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

MODELAGEM DAS EXIGÊNCIAS NUTRICIONAIS DE VALINA, LEUCINA E ISOLEUCINA PARA CODORNAS JAPONESAS NA FASE DE POSTURA

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Tese 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

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TÍTULO DA DISSERTAÇÃO: MODELAGEM DAS EXIGÊNCIAS NUTRICIONAIS DE VALINA, LEUCINA E ISOLEUCINA PARA CODORNAS JAPONESAS NA FASE DE POSTURA

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DADOS CURRICULARES DO AUTOR

KARLA NATHALY MEZA MARTINEZ – nascida na cidade de Pisco, estado de Ica – Perú, no dia 20 de outubro de 1988, filha de Carlos Pablo Meza Mozo e Rosa sabina Martinez Campos. Em fevereiro de 2009 ingressou no curso de zootecnia na Faculdade de Zootecnia da Universidad Nacional Agrária la Molina, Lima – Perú. Obtendo por esta instituição o título de Bacharel em Zootecnia em dezembro de 2014. Durante o período de graduação, foi bolsista na unidade experimental de aves sob orientação do Msc. Marcial Cumpa Garidia e do programa de Investigación y Proyección Social em Alimentos sob orientação do Msc. Victor Jesus Vergara Rubin. Em agosto de 2015, foi convidada pela professora Dra. Nilva Kazue Sakomura para o desenvolver um projeto de pesquisa em codornas Japonesas. Em março de 2016 iniciou o curso de Mestrado em Zootecnia na Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal sob orientação da Nilva Kazue Sakomura, sendo contemplado com bolsa de mestrado CAPES.

OFEREÇO...

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SUMÁRIO

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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.

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ABREVIATURAS

a – Ingestão de aminoácido por de massa de ovo; amino acid requirement for egg output

AAI – Ingestão de aminoácido; amino acid intake

AMEn – Energia metabolizável aparente corrigida para nitrogênio; nitrogen corrected apparent metabolizable energy

b – Ingestão de aminoácido para mantença; amino acid requirement for maintenance

BCAA – Aminoácido de cadeia ramificada; branched chain amino acid.

BW – Peso corporal; body weight.

BWG – Ganho de peso corporal; Body weight gain.

CP – Proteína bruta; crude protein.

CV – Coeficiente de variação; coefficient of variation.

DF – Graus de liberdade; degree of freedom.

EO – Massa de ovo; egg output.

EP – Produção de ovos; egg production

EW – Peso de ovo; egg weight.

FI – Consumo de ração; Feed intake.

Ile – Isoleucina; Isoleucine.

k – eficiência de utilização; efficiency of utilization.

Leu – Leucina; Leucine.

N-free – Livre de nitrogênio; Nitrogen free.

RB – Ave referência; reference bird.

RM – Modelo de Reading; Reading Model.

RR - Taxa de resposta; response rate;

SID -Digestibilidade ideal padronizada; standardized ideal digestibility

Val – Valina; Valine.

LISTA DE TABELAS

CAPÍTULO 2 – MODELLING JAPANESE QUAIL RESPONSES TO VALINE, ISOLEUCINE AND LEUCINE INTAKES ……………………………………………...12

CAPÍTULO 3 - MODEL TO ESTIMATE VALINE REQUIREMENT FOR JAPANESE QUAIL FOCUSED ON ECONOMIC ASPECTS …………………….……………..……36

LISTA DE FIGURAS

MODELAGEM DAS EXIGÊNCIAS NUTRICIONAIS DE VALINA, LEUCINA E ISOLEUCINA PARA CODORNAS JAPONESAS NA FASE DE POSTURA

RESUMO – Objetivou-se com esta pesquisa determinar as respostas de codornas japonesas e a elaboração de modelos matemáticos para determinar o nível ótimo dessas aves à ingestão de valina, isoleucina e leucina com base no modelo fatorial. Foram realizados três ensaios experimentais, utilizando 280 codornas japonesas em fase postura para cada ensaio. Os ensaios foram delineados inteiramente ao acaso, com oito tratamentos e sete repetições compostas por cinco aves. Os tratamentos consistiram de níveis crescentes de cada aminoácido teste. Os níveis propostos de valina variaram de 0,196 a 0,941%, isoleucina de 0,170 a 0,815% e leucina de 0,392 a 1,881% dos aminoácidos teste. Esses níveis foram estabelecidos para compreender todas as exigências (mantença, resposta e estabilidade ou platô de resposta). Para cada aminoácido foi estudado um nível controle com objetivo de confirmar a limitação do respectivo aminoácido teste. O período experimental foi de sete semanas, sendo três de adaptação e as outras quatro para coleta de dados. As variáveis estudadas foram, consumo de ração (g), consumo do aminoácido teste (g/ave/dia), peso médio do ovo (g), massa de ovos (g) e peso das aves (g). As respostas de produção foram ajustadas pelo modelo broken line e a função monomolecular para elaborar o modelo fatorial que fraciona as exigências em mantença e produção de ovos, bem como calcular o nível para otimizar a massa de ovos da população. Além disso foi estimado a exigência ótima econômica de valina pelo Reading Model. O modelo broken line estimou a máxima resposta para massa de ovos de 9,49; 9,34 and 9,54 g/d para Val, Ile e Leu, respectivamente. A eficiência de utilização estimado foram 75, 65 e 55% e os valores de *a* determinados foram 11, 10 e 221 mg/g de para Val, Ile e Leu, respectivamente. Os valores de *b* determinados pela função monomolecular foram de 86, 70 e 147 mg/BWkg^{0,67} por dia para Val, Ile e Leu, respectivamente. A ingestão ótima econômica de valina estimada pelo Reading model foi 167 mg/ave/dia, que atenderá ao 92% da população, gerando uma margem bruta de produção de 0.0153 ave/dia.

Palavras-chaves: aminoácidos, mantença, massa de ovos, reading model

MODELING OF THE NUTRITIONAL REQUIREMENTS OF VALINA, LEUCINE AND ISOLEUCINE FOR JAPANESE QUAILS IN PRODUCTION

ABSTRACT – The objective of this research was to determine the responses of Japanese quails and the elaboration of mathematical models based a factorial approach to predict the optimum intake using different levels of valine, isoleucine and leucine. Three experimental trials were carried out using 280 Japanese quails during the laying phase. The experimental design was completely randomized, with eight treatments and seven replicates composed of five birds. Treatments consisted of increasing levels of each amino acid test. Valine levels ranged from 0.196 to 0.941%, isoleucine from 0.170 to 0.815% and leucine from 0.392 to 1.881%. these levels were determined in order to analyze the utilization of these amino acids to meet the Japanese quails requirements (maintenance, response and stability or response plateau). For each amino acid trial, a control level was included in order to confirm the limitation of the respective amino acid test. The experimental period was seven weeks, being 3-week adaptation period and the other four data collection to elaborate the factorial model. The variables studied were feed intake (g), test amino acid intake (g/bird/day), egg weight (g), egg mass (g) and bird weight (g). The performance data were adjusted in the broken line and monomolecular models to determine the necessary parameters for the elaboration of the factorial model, as well as to calculate the amino acid level to optimize the egg mass of the population. The broken line model estimated the maximum response for egg output of 9.49; 9.34 and 9.54 g/day for Val, Ile and Leu, respectively. The efficiencies of utilization estimated were 75, 65 and 55% and the values of *a* determined were 11, 10 and 221 mg/g for Val, Ile and Leu, respectively. The values of *b* determined by the monomolecular function were 86, 70 and 147 mg/BWkg$^{0.67}$ per day for Val, Ile and Leu, respectively. The optimal economic intake of valine estimated by Reading model was 167 mg/bird/day, which will meet 92% of the population, resulting in a gross production margin of 0.0153 bird/day.

Keywords: amino acids, egg mass, maintenance, reading model.

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. INTRODUÇÃO

A coturnicultura brasileira vem se profissionalizando e, atualmente, pode ser vista como economia de escala, com granjas produzindo até 900 mil ovos por dia. Parte deste desenvolvimento atribui-se aos galpões climatizados com capacidade de até 200 mil aves. Esta tendência encontra-se em plena difusão no Brasil e as perspectivas para o segmento são positivas com crescimento exponencial nos próximos anos (IBGE, 2015). A melhoria na condição ambiental de criação possibilita que as aves aumentem o consumo de ração, o peso corporal, o peso dos ovos e aumentem o ganho de peso na fase adulta, e consequentemente o aumento da gordura corporal. O aumento no peso do ovo, além do padrão desejável (11-12 g, Silva Júnior, 2015) gera perdas nas esteiras de coletas de ovos, devido a maior sensibilidade da casca, proporcionada pela redução de sua espessura. Este fato é um forte indicativo de excesso de nutrientes, que contribui na desaceleração do crescimento desta atividade econômica. Esse cenário é piorado em sistemas de produção não automatizados afetando não somente os aspectos econômicos como também, a salubridade nas unidades produtivas, devido a emissão de amônia para o ambiente.

A viabilidade econômica e o sucesso da atividade dependem, dentre outros fatores, da qualidade nutricional da dieta, de forma que a nutrição deve garantir níveis nutricionais adequados para que as aves maximizarem a produção de ovos, bem como garantir a economicidade das dietas. Dentre os nutrientes, os aminoácidos participam de uma grande variedade de reações metabólicas no organismo animal, no entanto, tem-se observado que a ingestão desproporcional de aminoácidos (essenciais ou não essenciais) em quantidades ou padrões diferentes daqueles requeridos para máxima utilização pelos tecidos, resultam em efeitos adversos ao animal (SCHMIDT et al., 2011) e interferem diretamente na resposta produtiva das aves poedeiras (CUPERTINO et al., 2009).

Atualmente são utilizados os métodos dose-resposta e o fatorial para determinar as exigências ou o perfil ideal de aminoácidos (SAKOMURA & ROSTAGNO, 2007). Entretanto, a grande maioria dos estudos para definir exigências dos aminoácidos tem se baseado no método dose-resposta. Este também é um método bastante utilizado para fornecer componentes para os modelos fatoriais (D'MELLO, 2003), como a eficiência de utilização dos aminoácidos. Sendo que o método fatorial que têm como objetivo predizer as exigências das aves mantidas em diferentes condições (SAKOMURA & ROSTAGNO, 2007).

O conhecimento de como as aves respondem ao consumo de determinados aminoácidos é de fundamental importância para se determinar o consumo ótimo econômico dos mesmos, bem como permitir uma redução na excreção de nitrogênio para o ambiente. No entanto, a escassez de trabalhos neste tema para codornas japonesas impossibilita a determinação das exigências com maior acurácia e precisão. Este trabalho tem como objetivo estudar as respostas das codornas japonesas a diferentes níveis de valina, isoleucina e leucina, assim como determinar os coeficientes para massa de ovos e peso corporal na elaboração dos modelos fatoriais para estimar as exigências ótimas econômicas destes aminoácidos.

2. REVISÃO DE LITERATURA

Aminoácidos de cadeia ramificada

Os aminoácidos limitantes podem ser definidos como aqueles que estão presentes na dieta em uma concentração inferior à exigida para máximo desenvolvimento. A ordem de limitação depende dos ingredientes utilizados. No caso do Brasil, onde as rações são formuladas a base de milho e farelo de soja, os cinco primeiros aminoácidos limitantes para aves, em ordem de limitação, são metionina, lisina, treonina, valina e isoleucina.

A valina (Val), isoleucina (Ile) e leucina (Leu) são considerados aminoácidos hidrofóbicos de cadeia ramificada (BCAA – branched chain amino acids) que possuem estrutura química semelhante e compartilham as mesmas enzimas usadas para sua transaminação e descarboxilação oxidativa (HARPER et al., 1984). A importância destes aminoácidos está relacionada com o seu metabolismo na síntese proteica, e como sua regulação (HTOO & WILTAFSKY, 2011).

Segundo o NRC (1994) as concentrações dietéticas de valina, isoleucina e leucina para codornas japonesas em produção são 0,92%, 0,90% e 1,42%, respectivamente. De acordo com Silva e Costa (2009) as concentrações de valina e isoleucina são as mesmas 0,87% e para leucina 1,43%. Já Rostagno et al. (2011) recomendam concentrações de acordo a produtividade e consumo das aves, sendo 0,784, 0,679 e 1,568% para valina, isoleucina e leucina, respectivamente.

A diferença da leucina dos outros BCAA é por estar presente em maiores quantidades nos cereais e ingredientes comumente encontrados nas rações para aves. A soja, o principal alimento proteico utilizado nas rações possui baixos teores de valina e isoleucina (1,97 e 1,92%) quando comparados com leucina (3,19%) (ROSTAGNO et al., 2011).

