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

VALOR DE REFERÊNCIA PARA O PERFIL IDEAL DOS AMINOÁCIDOS ESSENCIAIS PARA CODORNAS JAPONESAS REPRODUTORAS

LIZIA CORDEIRO DE CARVALHO

Zootecnista

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Discente: Lizia Cordeiro de Carvalho

Orientador: Prof. Dr. Edney Pereira da Silva

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

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CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: VALOR DE REFERÊNCIA PARA O PERFIL IDEAL DOS AMINOÁCIDOS ESSECIAIS PARA CODORNAS JAPONESAS REPRODUTORAS

AUTORA: LIZIA CORDEIRO DE CARVALHO
ORIENTADOR: EDNEY PEREIRA DA SILVA

Aprovada como parte das exigências para obtenção do Título de Doutora em Zootecnia, pela Comissão Examinadora:

Prof. Dr. EDNEY PEREIRA DA SILVA (Participaçao Presencial)
Departamento de Zootecnia / FCAV UNESP Jaboticabal

Prof. Dr. NELSON JOSÉ PERUZZI (Participaçao Presencial)
Departamento de Engenharia e Ciencias Exatas DECEx / FCAV / UNESP - Jaboticabal

Profa. Dra. SILVANA MARTINEZ BARALDI ARTONI (Participaçao Presencial)
Departamento de Morfologia e Fisiologia Animal / FCAV UNESP Jaboticabal

Prof. Dr. RAMON DINIZ MALHEIROS (Participaçao Virtual)
Departamento de Avicultura / North Carolina State University

Dr. VITOR HUGO BRANDALIZE (Participaçao Presencial)
Cobb-Vantress Nutrição Avícola / Cascável/PR

Jaboticabal, 27 de abril de 2023

DADOS CURRICULARES DO AUTOR

LIZIA CORDEIRO DE CARVALHO – Filha de Vicente Bento de Carvalho e Maria Aparecida Cordeiro de Oliveira Carvalho, nasceu no dia 30 de outubro de 1993, na cidade de Turmalina, Minas Gerais. Em março de 2011 ingressou no curso de Zootecnia na Universidade Federal de Minas Gerais – Campus de Ciências Agrárias, Montes Claros, Minas Gerais, graduando-se em janeiro de 2015.

Em março de 2016 iniciou o curso de Mestrado em Produção Animal na Universidade Federal de Minas Gerais – Campus de Ciências Agrárias, Montes Claros, Minas Gerais, sob orientação do Prof. Dr. Raphael Rocha Wenceslau, defendendo sua dissertação em abril de 2018. Em agosto de 2018 iniciou o curso de Doutorado em Zootecnia na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista – Campus de Jaboticabal, São Paulo, sob orientação do Prof. Dr. Edney Pereira da Silva. De junho de 2022 a fevereiro de 2023, realizou parte do doutorado na North Carolina State University em Raleigh, Estados Unidos da América, sob orientação do Prof. Dr. Ramon Malheiro. Em março de 2023 defendeu sua tese de doutorado como requisito para obtenção do título.

EPÍGRAFE

“A maior recompensa para o trabalho do homem não é o que ele ganha com isso, mas o que ele se torna com isso.”

(John Ruskim)

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CERTIFICADO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS

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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

C E R T I F I C A D O

Certificamos que o projeto intitulado **"Modelagem das exigências de aminoácidos para codornas reprodutoras"**, protocolo nº 012203/17, sob a responsabilidade do Prof. Dr. Edney Pereira da Silva, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 03 de Agosto de 2017.

Vigência do Projeto	10/08/2017 a 10/08/2020
Espécie / Linhagem	*Coturnix coturnix*
Nº de animais	3090 = 210 + 2880
Peso / Idade	150g / 16 semanas
Sexo	Misto
Origem	Granja Vicami Ltda

Jaboticabal, 03 de Agosto de 2017.

Profª Drª Lizandra Amoroso
Coordenadora – CEUA

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

VALOR DE REFERÊNCIA PARA O PERFIL IDEAL DOS AMINOÁCIDOS ESSENCIAIS PARA CODORNAS JAPONESAS REPRODUTORAS

RESUMO – É inexistente publicações sobre perfil ideal de aminoácidos para reprodutoras de codornas japonesas. A falta dessas informações inevitavelmente, limita o poder de decisão dos nutricionistas sobre demandas no manejo alimentar e nutricional destas aves. Assim, resta para esses profissionais tomar atitudes conservadoras com grande cautela e empirismo, sobre o principal item do custo de produção do ovo fértil, o custo com alimentação e nutrição das aves. Dessa forma, o objetivo dessa tese foi estabelecer o perfil ideal de aminoácidos essenciais para reprodutoras de codornas japonesas. O primeiro estudo teve como objetivo descrever o potencial genético de codornas japonesas por meio da realização de uma meta-análise considerando estudos realizados em diferentes países. Apenas dados sobre a subespécie *Coturnix coturnix japonica* foram considerados. Os critérios investigados foram peso corporal (BW), idade (t), ano de publicação e local do estudo. Cada conjunto de material genético dentro de uma publicação foi codificado como um estudo. A função Gompertz foi utilizada para interpretar o crescimento de codornas comerciais assim, cada estudo foi representado por parâmetros da Gompertz. O BW e os dados t foram aplicados para estimar os valores dos parâmetros de crescimento da Gompertz, incluindo BW na maturidade (Wm), BW ao nascimento (Wi), taxa de crescimento (B) e ponto de inflexão (IP). A idade em que a taxa máxima de crescimento foi alcançada (t*) foi calculada considerando os parâmetros Wm, Wi e B. Os parâmetros estimados para cada ensaio foram utilizados em análises exploratórias, de agrupamento e de componentes principais. Os valores de Wi variaram de 4,1 a 11,6 g. Os valores de B variaram de 0,0393 a 0,1039/dia e, consequentemente, os valores de t* e IP variaram de 14 a 31 dias e 9,21 a 31,03 g, respectivamente. Esses resultados mostram que existe uma grande variabilidade no potencial de crescimento de codornas japonesas. Para entender melhor essa variação, dois grupos foram determinados: Brasil e outros países, segundo o agrupamento de Wi, Wm, B e t*; o parâmetro B foi a variável que apresentou maior especificidade, indicando que ambos os grupos modificaram a taxa de maturidade. Para a análise de componentes principais, o ano de publicação apresentou relação com os parâmetros de crescimento, mas apenas para estudos realizados no Brasil. Para estudos realizados em outros países, as mudanças nos parâmetros de crescimento não foram relacionadas ao ano de publicação. Em estudos brasileiros, houve uma diminuição na maturidade, mas o peso na maturidade foi maior. Portanto, diferentes estratégias de seleção genética foram adotadas no Brasil em comparação com outros países. No segundo estudo, o objetivo foi identificar as respostas das reprodutoras aos níveis de lisina (Lys), identificar as funções relacionadas a essas respostas, segregar as exigências de Lys e determinar a quantidade ideal de ingestão de Lys. Foram utilizadas 49 reprodutoras. Em um delineamento inteiramente ao acaso de sete tratamentos com sete repetições de uma ave cada. Os tratamentos consistiram na suplementação da dieta por Lys nas concentrações de 16,8, 11,8, 8,4, 6,7, 5,0, 3,4 e 1,7 g/kg. Foi utilizado um modelo misto para análise, sendo a unidade experimental o efeito aleatório e o nível de Lys o efeito fixo. Seis modelos exponenciais foram ajustados. O nível de suplementação de Lys afetou as respostas das aves ($P < 0,001$). A produção de ovos foi reduzida para 23,75% do nível normal com 1,7 g/kg de Lys e recuperada para 89,9% com 16,8 g/kg. As aves responderam aos níveis

fornecidos, permitindo a criação de uma curva de resposta de lisina. Uma função monomolecular com quatro parâmetros foi balanceada com as estatísticas de ajuste e seleção de modelos. Foi possível estimar o nível de lisina necessário para mantença em 133 ± 2 mg/$BW_{kg}^{0,67}$, e com base em uma eficiência média de 41%, 22 mg Lys produziu 1 g de massa de ovo (EO). A ingestão diária calculada pelo modelo fatorial não linear foi de 284 mg Lys para uma ave com 0,170 kg BW e produção de 10 g OE/dia. No terceiro estudo, teve como objetivo determinar o perfil ideal de aminoácidos para codornas Japonesa baseado na qualidade do ovo. Foram utilizadas 120 codornas Japonesas. Foi utilizado o delineamento inteiramente ao acaso, com 12 tratamentos e 10 repetições. Os tratamentos consistiram em uma dieta controle (CD) e outras 11 dietas obtidas a partir da deleção de 40% no aminoácido teste, usando como base a BD. Os aminoácidos estudados foram Lys, Met+Cys, Thr, Trp, Arg, Gly+Ser, Val, Ile, Leu, His e Phr+Tyr. O ensaio durou 25 dias e ao final foram mensurados peso do ovo (ES), altura, diâmetro e índice de albúmen e de gema, unidade Haugh, pH, peso da casca (ESW) e percentagem de casca. O cálculo da relação ideal foi aplicado quando detectado a diferença estatística pelo teste de Dunnett. Apenas as variáveis EW e ESW diferiram da BD. A relação ideal dos aminoácidos considerando a Lys como 100% para EW e ESW foram 82 e 83; 60 e 68; 18 e 21; 109 e 112; 99 e 102; 77 e 87; 61 e 67; 155 e 141; 34 e 37; 134 e 133, para Met+Cys, Thr, Trp, Arg, Gly+Ser, Val, Ile, Leu, His e Phr+Tyr, respectivamente. Finalmente no quarto estudo, dois ensaios foram conduzidos, com o objetivo de estimar pelo modelo de Goettingen os parâmetros que descrevem a máxima retenção teórica diária de nitrogênio (NRmaxT) e a necessidade diária de mantença de nitrogênio (NMR), que foram aplicadas para calcular a qualidade da proteína (b) e eficiência dietética do aminoácido limitante (bc-1), para determinar a exigência diária de Lys para codornas Japonesa reprodutoras, além de determinar o perfil ideal de aminoácidos (IAAR). Para o primeiro estudo, no balanço de nitrogênio foram utilizadas 49 codornas, distribuídas em sete tratamentos e sete repetições em delineamento inteiramente ao acaso. Os tratamentos consistiram em sete dietas baseadas em milho e farelo de soja, com níveis de proteína entre 70.1 a 350.3 g/kg. Os resultados da regressão linear entre nitrogênio ingerido e nitrogênio retido forneceu o valor estimado de 3386.61 mg/$PC_{kg}^{0,67}$ de $NR_{max}T$ diário. Além de, NMR diário de 425.27 mg/$PC_{kg}^{0,67}$ pela regressão exponencial entre nitrogênio excretado e nitrogênio ingerido. O que possibilitou estimar os parâmetros b e bc-1 para determinar a ingestão diária de Lys será de 291 mg/ave dia ou 1.164% de Lys na dieta considerando 25 g/ave, considerando 80% de $NR_{max}T$. No segundo, foi utilizado um delineamento inteiramente ao acaso, com 12 tratamentos e 10 repetições, totalizando 120 aves. Os tratamentos consistiram em uma dieta controle (CD) e os demais 11 tratamentos foram obtidos pela deleção em 40% da CD, para limitação de todos os aminoácidos essenciais. O IAAR foi determinado pela eficiência de utilização da proteína dietética (bc-1), e para Gly+Ser, não houve efeito sobre a qualidade proteica da ração e CD. Considerando a Lys como 100% foi determinado 87; 67; 21; 117; 96; 66; 142; 39 e 133 para Met+Cys, Thr, Trp, Arg, Val, Ile, Leu, His e Phr+Tyr, respectivamente.

Palavras-chave: Aminoácidos, balanço de nitrogênio, *Coturnix coturnix japonica*, relação ideal, reprodutoras.

