.0
Available phosphorus	3.5	3.5	3.5	03.5
Sodium	2.2	2.2	2.2	02.2
Potassium	7.2	7.2	6.0	05.6
Threonine	8.8	8.7	9.2	01.9
Tryptophan	2.5	2.6	2.3	-
Isoleucine	7.9	8.5	9.0	00.1
Valine	11.2	9.6	11.3	00.3
Leucine	14.3	14.1	21.7	00.3

*Analysed values. [1] Supplied per kg total diet: folic acid 2400 mg; pantothenic acid 30 g; biotin 160 mg; butylated hydroxytoluene 100 mg; niacin 84 g; Se 600 mg; Vit A 200000000 UI; Vit B1 5000 mg; Vit B12 36000 mcg; Vit B2 1300 g; Vit B6 7000 mg; Vit D3 5000000 UI; Vit E 375000 UI; Vit K3 4000 mg; Mn 150 g; Zn 140 g; Fe 90; Cu 15g; I 15 mg.

Table 2 – Means and standard deviation of feed intake (FI), amino acid intake (AAi), egg production (EP), egg weight (EW), egg output (EO) and body weight (BW) of laying hens fed diets with increasing levels of isoleucine, valine and tryptophan.

	Levels ($g.kg^{-1}$)	FI ($g.dia^{-1}$)	AAi ($g.dia^{-1}$)	EP (%)	EW (g)	EO (g)	BW (g)
				Isoleucine			
L1	2.80	61.0±12.1	149.4±29.7	30.9±15.6	49.6±2.8	15.2±7.5	1124±81
L2	3.64	76.5±13.3	250.3±43.4	71.1±14.7	51.7±2.9	36.7±7.2	1250±156
L3	5.11	95.3±22.0	441.3±102.1	89.3±19.3	58.4±4.1	52.6±12.9	1402±253
L4	6.34	103.4±9.6	591.2±55.1	96.4±3.6	60.4±3.1	58.2±3.0	1511±152
L5	6.95	100.6±5.9	633.0±37.4	97.9±2.5	60.9±3.0	59.5±2.7	1500±110
L6	7.80	101.7±8.3	720.9±58.6	97.5±4.8	61.1±3.3	59.5±3.1	1472±90
L7*	3.64*	71.6±12.4	234.0±40.5	35.7±12.7	50.8±2.6	18.3±6.9	1239±178
Mean		89.7±17.2	464.4±226.2	80.5±26.3	57.0±5.1	46.9±17.8	1376±156.7
P>F		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
				Valine			
L1	3.56	59.6±9.3	182.3±28.4	38.9±9.7	47.2±3.6	18.3±4.7	1128±237
L2	4.58	67.4±27.8	275.7±113.6	56.3±12.5	51.5±3.0	29.2±7.8	1198±195
L3	6.35	101.5±18.5	587.9±107.0	91.1±9.3	56.7±2.6	51.6±5.2	1422±177
L4	7.83	102.9±8.3	736.0±59.1	96.8±3.1	61.3±2.9	59.3±3.0	1480±122
L5	8.58	103.8±5.2	815.1±41.2	96.8±4.6	61.0±3.1	59.0±3.2	1475±148
L6	9.60	96.7±9.1	855.5±80.3	94.6±7.2	60.7±2.6	57.4±4.6	1426±115
L7*	4.58*	69.2±17.7	296.9±74.9	36.6±14.2	52.5±3.1	19.1±7.4	1153±148
Mean		88.7±19.8	575.6±285.0	79.1±25.1	56.4±5.9	45.8±17.6	1354±159.0
P>F		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
				Tryptophan			
L1	0.81	67.8±10.5	50.3±7.8	30.3±8.4	55.5±2.3	16.8±4.7	1208±106
L2	1.06	72.9±11.5	72.2±11.4	49.1±12.9	59.2±2.6	29.1±7.9	1220±78
L3	1.50	105.1±12.7	147.3±17.9	95.8±4.2	58.2±3.9	55.7±4.0	1483±157
L4	1.86	103.0±8.4	178.4±14.5	98.8±1.8	60.9±4.3	60.2±4.8	1500±88
L5	2.05	101.3±10.2	192.7±19.4	98.8±2.5	59.2±3.9	58.5±4.0	1436±80
L6	2.30	104.7±9.8	224.6±21.1	97.9±2.5	61.1±3.3	59.7±3.5	1479±153
L7*	1.06*	68.3±8.8	67.6±8.7	30.6±8.8	57.3±3.1	17.6±5.3	1221±103
Mean		89.0±17.2	133.3±69.2	71.6±30.6	58.7±2.1	42.5±18.8	1364±136.4
P>F		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

P= value associated with probability; F= f-test value;; *counter-proof diet.

Tabela 3 – Optimum intake of Ile, Val, and Trp according to different nutritional recommendations

Item	Parameters		Isoleucine (mg/bird/day)		Valine (mg/bird/day)		Tryptophan (mg/bird/day)	
Age (weeks)	BW[1] (kg)	EO[1] (g/bird/day)	FM[2]	BTPS[3]	FM[2]	BTPS[3]	FM[2]	BTPS[3]
22	1.51	42.0	334	481	475	573	103	142
28	1.62	56.8	443	628	625	748	138	185
34	1.65	58.2	454	642	640	766	141	189
40	1.66	57.8	451	639	636	762	140	188
46	1.67	57.6	450	637	635	760	140	188
52	1.67	57.1	446	633	630	754	139	187
56	1.68	56.5	442	627	624	748	137	185

[1]Genetic strain guideline, [2]Factorial model, [3]Brazilian Tables for Poultry and Swine (Rostagno et al., 2017)

Table 4. Optimal economic intakes of Val and Trp based on different economic scenarios

EO (g/bird/day)	BW (kg)	μ[1]	Cost-benefit ratio (k)		
Valine			**0.009**	**0.007**	**0.005**
			μ+y*z	μ+y*z	μ+y*z
50	1.3	545	744	764	789
	1.7	561	760	781	805
55	1.3	594	793	813	838
	1.7	610	810	830	855
60	1.3	643	842	863	887
	1.7	660	859	879	904
Tryptophan			**0.05**	**0.03**	**0.01**
50	1.3	121	162	171	189
	1.7	123	164	173	191
55	1.3	132	173	183	200
	1.7	134	175	185	202
60	1.3	143	185	194	212
	1.7	145	187	196	214

[1]μ= $a \times EOmax + b \times BW$; [2]Y=$\sqrt{a^2 \times \sigma^2 EOmax + b^2 \times \sigma^2 BW}$ and [3]k = cost-benefit ratio