Rações formuladas com imbalanço destes aminoácidos produzem efeitos de antagonismo, devido ao fato de competência pelo mesmo sítio de absorção (D'MELLO, 2003). A leucina, mais do que valina ou isoleucina, contribui significativamente para o aumento do catabolismo dos demais BCAA (UMIGI, 2009). Portanto, este antagonismo está relacionado com os níveis destes aminoácidos na dieta.

Este fato foi evidenciado em experimentos por Peganova & Eder (2002), que avaliaram a tolerância de poedeiras Lohmann Brown de 24 semanas de idade à suplementação em excesso de isoleucina. O estudo mostrou que o excesso de isoleucina (1,05%) levou à perda de peso e redução da massa de ovos linearmente com o aumento da isoleucina. O peso corporal se mostrou mais sensível ao excesso de isoleucina do que o consumo de ração. Os autores sugerem que o excesso de isoleucina pode ter causado deficiência de outros aminoácidos, presumivelmente valina e leucina, para o sítio de síntese da proteína.

Pesquisas realizadas por Allen e Baker (1972), avaliaram o efeito do excesso dietético de leucina sobre a eficiência de utilização de valina e isoleucina para frangos de corte em crescimento. Foram trabalhados níveis crescentes de L-valina nas rações com ou sem suplementação de L-leucina (0 e 3%). O aumento de leucina na dieta provocou redução linear da eficiência de utilização da valina. Assim, dietas deficientes em valina para frango de corte não só reduzem o ganho de peso e piora a conversão alimentar, como também determinaram anormalidades das pernas e empenamento (FARRAN; THOMAS, 1992b).

Atualmente, o uso de aminoácidos sintéticos como DL-Metionina, L-Lisina.HCl, L-treonina já formam parte de uma dieta codornas japonesas, no entanto, a suplementação de valina, isoleucina e leucina ainda é limitada, devido ao alto custo de produção em relação aos outros aminoácidos, porém, é necessário o desenvolvimento de pesquisas que avaliem a importância da suplementação dos aminoácidos L-Valina, L-Isoleucina e L-Leucina nas dietas, bem como a definição dos níveis mínimos exigidos para o melhor desempenho das aves. Aliado ao desempenho deve-se ter a preocupação com a redução da excreção de nitrogênio para o ambiente e com a melhoria da resposta imune da ave.

Modelos matemáticos para predizer as exigências de aminoácidos para codornas japonesas

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

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

Segundo Siqueira (2009) os primeiros modelos de predição das exigências de aminoácidos para aves foram desenvolvidos por Hurwitz et al. (1973), para aves de postura. As exigências totais de aminoácidos foram obtidas a partir da soma das necessidades para mantença, crescimento e produção de ovos, sendo os coeficientes que expressam cada uma destas frações determinados de maneira independente.

Os métodos utilizados para estudar as respostas das aves aos aminoácidos essenciais baseiam-se em duas abordagens principais: dose-resposta e fatorial (D’MELLO, 2003). O método dose-resposta determina as exigências com base na resposta de desempenho dos animais alimentados com dietas contendo níveis crescentes do nutriente estudado, Segundo Euclydes e Rostagno (2001), a adição de um nutriente limitante na ração, mantendo níveis adequados dos demais nutrientes, promove crescimento do animal até que sua exigência seja atendida. A partir daí, existirá uma faixa de estabilização no crescimento e, em seguida, dependendo do nutriente, poderá ocorrer uma perda de peso do animal. Este método é amplamente empregado em estudos para estimar as exigências de aminoácidos para aves, pois apresenta algumas vantagens como reduzido custo de operação, praticidade na execução e na análise estatística. Por outro lado, fatores como genética, idade, sexo, clima, podem causar imprecisão na determinação do nível ótimo aminoácidico (SAKOMURA e ROSTAGNO, 2007). Com isso, o uso deste método é restrito a condições idênticas àquelas em que as exigências foram estabelecidas.

O método fatorial constitui a base para a estimativa das exigências por meio de modelos matemáticos que levam em consideração a ingestão, retenção e a eficiência de deposição do aminoácido, além do peso corporal e da produção (SANTOMÁ, 1991). Este método é baseado no princípio de que a reposta do animal para determinado nutriente é que define a quantidade necessária do nutriente para manutenção, crescimento proteico, engorda e produção, levando em consideração as diferenças de peso, composição corporal, potencial de crescimento e produção de ovos (SAKOMURA e ROSTAGNO, 2007).

Segundo Hurwitz et al. (1978), este é o método mais adequado para estabelecer as exigências nutricionais para aves, sendo a base para a elaboração de modelos matemáticos que têm como objetivo predizer as exigências das aves mantidas em diferentes condições (SAKOMURA e ROSTAGNO, 2007).

Ajuste de respostas das aves por modelos matemáticos

A pressuposição básica para ajustar qualquer função matemática à resposta de um fenômeno ao um dado estímulo é a descrição detalhada do comportamento da resposta com a mudança do estímulo. Para aves, o comportamento da resposta ao aumento da ingestão do aminoácido (estímulo) pode ser dividido em quatro fases, ausência de resposta, linear de resposta, estabilidade de resposta e toxidez (SILVA et al., 2014). A técnica da diluição permite obter níveis de aminoácidos para descrever as quatro fases de resposta da ave (BENDEZU et al., 2015).

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

Um aspecto importante a ser considerado nos ensaios dose-resposta realizados para determinar o nível ótimo dos aminoácidos é a técnica de formulação das dietas experimentais, sendo ela: a suplementação gradativa (D'MELLO, 1982) e diluição (FISHER e MORRIS, 1970). A técnica da suplementação consiste na formulação de uma ração basal, para atender as exigências nutricionais de energia, minerais, vitaminas e aminoácidos essenciais, com exceção do aminoácido a ser estudado. Este é suplementado na dieta basal em níveis crescentes pela adição de uma fonte cristalina. A técnica da diluição consiste em diluir sequencialmente uma dieta alta em proteína e deficiente no aminoácido teste, com uma dieta isoenergética livre de proteína, obtendo-se os níveis intermediários do aminoácido a ser testado.

A maioria dos estudos realizados no continente americano são realizados utilizando a técnica da suplementação, no entanto, no continente europeu a técnica da diluição é mais difundida. A grande vantagem da técnica da diluição é a possibilidade de utilizar uma proteína cuja constituição é próxima a uma ração convencional, (milho e farelo de soja) na qual a maior parte da proteína da dieta é proveniente da proteína intacta dos ingredientes e não de aminoácidos sintéticos.

É desejável minimizar a inclusão de fontes sintéticas de aminoácido porque a velocidade dos processos digestivos, absortivos e metabólicos dos aminoácidos sintéticos é diferente dos aminoácidos vindo de proteína intacta (milho e farelo de soja). No processo de síntese proteica pode haver um comprometimento, uma vez que no processo de transcrição (mRNA) e tradução (tRNA) ocorre união de molécula de tRNA ao ribossomo, que transporta um aminoácido, por vez para se juntar aos outros dois, formando a trinca de base. Portanto, a falta de um aminoácido compromete o processo, que deveria continuar até que toda codificação de trinca de bases contida no mRNA tenham sido traduzidas no ribossomo, formando uma cadeia de aminoácidos e consequentemente a molécula de proteína.

Com base nessa pressuposição Fisher e Morris (1970) preconizaram que é possível criar um aminoácido limitante na síntese proteica da ave, por meio da deficiência relativa do aminoácido. Desta forma, independentemente da quantidade de proteína da dieta, a ave responderá ao primeiro limitante na síntese proteica. Assim, a técnica da diluição proporciona uma limitação na ingestão do aminoácido teste, que consequentemente limita decodificação de trinca de bases contida no mRNA, porque um dos aminoácidos estará em falta. Nesta técnica, faz-se necessário incluir um tratamento controle para comprovar se essa pressuposição foi verdadeira, o aminoácido teste. Além desses aspectos teóricos a redução da proteína é desejável para permitir obtenção de uma curva resposta bem definida, da fase de mantença à estabilidade. Um importante aspecto na técnica da diluição é que os níveis dietéticos formulados para provocar respostas na fase de mantença são baixos, por volta 5% de PB na dieta e facilmente pode ser obtido por essa técnica, que seria algo impraticável com a técnica da suplementação pela alta quantidade de aminoácido sintético que demandaria, implicando em o alto custo com os aminoácidos sintéticos.

Modelo de Reading

Todo modelo matemático além de apresentar bom ajuste da resposta média da população à ingestão do aminoácido, também deveria explicar a variação em torno da média. Baseado nisto os pesquisadores da Universidade de Reading (CURNOW, 1973; FISHER et al., 1973) desenvolveram um modelo matemático, cuja curva sigmoidal característica considera a resposta de um indivíduo como modelo fatorial simples, acrescida da derivação da resposta integrada média da população a partir de um grande número de respostas individuais.

Segundo Fisher et al., (1973), o Reading Model é capaz de se ajustar às condições de variabilidade de cada granja e estimar o nível ótimo econômico, concomitantemente. O modelo de Reading estima as exigências de aminoácidos considerando a variabilidade da população e os pequenos aumentos na ingestão de aminoácidos são previstos e embasados no ótimo econômico que se destinam às aves que ainda não atingiram sua capacidade máxima de produção, como também considera o custo benefício da suplementação em relação à taxa de produção e o valor marginal da produção de uma unidade de ovo (SILVA et al., 2014; 2015).

Entre as características do modelo de Reading pode mencionar-se que devido aos coeficientes estimarem os aminoácidos para mantença e produção de ovos, as estimativas não variam com os fatores ambientais, ou seja, independe da granja (SILVA et al., 2014; 2015). Estes coeficientes são obtidos da função resposta da massa de ovo e do peso corporal em relação à ingestão do aminoácido. Para estimar os coeficientes desse modelo faz-se necessário descrever a curva-resposta da ave à ingestão do aminoácido (SILVA et al., 2015). Na literatura, os estudos para definir os níveis dos aminoácidos na dieta de codornas japonesas em produção são escassos, sobretudo para valina, leucina e isoleucina.

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CAPÍTULO 2 - MODELLING JAPANESE QUAIL RESPONSES TO VALINE, ISOLEUCINE AND LEUCINE INTAKE

Abstract: This study was conducted to evaluate the responses of Japanese quails in the egg-laying phase to valine (Val), isoleucine (Ile) and leucine (Leu) intake and develop factorial models to estimate the requirements of these amino acids based on the maintenance and egg output coefficients determined by mathematical models. In order to fulfill such purposes, three feeding trials (one for each amino acid) were conducted using a completely randomized design, with eight levels of each amino acid studied, seven replicates of five birds. In each trial, an additional treatment was included to ensure that the amino acid studied in each trial was the first limiting. The trials started when the birds reached 28 weeks of age. The experiments lasted seven weeks, with the first three weeks for adaptation of birds to the experimental diets and the last four ones to collect data. Collected data were fitted to broken line and monomolecular model. To determine the Val, Ile and Leu requirements for maintenance (b), amino acid deposited was regressed against amino acid intake and maintenance was obtained when egg output (EO) = 0. Amino acid requirement for EO (a) was obtained by dividing the amino acid deposited in EO/efficiency of utilization. The maximum EO responses obtained were 9.49; 9.34 and 9.54 g/d for Val, Ile and Leu, respectively. The *b* values determined were 86, 70 and 147 mg/BWkg$^{0.67}$ per day and the values of *a* determined were 11, 10 and 21 mg/g for Val, Ile and Leu, respectively. Based on these models, we recommend the Val, Ile and Leu intakes of 148, 132 e 278 mg/d, respectively.

Keywords: egg output, model, maintenance.

Introduction

Factors which encompass the changes in Brazilian market demands; the affordable access to cereal grains and the modernization in bird housing and equipment have gradually led quail egg production towards a different scenario compared to the past, when the activity was basically focused on meeting a little and specific fraction of the consumer market. Nonetheless, despite these important advances, the sector still faces some challenges, which include the lack of reliable and solid information regarding Japanese quail amino acid nutrition (Santos et al., 2016). Since the policies of modern food industry production are not focused exclusively on obtaining maximum probability, but rather on producing high-quality affordable food under conditions of sustainability; it is indispensable to provide the ideal balance of amino acids to optimize the efficiency of nitrogen utilization.