REFERENCE VALUE FOR THE IDEAL PROFILE OF ESSENTIAL AMINO ACIDS FOR BREEDING JAPANESE QUAILS

ABSTRACT - There are no publications on the ideal essential amino acids ratio for Japanese quail breeders. In the absence of this information inevitably limits decision-making power of nutritionists on demands in the feed and nutritional management of these birds. Thus, these professionals to take conservative attitudes with caution and empiricism, about the main item of the production cost of the fertile egg, the cost with feed and nutrition of the birds. Therefore, the objective of this thesis was to establish the ideal essential amino acids ratio for Japanese quail breeders. The first study aimed to describe the genetic potential of Japanese quails by conducting a metaanalysis considering studies conducted in different countries. Only data about the subspecies Coturnix coturnix japonica were considered. The criteria investigated were BW (W), age (t), year of publication and location of the study. Each set of genetic material within a publication was coded as one study. The Gompertz function was used to interpret the growth of laying quails; thus, each study was represented by Gompertz parameters. The W and t data were applied to estimate the values of Gompertz growth parameters, including BW at maturity (Wm), BW at birth (Wi), maturity rate (B) and inflection point (IP). The age at which the maximum growth rate was achieved (t*) was calculated considering the parameters Wm, Wi and B. To estimate these parameters, random regression was used to randomize the parameter Wm. The parameters estimated for each assay were used in exploratory, grouping, and principal component analyses. The values of Wi ranged from 4.1 to 11.6 g. The values of B ranged from 0.0393 to 0.1039/day, and consequently, the values of t* and IP ranged from 14 to 31 days and 9.21 to 31.03 g, respectively. These results show that there is considerable variability in the growth potential of Japanese quails. To better understand this variation, two groups were examined: Brazil and other countries, according to the grouping of Wi, Wm, B and t*; parameter B was the variable that presented the highest specificity, indicating that both groups modified the maturity rate. For the principal component analysis, the year of publication showed a relationship with the growth parameters but only for studies performed in Brazil. For studies carried out in other countries, the changes in growth parameters were not related to the year of publication. In Brazilian studies, there was a decrease in the maturity rate, but the weight at maturity was higher. Therefore, different strategies of genetic selection were adopted in Brazil compared to other countries. In the second study, the objectives were to identify the responses of birds to various lysine (Lys) levels, identify the functions related to these responses, segregate the Lys requirements, and determine the ideal Lys intake amount for breeders. Forty-nine Japanese quails were used. A completely randomized design of seven treatments with one bird per experimental unit and seven replicates was used. Treatments consisted of diet supplementation by Lys in concentrations of 16.8, 11.8, 8.4, 6.7, 5.0, 3.4, and 1.7 g/kg. A mixed model was used for analysis, with the experimental unit being the random effect and Lys level as the fixed effect. Six exponential models were adjusted. The level of Lys supplementation was found to affect bird responses ($P < 0.001$). The egg production was reduced to 23.75% of the normal level with 1.7 g/kg Lys and recovered to 89.9% at 16.8 g/kg. The birds responded to the levels provided, allowing for the creation of a lysine response curve. A single-molecular function with four parameters was balanced against the statistics of adjustment and selection of models. It was possible to estimate the level of lysine

required for maintenance as 133 ± 2 mg/$BW_{kg}^{0,67}$, and based on an average of 41% efficiency, 22 mg Lys produced 1 g of egg mass (EO). The daily intake calculated by the non-linear factorial model was 284 mg Lys for a bird with 0.170 kg BW and production of 10 g EO/day. The third study aimed to determine the model parameters for maximum nitrogen retention ($NR_{max}T$), nitrogen maintenance requirement (NMR) and the efficiency of lysine utilization (bc^{-1}) to determine the Lys requirements for Japanese quail breeders. A completely randomized design of seven treatments with one bird per experimental unit and seven replicates was used. The trial lasted 21 days, with 6 days of adaptation and 15 days of excreta and egg collection. The treatments consisted of seven diets with protein levels ranging from 70.1 to 350.3 g/kg of feed, with Lys being limiting in the dietary protein (c = 4.80 g of Lys in 100 g of protein). The variables collected were: nitrogen intake (NI), nitrogen excretion (NEX), nitrogen in egg output (NMO), nitrogen deposition (ND) and nitrogen retention (NR, NR = ND + NMO + NMR). The NMR was calculated by the exponential relationship NEX and NI. The $NR_{max}T$ e b (slope related to protein quality) were estimated by the exponential fit between NR and NI. The bc^{-1} was obtained divinding *b* by *c*. The Lys intakes were estimated by the function: Lys = ($lnNR_{max}T$ – ln ($NR_{max}T$-NR)) / (16 × bc^{-1}). The $NR_{max}T$ was estimated as 3025.25 mg/$BW_{kg}^{0,67}$. The necessary lysine intake to reach 86% of $NR_{max}T$ was 1143 mg/$BW_{kg}^{0,67}$ per day, in other words, for a 0.16 kg bird the daily lysine intake was 335 mg/bird. The fourth study aimed to determine the ideal essential amino acids ratio for Japanese quail breeders based on egg quality. One hundred and twenty Japanese quails were used. A completely randomized design of 12 treatments and 10 replicates with one bird per experimental unit was used. The treatments consisted of a balanced diet (BD) and 11 other diets obtained from a 40% deletion in the test amino acid, using the BD as the basis. The amino acids studied were Lys, Met+Cys, Thr, Trp, Arg, Gly+Ser, Val, Ile, Leu, His and Phr+Tyr. The trial lasted 25 days and at the end egg weight (EW), height, diameter and index of albumen and yolk, Haugh unit, pH, eggshell weight (ESW) and shell percentage were measured. The calculation of the ideal ratio was applied when statistical difference was detected by the Dunnett test. Only the variables EW and ESW differed from BD. The ideal essential amino acids ratio considering Lys as 100% for EW and ESW were 82 and 83; 60 and 68; 18 and 21; 109 and 112; 99 and 102; 77 and 87; 61 and 67; 155 and 141; 34 and 37; 134 and 133, for Met+Cys, Thr, Trp, Arg, Gly+Ser, Val, Ile, Leu, His and Phr+Tyr, respectively. Finally, the aim of the fifith study was to apply the bc^{-1} data of the amino acids Lys, Met+Cys, Thr, Trp, Arg, Gly+Ser, Val, Ile, Leu, His and Phr+Tyr to obtain the ideal amino acid ratio (IAAR) for breeders. The nitrogen balance trials were conducted at 16 weeks of age. A completely randomized design of 12 treatments and 10 replicates with one bird per cage was used. A control diet (CD) was formulated to strictly meet the IAAR and the requirement of other nutrients. The limiting diets were formulated diluting DC with corn starch and supplemented with crystalline amino acids and other ingredients except the AA under study. In each period, NI, nitrogen NEX, NMO, ND and NR data were obtained in a 20-day trial. The *b* values were estimated by *b* = (ln ($NR_{max}T$) - ln ($NR_{max}T$-NR)) / (NI)). The bc^{-1} values were obtained by dividing *b* by *c*. The limitation was confirmed by the bc^{-1} values. The IAAR, determined by the Goettingen approach was estimated at (Lys 100%) was: Met+Cys 77, Thr 64, Trp 23, Arg 110, Gly+Ser 98, Val 98, Iso 66, Leu 144, His 38 and Phe+Try 129.

Keywords: Amino acids, breeders*, Coturnix coturnix japonica*, ideal ratio, nitrogen balance.

LISTA DE ABREVIATURAS

AA_{DC} – Amino acid in the control diet (Concentração do aminoácido na dieta controle)

AAI = Amino acid intake (Ótimo de ingestão do aminoácido)

AI – albumen index (Índice de albúmen)

AIC - Akaike information criterion (Critério de informação Akaike)

AICC - Corrected Akaike information criterion (Critério de informação Akaike corrigido)

Arg – Arginine (Arginina)

b – Slope of the exponential function that indicates the protein quality (Inclinação relacionada à qualidade da proteína)

B – Relative growth rate (taxa de crescimento relativo)

*b*0 - Estimates of the parameters (Estimativas dos parâmetros)

*b*1 - Estimates of the parameters (Estimativas dos parâmetros)

bc^{-1} – Eficiency of amino acid utilization (Eficiência de utilização de lisina)

CD – Control Diet (Dieta controle)

BIC - The Bayesian information criterion (Critério de informação Bayesian)

BW – Body weight (peso corporal)

c – Concentration of the lisine in the dietary protein (Concentração de lisina na proteína)

D – Diameter (Diâmetro)

dLys – Deposition of lysine in egg output (Deposição de lisina na massa de ovo)

ê - Regression error of the residues to the predicted values (erro da regressão dos resíduos sobre os valores previstos)

EO – Egg output (massa de ovo)

EP – Egg production (produção de ovo)

ESW – Eggshell weight (Peso de casca)

EW – Egg weight (Peso do ovo)

FI – Feed intake (consumo de ração)

Gly+Ser – Glycine + Serine (Glicina + Serina)

H – Height (Altura)

His – Histidine (Histidina)

HPD – High crude protein contente (Alto teor de proteína bruta)

HU – Unidade Haugh

IAAL – Limiting amino acid intake (Ingestão do aminoácido limitante)

IAAR – Ideal amino acid ratio (proporção ideal dos aminoácidos relacionados a Lisina)

Ile – Isoleucine (Isoleucina)

IP – Inflection point (ponto de inflexão)

K - The rate of decay of the function (taxa de decaimento da função)

LAAI – Limiting amino acid intake (Consumo do aminoácido limitante)

Leu – Leucine (Leucina)

Lys – Lysine (lisina)

Lys intake – Lysine intake (ingestão de lisina)

Lysm – Daily intake of lysine for maintenance (Ingestão diária de lisina para mantença)

M – Model (modelo)

MBW – Metabolic body weight (peso corporal metabólico)

Met+Cys – Methionine + Cysteine (Metionina + Cistina)

N – Nitrogen (Nitrogênio)

N – Level (Nível)

ND – Nitrogen deposition (Nitrogênio depositado)

NEX – Nitrogen excretion (Nitrogênio excretado)

NFD – Free of protein and amino acids (Livre de proteína e aminoácidos)

NI – Nitrogen intake (Nitrogênio ingerido)

NMO – Nitrogen in egg output (Nitrogênio na massa de ovo)

NMR – Nitrogen maintenance requirement (Exigência de nitrogênio para mantença)

NR – Nitrogen retention (Nitrogênio retido)

$NR_{max}T$ – Maximum theoretical nitrogen retention (Máxima retenção de nitrogênio teórica)

PC – Principal componente (componente principal)

PCA – Principal components analysis (análise de componentes principais)

Phe+Try – Phenylalanine + Tyrosine (Fenilalanina + Tirosina)

PYr – Standardized value of the reduction ratio (valor padronizado da proporção de redução)

RDP = Actual deletion ratio (Proporção real de redução)

R_{max} – Maximum response for deposition of lysine in egg output (máxima resposta para deposição de lisina na massa de ovo)

R_{min} – Minimum response for deposition of lysine in egg output (mínima resposta para deposição de lisina na massa de ovo)

S – Study (estudo)

t – Age (idade)

t* - Age of the maximum rate (idade de máxima taxa)

Thr – Threonine (Treonina)

Trp – Tryptophan (Triptofano)

Val – Valine (Valina)

W – Body weight at time (peso corporal no tempo)

*W*i – Body weight at birth (peso corporal no nascimento)

*W*m – Body weight at maturity (peso corporal na maturidade)

$Y_{control}$ – Mean value of the response of the control treatment (Valor médio da resposta do tratamento controle)

YI – Yolk index (Índice de gema)

Y_i – Response of each treatment (Resposta de cada tratamento)

$\bar{Y}_p$ - Average of the predicted values (Média dos valores previstos)

Y_p - Predicted value (valor predito)

Yr – Proportion of reduction (Proporção de redução)

σ – Standard devition (Desvio padrão)

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CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1.1. INTRODUÇÃO

A expansão industrial na produção de ovos de codornas Japonesas, atingiu um crescimento quatro vezes maior que a produção de ovos de poedeiras comerciais até o ano de 2019 (Silva et al., 2019). No entanto, ao longo da pandemia do vírus SARS-CoV-2 (2019-2021), houve decréscimo de 10% no alojamento de aves e 4,9% na produção de dúzias de ovos (70,6 milhões de dúzias ovos) (IBGE, 2022).

Devido, ao perfil do consumo de ovos de codornas japonesas é diferente dos ovos de postura comercial tradicional. Enquanto, que os ovos de postura comercial são consumidos dentro das residências, o consumo de ovos de codornas é feito por refeições em restaurantes, principalmente fora da residência (Piccinint et al., 2005). As consequências no setor de produção foi o descarte precoce de lotes de postura e diminuição do alojamento (IBGE, 2022), para equilibrar a relação entre oferta e procura no mercado. Já no setor de multiplicação, as medidas devem e são diferentes, devido ao trabalho de seleção das linhas genéticas, portanto, este setor teve que absorver todo prejuízo causado pela menor demanda do mercado por codornas de um dia de vida.

Além do período de crise, a falta dessa informação nutricionais tem acarretado limitações no manejo alimentar e nutricional de reprodutoras. A falta de uma recomendação referencial de requerimentos nutricionais e seus respectivos limites para codornas Japonesas reprodutoras, oneram os custos das rações, que via regra são formuladas considerando as recomendações da postura comercial, adicionado de uma margem de segurança, como garantia para não ter deficiência nutricional; portanto, os excessos de alguns aminoácidos são negligenciados. Este é o atual cenário da nutrição aminoacídica de reprodutores de codornas japonesas, e é um grande problema para o setor devido a representatividade da nutrição nos custos de produção das empresas, independente do cenário econômico. Dessa forma, esse estudo teve como objetivo determinar a relação ideal de aminoácidos essenciais para reprodutoras de codornas Japonesas.

1.2. REVISÃO DE LITERATURA

1.2.1. Reprodução e nutrição de codornas japonesas

Os ovos férteis são oriundos de matrizes que apresentam alta produtividade e qualidade de ovos, estas características são transferidas para prole, que é a pintainha de um dia, que gerará as codornas de postura comercial. Os reprodutores são responsáveis pela rápida produção, maturação, transporte de espermatozoides por meio do trato reprodutivo e fertilização dos oócitos (SANTOS et al., 2013).

No setor de reprodutores de frango de corte, a alimentação das aves é separada por sexo, baseada na diferença de altura entre machos e fêmeas, que permite o controle efetivo do nutriente ingerido e do peso corporal, evitando problemas de saúde nos machos e fêmeas, especialmente, nos machos, que demandam nutrientes apenas para mantença (NONIS e GOUS, 2008). Diferentemente, na nutrição de reprodutores de codornas japonesas, não há separação na alimentação por sexo, portanto, os reprodutores de codorna são criados em gaiolas junto com as matrizes na proporção de 1:1 e ambos consomem os níveis nutricionais estabelecidos para fêmea. No entanto, por serem animais adultos, a única demanda de aminoácidos é para mantença. Assim, deve ser dada atenção aos machos no processo reprodutivo, pois eles correspondem a 50% da fertilidade do plantel e são fundamentais para o sucesso da produção.

O problema a ser estudado aqui consiste em determinar as exigências aminoacídicas para fêmeas reprodutoras, e a forma mais simples e acessível aos produtores e nutricionista é o emprego do conceito de proteína ideal. A base desse conceito foi postulada na década de 60 por Mitchell e Scott, pesquisadores de Universidade de Illinois. O princípio fundamental consiste em formular uma dieta com o balanço exato dos aminoácidos essenciais, sem que tenha deficiência ou excesso; que atenda as exigências dos animais, para mantença, acréscimo tecidual e produção de ovos (EKMAY, 2011); que impeça o catabolismo desnecessário de aminoácidos como fonte de energia e diminua a excreção de nitrogênio (EMMERT; BAKER, 1997).

Muito embora, este conceito já esteja em uso na nutrição de codornas, os valores referência utilizados não correspondem aos demandados por codornas reprodutoras (NRC, 1994; Rostagno et al., 2011, 2017), que apresentam diferenças

nas sequências e pausas na postura dos ovos, durante o ciclo de produção. Portanto, é necessária essa determinação para essas aves que apresentam potencial genético diferente das codornas utilizadas na postura comercial.

Apesar da metionina ser o primeiro aminoácido limitante para aves em dietas a base de milho e farelo de soja, a lisina é considerada o aminoácido referência para estabelecer as proporções dos demais aminoácidos essenciais (BAKER; HAN, 1994). As razões para isso, baseia-se nos seguintes fatos: não há nenhuma síntese endógena; atuação no metabolismo proteico direcionado para síntese; não é utilizada para síntese de aminoácidos não essenciais; possui metodologia de fácil análise; para frangos e poedeiras é possível considerar que há disponibilidade de informações na literatura sobre a exigência, concentrações e digestibilidade nos alimentos. Apenas este fato, ao contrário das demais categorias, não é sustentável para codornas reprodutoras. Não foi encontrada até o presente momento nenhuma publicação sobre a exigência de lisina para reprodutoras.

A relação ideal dos demais aminoácidos essenciais como a lisina para codornas reprodutoras devem ser estabelecidos, com objetivo de aumentar a produção, tamanho e qualidade interna dos ovos, além de permitir uma formulação mais simplificada e assertiva, no que se refere aos limites que permitam formulações com níveis entre ótimo zootécnico e ótimo econômico. Com base na relação ideal, torna-se possível formular rações que possam maximizar a utilização de nitrogênio, com uso de rações com baixa proteína e que possibilite manter o desempenho produtivo (LEMME, 2003).

1.2.2. Aminoácidos essenciais para codornas Japonesas reprodutoras

Os aminoácidos essenciais destacam-se no processo reprodutivo desde o funcionamento adequando do trato reprodutivo de ambos os sexos, na qualidade final do ovo e da prole (LI, et al., 2019). Como comentado anteriormente a lisina, é utilizada na síntese proteica de tecidos do corpo e do ovo, e sua deficiência, ocasiona perda na produção (DOMINGUES et al., 2012). A deficiência de lisina na dieta afeta a expressão de genes relacionados a proteólise em músculos, com ativação das enzimas catepsinas B, H, L e D, calpaínas e a via dependente de ubiquitina-proteassoma (TESSERAUD et al., 2009), assim, os músculos atuam como depósito

de proteína na ingestão deficiente em lisina. Quando a lisina endógena não é suficiente para suprir as necessidades de produção, menores pesos de ovário e oviduto foram encontrados no estudo de Kim et al. (2020), e por consequência queda na produção de ovos. Excesso de isoleucina e lisina em matrizes pesadas levaram a diminuição da fertilidade (EKMAY et al., 2014). Uma possível causa foi atribuída a alcalinização sanguínea, que pode ser explicada pelo catabolismo desses aminoácidos em corpos cetônicos, onde alteram o microambiente das glândulas hospedeiras de espermatozoides, que tem uma faixa de pH natural levemente ácido, entre 6,72 e 6,94, conforme BOGDONOFF e SHAFFNER (1954).

Os aminoácidos sulfurados (metionina e cisteína) estão diretamente ligados na qualidade interna, no peso dos ovos (BERTECHINI et al., 1995) e são fundamentais para síntese proteica (CASTRO; KIM, 2020). Além do que, a metionina desempenha papel fundamental na doação do grupo metil ativo, pois com a liberação pode-se formar a S-adenosil-homocisteína e após à hidrolise forma-se a homocisteína (D'MELLO, 2003). Que pode ser transulfurada, para conversão junto com a serina em cisteína (STIPANUK, 2004), e posteriormente ser agrupada à uma proteína, ou utilizada na síntese de taurina e glutationa (MARTÍN-VENEGAS et al., 2006), molécula que atua de forma oxidativa no metabolismo celular (XIAO et al., 2017). A suplementação com DL-metionina na dieta aumentou a taxa de eclosão e taxa de fertilidade em codornas reprodutoras (8-16 semanas de idade), em 21,20 e 24,73% em relação as aves que não consumiram dietas com suplementação (REDA et al., 2020). Em outro estudo, também com reprodutoras de codornas, foi possível verificar que a suplementação de DL-metionina na dieta, foi capaz de melhorar desempenho, imunidade e status oxidativo, comparada as aves que não foram suplementadas com DL-metionina na dieta (KALVANDI, 2019).

Semelhante aos demais aminoácidos citados, os aminoácidos de cadeia ramificada (BCAA; leucina, isoleucina e valina), devem ser fornecidos na dieta.O desequilíbrio entre eles, causa interações negativas como o antagonismo (D'MELLO, 1974), por serem estruturalmente semelhantes, são degradados pela aminotransferase de cadeia ramificada (ACR), de forma irreversível (KIM et al., 2022). A leucina, tem maior estimulação enzimática da ACR, por tanto, excesso de leucina na dieta, leva ao catabolismo dos demais BCAAs. Presumisse que os BCAAs,

destacam-se na produção de ovos, pois são responsáveis pela regulação na síntese hepáticas de lipoproteínas para a gema, através do metabolismo de ácidos graxos no fígado (MACELLINE et al., 2021). Excesso de leucina na dieta, diminui a produção e qualidade dos ovos. Há o relato que este comprometimento na qualidade do ovo seja responsável por afetar as condições do meio interno e, consequentemente, o desenvolvimento do embrião, muito embora, seja reversível, quando restaurado com adição de isoleucina e valina na dieta (BRAY, 1970). Em estudo com codornas Japonesa reprodutoras, foi relatado que a produção de ovos, massa de ovo, peso da codorna de um dia e as características de qualidade do ovo foram melhoradas quando os autores utilizaram 18% de proteína bruta e 0,2% de L-valina na dieta das aves (HANAFY; ATTIA, 2018).

Dentre as espécies estudadas as aves são as mais exigentes em arginina, uma vez que, não é funcional a síntese de arginina pelo ciclo da ureia, portanto, é essencial o fornecimento desse aminoácido na dieta (FERNANDES e MURAKAMI, 2010). A arginina é a principal fonte de ornitina para síntese intestinal e de poliaminas, que são essenciais para crescimento celular na síntese de DNA e proteínas, na regulação genética e epigenética do crescimento celular (PEGG, 1986; WU et al., 2013; WU, 2014; SAGAR et al., 2021). A arginina, ainda tem um impacto positivo no desenvolvimento folicular e ovulação, pelo estímulo da secreção do hormônio luteinizante (YUAN et al., 2015).

A suplementação dietética de arginina em codornas reprodutoras, com 1,15, 1,30 e 1,45 vezes a necessidade para codornas pelo NRC (1994), criadas sob estresse térmico, apresentaram melhoras no peso do ovo, produção de ovos, unidade Haugh, fertilidade, eclodibilidade e na transmissão de anticorpos maternos para a prole, quando comparadas as aves sob as mesmas condições (KALVANDI et al., 2022). Apesar da importância da arginina no metabolismo, são poucas as referências para codornas japonesas (LIMA; SILVA, 2007; LOBATO; COSTA, 2009, REIS et al., 2012; SANTOS, 2013; MAURÍCIO et al., 2016; TUESTA et al., 2018). E dentre esses estudo citados, nenhum descreveu respostas mediante a redução da arginina na dieta. Uma hipótese aqui considerada é o referencial superestimado, junto com baixa amplitude dos níveis testados pelos autores. O que foi confirmado por Lima et al.

(2022), que níveis de arginina modificou respostas na produção, peso e massa de ovo, em codornas comerciais.

Segundo MACELLINE et al. (2021), poucos estudos são encontrados que determinem as exigências nutricionais em triptofano para poedeiras comerciais. O que se estende para codornas Japonesas. Rizzo et al. (2008) relataram que, níveis dietéticos de triptofano (0,23; 0,48; 0,73 e 0,98%) em dietas para codornas Japonesas comerciais, não apresentaram diferenças significativas nas respostas de desempenhos dessas aves. Em contraste, níveis dietéticos abaixo (0,14; 0,16; 0,18; 0,20 e 0,22%), foi suficiente para alterar respostas de peso do ovo, ganho de peso e conversão alimentar em codornas Japonesas (LIMA et al., 2020), os autores ainda fornecem a necessidade de 20,63 mg/ave dia, como recomendações nutricionais. O que foi 43% menor das recomendações de Sarcinelli et al. (2020), que recomendaram 55 mg/dia para máxima resposta em massa de ovo, para um consumo de 25 g/dia e peso corporal de 180 g/ave.

Nenhum estudo investigando as exigências nutricionais para reprodutoras de codornas Japonesas foi encontrado. No entanto, o triptofano desempenha papel importante na qualidade do ovo, os níveis de proteínas totais e cálcio no sangue são aumentados, quando o mínimo de 0,18 % de triptofano na dieta é utilizado (WEN et al., 2019). O triptofano regula positivamente a expressão do gene da ovalbumina, principal proteína do albúmen (FOUAD et al., 2021), com aumento da concentração do fator de crescimento semelhante à insulina I, que tem efeito positivo no desenvolvimento do oviduto (FU et al., 2001), e menor concentração de corticosterona (KIM; CHOI, 2014). Em revisão de Fouad et al. (2021), retrata que o triptofano, é capaz de equilibrar o metabolismo lipídico em poedeiras comerciais, citando como exemplo Rogers e Pesti (1992), onde todos os níveis utilizados foram capazes de reduzir os lipídeos no fígado. Na casca, a influência do triptofano está na melhoria da captação de Ca^{+2}, através da melatonina (Fouad et al., 2021).

Recomendações de treonina foram descritas para codornas Japonesas comerciais (UMIGI et al., 2007; UMIGI et al., 2012; LIMA et al., 2013). No entanto, nenhum estudo abordou a recomendação de treonina para reprodutoras. Em poedeiras e patas em postura níveis dietéticos de treonina tem efeitos na produção de ovos, peso de ovos e peso de albúmen (MARTÍNEZ-AMEZCUA et al., 1999; JIANG

et al., 2019). Em codornas Lima et al. (2013), encontraram efeito quadrático na produção, peso do ovo, massa de ovo, com o aumento de níveis de treonina na dieta. O autor, relata que codornas alimentadas com maior relação de treonina: lisina (86:100) em relação as aves que foram alimentas com menores relação (66, 70, 74, 78 e 82), apresentaram aumento de dobras e glândulas tubulares secretoras de albúmen no magnum, o que proporcionou maior produção de albúmen, e foi descrito o aumento de dobras secundária no útero, que pode ter relação direta com qualidade de casca. Em estudo com matrizes, níveis suplementares de treonina na dieta (0,12; 0,24; 0,36; 0,48 e 0,60%), não apresentaram efeito nos órgãos reprodutivos, porém esses autores relataram aumento da expressão do gene alvo da rapamicina em fígados e músculos do peito de embriões de poedeiras, indicando efeito benéfico na suplementação de treonina para proles (JIANG et al., 2019), na tradução proteica e da biogênese proteica (COTA et al., 2016).

O resultado do catabolismo de treonina gera energia ou glicina, aminoácido chave em processos metabólicos na síntese de proteína, serina, colina, creatinina, ácido úrico, ácido guanidino acético, glutationina e sais biliares (KIDD; KERR, 1996; TAYLOR; MEYERS, 2012; KIDD; LOAR, 2021). Dentre os dois aminoácidos produzidos através de treonina (glicina e serina), a glicina é considerado um aminoácido insuficientemente sintetizado, pois sua exigência não pode ser totalmente sanada por seus precursores (QAID; AL-GARADI, 2021), a glicina é um percursor de serina, assim, o valor dietético dos dois aminoácidos é expresso em equivalente de glicina (DEAN et al., 2006).

Sabe-se que a histidina é um aminoácido essencial em aves desde 1959 (LEVEILLE; FISHER, 1959). Porém, limitações ou excessos de histidina na dieta, não foram elucidados em aves na produção de ovos. A histidina, tem função fisiológica importante, atuando como peça-chave na geração de carnosina, histamina e 3-metilhistidina, sendo o último relacionado ao turnover proteico muscular (TAYLOR; MEYERS, 2012). A carnosina, desempenha importante funções fisiológicas, como antioxidação, antiglicação e tamponamento potente de pH (KIM; CHOI, 2014), que são encontradas em músculos esqueléticos, coração e sistema nervoso (TIAN et al., 2007).

Entre a sequência de aminoácidos citados, e suas devidas contribuições na nutrição avícola, alguns aminoácidos também essenciais não impactaram na literatura científica. Com o constante refinamento das dietas de aves, entender e quantificar as necessidades nutricionais torna-se necessário, no entanto, nenhum estudo foi encontrado com fenilalanina dietética para codornas. A fenilalanina e tirosina são considerados pares, devido a habilidade de interconverter, segundo Sasse e Baker (1972), cerca de 42,5% da necessidade de fenilalanina é composta por tirosina, para pintos na fase inicial. A fenilalanina está diretamente envolvida na síntese de hormônios tireoidianos, que controlam os processos metabólicos de crescimento e influenciam no crescimento, na eficiência nutricional, consumo de oxigênio, proteogêneses e metabolismo de proteínas, carboidratos e lipídeos, além de, estarem envolvidos na termorregulação (TAYLOR; MEYERS, 2012). Após sua conversão em tirosina, neurotransmissores são sintetizados tendo a tirosina como substrato, dentre eles: dopamina, norepinefrina e epinefrina (FERNSTROM; FERNSTROM, 2007; TAYLOR; MEYERS, 2012).