Efforts put forwards on establishing the ideal amino acid profile for broiler breeders, laying hens and broilers allowed manipulating dietary amino acid supply in order to mitigate environmental nitrogen excretion and therefore to reduce the long-term impact of livestock in natural resources (Donato et al., 2016). Extrapolating this reality to Japanese quail production is, however, not a simple task, since some amino acid requirements remain unclear. Literature on quail responses to lysine (Ribeiro et al., 2013), methionine + cysteine (Reis et al., 2011), threonine (Umigi et al., 2012) and tryptophan intake (Pinheiro et al., 2008) have contributed to guide nutritionists to manipulate the dietary concentration of these amino acids and reduce crude protein supply without compromising performance objectives. However, unless the essential amino acid profile is completely determined, further reductions on dietary crude protein supply will remain limited.

Branched chain amino acid (BCAA) group include valine (Val), isoleucine (Ile) and leucine (Leu). Despite their essentiality for body and egg protein synthesis, little data exist concerning the BCAA requirement of egg-laying Japanese quails. Allen and Young, (1980) estimated the optimum intake of Val, Ile and Leu in 183, 178 and 282 mg/bird, respectively, based on a daily feed intake of 22 g/bird. Recently, Santos et al. (2016) estimated the optimum daily intake of Ile in 191 mg/bird. All the estimates aforementioned are originated from models, which are restricted in providing a single estimate of the nutrient dose necessary to optimize performance throughout the entire egg-laying phase (Silva et al., 2015). Contrary to such approach, factorial model partitions amino acid nutritional needs into maintenance and production, beyond isolating the effects of environment, genetic and body composition on the predicted estimate (Sakomura et al., 2015). Given the need to establish the BCAA requirements for Japanese quails and the advantages offered by factorial approach in predicting amino acid requirements, three trials were conducted to evaluate egg-laying Japanese quail responses to Val, Ile and Leu intake and develop factorial models to estimate the requirements of these amino acids based on determined coefficients for maintenance and egg output.

Materials and Methods

Ethics Committee approval

The Ethics Committee on Animal Care and Use (CEUA) of Faculty of Agriculture and Veterinary Sciences (FCAV) of the Universidade Estadual Paulista "Julio de Mesquita Filho" (UNESP), Jaboticabal, Brazil approved all experimental procedures used in the current study under the Protocol number 9999/14.

Bird husbandry and experimental design

Twenty-five-week-old Japanese quails were obtained from a local commercial flock and housed in a curtain-sided room. Quails were allotted into 35 cm × 34 cm × 15 cm (length × width × height) steel cages equipped with one nipple drinker and one trough feeder. In total, eight hundred forty Japanese quails were used in three different simultaneous dose-response trials (280 quails/trial). At 28 weeks of age, all quails were weighed and assigned to dietary treatments, so that initial weight and egg production (recorded from 25 to 28 week of age) were similar among treatment groups. In each trial, seven replicate cages of five quails were randomly assigned to each of the 8 treatments. Throughout the 7-week feeding trials, quails had free access to water and feed (mash form). Lighting program was set at 16L:8D. Average minimum and maximum room temperature recorded throughout the trials were 20 ± 2°C and 34 ± 4°C, respectively.

Experimental diets: valine, isoleucine and leucine trials

All the experimental treatments administrated in the three different trials were obtained from a summit diet and a nitrogen-free diet based on cornstarch, sugar and rice husk (Table 1). Both diets were isonutritive and formulated to meet or exceed Rostagno et al. (2011) nutritional recommendations, except for crude protein and amino acid content.

The amino acids studied in each trial (Val, Ile or Leu) were provided in summit diet in a concentration which exceeded 20% Rostagno et al. (2011) recommendations, whereas the remaining essential amino acids were supplied to exceed approximately 40% of the levels recommended by these authors. Thus, in Val, Ile and Leu trial, summit diet contained 9.41 g/kg of Val, 8.15 g/kg of Ile and 18.81 g/kg of Leu, respectively. This procedure was used to ensure that amino acid was the first limiting amino acid in the dietary protein with a relative deficiency of 20% as recommended by Fisher and Morris (1970).

Dietary treatments (Table 2) were obtained through the dilution technique (Fisher and Morris, 1970), where the nitrogen-free diet was graded replaced by the summit diet to produce levels Val (1.96, to 9.41g/kg), Ile (1.70 to 8.15g/kg.) and Leu (3.92 to 18.81g/kg). In all of the trials, the tested amino acid levels ranged among treatments from 25% to 120% of amino acid recommendation (Rostagno et al., 2011).

Additionally, to confirm whether the tested amino acid was indeed limiting in diets, a control treatment was produced by adding 1.60g L-Val/kg diet (Val trial), 1.37g L-Ile/kg diet (Ile trial), and 3.16g L-Leu/kg diet (Leu trial) to the diet with the lowest level of the amino acid tested (Table 2). The crystalline amino acids were supplied in order to equal the Val, Ile and Leu content control diet to the diet containing the second-lowest level (3.53g Val/kg, 3.06g Ile/kg and 7.05g Leu/kg).

Prior to diet formulation all feedstuffs were analyzed for total amino acid content. The standardized ideal digestibility (SID) amino acids were calculated using AMINODat 4.0 (Evonik Industries, Hanau, Germany). The coefficient of digestibility for crystalline amino acids were assumed to be 100% SID.

Table 1 - Ingredients (%) and nutrient composition (g/kg) of the summit and N-free diets.

Ingredients	Summit			Nitrogen-free
	Valine	Isoleucine	Leucine	
Corn	50.13	52.36	40.51	-
Soybean meal 45%	26.37	23.47	43.68	-
Corn gluten meal 60%	9.61	10.40	-	-
Corn starch	-	-	-	50.00
Sugar	-	-	-	26.46
Rice husk	-	-	-	9.66
Limestone	7.18	7.18	7.19	7.09
Dicalcium phosphate	1.26	1.29	1.12	1.75
Soybean oil	1.79	1.26	4.93	2.53
Biolys L-lysine sulfate (56.4%)	1.06	1.18	0.46	-
L-Arginine (100%)	0.51	0.58	0.17	-
DL-methionine (99%)	0.52	0.53	0.58	-
Salt	0.36	0.36	0.36	0.36
Choline chloride 60%	0.26	0.26	0.28	0.34
Potassium chloride	-	-	-	1.40
L-Threonine (98.5%)	0.18	0.21	0.09	-
Vitamin mineral premix[a]	0.40	0.40	0.40	0.40
L-Valine (96%)	-	0.19	0.09	-
L-Tryptophan (98.5%)	0.11	0.12	0.03	-
L- Isoleucine (100%)	0.11	-	-	-
L-Histidine (100%)	0.09	0.11	0.03	-
L-Phenylalanine (100%)	0.05	0.09	0.09	-
B H T (Antioxidant)	0.01	0.01	0.01	0.01
Nutrient (g/kg)				
Metabolizable energy (kcal/kg)	3000.00	3000.00	3000.00	3000.00
Crude Protein	246.85 (220.16)[b]	244.19 (213.97)[b]	244.19 (229.89)[b]	-
Methionine+Cysteine	12.00 (11.84)[b]	12.00 (11.89)[b]	12.00 (11.72)[b]	-
Methionine	8.74 (8.75)[b]	8.80 (8.84)[b]	8.78 (8.78)[b]	-
Lysine	14.63 (14.38)[b]	14.63 (14.48)[b]	14.63 (14.12)[b]	-
Tryptophan	3.07 (2.98)[b]	3.07 (2.96)[b]	3.07 (2.85)[b]	-
Threonine	8.78 (8.59)[b]	8.78 (8.64)[b]	8.78 (8.44)[b]	-
Arginine	16.97 (16.59)[b]	16.97 (16.63)[b]	16.97 (16.34)[b]	-
Valine	9.41 (9.13)[b]	10.97 (10.68)[b]	10.97 (10.54)[b]	-
Isoleucine	9.51 (9.24)[b]	8.15 (7.84)[b]	9.51 (8.99)[b]	-
Leucine	21.95 (21.45)[b]	21.95 (21.49)[b]	18.81 (17.28)[b]	-
Histidine	6.14 (6.13)[b]	6.14 (6.15)[b]	6.14 (6.09)[b]	
Phenylalanine	11.28 (11.01)[b]	11.41 (11.16)[b]	11.93 (11.45)[b]	
Calcium	30.99	30.99	30.99	30.99
Sodium	1.55	1.55	1.55	1.55
Available Phosphor	3.23	3.23	3.23	3.23

[a] Containin g/kg: Vit. A 1.750.000 U. I; Vit. D3 500.000 U. I; Vit. E 2.000 U. I; Vit. K3 500 mg; Vit. B1 250 mg; Vit. B2 875 mg; Vit. B6 500 mg; Vit. B12 1.250 mcg/kg; Niacin 6.250 mg; Choline 65 g; pantothenate acid 2.500 mg; Methionine 272.500 g; Copper 2.000 mg; iron 12.500 g; Manganese 17.500 g; Zinc 12.500 g; Iodine 300 mg; Selenium 50 mg.

[b] Values in parentheses indicate analyzed dietary concentration of the amino acid studied in the respective experiments.

Data collection

Egg production (EP) was daily recorded and all the eggs laid at the last 3 days of each week were weighed to obtain average egg weight (EW). Feed intake (FI) were weekly measured, whereas body weight (BW) was measured at the 1st, 3rd and 7th week of the trials. Egg output (EO) was calculated multiplying egg weight by egg production.

Table 2 - Proportions of the summit diet diluted with the corresponding nitrogen-free diet in the valine, leucine and isoleucine trials and the resulting concentrations of the limiting amino acids in the diets.

Valine			Leucine			Isoleucine		
Summit %	N-free %	Valine g/kg	Summit %	N-free %	Leucine g/kg	Summit %	N-free %	Isoleucine g/kg
20.8	79.2	1.96	21.7	78.3	3.92	20.9	79.1	1.7
37.5	62.5	3.53	39.1	60.9	7.05	37.5	62.5	3.06
54.1	45.9	5.09	56.5	43.5	10.19	54.2	45.8	4.42
70.8	29.2	6.66	73.8	26.2	13.32	70.8	29.2	5.77
83.3	16.7	7.84	87.9	12.1	15.68	83.3	16.7	6.79
91.6	8.4	8.62	95.5	4.5	17.24	91.7	8.3	7.47
100	0	9.41	100	0	18.81	100	0	8.15
20.8	79.2	3.53[a]	21.7	78.3	7.05[b]	20.9	79.1	3.06[c]

[a] Addiction of 1.602 g of L-valine/kg of diet; [b] Addiction of 3.194 g of L-leucine/kg of diet; [c] Addiction of 1.374 g of L-ileucine/kg of diet. a, b, c Control diet.

Quail Responses to changes in dietary amino acid levels

The linear broken-line regression model was used according to Robbins et al. (2006) as follows:

$$Y = L+U(R-X), \text{ where } (R-X) \text{ is defined as zero when } X > R. \qquad \text{[Eq. 1]}$$

Where Y is the variable response (feed intake, egg production, and egg weight); X is the intake of the digestible amino acid studied (mg/bird day), L is the response estimated at the plateau of the function; R is the breakpoint or the optimal digestible amino acid intake; and U is the slope of the function. In order to evaluate the sensibility witch which birds responded to changes in dietary limiting amino acid supply, we correlated the relative responses of feed intake (FI), egg weight (EW), and egg production (EP) with the relative amino acid intake.

The relative response (Yr) was obtained by dividing the observed response (Y) by the maximum response estimated (Ymax), (Yr = feed intake/maximum feed intake, egg weight/maximum egg weight or egg production/maximum egg production). The value of Xr was obtained by dividing X by maximum X (Xmax), where X = amino acid intake and Xmax is amino acid intake for the diet which supported the maximum response. The maximum response (Ymax) was considered the breakpoint estimated by the broken line model (L parameters), estimated from the regression of variables in the original units against amino acid intake. The Xmax is the R parameters estimated by broken line model. These values Yr and Xr were plotted to analyze the behavior of the relative responses to the amino acid intake. In order to calculate how quail response rate (RR) varied when amino acid supplied increased, we divided the variation in response when amino acid intake increased at one level by the variation in the intake of the amino acid as follows:

$$RR = \Delta Y(Y_n - Y_{(n-1)})/\Delta X(X_n - X_{(n-1)}), \qquad \text{[Eq. 2]}$$

Where Y is the variable response assessed, X is the intake the amino acid studied, and n is the level of the amino acid studied.

Estimate of valine, isoleucine and leucine intake for maintenance

The amino acid intake and the amount of amino acid deposited in the egg output were both expressed as a function of metabolic weight in a daily basis (i.e., mg amino acid/kg $BW^{0.67}$ and g egg output/kg $BW^{0.67}$). The amino acid deposition (D) was obtained as follows:

$$D = (d \times EO), \qquad \text{[Eq. 3]}$$

Where d is the amount of Val (0.90% or 8.44 mg/g egg), Ile (0.65% or 6.20 mg/g egg), and Leu (1.24% or 11.66 mg/g egg) in quail egg (Genchev, 2012), EO is the egg output expressed in mg/kg$BW^{0.67}$. Then, the monomolecular function (Kebreab et al., 2008) was applied to estimate maintenance requirements as follows:

$$Y = Ymax(1-\exp^{-s(X-b)}), \qquad \text{[Eq. 4]}$$

Where Y is the amino acid deposited in the EO (mg/kg $BW^{0.67}$); X is the amino acid intake (mg/kg $BW^{0.67}$); Ymax is the maximum amino acid deposition in the EO (mg/kg $BW^{0.67}$); s is the slope of the function; and b is the maintenance requirement (mg/kg$BW^{0.67}$). Finally, maintenance was calculated by equalizing egg mass to zero (EO = 0).

Efficiency of utilization and amino acid required per unit of egg output

The efficiency of utilization for each amino acid *(k)* was calculated by dividing the deposition of the amino acid in the quail egg by the amino acid intake discounting the intake for maintenance as the following equation:

$$k = [D/(X–b)]×100, \qquad \text{[Eq. 5]}$$

Where k is the efficiency of amino acid utilization, X is the amino acid intake (mg/kgBW$^{0.67}$) and b is the amino acid requirement for maintenance (mg/kgBW$^{0.67}$), estimated by monomolecular function. The k was obtained by the ratio between the deposition and the respective intake corrected for maintenance of Val, Ile and Leu. The value a, mg of amino acid per g egg output, was obtained by dividing the d/k. This procedure was applied to each amino acid.

Efficiency data were fitted to broken-line-line model (Robbins et al., 2006) as follows:

$Y=L+U(R−X)+V(X−R)$, where $(R − X)$ is defined as zero at values of $X > R$, and $(X − R)$ is defined as zero when $X < R$, [Eq. 6]

The parameters by definition are the breakpoint on the axis X value (R), an asymptote for the first segment (L), and slopes for the 2-line segments (U, V), Y is the response variable, X is the digestible amino acid intake (mg/bird day).

Factorial model to predict amino acid intake (AAI) based on the potential for egg output production and body weight

Based on the coefficients *a* and *b*, the following factorial model was generated to predict Japanese quail requirements for amino acids:

$$AAI = aEO + bBW^{0.67} \qquad \text{[Eq. 7]}$$

Where egg output (EO) and body weight (BW) are the input variables. The inversion of the factorial model was used to calculate the EO response as a function of the amino acid intake as follows:

$$EO = (AAI− (bBW^{0.67}))/a. \qquad \text{[Eq. 8]}$$

Evaluation of the model

In order to evaluate the factorial models developed for Val, Ile and Leu, the residuals (observed values – predicted values) were regressed against the predicted values as described by St-Pierre (2003):

$$ei = b0 + b1\times(EOi - EO\mu) + \check{e}i, \qquad \text{[Eq. 9]}$$

Where ei is the residual for the observation i; b0, b1 are the estimates of the parameters; EOi is the predicted value for i; EOμ is mean of predicted values; ěi is the error when residual values were regressed against predicted values.

Results

Responses of feed intake, egg production and egg weight to changes in dietary amino acid levels

The responses of Japanese quails to different digestible levels of Val, Ile and Leu are shown in Table 3. The levels studied affected all variables ($P<0.001$) regardless of the amino acids studied. Quails fed the control diet (counter-proof treatment) showed intermediary egg production and egg weight responses, exhibiting values of means between the first and second lowest levels of Val, Ile and Leu, confirming that these amino acids were indeed limiting in diets.

As detailed in Table 3, the dietary Val levels of 1.96, 3.53, and 5.09 g/kg diet elicited a higher response rate ($\Delta y/\Delta x$ or y_n-$y_{(n-1)}$/x_n-$x_{(n-1)}$). The calculated values were 3.3, 2.6 and 2.8, respectively. Between the Val levels of 6.66 and 7.84 g/kg diet, this rate was positive (0.25 g of intake/unity of concentration in diet), whereas between the levels of 7.84, 8.62 and 9.41 g Val/kg diet, the rates were negative with the reduction of –0.26 and -0.51 g in feed intake/unity of dietary Val. In general, the same pattern was observed for Ile. For Leu, the rates calculated among the increasing levels were 3.92, 7.05, 10.2, 13.3, 15.68, 17.24 and 18.81 g Leu/kg diet were 1.8, 1.6, 0.9, -0.3, 0.1 and 0.4 g, respectively.

Table 3 - Responses to levels of valine, isoleucine and leucine (g/kg) for feed intake (g/bird d), amino acid intake (mg/bird d), egg production (per 100 bird d), egg weight (g), egg output (g/bird/d), body weight (g/bird) and change in body weight (g/bird d) of Japanese quails (28 to 35 weeks of age) to different dietary valine, isoleucine and leucine (g/kg). Mean (± Standard deviation).

Levels	Feed Intake	AA intake	Egg production	Egg weight	Egg output	Body weight	Change in body weight
Valine trial							
1.96	9±2	19±3	1±1	7.8±0.6	0.2±0.1	139±5	-1.1±0.2
3.53	14±1	51±2	27±5	8.7±0.6	2.3±0.4	157±4	-0.6±0.1
5.09	19±1	95±2	66±8	9.6±0.4	6.3±0.6	162±1	-0.5±0.1
6.66	23±2	153±1	89±2	10.7±0.2	9.5±0.3	170±6	-0.2±0.2
7.84	23±1	183±7	90±6	10.7±0.1	9.7±0.7	173±6	-0.1±0.1
8.62	23±1	199±7	86±7	11.0±0.2	9.5±0.9	171±4	-0.1±0.1
9.41	23±1	214±7	84±8	11.0±0.2	9.3±0.9	173±5	-0.1±0.1
3.53[a]	10±1	34±3	4±2	8.1±0.3	0.4±0.2	147±7	-1.1±0.3
SEM[d]	0.73	10.60	4.78	0.17	0.52	1.73	0.05
DF[e]	41	41	41	40	40	41	41
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Isoleucine trial							
1.70	9±1	16±2	2±1	7.9±0.4	0.2±0.1	143±7	-1.1±0.1
3.06	14±2	43±5	25±4	8.5±0.2	2.1±0.3	154±5	-0.8±0.1
4.42	19±1	83±4	67±9	9.3±0.3	6.3±1.1	162±4	-0.5±0.1
5.77	22±1	128±3	87±4	10.8±0.3	9.4±0.4	173±4	-0.2±0.2
6.79	22±1	148±6	87±3	10.7±0.2	9.3±0.4	173±5	-0.2±0.1
7.47	23±1	172±6	87±4	10.8±0.3	9.4±0.6	170±5	-0.1±0.1
8.15	23±1	185±4	84±6	11.1±0.1	9.3±0.6	173±3	-0.1±0.1
3.06[b]	10±1	31±4	6±2	8.1±0.4	0.5±0.1	146±6	-1.0±0.3
SEM[d]	0.74	9.17	4.78	0.18	0.54	1.78	0.06
DF[e]	42	42	40	40	40	42	42
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Leucine trial							
3.92	10±1	40±4	3±2	7.9±3.6	0.3±0.2	140±3	-1.2±0.2
7.05	16±1	110±6	38±9	8.9±0.7	3.4±0.7	156±6	-0.7±0.1
10.19	21±1	211±9	78±7	10.2±0.3	8.0±0.7	167±5	-0.3±0.1
13.32	24±1	314±8	89±7	10.9±0.4	9.7±1.0	169±5	-0.2±0.1
15.68	23±1	361±9	85±3	10.9±0.2	9.4±0.3	172±4	-0.1±0.2
17.24	23±1	401±9	86±7	11.0±0.2	9.4±0.9	173±5	-0.1±0.1
18.81	24±1	448±9	87±6	11.1±0.3	9.6±0.7	175±5	0.0±0.1
7.05[c]	11±1	80±5	5±4	8.5±0.6	0.4±0.4	143±6	-1.1±0.2
SEM[d]	0.73	21.59	4.38	0.17	0.50	1.85	0.06
DF[e]	42	42	41	41	41	42	42
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a] 3.53 = 1.96 + 1.602 g L-valine/kg. [b] 3.06 = 1.70 + 1.374 g L-isoleucine/kg. [c] 7.05 = 3.92 + 3.194 g L-leucine/kg. [a, b, c] Control diet. [d] SEM = standard error of the mean. [e] DF = degrees of freedom.

Table 4 - Chemical composition of whole carcass (with bones).

	Water %			Protein %			Fat %			Ash %		
RB	69.9 ± 0.9			20.2 ± 0.5			6.6 ± 0.7			4.1 ± 0.9		
	Val	Ile	Leu	Val	Ile	Leu	Val	Ile	Leu	Val	Ile	Leu
1	70.3	70.1	68.9	18.0	18.4	17.8	7.0	6.8	7.7	5.4	5.5	5.5
2	68.1	68.9	68.0	18.5	19.1	18.0	7.1	6.8	7.7	5.7	5.3	5.5
3	69.9	68.0	68.0	18.8	19.2	18.9	6.5	6.9	7.4	4.8	5.3	5.4
4	68.8	69.8	69.8	19.2	18.9	18.9	6.3	6.5	6.3	5.5	4.9	5.0
5	68.4	69.3	70.9	19.5	19.1	18.6	6.3	6.3	6.1	5.3	5.2	4.8
6	69.2	69.1	69.4	19.4	19.8	19.0	6.0	5.8	6.1	5.3	4.9	5.1
7	68.4	70.5	70.2	19.7	19.4	18.9	6.0	5.8	5.8	5.4	4.9	5.0
8*	69.8	68.4	70.1	18.5	18.4	18.1	7.1	7.3	7.6	5.2	5.7	4.9

*Control diet. RB: reference bird. The average body composition for Val, Ile and Leu were 69.3% ± 0.9 for water, 18.8 ± 0.6 for protein, 6.6 % ± 0.6 for fat and 5.2 % ± 0.3 for ash.

From the fifth level of Leu, it was observed an improvement in response rates indicating an improvement in quail intake, whereas in the highest levels of Val and Ile an opposite response was observed indicating a reduction on feed intake. Considering all amino acids, the correlation between the response rate ($\Delta y\text{-}y/\Delta x\text{-}x$) of the feed intake *vs* egg production and the feed intake vs egg weight was 0.906 and 0.678, respectively. The Ymax for feed intake (g/bird d), egg production (per 100 bird d) and egg weight (g) were 23, 87 and 11 for all amino acids, respectively. The Xmax varied according to limiting amino acid (Table 5). Based on the parameter R of the broken-line model, it was possible to evaluate the amplitude of the dietary amino acid levels, and based on results, the minimum and maximum levels were 12 and 142% for Val, 15 and 172% for Ile, and 16 and 179% for Leu. By adopting the scale from 0 to 1 in the X axis, we could analyse how sensible feed intake, egg production, and egg weight were in responding to increment in amino acid doses (Figure 1).

The responses of feed intake ($FI = 1.0–0.67*[1.0\text{- }Xr]$, $R^2 = 98.9$), egg production ($EP = 1.0–1.16*[1.0\text{-}Xr]$, $R^2 = 99.7$), and egg weight ($EW = 1.0–0.32*[1.0\text{- }Xr]$, $R^2 = 98.8$) changed with different intensity. According to the equation above described, egg production was close to zero when amino acid intake was reduced in 0.85, i.e., Xr was equal to 0.15, for all the three amino acids. For the same level of reduction feed intake decreased by 0.58 and egg weight by 0.28.

Table 5 - Estimated parameters of the models broken line (BL), for feed intake (g/bird d), egg production (per 100 bird d), egg weight (g), egg output (g/bird/d) and change in body weight (g/bird d) of Japanese quails (28 to 35 weeks of age) according to valine, isoleucine and leucine (mg/bird d).

Amino acid	L	U	R	R^2	P-value
Valine					
Feed Intake	23.25	-0.096	150.2	0.95	***
Egg Production	87.38	-0.853	121.0	0.96	***
Egg weight	10.86	-0.024	147.2	0.91	***
Egg output	9.49	-0.084	134.2	0.96	***
Change in body weight	-0.114	-0.007	144.9	0.84	***
Isoleucine					
Feed Intake	22.38	-0.137	107.5	0.97	***
Egg Production	86.13	-0.988	103.1	0.97	***
Egg weight	10.85	-0.026	133.8	0.94	***
Egg output	9.34	-0.094	116.7	0.97	***
Change in body weight	-0.127	-0.008	127.5	0.89	***
Leucine					
Feed Intake	23.50	-0.062	250.5	0.96	***
Egg Production	86.77	-0.426	229.7	0.96	***
Egg weight	10.99	-0.013	266.7	0.91	***
Egg output	9.54	-0.045	245.5	0.97	***
Change in body weight	-0.095	-0.005	250.5	0.87	***

*** *P*<0.001 - Broken-Line Regression Model: Y = L+U(R−X), where (R−X) is defined as zero when X > R. Where: Y is the response variable, feed intake, egg production and egg weight; X is the digestible amino acid intake (mg/bird day), L is the response estimated at the plateau of the function; R is the break point or the optimal digestible amino acid intake; and U is the slope of the function.