1.2.3. Métodos utilizados para obter as recomendações aminoacídicas

Comumente, as Tabelas de recomendação nutricionais utilizam a mesma base teórica (NRC, 1994; ROSTAGNO et al., 2011, 2017). Que consiste resumidamente, em três passos: primeiro, conhecer a exigência de lisina; segundo, conhecer a relação da lisina com os demais aminoácidos essenciais e; terceiro, associar às curvas de produção para obter as recomendações de forma dinâmica, diariamente ou por fase de criação.

O método dose resposta ou empírico, denominado por alguns pesquisadores (HAUSCHILD et al., 2010), é o mais difundido e simples para se estudar a relação entre nutriente ingerido e resposta da ave. Devido sua simplicidade foi preferido por várias décadas e poucas alternativas foram desenvolvidas. Especificamente, para o primeiro passo, que consiste em encontrar a exigência de lisina, ainda é sustentável e robusto o uso do método dose resposta, devido a modelagem empregada na resposta da ave à ingestão da lisina.

Algebricamente a “exigência” ou ingestão de um aminoácido pode ser expressa da seguinte forma: $I=a.R+b.P$, quando I é ingestão, *a* e *b* são as exigências

por unidade de resposta R e peso P. Desta forma, torna-se possível predizer a resposta (R=I-[*b*.P]/*a*), quanto foi utilizado para mantença (*b*=I-[*a*.R]/P) ou a eficiência com que os animais respondem (*a*=I-[*b*.P]/R), por isolamento dos parâmetros da equação básica (I=*a*.R+*b*.P) (Silva et al., 2014). O aprofundamento desse modelo permite avançar o entendimento do sistema biológico, da seguinte forma: $I=a.Pm^{0.73}.u+b.PP+c.DPc/k+d.DPp/k+e,DPo/k$, quando Mc = [$a.Pm^{0.73}.u$], em que, *a* é a exigência para peso metabólico proteico do corpo depenado ($Pm^{0.73}$); Pm é peso proteico na maturidade do corpo depenado; *u* é grau de maturidade da proteína corporal (Pm/Pt) representado em uma escala que considera o estado fisiológico do animal, entre o peso proteico atual ou no tempo (Pt) e peso na maturidade (Pm), desta forma, o crescimento varia de 0 a 1, e qualquer restrição é considerada pela massa atual do animal (Pt). O conceito de mantença de pena é representado por Mp = [*b*.PP], em que *b* é o coeficiente de perda de penas; PP é peso proteico de penas, desta forma, a mantença das penas tem a finalidade de repor a quantidade perdida. As exigências para deposição de proteína no corpo depenado [*c*.DPc/k] e nas penas [*d*.DPp/k] são calculadas separadamente. Os parâmetros *c, d* e *e* representam o conteúdo do aminoácido na proteína do corpo, nas penas e no ovo, o *k* é a eficiência de utilização para deposição na proteína do corpo (DPc) e na proteína das penas (DPp) e no ovo (DPo). Com base nesse modelo é possível calcular a ingestão do aminoácido atendendo precisamente a demanda para deposição de proteína.

O segundo passo, consiste na determinação da relação ideal. Para se obter as relações dos aminoácidos essenciais com a lisina há diferentes opções metodológicas. O mais comum é o método dose reposta, mas é necessário realizar um ensaio para cada aminoácido, portanto, em relação ao tempo, é considerado um método que delonga para se obter as relações e, por isso tem alto custo (SAKOMURA; ROSTAGNO, 2017). As relações podem ser extraídas por meio de metanálise, entretanto, pressupõe que há na literatura informações e publicações suficientes para filtrar as coerências e estabelecer as relações aminoácido:lisina (KIEFFER et al., 2009; HAUSCHILD et al., 2010). Mas, este método é impraticável com reprodutoras de codornas, uma vez que, não há publicações sobre esse assunto. Uma outra opção é estabelecer com base na composição aminoacídica do tecido alvo, ou seja, composição aminoacídica do ovo. O problema neste procedimento é admitir que não

há ineficiência, nem perdas no processo e toda relação aminoácido:lisina utilizada na ração será integralmente, encontrada no ovo (MERTZ, 1972). Este pressuposto não é sustentável, porque há perdas e há ineficiência, como já dito em seções anteriores.

Uma técnica desenvolvida por Wang e Fuller (1989), pressupõe que a remoção de um aminoácido não limitante não tem efeito sobre a retenção de nitrogênio. Portanto, as mudanças na retenção de nitrogênio pela remoção proporcional de cada aminoácido podem ser usadas para calcular um perfil de aminoácido, na qual, todos os aminoácidos são limitantes. A remoção do primeiro aminoácido limitante poderia reduzir o nitrogênio retido na maior proporção. Se a remoção de um aminoácido não reduzir a retenção de nitrogênio, então a quantidade removida estava em excesso relativo ao primeiro aminoácido limitante. Se a remoção de um aminoácido resultou na redução do nitrogênio retido, intermediário, então à proporção que poderia ter sido removida sem reduzir o nitrogênio retido pode ser interpolada proporcionalmente (WANG; FULLER, 1989).

Baseando-se que a redução de um aminoácido não limitante, não tem efeito sobre a retenção de nitrogênio, o método é conceituado, e denominado de deleção (WANG; FULLER, 1989; GRUBER et al., 2000; WECKE; LIEBERT, 2013; DORIGAM et al., 2017; SOARES et al., 2019). Este possibilita a determinação da exigência e da relação de todos os aminoácidos essenciais em, apenas um único ensaio, utilizando o mesmo grupo de animais, o que intensifica a uniformidade e coerência nos resultados (DORIGAM et al., 2017). O diferencial desta técnica é a possibilidade de determinar as relações em curto período, para essas aves, aproximadamente 21 dias. Para determinar um perfil de aminoácidos pelo método da deleção, uma dieta basal é formulada para atender 100% de exigência dos aminoácidos essenciais. Para que toda a exigência seja atendida utiliza-se fontes industriais dos aminoácidos. Em seguida é feita diluição da dieta, em percentagem determinado para ser limitante e novamente todos os aminoácidos são atendidos com utilização de fontes industriais de aminoácidos, exceto o aminoácido em estudo, que será o limitante. Sendo assim, é formulada uma dieta para cada aminoácido testado, conferindo um tratamento, ou seja, de cada tratamento se obtém uma relação ideal (DORIGAM et al., 2017). Esta característica é bastante oportuna para ser utilizada na determinação da relação ideal para codornas reprodutoras, que precisa de informações consistente.

O terceiro passo, consiste na integração e estruturação do modelo matemático para determinar a ingestão da lisina diariamente, utilizando as curvas de produção de massa de ovo fértil. Com base nas estimativas diárias de ingestão de lisina aplica-se a relação ideal e obtém-se as exigências dos demais aminoácidos. Para isso, é necessário descrever o potencial de produção de ovos das reprodutoras. Adicionalmente, para uma maior aproximação da realidade de cada ambiente produtivo, faz-se necessário ajustar o padrão de consumo de ração. Estas informações são facilmente modeladas e permitem estimar a produção diária e o consumo de ração diário. Compreender a exigência nutricional durante o ciclo de reprodução é uma importante ferramenta que possibilita, em sequência implantação de programas de alimentação, com maior produtividade e retorno econômico. Diante disso, objetiva-se com esta pesquisa estabelecer o perfil de aminoácidos essenciais e seus respectivos limites para matrizes de codornas japonesas, por meio da modelagem das respostas das aves à ingestão de lisina e determinação da relação ideal do aminoácido.

1.2.4. Caracterização do material genético utilizado

O desenvolvimento de linhagens de codornas comerciais de alto rendimento (300 ovos), juntamente com o crescimento estrutural e operacional na produção, caracteriza a coturnicultura atualmente. O valor genético é determinado por características primárias e correlatas de seleção (RAJKUMAR et al., 2021). Assim, o conhecimento aprofundado da base genética é essencial para um bom planejamento onde estratégias de reprodução sejam eficazes para programas de melhoramento genético.No entanto, as respostas fenotípicas dessas aves só podem ser expressas caso nutrição, ambiente e sanidade sejam atendidos com precisão. Como já caracterizado anteriormente nessa revisão informações nutricionais para reprodutoras são escassas na literatura. As reprodutoras utilizadas nos estudo posteriores pertence a linhagem maternal masculina, como descrito na figura 1 e as fêmeas da linha B da segunda geração foram as aves utilizadas nas pesquisas que serão descritas no decorrer na tese.

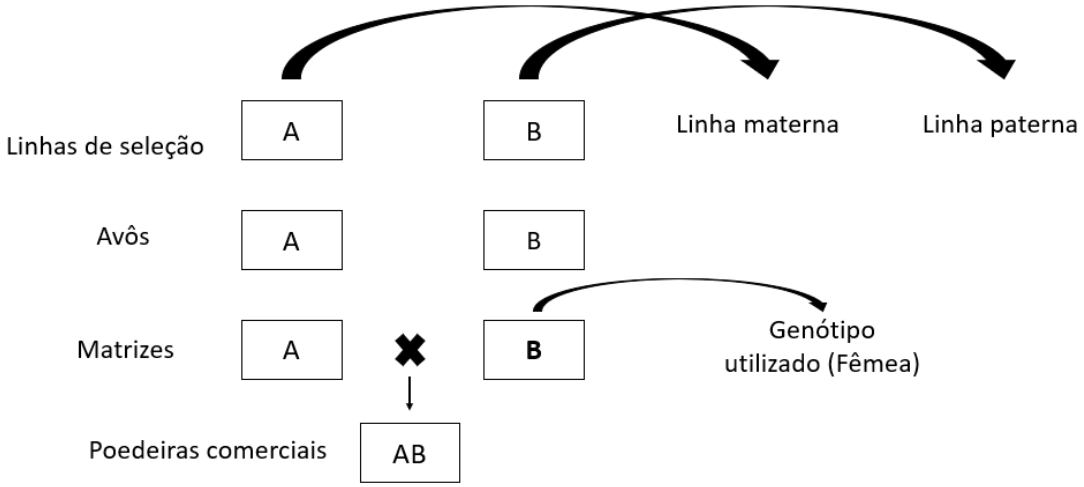

Figura 1. Representação esquemática da estrutura populacional de codornas Japonesas. aves puras (A e B) e aves híbridas (AB) de dois programas de cruzamento, sendo A linha fêmea e B macho.

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CAPÍTULO 2 - GENETIC GROWTH POTENTIAL CHARACTERIZATION IN THE JAPANESE QUAIL: A META-ANALYSIS

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Genetic growth potential characterization in the Japanese quail: a meta-analysis

L. C. Carvalho [1], H. S. Nogueira [1], A. R. T. Minussi [1], M. B. Lima[1], D. P. Munari[1], N. J. Peruzzi[1] and E. P. Silva [1]

[1] *UNESP – Univ Estadual Paulista, College of Agriculture and Veterinary Sciences, Jaboticabal, SP, Brazil.*

Corresponding author: Lizia Carvalho. Email: liziatur@hotmail.com

Short title: Characterization of growth in Japanese quail

Abstract

The description of the growth of the Japanese quails is necessary to characterize the genetic potential of these birds raised in different countries. Thus, the aim of this study was to describe the genetic potential of Japanese quails by conducting a metaanalysis considering studies conducted in different countries. Only data about the subspecies Coturnix coturnix japonica were considered; studies regarding Coturnix coturnix coturnix were not examined. The criteria investigated were BW (W), age (t), year of publication and location of the study. Each set of genetic material within a publication was coded as one study. The Gompertz function was used to interpret the growth of laying quails; thus, each study was represented by Gompertz parameters. The W and t data were applied to estimate the values of Gompertz growth parameters, including BW at maturity (*W*m), BW at birth (*W*i), maturity rate (B) and inflection point (IP). The

age at which the maximum growth rate was achieved (t*) was calculated considering the parameters *W*m, *W*i and *B*. To estimate these parameters, random regression was used to randomize the parameter Wm. The parameters estimated for each assay were used in exploratory, grouping, and principal component analyses. The values of *W*i ranged from 4.1 to 11.6 g. The values of B ranged from 0.0393 to 0.1039/day, and consequently, the values of t* and IP ranged from 14 to 31 days and 9.21 to 31.03 g, respectively. These results show that there is considerable variability in the growth potential of Japanese quails. To better understand this variation, two groups were examined: Brazil and other countries, according to the grouping of *W*i, *W*m, *B* and t*; parameter B was the variable that presented the highest specificity, indicating that both groups modified the maturity rate. For the principal component analysis, the year of publication showed a relationship with the growth parameters but only for studies performed in Brazil. For studies carried out in other countries, the changes in growth parameters were not related to the year of publication. In Brazilian studies, there was a decrease in the maturity rate, but the weight at maturity was higher. Therefore, it appears that different strategies of genetic selection were adopted in Brazil compared to other countries.

Keywords: *Coturnix coturnix japonica*, growth curve, Gompertz function, principal components, random regression

Implications

In this study, different strategies applied in the selection of Japanese quails were verified. In some countries, it was possible to verify birds with greater weight at maturity and this may imply an increase in the size of the egg and better use of the female

carcass at the end of the egg production cycle. In addition, males can be better used for meat production. In other countries, like Brazil, the change in growth was directed for egg production. In addition, there was a decrease in body weight at maturity, and it is expected that the egg size will be reduced and that this combination favors the lower incidence of cloacal eversion.

2.1 Introduction

Egg production from Japanese quails has increased since its introduction in Japan in 1920, with the first specialized genotype for egg production (Wakasugi, 1984). The modernization of production structures, large-scale breeding, different forms of processing and presentation of the eggs for consumption allowed greater acceptance and consumption of quail eggs in Brazil. Currently, large Brazilian farms house more than 100 000 quails in automated production systems that provide a regular supply of good quality eggs to the market (Silva et al., 2019).