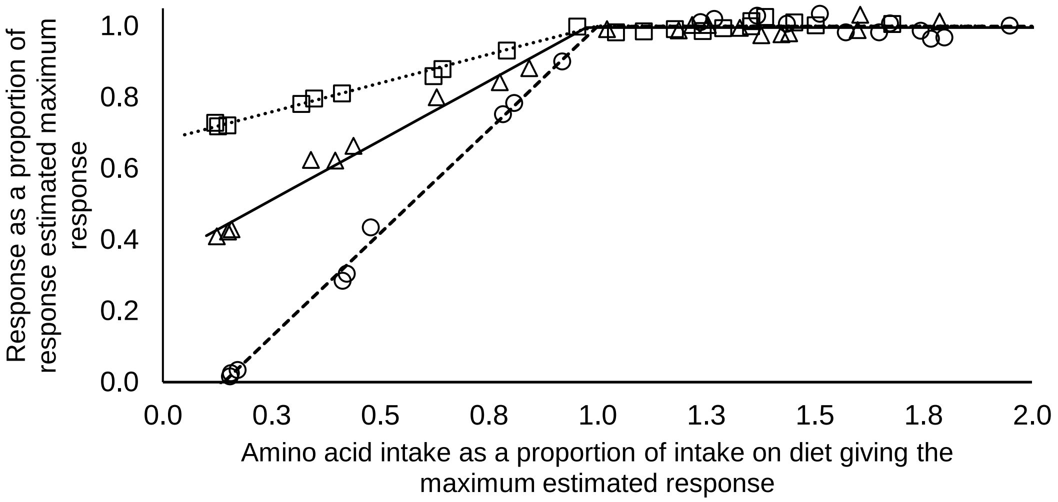

Figure 1. Performance variables relative to amino acid intake for each amino acid (Val, Ile and Leu). Performance variables were: feed intake (▲ observed, __ predicted), egg production (○ observed, ---- predicted) and egg weight (□ observed, …. predicted). The observed values represent the mean responses for each treatment of Val, Ile and Leu.

Estimate of valine, isoleucine and leucine intake for maintenance

For Val, the model parameters of Ymax and s were estimated at 324 mg/kgBW0.67/day and 0.0037, respectively. The daily valine intake for maintenance was estimated as 86 mg/kgBW$^{0.67}$. Errors associated with Ymax, *s,* and valine intake for maintenance were: 6%, 16% and 12%, respectively. For isoleucine, the model parameters of Ymax and *s* were estimated at 234 mg/kgBW$^{0.67}$/day and 0.0042, respectively. The estimated daily maintenance requirement was 70 mg/kgBW$^{0.67}$. Errors associated with Ymax, *s*, and isoleucine intake for maintenance were: 6%, 15% and 11%, respectively. For Leu, the model parameters of Ymax and *s* were estimated at 418 mg/kgBW$^{0.67}$/day and 0.0024, respectively. The estimated daily maintenance requirement was 147 mg/kgBW$^{0.67}$. Errors associated with Ymax, *s*, and leucine intake for maintenance were: 3%, 12% and 11%, respectively.

Efficiency of utilization and amino acid required per unit of egg output

The efficiency corresponds to the estimate of the parameter L of the two-slope broken-line model. The value of *k* estimated for Val, Ile, and Leu were 75, 65 and 55%, respectively (Table 6). Errors associated with L, U, R, and V were, respectively, 3, 5, 14, and 4% for Val; 2%, 7%, 7% and 3% for Leu; and 3%, 8%, 14% and 4% for Ile. Even though L is the parameter of interest, the errors associated with the other parameters, which are part of the model, can be considered acceptable. The fit of the two-slope broken-line model adjusted with efficiency of amino acid utilization as a function of the amino acid consumed over the maintenance, is assumed to estimate values that represent the maximum amino acid efficiency of utilization, after which, the efficiency decrease in a “V” ratio. In this sense, the efficiency of utilization (Table 6) for Ile (-0.30) decrease in a faster ratio, followed by Val (-0.27) and Leu (-0.11). According to the efficiency of utilization estimated (*k*), the requirement for egg production in mg of amino acid per g of EO were 11.25, 9.54, and 21.20 for Val, Ile, and Leu, respectively.

Table 6 - Estimated parameters of the models broken line (BL) with two-slope, of efficiency of utilization (*k*, %) and for amino acid requirement per unit of egg output (a, mgg-1) in function to valine, isoleucine and leucine (mg/bird d) of Japanese quails (28 to 35 weeks of age).

Amino acid	L	U	V	R	R^2	*P-value*
Valine *(k, %)*	75.21 ± 2.45	-0.69 ± 0.04	-0.27 ± 0.04	99.74 ± 3.95	0.92	***
Isoleucine *(k,*	65.31 ± 2.19	-0.64 ± 0.05	-0.30 ± 0.04	89.96 ± 4.10	0.85	***
Leucine *(k, %)*	54.97 ±0.98	-0.18 ± 0.01	-0.11 ± 0.01	212.60 ± 6.84	0.92	***

*** *P*<0.001 - Broken-Line Regression Model: Y=L+U(R−X)+V(X−R), where (R−X) is defined as zero at values of X>R, and (X−R) is defined as zero when X<R. Where: Y is the response variable, *k*; X is the digestible amino acid intake (mg/bird day), L is asymptote for the first segment; R is the break point or the optimal digestible amino acid intake; U and V are the slopes of the function.

Prediction of amino acid intake (AAI) based on the potential for egg output production and body weight: factorial model

Factorial models were developed using the EO and BW as input, according to "*a*" and "*b*" coefficients estimated herein. In this model, a linear relation between EO and $BW^{0.67}$ is set, maintaining the EO ≤ to the maximum potential for EO (11g), avoiding an overestimation of amino acid requirement. Another constraint was made when the objective was to predict the EO from the inverted equation, i.e. when AAI is < than $b\text{x}BW^{0.67}$, EO must be considered zero in order to avoid negative values for EO (Fisher et al. 1973; Silva et al., 2015).

The models obtained were:

Val intake: $AAI = 11EO + 86BW^{0.67}$;

Ile intake: $AAI = 10EO + 70BW^{0.67}$;

Leu intake: $AAI = 21EO + 147BW^{0.67}$.

Evaluation and application of models

The models were analyzed for their impartiality by associating the errors with the prediction of EO. The following equations explain these relations for the models developed by Val, Ile, and Leu. For Val, only the intercept was significant: $ei = 0.0935\ (\pm 0.2029^{NS}) - 0.0016(\pm 0.0283^{NS}) \times (EOi - 6.239)$, $R^2 = 0.0001$. For Ile none of parameters were significant, which indicates that the model obtained are impartial: $ei = -0.0159\ (\pm 0.0301^{NS}) - 0.0112(\pm 0.2096^{NS}) \times (EOi - 5.943)$ $R^2 = 0.0103$. Regarding Leu, only the slope was significant, and therefore, the model do not meet completely the impartiality condition: $ei = 0.2845\ (\pm 0.2097^{NS}) - 0.0231(\pm 0.0285^{NS}) \times (EOi - 6.435)$ $R^2=0.0246$.

The precision was calculated by subtracting the R^2 of 1 ($1 - R^2$). Based on the R^2 values of 99.9, 99.0 e 97.5 for Val, Ile, and Leu, respectively, the models can be said to shown an acceptable precision. The model was applied to predict the intake of Val, Ile, and Leu, based on EOmax of 11 g/day and BW of 0.168 kg. The intake of Val, Ile, and Leu calculated were 148, 132 e 278 mg/bird/day, respectively.

Discussion

The current research was centered on establishing the ideal dietary BCAA intake for Japanese quails in the egg-laying phase. In this context, the trials described herein were designed with the objective of quantifying optimum Val, Ile, and Leu intake and to understand the quail responses to the increasing dietary concentration of these amino acids, a phenomenon that until the present is not well described in literature. Given the performance responses of quails fed counter-proof diets in comparison with those fed the lowest levels of Val, Ile, and Leu, we could conclude that all of these amino acids were limiting in their respective trials.

The supplementation of 1.60 g L-Valine, 1.37 g L-Isoleucine, and 3.19 g L-Leucine/kg in counter-proof diets increased quail feed intake by 4.80, 6.65, and 6.93% when a comparison is made with the group of birds fed the lowest dietary concentration of these amino acids. Feed intake responses exhibited a slight increase in response to crystalline amino acid supplementation. Nonetheless, despite such low increments, additional Val (3.53 g/kg), Ile (3.06 g/kg), and Leu (7.04 g/kg) supply supported an egg production rate 196% [(4.23/1.43)-1×100], 171% [(6.03/2.22)-1×100] and 63% [(4.82/2.96)-1×100] higher than those supported by diets containing 1.96 g Val, 1.7 g Ile e Leu 3.92 g/kg, respectively. Additional responses of egg weight by providing quails the counter-proof diets were characterized by the increase in 3% [(8.06/7.80)-1×100], 3% [(8.14/7.90)-1×100] and 6% [(8.45/7.93)-1×100] in comparison with the diets containing the lowest Val, Ile and Leu content, respectively. Such improvements in performance responses corroborate that the relative amino acid deficiency was not correlated to the crude protein content of diets and was not influenced by dilution technique. The occurrence of these responses is crucial, and only by meeting such assumption it is possible to infer that the method used herein supports the results obtained (Fisher and Morris; 1970).

Allen and Young, (1980), Santos et al. (2016), and Paula et al. (2017) studied the effects of the increment in Leu, Ile and Val doses for Japanese quails in the egg-laying phase, respectively. Even though these studies have fulfilled the purpose of determining the amount of dietary Val, Ile, and Leu, which optimized performance traits assessed (e.g. egg production, egg output and feed conversion ratio), they were relatively unhelpful in providing information, which allows a better understanding about the way with which bird organism uses these amino acids. Such fact can be attributed to the narrow range between the minimum and maximum responses to amino acid intake observed by the authors. Conversely, in the current research, we observed a wider amplitude in quail responses to increasing amino acid intake compared to the aforementioned studies, since some responses such as egg production rate reached values close to zero in birds fed the lowest levels of Val, Ile, and Leu. By observing this behavior, we could understand better and make inferences on efficiency of amino acid utilization, maintenance and egg production requirements, and hence, modelling such responses in order to develop factorial models for the amino acids studied herein.

Understanding the mechanisms involved in animal feed intake modulation is a challenging task (Emmans, 1997). Our outcomes clearly demonstrate that regardless of the amino acid studied, feed intake was decreased as dietary amino acid concentration reached the lowest values. These outcomes support previous findings that lowest concentrations of amino acid in diets formulate based on dilutions technique trigger off a suppression on bird voluntary feed intake. (Fisher and Morris, 1970, Gous and Morris, 1985; Dorigam et al., 2014; Silva et al., 2015; Donato et al., 2016; Ferreira et al., 2016). Feed intake decrease may be presumably associated with changes in nitrogen corrected apparent metabolizable energy and crude protein (AMEn:CP) ratio of diets. Based on the coefficients of maintenance needs for AMEn (92.34 kcal/kg $BW^{0.75}$) and CP (6.17 g/kg $BW^{0.75}$) of quails described by Jordão Filho et al. (2011), we estimated quail maintenance requirements for AMEn in 24 kcal/bird/day and CP in 1.6 g/bird/day, which corresponds to AMEn:CP ratio of 15:1.

The quails fed the lowest level of Val, Ile and Leu consumed 28.6, 28.4 and 30.3 kcal/bird day, respectively, indicating that quails were fed above AMEn maintenance needs. Regarding CP intake, birds consumed 0.49, 0.48, and 0.54 g CP/bird day in

Val, Ile and Leu trials, resulting in an average AMEn:CP ratio intake of 58:1. As summit diet was diluted, dietary CP and amino acid supply decreased, whereas energy and the remaining nutrients are equally supplied in all experimental diets. In the most diluted diets, the poorer amino acid concentration was not effective in supporting egg protein synthesis, and as result of such deficiency, birds exhibit minimum egg production rates and stopped the egg production. The energy required by these birds is very small and the excess is deposited in body as lipids or must be lost as heat if the birds were continuously eating. As a means to avoid possible imbalances, birds reduce voluntary feed intake (Bowmaker and Gous, 1991).