The characterization of the genetic potential of these birds is the first step in understanding their growth profile, including the rates of weight gain and daily nutrient intake requirements for maintenance and desired weight gain (Gous et al., 1999) as well as in analyzing the best feeding program (Silva et al., 2015, 2016 and 2019). The first characterization of the genetic potential of Japanese quails was published in 1960 (Wilson et al., 1960), and from this date forward, some publications began using the Japanese quail as amodel for various purposes. Several studies were published with incomplete information, so there is a desire to reproduce these studies.

Due to the availability of the data, systematic analysis is of paramount importance for characterizing the variability in growth parameters. The Gompertz function is a

mathematical tool used by nutritionists to characterize the genetic potential of birds (Silva et al., 2015 and 2016). This function has three parameters related to biology and easy interpretation of the growth response of an animal: initial BW or BW at birth (Wi), BW at maturity (Wm) and relative growth rate (B), which represent the rate of growth from Wi to Wm.

This function was used by Silva et al. (2015) to characterize the modifications in the genetic growth potential of broiler chickens and commercial laying hens. According to Silva et al. (2015), few data are available on the reevaluation of these growth parameters for broiler chickens and commercial laying hens. For broiler chickens, the Wm was not significantly altered, but there was an early expression of the genetic potential as an anticipation of the age of the maximum rate (t*), causing an increase in the maturity rate B (Silva et al., 2015).

The analysis conducted by Silva et al. (2015) on commercial laying hens showed a decrease in the Wm value and an increase in B to cause the poultry birds to weigh less and mature more quickly (Silva et al., 2015). Thus, the question that needs to be answered is: what happened to the genetic potential of Japanese quails? Based on the Gompertz function, it is possible to obtain data to answer this question and to use it for calculating the BW and weight gain rate as well as to provide information for nutritional purposes such as nutrient intake calculations and feeding program simulations. The aim of this study was to characterize the genetic potential of Japanese quail growth by conducting a meta-analysis of studies published in the literature. The preliminary results were published in an abstract form in Advances in Animal Biosciences, according to Carvalho et al. (2019).

2.2 Material and methods

2.2.1 Literature search: search strategy. The bibliographic survey, following instructions from Koutsos et al. (2019), was carried out without distinction of publication date or place of publication. We considered relevant information to be published in scientific articles, theses, dissertations and unpublished studies. Publications in summaries of proceedings of events were not considered. The indexing terms used on Scopus platforms, in Science Direct and in the Web of Science database were TITLE-ABS-KEY (quail * AND (growth * AND (OR *) OR Japanese *), KEY (‘Japanese’, ‘quail’, ‘growth’, ‘Curve’ growth, Curve) and (quail * AND (growth * AND (OR curve *) OR Japanese *), respectively.

Six inclusion criteria were considered for studies in the databases. They were:

Criterion 1: The study reported information on Coturnix coturnix japonica as a species, and no other subspecies have been accepted.

Criterion 2: The study included the variable BW in relation to time.

Criterion 3: The study included a control group of birds, with no changes due to any treatment that may interfere with BW.

Criterion 4: For paired comparisons, it is possible to identify the location of the experiment (country) and the year of publication.

Criterion 5: It was assumed that the experimental tests were designed to allow the expression of the bird’s maximum genetic potential.

Criterion 6: The study provided non-limiting conditions for average growth, extending to management practices during rearing, diet and applied nutritional levels.

To obtain robust and impartial results, information about the environment, such as temperature, humidity and ventilation, was not considered to have an effect on the statistical analyses, as the authors admitted that the tests were adequately designed for the study of growth. Even with these restrictions, some food evaluation studies were used; although the original objective of these studies was not to describe growth, if the publication fit the conditions, it was partially considered, adding only the control group to the base. Each article was carefully analyzed, and several studies included more than one set of genetic material; in these cases, the experiments in the same article received the study codes S1, S2, S3, : : : , S52, separating the general average effect that could dilute the differences between the different genetic materials. More information about the criteria to include studies is provided in Supplementary Material S1 (Supplementary Table S1). In addition to the information collected in the literature, three studies were carried out at Universidade Estadual Paulista, Campus de Jaboticabal, SP, Brazil.

2.2.2 Data extraction: Both W and t data were obtained from the publications for analysis. Initial t at the start of the test was considered ‘day 1’ and the starting age for all assays. The studies were interpreted using Gompertz function parameters. The values of W and t were used to estimate the parameters of the Gompertz function as described by Emmans (1981): $W = W_m \times e\{[-e((\ln(-\ln(W_i / W_m)))-B \times t]\}$, where W is the BW (g) at time t, t is the age of the bird (day), W_m is the BW at maturity (g), W_i is the BW at birth (g), B is the maturity rate (per day), e is Euler’s number and ln is the natural log. In addition to the parameters of the function, three pieces of information were obtained from the adjusted models for each study: the age of maximum growth

rate (t*, day), absolute growth rate (dW/dt, g/day) and weight at inflection point (IP, g), which were calculated according to Emmans (1981). The t* was calculated for each study using the adjusted parameters (*W*m, *W*i and *B*) as follows: $t^* = \ln[-\ln(Wi/Wm)]/B$. The absolute growth rate was calculated as $dW/dt = B \times W \times \ln(Wm/W)$. The IP was also calculated for each study using *W*m, *B* and t*: $IP = Wm \times e\{[-e((-B(t^*-t^* t))]\}$.

2.2.3 Strategy for interpreting extracted data: The variables *W*m, *W*i, *B*, t* and IP were analyzed using a non-linear model of comparison techniques to verify possible differences in Gompertz parameters adjusted with data from studies performed in Brazil v. studies conducted in other countries. We used exploratory techniques, cluster analysis and principal component analysis (PCA) to interpret the relationmof the parameters with the year of publication.

2.2.4 Statistical analysis: The estimated parameters and their descriptive statistics were used to represent the studies in the subsequent PCA. For this analysis, we used only the average of the variables (*W*m, *W*i, *B*, t* and IP) after standardization of all observations ($\mu = 0$ and $\sigma = 1$). This analysis was applied to both studies conducted in Brazil and studies from other countries. Following this, additional analyses with data interpretation were completed using PCA through the Pearson correlation matrix. Utilizing linear combinations of the original variables, we aimed to determine which variables were the most important and better explained the interpretation of the studies in each principal component (PC). This method implies that the first PCs retain greater variability of the original data and reduce the PC for the other components. Data

analysis was performed using SAS 9.4 (SAS Institute Inc., 2014) with the PROC NLMIXED tool to adjust the parameters of the Gompertz function for each study and accounting for randomization. Multivariate analysis was performed using STATISTICA PL (2011) 10.0 software (Statistica version 10.0; StatSoft, Inc., PL, Tulsa, OK, USA, 2011).

2.3 Results

2.3.1 Data base: The database was composed of 57 trials or experiments. These experiments aimed to describe the genetic growth potential of Japanese quails and were obtained from 19 papers, 2 doctoral theses, 1 master's, and 3 assays carried out at UNESP (Jaboticabal), which are unpublished. After careful examination, some papers were included in the database despite not being originally designed for studying the genetic potential of these birds; some of these studies exhibited the minimum data and characteristic requirements for composing the database and thus were included. The database represents the relationship between BW and the age of 10 969 thousand birds of the subspecies Coturnix coturnix japonica.

The oldest study used in the database was published in 1960, and the most recent was carried out at UNESP (Jaboticabal) in 2019. Although the database holds 58 years of research on Japanese quails, only one study was found from the 20th century; the other studies were published between 2002 and present, concentrating 95.6% of the data in the last 17 years.

2.3.2 Interpretation of obtained data: The Gompertz parameters estimated for each trial are represented in Table 1. Generally, the values of estimated *Wi* for the 57 studies ranged from 4.1 to 11.6 g, with amplitude of 7.5 g, corresponding to 241%. The lower

estimated *W*m was 116.6 g for the study published in 1960, while the highest estimated value was 341.9 g for the study published in 2019, with amplitude of 225 g, corresponding to 193.9% in relation to the minimal value. In numerical order, the *B* values ranged from 0.0393 to 0.1039 day-1. The amplitude between the values was 164.3%. The obtained values of *t** were 9 and 31 days, for minimum and maximum, respectively, with 237% of amplitude in relation to the lowest value.

Table 1 *Estimates of means (μ)and standard deviation (σ) values for growth parameters of Japanese quail obtained from 57 studies published in the literature and unpublished data.*

Study	Country	Year	Wi	Wm	B	t*	σWi	σWm	σB	σt*
S1[2]	Brazil	2016	5.5	144.4	0.0826	14.3	1.0	3.3	0.0045	0.4
S2[2]	Brazil	2012	5.1	153.0	0.0706	17.3	0.5	2.8	0.0023	0.4
S3[2]	Brazil	2012	4.7	167.3	0.0712	17.9	0.6	4.2	0.0031	0.5
S4	Nigeria	2014	11.3	166.1	0.0499	19.8	3.4	6.3	0.0058	1.4
S5	USA	1960	8.6	116.6	0.1039	9.2	30.3	7.6	0.0176	1.0
S6	Turkey	2017	7.5	228.9	0.0739	16.7	0.9	4.9	0.0029	0.4
S7	Slovac[1]	2004	5.7	175.8	0.0638	19.3	0.8	3.4	0.0027	0.5
S8	Nigeria	2014	10.1	148.0	0.0540	18.3	2.6	3.0	0.0047	1.0
S9	Iran	2018	6.5	305.1	0.0550	24.5	1.2	18.0	0.0038	1.3
S10	Iran	2018	6.3	300.5	0.0577	23.4	0.6	8.2	0.0020	0.6
S11	Iran	2018	4.5	249.1	0.0691	20.1	1.4	13.4	0.0059	1.0
S12	Iran	2018	4.4	290.4	0.0607	23.7	1.3	20.3	0.0055	1.4
S13	Iran	2018	5.0	269.8	0.0528	26.1	1.0	18.5	0.0040	1.5
S14	Iran	2018	5.5	286.0	0.0535	25.7	1.2	20.7	0.0044	1.6
S15	Iran	2018	4.5	270.1	0.0582	24.2	1.4	21.9	0.0058	1.7
S16	Turkey	2005	7.4	304.5	0.0631	20.8	1.1	18.0	0.0042	1.1
S17	Turkey	2005	4.1	210.8	0.0664	20.6	1.1	5.5	0.0040	0.6
S18	Turkey	2005	5.1	231.7	0.0646	20.7	2.3	11.7	0.0071	1.2
S19	India	2017	6.7	296.1	0.0572	23.3	2.0	26.5	0.0064	1.9
S20	India	2017	7.1	303.0	0.0560	23.6	1.8	24.2	0.0055	1.7
S21	India	2017	10.1	273.1	0.0596	20.0	3.8	31.9	0.0100	2.5
S22[2]	Brazil	2002	5.8	126.4	0.0753	14.9	0.7	4.5	0.0043	0.6
S23	Turkey	2016	5.6	250.3	0.0649	20.6	3.1	19.8	0.0097	1.6
S24	Turkey	2015	5.3	250.1	0.0737	18.3	1.0	6.2	0.0036	0.4
S25	Turkey	2015	5.2	269.5	0.0669	20.5	0.6	4.9	0.0020	0.3
S26	Turkey	2015	7.3	208.7	0.0609	21.2	0.6	5.2	0.0017	0.4
S27	Turkey	2015	6.0	264.7	0.0717	18.6	1.7	10.6	0.0054	0.7

S28	Romania	2014	6.2	239.9	0.0715	18.1	2.3	15.9	0.0082	1.2
S29	India	2002	6.5	234.6	0.0644	19.8	1.4	24.1	0.0080	1.9
S30	Bulgaria	2005	7.9	172.9	0.0800	14.0	0.1	2.9	0.0030	0.3
S31	Bulgaria	2005	6.9	172.7	0.0830	14.1	0.8	2.8	0.0030	0.3
S32	Turkey	2005	6.5	260.2	0.0714	18.3	0.8	15.6	0.0045	1.0
S33	Turkey	2005	5.6	227.9	0.0593	22.1	0.6	6.7	0.0023	0.6
S34	Turkey	2005	8.3	289.4	0.0499	25.4	1.0	15.4	0.0029	1.2
S35	India	2016	4.4	211.2	0.0708	19.1	1.3	10.2	0.0057	0.9
S36	India	2016	5.2	220.3	0.0624	21.0	1.2	11.6	0.0047	1.1
S37	India	2016	3.4	198.0	0.0797	17.6	1.8	12.6	0.0099	1.1
S38	Iran	2009	7.7	236.1	0.0550	22.3	1.6	18.2	0.0054	1.7
S39	Turkey	2005	8.0	241.0	0.0742	16.5	1.4	10.9	0.0050	0.8
S40	Turkey	2017	6.2	222.3	0.0810	15.7	0.8	3.6	0.0027	0.3
S41	Slovak[1]	2004	6.5	186.9	0.0591	20.5	1.0	9.1	0.0040	1.0
S42	Bangladesh	2018	6.4	201.4	0.0450	27.6	1.1	20.5	0.0045	2.5
S43	Bangladesh	2018	6.2	187.9	0.0509	24.1	1.5	20.1	0.0063	2.5
S44	Bangladesh	2018	7.3	214.1	0.0437	27.8	2.0	40.3	0.0079	4.8
S45	Bangladesh	2018	7.0	183.4	0.0503	23.6	1.6	19.8	0.0063	2.5
S46	Bangladesh	2018	8.9	256.7	0.0393	30.8	2.0	51.3	0.0068	5.4
S47	Bangladesh	2018	7.4	230.1	0.0397	31.0	2.3	61.4	0.0091	7.2
S48	Iraq	2019	9.4	240.2	0.0581	20.2	2.1	27.7	0.0076	2.3
S49	Iraq	2019	9.0	223.0	0.0617	18.9	1.7	19.7	0.0066	1.7
S50	Iraq	2019	11.6	341.9	0.0453	27.0	3.5	100.5	0.0110	6.9
S51[2]	Brazil	2007	7.17	190.1	0.0641	18.5	1.0	7.2	0.0038	0.7
S52[2]	Brazil	2007	7.75	192.8	0.0649	17.9	0.03	5.6	0.0030	0.5
S53[2]	Brazil	2007	6.94	189.8	0.0673	17.7	0.7	4.8	0.0028	0.5
S54[2]	Brazil	2007	7.38	189.4	0.0665	17.6	1.2	7.4	0.0043	0.7
S55[2]	Brazil	2019	6.02	158.6	0.0694	17.0	1.8	4.5	0.0056	0.7
S56[2]	Brazil	2019	5.90	167.3	0.0681	17.7	1.8	5.0	0.0057	0.7
S57[2]	Brazil	2019	5.69	174.6	0.0647	19.0	1.4	4.5	0.0043	0.6

Slovac[1] Slovac Republic;

[2] Brazilian studies.