Considering the average responses of the 3 feeding-trial conducted herein, the amount of Val, Ile, and Leu required for maximum egg weight was 23% higher than that which optimized egg production (22% Val, 30% Ile and 16% Leu), as shown in Table 5. Nonetheless, as detailed in Figure 1 the deleterious effects of poorer amino acid intake on egg weight seems to be more easily recovered compared with egg production. Our outcomes demonstrate that the reduction on amino intake from the optimum level estimated to the lowest level studied impaired egg weight in 28%, whereas the same reduction on amino acid intake decreased egg production by approximately 100%. Comparing the results found herein with the published literature, we noticed that both commercial laying hen (Morris and Gous, 1988; Silva et al., 2015) and broiler breeder hen egg weight (Bowmaker and Gous, 1991) are less sensitive in responding to changes in amino acid dietary concentration compared with Japanese quails.

Changes in body weight and body chemical composition are often noticed when dilution technique is the method elected to produce a variation in dietary amino acid levels. It is well acknowledged that lipids represent the main component of body weight gain in birds, which had already reached mature protein weight. Such lipid gain has been a common response exhibited by broiler breeders and laying hens to dilution series is the graded decrease in carcass lipid content as nitrogen in diets increases **(**Gous and Morris, 1985; Burnham et al., 1992; Ferreira et al., 2016**).** Even though quails given diets containing the lowest content of Val, Ile, and Leu have exhibited a more severe weight loss, as expected, all the levels tested here including the highest ones, promoted a negative variation in BW regardless of the amino acid studied (Table

3). Understanding BWG responses requires an analysis of quail carcass chemical composition (Table 4).

In order to maintain the egg protein synthesis, birds fed amino acid-deficient diets enhance body protein catabolism so that amino acids originated from muscles can support egg production. Our findings support such hypothesis, since in the assays conducted here the lowest was the supply of amino acids, i.e. nitrogen; the more severe was quail weight loss. Our outcomes also support the statement that birds fed diets with low levels of amino acids tend to show higher lipid content in body compared with those fed higher protein levels. The weight loss observed in quails fed the highest amino acid levels may be attributed to the excessive amount of nitrogen consumed which contrary to carbohydrates and lipids, cannot be stored in body as reserve, and thus must be excreted. Aware of the fact that nitrogen deamination and excretion is an energy-expensive process, we believe that changes in weight loss in quails fed diets with high Val, Ile, and Leu content may be due to the mobilization of energy from the of body lipid reserves to excrete the excess of nitrogen in organism. We noticed that protein content in quail carcasses of the reference group decreased regardless of the amino acid supply (Table 4). Although unexpected, these responses are coherent with those exhibited in BW variation. Birds fed diets in which amino acid supply met or exceeded their requirements for maintenance and egg output should theoretically present body protein content similar to the birds of the reference group, given the fact that they reached maturity in protein weight prior to the beginning of the trials. Why this occurred with body chemical composition, it is unknown, and this fact reinforces the needs of further investigations to elucidate bird responses to CP and AMEn. The content of CP of 20% determined in quail carcass of the reference group was very similar to that determined by Genchev et al. (2008) (19.45%).

Previous studies have applied the monomolecular function to determine the maintenance requirements of broilers for amino acid (Kebreab et al., 2008; Darmani Kuhi et al., 2009). However, to our knowledge, there are no studies, which determined quail amino acid requirements for maintenance. Assuming the average body weight of 168 g, we found the maintenance requirements of Val, Ile, and Leu as 155, 126 e 265 mg/kg BW/day, respectively. The wide amplitude in egg output responses allowed us estimating accurately the maintenance status of birds, which was considered when EO values were equal to zero. In the current study, the maintenance requirement of Val for quails was approximately 2.8 and 3.8 times higher than the values found for roosters by Leveille and Fisher (1960) and Lima et al. (2016). The estimated Ile requirement for maintenance of Japanese quails found in this study was 126 mg/kg BW/day, which is higher than that determined by Burnham and Gous (1992); Leveille and Fisher (1960) and Lima et al. (2016). Leucine requirement for maintenance determined here was about five times higher than the values found for roosters by Leveille and Fisher (1960). These authors used roosters to determine maintenance because in these birds all the requirements for amino acids are associated with the inevitable losses (maintenance). According to Burnham and Gous, (1992), these method increases the accuracy with which maintenance is determined.

Another important step in determining bird amino acid requirements is to know the efficiency with which they are used by the organism. In this research, efficiency of amino acid utilization was obtained by the ratio between the amino acid retention and amino acid intake. However, contrary to other studies, beyond discounting the intake of amino acids for maintenance, we also considered the amino acid mobilization in quail muscle tissue taking into account the loss of weight. This correction of amino acid mobilization was calculated based on amino acid composition of quail carcasses described by Genchev et al. (2008). Despite this correction, the efficiency of amino acid utilization found in this research was lower compared with literature. Here, we estimated the efficiency of Val, Ile, and Leu utilization in 75, 65, and 55%, respectively. After reaching the maximum value, the efficiency of utilization decreases, since no increments in BW retention is observed in response to increasing amino acid intake. Growth and protein deposition is determined by a genetic potential. Then, increases in protein or amino acid supply will not result in increments in performance of birds which have already consumed to satisfy their genetic potential. Contrary to this idea, the excessive amount of amino acid supply may decrease productivity because the organism must eliminate the extra nitrogen consumed (Aletor et al., 2000). The requirement of Val for egg output (mg/g) determined in this assay was 11.25 mg/g, being superior to that determined by McDonald and Morris, (1985) with laying hens (8.9 mg/g output). On the other hand, the same coefficient determined for Ile was 9.54 mg/g, which is very close to values reported by Gous et al. (1987) and Huyghebaert et al. (1991), who estimated these coefficients in 8.45 and 9.48 mg/g egg output for laying hens respectively. There are no studies published in literature regarding egg-laying bird Leu requirements for egg output, which makes comparisons impossible.

Regarding the evaluation of the models, the analysis of the errors performed here showed a high goodness of models in predicting quail amino acid intake. It is interesting to highlight that each model has random errors associated with measures and parameter estimates (St-Pierre, 2005). We performed some simulations with data available in literature. Paula et al. (2017) estimated an optimum daily Val intake of 212.4 mg/day for quails with 9.96 g of EO, whilst Santos (2016) estimated the daily Ile intake of 190.8 mg/bird de Ile for an EO of 8.89 g. Due to the lack of studies involving Leu, we considered the Leu intake of 414 mg for an EO of 10.3 g (Rostagno et al., 2011).

Taking into account the recommended intake of Val (Paula et al., 2017), Ile (Santos et al., 2016) and Leu (Rostagno et al., 2011), the BW of 0.165 kg (Rostagno et al., 2011), and our coefficients a and b, the expected EO production for both Val and Ile is 16.9 g/bird and 17.1 g/bird for Leu. As a way to maintain the aforementioned EO production birds should exhibit an egg production rate close to 100%, whilst the eggs should weight approximately 17 – 19 grams, which biologically cannot be condoned considering Japanese quail genetic potential. Therefore, according to our coefficients, it is recommended a lower intake of BCAA for Japanese quails than those, which has been published. This difference may be presumably due to the methodology used to estimate such intakes. In this research the we aimed to obtain models with coefficients with physiological meaning, whilst the other studies focused on estimating optimum amino acid ratio relative to lysine using empirical models which estimate optimum doses. Some of these models are curvilinear, which are characterized by estimating amino acid requirements for the superior individuals in a population, which not always results in optimum economic return (Baker, 1986). Factorial models, in turn, estimate the requirements for average individual of population as suggested by Hauschild et al. (2010), and therefore produce lower estimates of requirements.

Understanding how Japanese quails respond to increasing Val, Ile, and Leu intake, as well as, determining the efficiency with which these amino acids are used in organism is critical for nutritional decisions. Our results generated important information such as amino acid efficiency of utilization and coefficients for maintenance and egg output production, which allowed the development of factorial models to predict quail BCCAs requirements. By inputting daily or weekly information of quail body weight and egg output, the nutritionist can become these factorial models dynamic and therefore to use this tool to elaborate feeding programs which represent more accurately quail needs for amino acids instead of working with single values of requirements throughout the entire egg-laying cycle.

Statement of conflict of interest

The authors declare that there is no conflict of interest for the publication of this work.

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CAPÍTULO 3 - MODEL TO ESTIMATE VALINE REQUIREMENT FOR JAPANESE QUAIL HENS FOCUSED ON ECONOMIC ASPECTS

Abstract: The current study aimed to estimate the optimal economic intake of valine using the Reading Model and to make an economic evaluation of the estimated value of valine for Japanese quail in the laying phase. Two hundred and eighty Japanese quails were allocated in eight treatments randomly distributed into seven replications of five quails each. The experimental diets were formulated using the dilution technique with one summit diet and one nitrogen (N)-free diet, resulting in levels that ranged from 1.97 to 9.85 g/kg of valine. The amino acid intake, egg output (EO) and body weight (BW) were adjusted using a Reading Model (RM). Using minimum cost approach, 8 diets with crescent levels of Val were simulated to evaluate the results found in RM and the cost of the addition of Val as a nutrient in a diet. The responses of Japanese quails were affected to different dietary levels of valine ($P < 0.001$). According to the adjusted RM, the optimum economic intake for Val was calculated at 167 mg/bird/d. Based on Val intake estimated by the model and considering the feed intake of 25g/bird/d, the cost of feeding was 0.0069 US $/bird/d generating an income of 0.0222 US $/bird/d giving a gross margin of 0.0153 US $/bird/d. The RM predict an economic intake of an amino acid; however, in this study and considering the economic scenario applied, RM estimate an intake of amino acid that is above the genetic potential of the bird. The issue might be controlled using a constraining into the model.

Keywords: amino acid, economic, egg output, Reading model

Introduction

Since the 40s of the last century, there is a perception that amino acid requirements should be also considered under an economic perspective and not only defined as a single point on a smooth response curve which relate collected output to dietary amino acid supply (Hauser, 1940; Fisher, 1994). Unconsidered until the 70s, such assumption gained force and from such decade onward and several research efforts have been put forward developing tools, whose application could allow abolishing the traditional and old-fashioned idea that broiler breeder and laying hens requirements for amino acid are static and “fixed”. In such context, the Reading model

(RM), a flexible approach, was developed to predict the optimal biological and economic intake of amino acids based on the mean and the standard deviations of egg output and body weight, and on the ratio between the price of the amino acid under study and the price of eggs (Fisher et al. 1973). Based on the most productive individual in the flock, RM assumes that the optimal dietary amino acid intake might be exceeded, and a consequent improvement on egg output is expected leading to maximum profit.

Contrary to breeders and laying hens, there is still so much work to be done in regard to Japanese quail nutrition in the sense of establishing the ideal pattern of essential amino acids, which ensure proper bird productivity. An attempt of modelling Japanese quail requirement for essential amino acids has succeeded in determining optimal intake of lysine, methionine + cysteine, threonine, and tryptophan using Reading model (Sarcinelli, 2016). Among essential amino acids, whose insufficient supply limits egg protein synthesis; Val is the fourth limiting in poultry diets and has an important role on protein metabolism either by participating on bodily protein accretion or by influencing the metabolism and requirements of other large neutral amino acids such as leucine, isoleucine, tryptophan, methionine, histidine, phenylalanine, and tyrosine (D’Mello, 2003).

For the fact of Val being a feed grade amino acid trade product, nutritionists can easily manipulate its content in poultry diets to achieve specific purposes, as for example to provide an amount of Val to hens guided by an economic point of view. Modelling quail biological responses taking into account economic factors allows maximizing productive performance parallel with the optimization of profit, which in turn, is the final objective of any industrial activity. Given this previous, we conducted the current study in order to develop models, which allows estimating the optimal economic intake of valine based on biological and economic aspects.

Methods

Bird husbandry and experimental design

One dose response trial was performed using Japanese quails in egg-laying phase. The Animal Ethics and Welfare Committee of Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista, Jaboticabal Campus in Sao Paulo, Brazil approved all experimental procedures used in this study under the Protocol number 9999/14.

Two hundred eighty Japanese quails in the egg-laying period at 28 weeks of age were used in this trial. The experimental units were represented by cages of 35 cm long, 34 cm wide and 15 cm high, equipped with feeders and nipple drinkers. In total, eight treatments were randomly distributed in seven replicates of five quails per replicate. The experiment lasted seven weeks, in which only the last four were used for statistical analysis. The maximum and minimum temperature and humidity recorded were 33.85±3.65 ºC and 20.43±1.97 ºC and 73.56±19.11% and 29.75±14.13%, respectively. It was offered 16 hours of light each day for the entire experiment; feed and water were provided ad libitum.