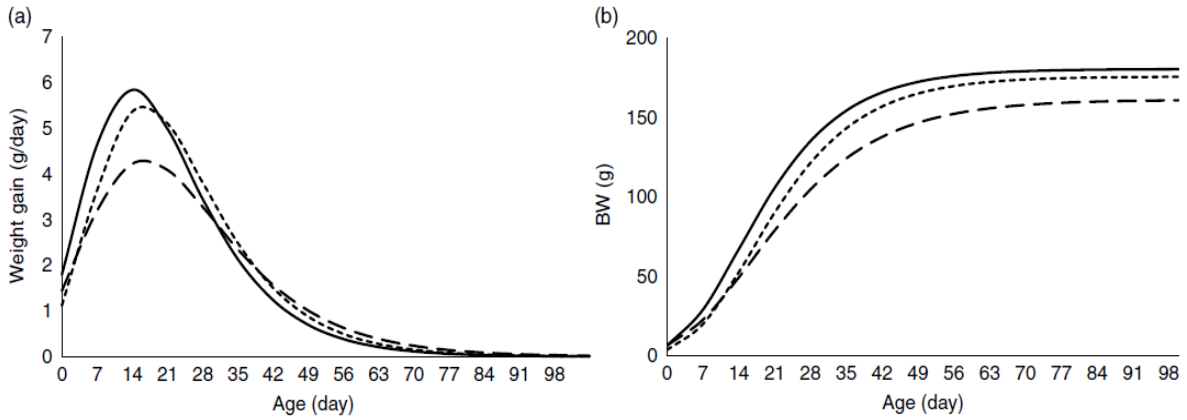

Figure 1 Growth rate of Japanese quails, in Brazilian studies— — –, studies conducted ____ In other countries, except Brazil, ------considering all studies. **(a)**: Relation between age (day) and weight gain (g/day). **(b)**: relation between age (day) and BW (g).

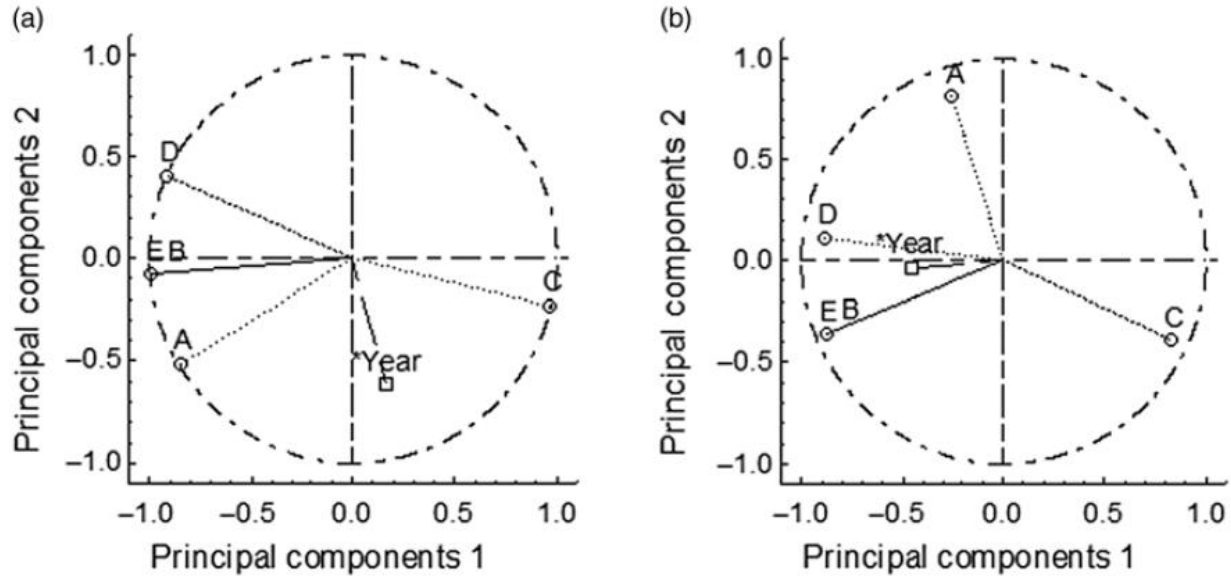

Figure 2 Relation of first (X-axis) and second (Y-axis) rotated (Varimax rotation technique) extracted principal components estimated by multivariate analysis of the Japanese quail growth parameters. Symbols are (∘) origin variable and supplementary variable (▫). (a) Brazilian studies. (b) Studies conducted in other countries, except Brazil. 'A' = *W*i is the BW at birth (g); 'B' = *W*m is the BW at maturity 9g); 'C' = B is the

maturity rate (per day); ‘D’ = t* is the age of maximum growth rate (day); ‘E’ = IP is weight at inflection point (g).

Based on the parameters of the adjusted model for S5 study published in 1960 ($W = 116.6 \times e\{[-e^{((ln(-ln(8.6/116.6)))-0.1039 \times t}]\}$), the growth profile can be considered as a reference of the growth potential of these birds.

To present the curves:

Brazilian studies: $W = 161.2 \times e\{[-e^{((ln(-ln(6.0/161.2)))-0.0722 \times 16.4}]\}$

Other countries: $W = 180.5 \times e\{[-e^{((ln(-ln(6.0/180.5)))-0.0878 \times 16.3}]\}$

General: $W = 175.7 \times e\{[-e^{((ln(-ln(3.33/175.7)))-0.0845 \times 16.3}]\}$

The growth curves adjusted by the Gompertz equations are shown in Figure 1 a and b, distinguishing the Brazilian studies from both the studies conducted in other countries and the total population and indicating the variability of growth in the curves.

2.3.3 Exploratory analysis: Figure 2 portrays the PCs obtained from the original variables (*Wi*, *Wm*, *B*, t* and IP) for both the Brazilian studies (Figure 2a) and the studies conducted in other countries (Figure 2b). For the Brazilian studies, all characteristics showed discriminatory power for the two components. The correlations between PC1 and *Wi*, *Wm*, *B*, t* and IP were −0.84, −0.98, 0.96, −0.91 and −0.98, respectively. In PC2, the correlations were −0.51, −0.07, −0.23, 0.40 and −0.07 for *Wi*, *Wm*, *B*, t* and IP, respectively. The additional variable of the year of publication presented a correlation of 0.19 in PC1 and 0.60 in PC2. For the studies conducted in other countries, the variables *Wi*, *Wm*, *B*, t* and IP showed discriminatory power in both PCs. The correlation values for the variables *Wi*, *Wm*, *B*, t* and IP were estimated

to be −0.25, −0.87, 0.83, −0.88 and −0.87, respectively, in PC1 and 0.81, −0.36, −0.38, 0.11 and −0.36, respectively, in PC2. For the year of publication, the correlation was estimated to be −0.46 for PC1 and −0.03 for PC2.

2.4 Discussion

This study was designed to determine whether there has been a change in the genetic growth potential of Japanese quails. There is a need for a reference to contrast with the other available data. In this review, we were pleased to find the study by Wilson et al. (1960). The growth profile of Japanese quails in the 1960s was described by the model $W = 116.6 \times e\{[-e^{((\ln(-\ln(8.6/116.6)))-0.1039 \times t}]\}$, which was adjusted for the study by Wilson et al. (1960) and considered a reference to answer the fundamental question of this study. In this way, the estimated growth parameters characterize the growth of Japanese quails in 1960 as light and early birds, indicating that they weighed less at maturity and matured more quickly. Another observation obtained by the calculated CV ($CV = \sigma/\mu \times 100$, Table 1) was the likely heterogeneity in growth, especially when the parameter Wi was considered, which registered the largest CV (352%). This value can be considered significant when compared to the CV values obtained from studies conducted in 2019, which ranged from 25% to 31% (Table 1).

The Japanese quail growth profile of the Brazilian studies was described by the following model: $W = 161.2 \times e\{[-e^{((\ln(-\ln(6.0/161.2)))-0.0722 \times t}]\}$. For the studies conducted in other countries, the growth of these birds can be interpreted by the model: $W = 180.5 \times e\{[-e^{((\ln(-\ln(6.0/180.5))) -0.0878 \times t}]\}$. The overall average of all studies was illustrated by the following model: $W = 175.7 \times e\{[-e((\ln(-\ln(3.33/175.7))) -0.0845 \times t]\}$. These models indicate that Japanese quail growth was manipulated, and the Wm and B parameters

were negatively correlated. Thus, the increase in the *W*m values resulted in lower *B* values (Silva et al., 2016). The mean weight of eggs reported by Wilson et al. (1960) was 9.5 g, or approximately 8% of the Wm, reaching 7% when the bird was at peak production. The average egg weight in recent studies was 11.2 g (Silva et al., 2019). Although the average egg weight increased from 9.5 to 11.2 g, the percentage of *W*m only changed slightly from 8 to 7. This relation can be explained by manipulation in the growth of these birds, more specifically in the increase of the *W*m, since egg weight correlates positively (r = 0.953) with *W*i, according to Silva et al. (2019).

The approach endorsed by this study offers comparative data on the characterization of Japanese quail growth in Brazil vs. that in other countries. Although the purpose of the application of Japanese quails worldwide is egg production, the differences in the parameters suggest that different strategies were adopted in Brazil compared to other countries. The adjusted models highlight the differences, including reduced *W*m and *B* values in the Brazilian studies when compared to the growth profile described by studies from other countries.

Considering the reference studies chronologically and comparing Wilson et al. (1960) to Aljumaily and Taha (2019), the difference between them can be summarized in the values of *W*m (117 g v. 342 g) and *B* (0.1039/day v. 0.0453/day). In this comparison, there is an indicator of the strategy adopted by other countries: clearly the objective was to increase the *W*m, and because of the negative correlation, the *B* value decreased. This scenario also supports the probable use of cutting genotypes (*Coturnix coturnix coturnix*) in the production lines for egg production from Coturnix coturnix japonica, thereby promoting an increase in BW because the growth potential of the cut birds (*Coturnix coturnix coturnix*) is significantly larger, as shown by Grieser

et al. (2018). The hypothesis of the greater use of cut birds as a way to increase the *W*m of the poultry can be supported by analyzing the similarity between the cutting lineage studied by Grieser et al. (2018) and poultry studied by Aljumaily and Taha (2019), as shown in the Wm value of 369 g described by Grieser et al. (2018) v. 342 g described by Aljumaily and Taha (2019).

To understand the changes in the parameters and considering the variability of the database, we used PCA and added the variable 'year of publication' (Figure 2). This variable can be interpreted as the consistency or targeting of a selection goal. Figure 2a shows that for Brazilian studies, there was a relationship with the year of publication. Although it brings together approximately 20 years of publication, the Brazilian database included few studies; however, it was possible to obtain data that features the use of the Japanese quail as a typical egg production bird, that is, a light bird. Conversely, Figure 2b shows the main effect of the relationship between *W*m and *B*, in which the year of publication did not show consistency. The low correlation can be interpreted in two ways: first, as random variability, since without targeting, publications that were featured used animal models for experimentation (Minvielle, 2004), and second, as an animal sciences purpose, which is audacious, but it is possible that there is an intention to develop an improvement in the use of the carcass of the bird at the end of the laying period, that is, at the time of discarding the bird.

Although it seems audacious to print 'double fitness' in the animal sciences function for laying quails, it may be strategic to increase carcass traits for *Coturnix conturnix japonica* rather than improve reproductive traits for Coturnix coturnix coturnix. However, this way of interpreting the increase in *W*m observed in other countries' studies must be supported by market demand. Despite the lack of a statistical

foundation that relates the facts ‘increase of *W*m’ and ‘consumer market’, the majority of the studies are from Asian countries, and it is known that Asia has been registering population growth and demand for food; many studies have been published on raising Japanese quail for meat and egg production (Minvielle, 2004; Kayang et al., 2004; Vali, 2008).

A market exists in Brazil for quail eggs, and a productive chain has been structured parallel to that for the chicken egg. The same cannot be said of quail meat, and there is no full acceptance in the consumer market, with only a few niches explored. Therefore, it does not seem of interest for Brazil to increase theWm, and consequently the BW at disposal, since this increase would also increase the demand for nutrients and the costs of food. Taking into account that at least currently the quails would not be used for human consumption, this method would have low added value.

Lastly, the publications used in the review date from 1960 to 2019. By analyzing the chronology of the results, it was not possible to establish the same relationship among the growth parameters (Wi, Wm, B and t*) that Silva et al. (2015) found for commercial poultry and broilers. Thus, it is possible to infer that there were different research groups that genetically improved the Japanese quails to obtain different characteristics of the final product.