Experimental diets

The experimental diets were formulated according to dilution technique (Fisher and Morris, 1970). Using the Brazilian Table for Poultry and Swine (Rostagno et al., 2011) as a reference for quail's amino acid requirement, a high protein diet (summit) was formulated to contain approximately 1.2 times the digestible amino acid tested; all other essential amino acids were set at a minimum of 1.4 times their recommended levels (Table 1).

A high protein summit diet, based on corn and soybean meal, was formulated containing 9.41 g/kg of valine and other diet (N-free) was formulated to meet the same nutritional level as the summit diet, except for protein and amino acids. The nitrogen-free diet was used to dilute the summit diet, in appropriate proportions, to obtain intermediate levels of valine, according to Fisher and Morris (1970). In total, seven feeds with crescent levels of tested amino acid and one control diet was produced, performing 8 treatments. The control diet was used to confirm that the amino acid tested was limiting in the feed. Moreover, a small quantity of the crystalline amino acid (L-Valine 96%) was added to the diet with the lowest level of the amino acid tested (D1), which was sufficient to meet the level of the amino acid in the second-lowest level in the dilution series. (Table 2).

Table 1 - Ingredients (%) and nutrient composition (g/kg) of the summit and N-free diets.

Ingredients	Summit	Nitrogen-free
Corn	50.13	-
Soybean meal 45%	26.37	-
Corn gluten meal 60%	9.61	-
Corn starch	-	50.00
Sugar	-	26.46
Rice husk	-	9.66
Limestone	7.18	7.09
Dicalcium phosphate	1.26	1.75
Soybean oil	1.79	2.53
Biolys L-lysine sulfate (56.4%)	1.06	-
L-Arginine (100%)	0.51	-
DL-methionine (99%)	0.52	-
Salt	0.36	0.36
Choline chloride 60%	0.26	0.34
Potassium chloride	-	1.40
L-Threonine (98.5%)	0.18	-
Vitamin mineral premix[a]	0.40	0.40
L-Valine (96%)	-	-
L-Tryptophan (98.5%)	0.11	-
L- Isoleucine (100%)	0.11	-
L-Histidine (100%)	0.09	-
L-Phenylalanine (100%)	0.05	-
B H T (Antioxidant)	0.01	0.01
Nutrient (g/kg)		
Metabolizable energy (kcal/kg)	3000.00	3000.00
Crude Protein	246.85 (220.16)[b]	-
Methionine+Cysteine	12.00 (11.84)[b]	-
Methionine	8.74 (8.75)[b]	-
Lysine	14.63 (14.38)[b]	-
Tryptophan	3.07 (2.98)[b]	-
Threonine	8.78 (8.59)[b]	-
Arginine	16.97 (16.59)[b]	-
Valine	9.41 (9.13)[b]	-
Isoleucine	9.51 (9.24)[b]	-
Leucine	21.95 (21.45)[b]	-
Histidine	6.14 (6.13)[b]	
Phenylalanine	11.28 (11.01)[b]	
Calcium	30.99	30.99
Sodium	1.55	1.55
Available Phosphor	3.23	3.23

[a] Containing/kg: Vit. A 1.750.000 U. I; Vit. D3 500.000 U. I; Vit. E 2.000 U. I; Vit. K3 500 mg; Vit. B1 250 mg; Vit. B2 875 mg; Vit. B6 500 mg; Vit. B12 1.250 mcg/kg; Niacin 6.250 mg; Choline 65 g; pantothenate acid 2.500 mg; Methionine 272.500 g; Copper 2.000 mg; iron 12.500 g; Manganese 17.500 g; Zinc 12.500 g; Iodine 300 mg; Selenium 50 mg.

[b] Values in parentheses indicate the result of nutrients analyses of the amino acid studied.

Data collection

The feed waste was weighed weekly to measure feed intake (FI). The valine intake was calculated multiplying FI by the digestible amino acid content in the diets. The body weight (BW) was measured in the first, third and seventh week. The mean BW (mBW) was obtained considering the difference between the last and first weighting. The egg production (EP) was recorded daily and the egg weight (EW) were measured three times per week. The egg output (EO) was obtained multiplying EP by EW.

Table 2 - Proportions of the summit diet diluted with the corresponding nitrogen-free diet and the resulting concentrations of the limiting amino acids in the diets.

Diet	Summit %	N-free %	Val g/kg
D1	20.8	79.2	1.96
D2	37.5	62.5	3.53
D3	54.1	45.9	5.09
D4	70.8	29.2	6.66
D5	83.3	16.7	7.84
D6	91.6	8.4	8.62
D7	100	0.0	9.41
D8*	20.8	79.2	3.53[a]

[a]Addition of 1.602 g of L-valine/kg of diet: *, Control diet.

Statistical analysis and mathematical models

The response variables analyzed were: amino acid intake (AAI), FI, EP, EW, EO and mBW. To perform the analyses of variance (ANOVA), all assumptions were a priori confirmed. Treatment eight was used to confirm if valine was the first limiting in the diets, hence, only treatments from 1 to 7 were used in statistical analyses (Fisher and Morris, 1970; Gous et al., 1985). All analyses were performed using the PROC GLM procedure of SAS (2010).

Optimum economic intake of amino acid

The economic intake of amino acid (OptAAI) was calculated using the model developed by Pilbrow and Morris (1974). The predicted value (output) of the model is the OptAAI in mg/bird d. There are six variables necessary to set the model (input): 1- maximum EO (EO_{max}, g/day); 2 – standard deviation of EO_{max} (σEO_{max}, g/day); 3 – *BW* (kg); 4 – standard deviation of BW (σBW, kg); 5 – nutritional constant for EO production (a, mg/g of EO); and 6 – nutritional constant for BW (b, mg/kg of W). The simple form of this model is:

$$\text{OptAAI} = a\,\overline{EO}_{\text{max}} + b\,\overline{BW} + Z\sqrt{a^2\,\sigma^2\overline{EO}_{\text{max}} + b^2\,\sigma^2\overline{BW} + \text{r}EOBW} \qquad \text{[Eq. 1]}$$

Where:

OptAAI is the optimal economic intake (mg/bird day);

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

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

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

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

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

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

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

This model works in two parts

The first part makes reference to optimum Val intake for average individual of the population:

$$\text{OptAAI} = a\,\overline{EO}_{\text{max}} + b\,\overline{BW} \qquad \text{[Eq. 2]}$$

The second part makes reference to optimum economic Val intake, where y represents the economically viable quantity of extra Val intake to the most productive individual and this point is represented in the curve where the slope of the tangent is the same for output and input value:

$$y = \sqrt{a^2\,\sigma^2\overline{EO}_{\text{max}} + b^2\,\sigma^2\overline{BW} + \text{r}EOBW} \qquad \text{[Eq. 3]}$$

The Z value is a modifications factor of y parameter, which take into account the economic value Z=ak, where k represents the marginal cost of 1 mg amino acid input (US$/mg) /marginal value of 1 g egg output (US$/g). The Z value of the standard normal distribution, corresponding to the value of the expression (1.0-ak). Various software could calculate Z, in this study was used the MS Excel 2016, using the formula Z = NORM.INV(1-ak).

The coefficient a (11 mg/g) was obtained by dividing a = d/$k$max, where d is the deposition of valine (0.90% or 8.44 mg/g egg) in quail egg (Genchev, 2012), and *kmax* is the maximum efficiency of utilization estimated through the variables: efficiency and valine intake adjusted to model broken line: $k = k\text{max} + U(R-X)$, Where k is the efficiency; $k$max is the efficiency estimated at the plateau of the funtion X is the amino acid intake (mg/bird day); R is the breakpoint or the optimal amino acid intake; and U is the slope of the function. The coefficient b (155 mg/kg) was obtained by adjusting the variables: valine intake and egg output in the monomolecular function: $EO= EOmax(1\text{-}exp^{-s(X-b)})$, Where EO is egg output (g); X is the amino acid intake (mg/kg); *EOmax* is the maximum EO (g); s is the slope of the function; and b is the maintenance requirement (mg/kg). Finally, maintenance was calculated by equalizing egg output to zero (EO = 0).

Application of the model on different scenarios

To evaluate the model determined herein, 63 economic scenarios were simulated in order to mime different conditions obtained in real quail production farms. The simulations were generated applying variation to EO_{max}, *BW*, and k obtained by the combination of three $EO_{\text{max},}$ three W and seven k. Firstly, it was used the values of 11 and 0.170 for EO_{max} and *BW*, respectively, determined in previous studies conducted in Unesp/Jaboticabal (Unpublished data). Further, the variation of ± 10% of the mean was applied to the average, obtaining the values of 9.9 and 12.1 g/day for EO_{max} and 0.153 and 0.187 kg for *BW*, respectively.

The values of k were obtained varying only the cost of the crystalline amino acid, considering 70%, 80%, 90%, 100%, 110%, 120%, and 130% over the mean value. The mean price of Val was determined as 14 US$ per kg of diet. The price per unit of egg was considered as US$ 0.021 for an egg of 11g. Thus, the values of $k1$, $k2$ $k3$, $k4$, $k5$, $k6$, and $k7$ were 0.0051, 0.0059, 0.0066, 0.0073, 0.0080, 0.0088 and 0.0095, respectively.

Economic evaluation of feeds formulated to meet different proportions of the population

Based on Equation 1, various amounts of valine intake were obtained to meet different percentage of the population (50.00, 69.15, 84.13, 93.32, 97.72, 99.38, and 99.87%). In order to obtain this variation, a standard deviation of 0, 0.5, 1, 1.5, 2, 2.5, and 3 was applied, obtaining the intake of valine (150, 156, 162,168, 175, 180 e 187 mg/bird per day). The feed intake was considered as 25 g/bird day, which gives the possibility to determine the requirement of the amino acid in percentage. Finally, a total of 200 feeds were formulated with increasing levels of Val, starting from 0.600% of Val in the feed, that correspond to 0 standard deviation from the mean. The last feed was formulated with 0.748% of Val, which was equal to 3 standard deviation from the mean. All feeds were produced using linear programing (Solver – MS Excel) and considering minimum cost as the objective. The prices of each ingredient used to formulate the feed was obtained on local market (0.15 U$/kg corn, 0.32 U$/kg soybean meal 45%, 0.51 U$/kg soybean oil, 3.36 U$/kg L-lysine 78%, 4.00 U$/kg DL-methionine (99%), 4.98 U$/kg L-Threonine (98.5%), 18.28 U$/kg L-Tryptophan (98.5%) and 14 U$/kg L-Valine (96%). Crude protein (17.22%) and all amino acids were hold constant, except Val. The feed formula that meet 50% of the population (0 standard deviation), was corn and soybean meal based, without the addition of L-Val.

The equation 1 was inverted to obtain EO for each Val level. The values of EO were multiplied by the price of egg (1.91 U$/kg of egg) to determine the revenue. The expenses were obtained multiplying feed cost with feed intake. Finally, gross margin from each diet were obtained by the difference among revenue and expenses. In this simulation is possible to identify the feed that will return the maximum profit, with quite accuracy since 200 feeds where simulated. The results in this simulation were compared with the predictions obtained by reading model.

Results and Discussion

Quail responses to Val intake

Quail responses to different dietary levels of Val are shown in Table 3. The levels of the amino acid affected all variables assessed ($p<0.01$). The improvement the performance responses of quails fed counter-proof treatment compared with the performance of quails fed the lowest level of Val (3.53 g/kg) confirmed that the amino acid tested was the first limiting in the diet, and that the responses were not affected by dietary protein content (Fisher and Morris, 1970). All the performance traits herein assessed were impaired as dietary Val supply decreased ($p<0.01$). In general, a decrease in food intake is observed as dietary Val content increase (Corzo et al., 2005). In literature, such response is commonly attributed to interactions with tryptophan, whose uptake into brain is impaired when plasmatic levels of Val increase. Since Trp is the key precursor for the synthesis of serotonin, an orexigin neurotransmitter, voluntary food consumption may indeed be impaired by excessive dietary Val. Conversely, in the current research, we noticed an opposite behavior in food consumption responses, where the lowest Val supply led to the poorest food consumption means. The discrepancies between our outcomes and literature may be presumably associated with the technique used to produce the variation dietary Val levels. Since the dilution technique used in our study warrants an equal balance among essential amino acids regardless of the concentration with which he amino acid under ranges, the possibility of the occurrence of antagonism are minimal. Bowmaker and Gous (1991) highlight that birds fed diets, which cannot underpin proper rates of egg protein synthesis, due to a poor amount of nitrogen, tend to decrease food consumption as a way to avoid an over intake of energy. Egg production in the most Val-deficient diet was almost suppressed, reaching the rate of 1.4%. However, if the same analysis were performed for egg weight, it is possible to notice that such variable was less severely impaired in response to the decrease in dietary Val supply. These outcomes support Morris and Gous (1988) findings that egg production is affected in a greater extent compared with egg weight. According to Bowmaker and Gous, (1991) the nature of such responses has a biological basis and relies on the fact that although decreased, eggs are laid with a minimum weight to ensure embryo development.