In the end, for the Brazilian producer, it is believed that an assessment of the growth potential of the lineage used is necessary in locus. The information found in this research does not support generalizations for use in factorial models for the calculation of nutrient intake, even in the 2019 studies (Table 1). There are genotypes with specific characteristics that should be considered in the expansion of nutritional programs.

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Declaration of interest

The authors declare that trere is no conflicts of interest regarding the publication of this article.

Ethics statement

Not applicable.

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CAPÍTULO 3 - ESTIMATE OF LYSINE NUTRITIONAL REQUIREMENTS FOR JAPANESE QUAIL BREEDERS

Este capítulo corresponde ao artigo científico submetido à revista Peer Journal e encontra-se em avaliação para publicação.

Estimate of lysine nutritional requirements for Japanese quail breeders

Lizia Cordeiro de Carvalho[1], Jaqueline Aparecida Pavanini[1], Manoela Garcia Borgi Lino de Sousa[1], Tadia Emanuele Stivanin[2], Michele Bernardino de Lima[1], Nelson José Peruzzi[1], Alan Rodrigo Panosso[1], Edney Pereira da Silva[1]

[1] Department of Animal Sciences, Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil
[2] Genetic improvement of Japanese quails, Vicami Codornas, Assis, São Paulo, Brazil

Corresponding Author:
Edney da Silva[1]
Via de Acesso Prof. Paulo Donato Castellane s/n, Jaboticabal, São Paulo, 14884-900, Brazil
Email address: edney.silva@unesp.br

Abstract

Background. Japanese quail breeders are the basis for genetic improvement and multiplication for commercial layers, however, there have been no known studies on the optimal lysine level for these birds. Thus, study the egg output response to the Lys supply using different e-functions and evaluate the that best fit, have allowed the partition the lysine requirements for maintenance, both weight and egg output maximum.

Methods. The objectives of this study were to identify the responses to various Lysine (Lys) levels, identify the functions related to these responses and determine the ideal Lys intake amount for Japanese quail breeders. A completely randomized design of seven treatments with seven replicated was used. Treatments consisted of diet supplementation by Lys in concentrations of 16.8, 11.8, 8.4, 6.7, 5.0, 3.4, and 1.7 g/kg. Six exponential models were adjusted.

Results. The level of Lys was found to affect bird responses ($P < 0.001$). The birds responded to the levels provided, allowing for the creation of a lysine response curve. A monomolecular function with four parameters was balanced against the statistics of adjustment and selection of models. It was possible to estimate the level of lysine required for maintenance as 133 ± 2 mg/kg $BW^{0.67}$, and based an average of 41% efficiency, 22 mg Lys produced 1 g of egg output (EO). The daily intake calculated by the monomolecular factorial model was 284 mg Lys for a bird with 0.170 kg body weight and production of 10 g EO/day. The four-parameter monomolecular function proposed in this study is adequate for interpreting the animal response and calculating lysine intake for breeders.

Keywords. Dilution technique, efficiency, maintenance, models, requirement.

3.1 Introduction

Japanese quail breeders is the basis for genetic improvement and multiplication for commercial layers. Through the selection process, in each generation, genetically superior animals are used to form the breeding stock. Approximately 4,444 breeder birds are required to produce one million Japanese quails, and in Brazil, more than 111,000 were necessary to yield the 25 million currently housed (Silva et al., 2020). The breeders used in this study were selected for egg production and belong to the male line of laying quails. There have been no known studies on the optimal amino acid level for Japanese quails breeders. Lysine (Lys) is the reference amino acid for establishing the ideal relationship, and it is the second limiting amino acid in maize and soybean diets of birds. Lys acts on protein and lipid metabolism, and in reproduction, subdoses are related to atrophy of reproductive organs and the liver (Ruan et al., 2019; Tian et al.,2019).

Among the main methods used to establish amino acid intake are dose response and factorial analysis. The factorial method is a reasonable option to establish amino acid intake by using variables such as body weight (BW) and egg output (EO) (Silva et al., 2019). This method is based on using the linear relationships between amino acid intake and these variables (mg per kg BW and mg per g EO) to partition the BW maintenance requirement and EO production (Sakomura et al., 2015; Reis et al., 2018). A reduction in feed conversion (Basaglia et al., 2005) is possible because the input values for BW and EO correspond to the average population potential (Hauschild, Pomar & Lovatto, 2010).

Although feed conversion reduction in the egg production industry can be useful, the effectiveness of this reduction is based on the average production potential of the batch (Basaglia et al., 2005, Silva et al., 2015a), and thus individuals above the average population potential would inevitably receive a subdose (Hauschild, Pomar & Lovatto, 2010; Silva et al., 2019; Silva et al., 2015a). This characteristic of the factorial method may be a limitation for nutritionists in genetic improvement programs and multiplication systems, in terms of how individuals performing above the population average should be adequately identified and nurtured. Therefore, since linear relationships are limited because they are infinite in all directions, empirical constraints should be used to obtain more precise estimates that represent a closer approximation to the actual condition (Silva et al., 2015a; Silva et al., 2019).

Nonlinear factorial models are alternatives for breeders (Kebreab et al., 2008; Ekmay et al., 2014) especially for birds that prioritize reproduction by mobilizing body reserves to maintain egg laying (Lima et al., 2018; Lima et al., 2020). Exponential functions allow for the consideration of maintenance and production partitioning (Samadi & Liebert, 2008; Dorigam et al., 2017) while catering to the most productive animals of the population, since curvilinear adjustment can change the response rate (α/β x) with the approximation of the maximum genetic potential (Fuller & Garthwaite, 1993; Silva et al, 2019). Factorial models based on e-functions are available that have parameters with biological significance that can be improved, such as the requirement to maintain unity on the axis of the ordinate (Samadi & Liebert, 2008; Dorigam et al., 2014; Dorigam et al., 2017) when the ideal would be on the axis of the abscissa (Kebreab et al., 2008; Sarcinelli et al., 2020; Silva et al., 2020), thereby avoiding confusion between the minimal response on the ordinate axis (Lima et al., 2013; Dorigam et al., 2017) and the

requirement of nutrient maintenance on the abscissa axis. Therefore, this study aimed to (1) study the EO response to the Lys supply using different e-functions, (2) evaluate the e-functions that best fit the EO responses, (3) partition the Lys requirements for BW maintenance and EO production, and (4) the Lys intake level that maximizes EO.

3.2 Materials & Methods

The study was conducted in the Laboratory of Poultry Sciences of the Departament of Zootecnia da Faculdade de Ciências Agrárias e Veterinárias of the Universidade Estadual Paulista, Campus of Jaboticabal, São Paulo, Brazil. The procedures used in this study were approved by the Committee on Animal Use Ethics, under protocol 012203/17.

3.2.1 Birds, housing, and experimental design

Forty-nine VICAMI® Japanese quails breeders were used at 14 weeks of age, when they are at their peak performance. The experiment was conducted in a temperature-controlled climate chamber containing galvanized wire cages measuring 0.26 m × 0.37 m × 0.36 m, with channel feeders and nipple drinkers. The temperature during the experimental period was maintained at 24 °C, with a 16:8 h (L:D) photoperiod. Water was provided ad libitum. A completely randomized design was used, with seven treatments and seven repetitions. Each experimental unit consisted of one bird per cage. All cages were identified with different colored labels, according to the treatments. The treatments consisted of seven levels of Lys in the diet as follows: D7 – 16.8 g/kg; D6 – 11.8 g/kg; D5 – 8.4 g/kg; D4 – 6.7 g/kg; D3 – 5.0 g/kg; D2 – 3.4 g/kg, and D1 – 1.7 g/kg. After the trial the animals remained in the university's herd for egg production.

3.2.2 Experimental treatments and diets

The level of Lys in the dietary protein profile and experimental diets were formulated as described by Fisher and Morris (1970). A formulation with a high crude protein content (HPD) and a relative deficiency in Lys compared to the other amino acids, and a second formulation that was free of protein and amino acids (NFD) were prepared (Table 1). The nutritional levels of the essential amino acids in the HPD were based on the recommendations described previously by Rostagno et al. (2011) (Table 2). The Lys level was established by multiplying the recommended amount by 1.5, and that of the other amino acids by 2.0 to maintain a minimum Lys deficiency of 50% compared to the other amino acids. For energy and other nutrients (vitamins and minerals), the minimum recommendations were followed by Rostagno et al. (2011). NFD was formulated to provide energy and the other nutrients with no amino acids. The intermediate experimental levels of Lys were obtained by diluting the HPD with NFD in the following proportions (HPD:NFD): 100:0; 70:30; 50.1:49.9; 40:60; 30:70; 20.1:79.9; and 10:90; thus obtaining Lys concentrations of 16.8, 11.8, 8.4, 6.7, 5.0, 3.4, and 1.7 g/kg respectively.

Table 1. Composition (g/kg) of the diets used in the lysine assay

Ingredient (g/kg)	HPD[a]	NFD[b]
Corn	356.97	-
Soybean meal	315.97	-
Corn gluten meal (60% CP)	181.22	-
Soybean oil	20.00	24.84
Dicalcium phosphate	10.13	15.02
Limestone	69.81	69.81
Salt	3.34	3.67
Choline chloride (60%)	0.84	3.40
Mineral premix[c]	0.25	0.25
Vitamin premix[c]	0.25	0.25
DL-Met (99%)	4.88	-
L-Lys HCl (78%)	5.72	-
L-Thr	2.71	-
L-Val	3.21	-
L-Ile	2.00	-
L-Arg	10.72	-
LTrp	1.81	-
Potassium chloride	-	11.95
Corn starch	-	249.03
Sugar	-	496.74
Rice husks	-	125.00

[a]HPD, high protein diet

[b]NFD, nitrogen free diet

[c]Content per kg of the diet - vit A 6.668 IU; vit D3 1.668 IU; vit E 8 IU; vit K 3.2 mg; vit B1 1 mg; vit B2 3.34 mg; vit B6 2 mg; vit B12 5 mcg/kg; niacin 21 mg; chlorine 0.13 g; pantothenate acid 8 mg; folic acid 0.46 mg/kg; biotin 0.05 mg/kg; copper 8 mg/kg; iron 60 g; manganese 70 g; zinc 25 g; iodine 6.25 mg; selenium 0.12 mg.

Table 2. Nutritional levels of experimental diets

Itens	HPD[a]	NFD[b]
Calculated composition (g/kg)[c]		
Metabolizable energy (MJ/kg)	12.5	12.5
Calcium (g/kg)	30.0	30.0
Avaliable phosphorus (g/kg)	3.0	3.0
Analyzed composition (g/kg)		
Crude protein	350.0	NI[e]
[d]Digestible Lys	16.8	NI
Digestible Met + Cys	17.1	NI
Digestible Met	1.1	NI
Digestible Trp	0.3	NI
Digestible Thr	1.5	NI
Digestible Arg	2.5	NI
Digestible Val	1.7	NI
Digestible Ile	1.5	NI
Digestible Phe	1.9	NI

[a]HPD, high protein diet
[b]NFD, nitrogen free diet
[c]The nutrient content of the ingredients used in the formulation was analyzed using a near-infrared spectrometer (NIR).
[d]The total amino acid content of the diets were analyzed HPLC and digestible content calculated using coefficients from Rostagno et al. (2011)
[e]NI, Not identified

3.2.3 Measurements and variables analysed

The experiment occurred over 22 days, with 7 days of adaptation and 15 days of data collection. The maximum consumption per kilogram of metabolic weight ($BW^{0.67}$) was determined during the adaptation period, when the birds were fed ad libitum. In the subsequent period, the diets were supplied based on the kg of $BW^{0.67}$ of each bird. The supply of feed was adjusted after each weekly weighing of the birds. To avoid waste, the feed for each experimental unit was divided into two meals per day.

The variables evaluated were: daily feed intake (FI, g/bird), daily Lys intake (LysIntake, mg/bird), body weight (BW, kg), body weight change (BW, g/bird), daily egg production (EP, %/bird), egg weight, and daily deposition of Lys in egg mass (dLys, mg/bird), which was achieved by considering the concentration of 13% protein (Ali, 2019) and the 6.89% level of Lys in egg protein (Ali, 2019). Lys mobilization was calculated from the change in BW, considering the mobilized protein fraction and, consequently, the proportion of Lys in the mobilized protein. Protein and Lys concentrations in the body were obtained from the method of a previous study (Siqueira et al., 2021).

3.2.4 Description of responses by different mathematical functions

The variables dLys and LysIntake were related to the metabolic weight of the bird ($BW^{0.67}$). Two linear functions were used: linear regression and broken-line regression (Table 3).

To interpret the relationship between dLys and LysIntake, six e-functions were used, one of which was proposed in this research and the other five were obtained from the literature, considering the interpretation and biological meaning of the parameterization of the model (Table 3). The adjusted functions consisted of a monomolecular parameterized model with three (Kebreab et al., 2008; Samadi & Liebert, 2008) and four parameters (Kops & Lamberson, 2006; Strathe et al., 2011).

Table 3. The functional forms used to describe the relationship between deposition of lysine (dLys) and lisine intake (LysIntake) daily.