Table 3 - Means and standard deviations (SD) of the feed intake, amino acid intake, egg production, egg weight, egg output and body weight for Japanese quails (28 weeks).

Levels g/kg	Feed Intake (g/bird/d)		AA intake (mg/bird/d)		Egg Production (%/bird/d))		Egg weight (g)		Egg output (g/bird/d)		Body weight (Kg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.96	9.5	1.7	18.7	3.3	1.4	1.1	7.8	0.6	0.2	0.1	0.139	0.005
3.53	14.5	0.5	51.2	1.8	26.7	5.2	8.7	0.6	2.3	0.4	0.157	0.004
5.09	18.6	0.5	94.7	2.5	65.9	7.6	9.6	0.4	6.3	0.6	0.162	0.001
6.66	23.0	2.3	153.5	15.2	89.2	2.0	10.7	0.2	9.5	0.3	0.170	0.006
7.84	23.3	0.9	182.9	7.2	90.0	6.3	10.7	0.1	9.7	0.7	0.173	0.006
8.62	23.1	0.8	199.4	6.9	85.9	9.3	11.0	0.2	9.5	0.9	0.171	0.004
9.41	22.7	0.8	213.8	7.1	84.5	7.7	11.0	0.2	9.3	0.9	0.173	0.005
3.53	9.6	1.0	33.9	3.5	4.2	2.3	8.1	0.3	0.4	0.2	0.147	0.007
P-Value	<.0001		<.0001		<.0001		<.0001		<.0001		<.0001	
CV[b]	6.37		5.82		10.14		11.94		10.68		3.37	
DF[c]	41		41		41		40		40		41	

[a] Addiction of 1.602 g of L-valine/kg of diet; [b] CV; Coefficient of variation. [c] DF; degrees of freedom. [a] Control diet.

Applicability of the Reading Model

Reading model was fitted to the collected data of Val intake, EO, and BW. The parameters of the model are described as follows: EO_{max} = 11 g/bird d; σEO_{max}: 1.1 g/day (0.10 × EO_{max}); *BW* = 0.170 kg; and σ*BW* = 0.02 kg (0.12 × *BW*). The economic factor considered in the Reading model is the ratio between the price of the amino acid under study as a nutrient (U$$ mg of Val) and the selling price of the quail egg (U$$ g of eggs). As detailed in Table 4, we performed serial simulations to observe how the optimal economic intake of Val behaves in response to changes in Val price. Considering quail genetic potential above described (*BW* = 0.170 kg and EO = 11 g/bird/day), we estimated the optimum Val intake for the average individual (μ) in 150 mg/quail/day. Considering the estimates for the average individual, we clearly notice an increase in Val requirement as EO and *BW* increase. These outcomes were already expected, since these are these inputs are multiplied by the *a* and *b* coefficients to estimate requirements, and the highest genetic potential (protein synthesis rates), the highest are bird requirements for amino acids.

Table 4. Optimum economic amino acid intake (mg/bird per day) as influenced by potential egg output (g/bird per day), mean BW (kg), and cost ratios (*k*).

Egg output	BW			Cost ratio (*k*) ^{1}						
Valine		μ	*y*	0.0051	0.0059	0.0066	0.0073	0.0080	0.0088	0.0095
9.9	0.153	135	11	152	151	151	150	150	149	149
	0.170	138	11	155	154	153	153	152	152	151
	0.187	140	11	157	157	156	155	155	154	154
11.0	0.153	147	12	166	166	165	164	164	163	162
	0.170	150	12	169	168	167	167	166	166	165
	0.187	153	12	172	171	170	169	169	168	168
12.1	0.153	160	13	181	180	179	178	178	177	176
	0.170	162	13	183	182	182	181	180	180	179
	0.187	165	13	186	185	184	183	183	182	182

k, Cost benefit ratio,
μ Is the requirement for the mean population,
y Is the standard deviation from the mean requirement.
[1] Values of k according to each valine cost as nutrient, fixing the egg cost.

Based on the current market conditions previously described ($k = 0.0073$), the extra intake of Val, which would reflect the best economic scenario (y x Z) was estimated in 17 mg/bird/day. Thereby, taking into account biological and economic purposes ($\mu + y$ x Z), the Val intake estimated to optimize profit was 167 mg/bird d. Based on the quail average food intake of 25 g/bird/day described by Rostagno et al. (2017), our estimate would equivalent to 0.666% of Val. Beyond, considering genetic potential when predicting requirements (as well as any factorial model), Reading model offers the flexibility of estimating either the amount of the individuals in population, which are fed by the optimal economic intake amino acids, or the possibility of choosing which fraction of these individuals will be fed by a specific amino acid level previous determined. In possess of such tool, poultry nutritionist can achieve the most varied performance or economic objectives. The population fed by the optimal economic Val intake herein estimated (167 mg/bird/day) was determined based on the normal standard deviation (Z) of the proportion of the population, i.e., 1.39. Based on such standard deviation, the extra amount of Val meets 92% of the population. It is worth highlighting that this discussion focuses on the estimates of optimal Val intake based on the current market conditions. However, these values may change in function of the dynamic in both amino acid and egg price. Analyzing the simulations performed, we notice that regardless of the BW and EO, when amino acid price decreased, i.e. k values decreased, the estimates of optimal economic intake increased, suggesting that more Val should be added to diets to achieve optimal economic return, whilst when amino acid price increased, i.e. k value increased, the opposite response was observed.

It is worth recalling that although Reading model estimates amino acid requirements dynamically, its estimates must be interpreted with criteria, since depending on the spread in egg and amino acid price, the model may produce estimates, which cannot be biologically condoned, since the values would not reflect in performance increments due to biological limits in bird genetic potential. Furthermore, although some levels do not fit in the case above described, when excessive, some estimates of optimal intake may interfere in the metabolism of other amino acids. In Val case, for example, it is essentially important to consider that when supplied in excessive amounts, other large neutral amino acids such as lysine, tryptophan, and tyrosine may have the intestinal uptake impaired (D’Mello, 2003;

Wiltafsky et al. 2009), which could lead to an unbalanced amino acid pattern in plasma and hence impair proper protein synthesis.

Economic evaluation of the Model

Although models are useful tools in animal nutrition, it is crucial to evaluate how accurate are their predictions. In the current research, we estimated the optimal economic intake of Val in 167 mg/bird/day, which corresponded to the normal standard deviation on the normal curve distribution of 1.39. The first step to evaluate Reading model estimate was to formulate diets containing levels of Val, which fed the individuals, placed on the zero-standard deviation (*Z*) from the mean of the normal curve i.e. the average individuals to the highest superior individual, i.e. 3 standard deviations (*Z*) from the normal curve. Then, eight diets were formulated to meet quail requirements (minimum cost technique) considering the following standard deviations *Z*: 0.0, 0.5, 1.0, 1.39, 1.5, 2.0, 2.5, and 3.0. Once obtained the feed cost (diet cost x food intake (25g/bird/day)), the next step involved using the Val intake estimated for the different individuals to predict EO by inverting the model. Finally, based on the egg output production predicted for the different individuals Z (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0), and the price of the eggs, we calculated revenue, whilst the feed cost, as mentioned above, was obtained from the diets formulated. In possess, of these data, we calculated the profit (revenue – feed cost) generated by the different Val intake as detailed in Table 5.

The optimum economic amino acid intake is achieved when the difference between revenue and the cost is maximized (Kleyn and Gous, 1988). Firstly, from this simulation, we noticed that the maximum profit was achieved without the dietary supplementation of L-Val (Table 5). According to the results, the feed cost reached the higher value when the level of Val was the lowest tested 0.598% (Z= zero). Conversely, as Z values (0.5, 1.0 1.5, 2.0, 2.5) increased, i.e., increase of dietary Val, feed cost was decreased. For the concentration of Val 0.744% (Z = 3.0), the feed cost increased, which might be consequence of L-Valine supplementation. It widely recognized that the supplementation of crystalline amino acids allows meeting with a higher accuracy the requirements of poultry for essential amino acids. Indeed, there is a truth in such statement; however depending on the amino acid supplemented such reality cannot be achieved.

Table 5 - Economic evaluation of production costs in relation to the levels of % Val.

Z	Population (%)	Val (%)	Cost of diet (U$/kg)	EO (g)	Cost of feeding[2] (U$/bird/day)	Revenue[3] (U$/bird/day)	Gross margin (U$/bird/day)	EO (g) (Constrained)	Revenue[3] (U$/bird/day)	Gross margin (Constrained) (U$/bird/day)
0.00	50	0.60	0.2795	11.24	0.0070	0.0215	0.0145	11.24	0.0215	0.0145
0.50	69	0.62	0.2782	11.79	0.0070	0.0225	0.0156	11.6	0.0222	0.0152
1.00	84	0.65	0.2769	12.33	0.0069	0.0236	0.0166	11.6	0.0222	0.0152
1.39[1]	92	0.67	0.276	12.78	0.0069	0.0244	0.0175	11.6	0.0222	0.0153
1.50	93	0.67	0.2756	12.87	0.0069	0.0246	0.0177	11.6	0.0222	0.0153
2.00	98	0.70	0.2744	13.51	0.0069	0.0258	0.0189	11.6	0.0222	0.0153
2.50	99	0.72	0.2738	13.97	0.0068	0.0267	0.0198	11.6	0.0222	0.0153
3.00	100	0.74	0.2771	14.51	0.0069	0.0277	0.0208	11.6	0.0222	0.0152

Z: standard deviation; EO: egg output.

[1] Data estimated by Reading Model.

[2] Feed intake: 25 g/bird/day.

[3] Egg price: 0.00191 U$/g.

Feed grade Lys, Met, and Thr are produced by industry for a longer time compared with Val. Although, L-Val may potentially decrease feed cost in a further scenario, our simulation demonstrate the opposite idea, since the unique diets, whose Val intake decreased profit were those in which L-Val was supplemented. It clearly indicated that it is still better provide Val bounded to peptides instead of providing it in the free form.

Reading model should estimate the optimal economic intake of amino acids. Therefore, the higher profit should be originated from Reading model estimate. However, we noticed that the greater profit was generated when Val was supplied to meet the requirements of the individuals placed from 2.5 standard deviation ($Z = 2.5$) of the mean ($Z = 0$) considering the normal curve distribution. In theory, the optimal profit should be generated by feeding the standard deviation estimated by the Reading model ($Z = 1.39$). Such unexpected framework occurred because the predicted values of egg output from the Val intake of the superior individuals. In the Table 5, it is possible to notice that the Val required by the superior individuals predicted an egg output, which do not respect the limits of quail genetic potential. Therefore, it attributed a revenue from egg production, which could never exist. The feed formulated to meet the optimum economic intake of Val, determined by RM (0.666%), had a minimum cost of 0.276 U$/kg of feed. Considering a feed intake of 25 g/bird/day, the feed cost estimated was 0.0069 U$/bird/day. Inverting the equation 1, a value of 12.8 g for EO is determined, which is higher than the expected quail genetic potential. Since in this scenario the extra Val intake will not reflect increments in productivity, nitrogen will be in excess in the organism.

Among deleterious impacts of excessive nitrogen supply in diets, we can list the reduction of feed intake due to ammonia blood concertation, which may negatively affect the egg production. Moreover, nitrogen excretion is a high-energy expenditure process (Aletor et al., 2000). All of this reinforces the statement above mentioned about interpreting reading model estimates with criteria. In order to estimate the optimal intake of Val for individuals, which exist under a genetic point of view, we are introducing a constraining factor in our simulation by limiting the egg output in a genetic potential possibly achieved, in this case 11.6 g/bird/day. Such egg mass values were elected based on the expected genetic potential of a quail with an egg production of 94% and egg weight of 12.3 g (Moura et al., 2008). Fixing such maximum genetic potential for all individuals, we performed the same simulations again. In this reality, we found that the estimates originated from Reading model warranted the maximum profit. Two conclusions may be obtained from these exercises. Firstly, Reading model inputs, in this case egg output, must be constrained in order to avoid misguided assumptions of quail genetic potential, and secondly that this model is a useful and powerful tool to predict optimal economic intake of amino acids.

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