Functional form	Function	Characteristic	Reference
M1 = dLys = [LysIntake-Lysm×$BW^{0.67}$]/a	Linear	Linear model, estimates the average requirement of the population.	Silva et al. (2019)
M2 = dLys = R_{max}+U×(R-LysIntake), for LysIntake<R	Linear	Broken line, estimates the average requirement of the population.	Reis et al. (2018)
M3 = dLys = (R_{max} - R_{min})[1-$e^{-k(LysIntake-Lysm)}$]	Exponential	Addition of the R_{min} parameter with the response on the ordinate axis.	Sousa et al. (2022)
M4 = dLys = R_{max}[1-$e^{-k(LysIntake-Lysm)}$]	Exponential	The function does not provide the parameter of R_{min}.	Kebreab et al. (2008)
M5 = dLys = R_{max} [1-$e^{-kLysIntake}$] - R_{min}	Exponential	The R_{min} parameter with the response on the abscissa axis.	Samadi & Liebert (2008)
M6 = dLys = R_{min} + Range [1-$e^{(-e-k(LysIntake-Lysm)}$)]	Exponential	It was a dual exponential model developed for the optimal response as a proportion of the asymptote.	Strathe et al. (2011)
M7 = dLys = R_{max}[1-$e^{(-e-k(LysIntake-Lysm)}$)]	Exponential	It is similar to model 6, with modified parameters.	Strathe et al. (2011)
M8 = dLys = R_{max} - (R_{max} - R_{min}) [$e^{-k(LysIntake-Lysm)}$]	Exponential	This function was used to repair the Brody model.	Kops & Lamberson (2006)

[M] Model. [Lysm] The daily intake of lysine for maintenance. [BW] Body weight. [a] The deposition of 1 mg Lys in the egg mass. [Rmax] The maximum response for dLys (mg/kg $BW^{0.67}$). [U] The rate of function growth. [R] The estimated value of LysIntake for Rmax (mg/kg $BW^{0.67}$). [Rmin] The minimum response for dLys (mg/kg $BW^{0.67}$). [k] The rate of decay of the function.

3.2.5 Model Adjustment and Selection Statistics

The adjustment and selection statistics used were the determination coefficient (R^2), determination coefficient adjusted for the number of parameters (R^2 Adjust), Akaike information criterion (AIC), corrected Akaike information criterion (AICC) and the Bayesian information criterion (BIC), model quality was based on the lowest score for AIC, AICC and, BIC.

3.2.6 Structure and assessment of linear and non-linear factorial models to estimate Lys intake based on BW and EO values

The factorial model calculated the nutrient Lys according to its partition, maintenance, and production. The nonlinear factorial model was based on the logarithmic transformation of Samadi and Liebert (2008), according to Equation 9 (M9). In this model, the maintenance parameter was added after calculating the requirements for egg mass production.

$$\text{LysIntake} = BW^{0.67} \times [\text{Lysm} + (\ln R_{max} - \ln(R_{max} - 8.853 \times (EO/BW^{0.67})))/k] \quad (9)$$

The parameters necessary to calculate LysIntake were the R_{max}, Lysm, and k that were obtained from M3, M5, and M8, generating the predicted values and the respective prediction errors for each monomolecular function.

To compare the LysIntake estimates by the nonlinear factorial model M9, the traditional factorial model (Sakomura et al., 2015; Silva et al., 2019) was used to estimate LysIntake according to Equation 10 (M10).

$$\text{LysIntake} = BW^{0.67} \times [\text{Lysm} + a \times (8.853 \times EO)] \quad (10)$$

The parameters required to calculate LysIntake were Lysm and a, which were obtained from linear models M1 and M2.

The input variables in Equations 9 and 10 were BW and EO expressed in $kg^{0.67}$ and g/kg $BW^{0.67}$, respectively, and the value of 8.853 is the relationship between dLys and EO. LysIntake in Equations 9 and 10 is the model output of daily intake in mg/bird.

Assessment of dLys response prediction error as a function of LysIntake estimated by non-linear and linear factorial models. The prediction error was determined as the difference between the observed and predicted values of dLys. The errors were subjected to linear regression analysis according to the predicted value of a previous study (St-Pierre, 2003), according to Equation. 11 (M11).

$$ep = b0 + b1\ (Yp - \bar{Y}p) + \hat{e} \quad (11),$$

where ep was the residual value for all observation; b0 and b1 were the estimates of the parameters, Yp was the predicted value, $\bar{Y}p$ was the average of the predicted values, and $\hat{e}$ was the regression error of the residues to the predicted values. The decision rule was based on the assumption that the model is impartial when the correlation approaches 1 and R^2 approaches 0.

Therefore, the residues are not correlated with the predictions, and consequently, the value of b1 is close to zero for the unbiased model. The ratios of the parameters (b0 and b1) to regression error (ê), scalar error (b0/ê), and prediction bias (b1/ê) were obtained for the model.

3.2.7 Statistical analyses

The assumptions of homoscedasticity and residual normality were tested. Subsequently, the data were subjected to analyses of variance, and when an invalid hypothesis was verified, the data were analyzed for linear and quadratic effects of the Lys levels, considering a significance of 0.05. The parameters of the models were estimated by the maximum probiosimilarity, using the NLMIXED procedure of SAS, considering the maximum random effect of the model (Robbins, Saxton & Southern, 2006). The values were calculated using SAS software (SAS Institute Inc., Cary, NC, USA, 2014, version 9.4).

3.3 Results

The Lys level in the diet affected the performance of Japanese quails breeders (Table 4), thereby rejecting the null hypothesis, where the variables do not differ with the levels of Lys in the diet ($P < 0.05$). The contrast analysis was significant for the linear and quadratic effects of Lys levels on bird replenishment, except for BW, which responded linearly to Lys levels in the diet. The homoscedasticity and residual normality were tested by the Shapiro-Wilk test, the data were normal, and the residuals are randomly distributed around zero ($p>0.05$).

The quails that were fed a lower level of Lys (1.7 g/kg) reduced their daily consumption by 39%, when compared with 8.4 g Lys per kg consumed the maximum value of 25.1 g/bird. The daily Lys intake at 1.7 g/kg was 13% of that of the highest level of Lys in the diet (16.8 g/kg). Therefore, the egg production and egg weight were reduced in different proportions. In 1.7 g/kg of Lys diet, the egg production decreased by 77% from the maximum value of 94%, while egg weight reduced only 25% of the maximum value of 11.2 g obtained at 16.8 g/kg of Lys diet (Table 4).

Egg mass and Lys deposition decreased by 82% in response to the limitation of Lys intake in the diet. Birds exhibited greater weight loss and consequently higher daily Lys mobilization values in diets with a greater degree of limitation in daily Lys intake. Increased intake of Lys linearly decreased its mobilization. Although consumption decreased, Lys limitation was responsible for low feed efficiency and consequently higher feed conversion values (Table 4). The feed conversion presented the largest amplitude (6.04) between the maximum (8.4 g/g at the level of 1.7 g/kg) and minimum (1.7 g/g at 16.8 g/kg) values corresponding to a change of 356% (Table 4). This result shows that the daily consumption of 15.2 g/bird would support a larger egg production, but Lys was limiting for protein synthesis.

Table 4. Responses to lysine levels for daily feed intake, lysine intake, egg production, egg weight, egg mass, feed conversion ratio, lysine deposition, body weight, change body weight and lysine mobilization

Lysine In Diet	Feed Intake	Egg Production	Egg Weight	Lysine intake	Egg mass	Feed Conversion Ratio	Lysine Deposition In egg	Body Weight	Change In Body Weight	Lysine Mobilization
g/kg	g/bird	%	g	mg/bird	g/bird	g/g	mg/bird	kg	g/bird	mg/bird
1.7	15.2	21.9	8.4	45.2	1.8	8.40	16.4	0.136	-6.0	-4.0
3.4	18.7	45.8	8.6	83.4	3.9	4.81	34.7	0.145	-5.9	-4.0
5.0	22.9	62.5	9.9	136.6	6.3	3.81	55.7	0.159	-1.6	-1.0
6.7	23.2	89.1	10.5	173.0	9.5	2.51	83.8	0.165	0.7	0.5
8.4	25.1	93.8	10.6	261.5	10.0	2.53	88.5	0.165	-3.4	-2.3
11.8	24.3	93.8	10.5	289.3	9.8	2.48	86.4	0.172	-1.1	-0.7
16.8	23.5	91.3	11.2	350.1	10.1	2.36	89.5	0.173	7.3	4.9
General	22.0	71.9	10.0	200.1	7.4	3.77	65.6	0.160	-1.1	-0.727
SEM	0.5	4.8	0.2	16.7	0.6	0.37	5.1	0.002	1.4	0.971
P-Value										
Treatment	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0683	0.1480
Linear	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0043	0.0191
Quadratic	<.0001	0.0002	0.0032	0.0051	0.0003	<.0001	<.0001	0.0283	0.5759	0.5877

General General average

SEM The standard error of measurement

3.3.1 Analysis of adjustment and selection functions and statistics

The selection of Lys intake models is shown in Table 5. M2 presented better adjustment when considering only the values of the adjacent R^2. Model selection statistics (AIC, AICC, and BIC) indicated that the broken-line model (Table 5: M2) and the double exponential function (Table 5: M7) best adjusted the relationship between dLys and LysIntake.

The maintenance requirements of 36 and 139 mg/kg values of $BW^{0.67}$ obtained with M1 and M8, respectively, revealed no information on variability. A value of 52 mg/kg was the $BW^{0.67}$ obtained for maintenance using M2 (Table 5) at dLys = 0; therefore, this also showed no variability. However, this model (Table 5: M2) presented a better adjustment and lower AIC, AICC, and BIC values (Table 5). In contrast, the M6 and M7 models (Table 5) estimated retention requirement values between 10 and 7 times greater than the M2-based value, respectively. The M7 (Table 5), along with the M2 presented better adjustments and lower values of AIC, AICC, and BIC (Table 5), while the R_{max} estimates revealed the lowest determined value, which underestimated the genetic potential value, since the maximum response that was estimated as 269 mg/kg $BW^{0.67}$ was lower than the values obtained in the treatments with 6.7 – 16.8 g of Lys per kg.

For Models 3 and 4, the results of the adjustment and selection statistics support M4 as superior (Table 5). This model presented the highest maximum response value, which was estimated at 444 mg/kg $BW^{0.67}$. The maximum observed response of 297 mg/kg $BW^{0.67}$, was 67% of the estimated value for R_{max}. R_{min} showed a variation of 43%, indicating a limited power of inference to interpret the animal response.

M3 presented estimates for R_{max}, R_{min}, k, and Lysm with smaller error values, supporting the biological significance in interpreting the bird response (Table 5). The maximum estimated response of 357 mg/kg $BW^{0.67}$ was 17% greater than the maximum observed value. The value of Lysm was 133 mg/kg $BW^{0.67}$, with a range of 128 to 137 mg/kg $BW^{0.67}$. Among the adjusted models with Lysm as a parameter, M5 returned 114 mg/kg $BW^{0.67}$ with a range of 25 to 203 mg/kg $BW^{0.67.}$ For M8, 139 mg/kg was the estimated $BW^{0.67}$; therefore, the 133 mg/kg $BW^{0.67}$ value of the M3 was similar to those estimated in M5 and M8 (Table 5).

Table 5. Fit statistics for the linear models, linear plateau and monomolecular functions for the relationship between deposition (Y) and lysine intake (X) of Japanese quail breeders

Models		Regression	R^2	R^2adj	AIC	AICC	BIC
M1	Multiple linear	Linear	0.710	0.690	215	216	212
M2	Linear plateau	Linear	0.902	0.888	207	209	203
M3	Exponencial	Non linear	0.840	0.810	219	221	213
M4	Exponencial	Non linear	0.866	0.847	217	218	212
M5	Exponencial	Non linear	0.866	0.846	217	218	212
M6	Exponential double	Non linear	0.847	0.819	222	224	216
M7	Exponential double	Non linear	0.841	0.818	207	208	203
M8	Exponencial	Non linear	0.876	0.854	216	219	210

R^2 = R-Square; R^2adj = R-square adjust; AIC = Akaike Information Criterion; AICC = Corrected Akaike Information Criterion; BIC = Bayesian information criteria.

M1, Model 1: $dLys = (LysIntake - 36 \times BW^{0.67})/3.69$

M2, Model 2: $dLys = 293 - 0.47 \times (682 - LysIntake)$

M3, Model 3: $dLys = (357 - 4) \times [1 - e^{(-0.0021 \times (LysIntake - 133))}]$

M4, Model 4: $dLys = 444 \times [1 - e^{(-0.0027 \times LysIntake}] - 117$

M5, Model 5: $dLys = 327 \times [1 - e^{(-0.0027 \times (LysIntake - 114))}]$

M6, Model 6: $dLys = (119+170) \times [1 - e(^{-e(-1.025 \times (LysIntake - 511))})]$

M7, Model 7: $dLys = 269 \times [1 - e^{(-e(-0.0041 \times (LysIntake - 374))})]$

M8, Model 8: $dLys = 314 - (314 - 17) \times e[^{-0.0028 \times (LysIntake - 139)}]$

3.3.2 Structure and assessment of linear and non-linear factorial models

The observed averages for LysIntake and dLys, in mg/kg $BW^{0.67}$ for each treatment and the respective estimated values are shown in Table 6. The estimates of the linear factorial models differed, especially in relation to the prediction of animal replenishment. M2 (Table 6) overestimated the response after ingestion of 286 mg/kg $BW^{0.67}$, while M1 presented better response estimates.

The M6 and M7 exponential models (Table 6) showed a discrepancy between the response estimates, where in M6, the estimated between levels did not differ and for M7 it was not possible to estimate for the level 6.7 g/kg of Lys per diet. While M3, M4, M5, and M8 revealed errors of 33.4, 33.4, 33.5, and 32.2 mg/kg $BW^{0.67}$, respectively (Figure 2). The prediction of nonlinear factorial models could only be reasonably evaluated with the aid of residue analysis (Figure 1, 2). Residue analysis statistics (Figure 1) show that M5 and M8 presented lower values for scalar error and prediction bias (Figure 2). However, this analysis considers only the lines with observations, and some experimental units had dLys values greater than the R_{max} of these models, resulting in negative values and therefore no solution, which decreased the number of observations for the analysis of the association between the residue and the predicted value, thereby limiting the use of these models in the factorial calculation of LysIntake.

M3 with the values of R_{max}, k and Lysm (LysIntake = 133+(ln(357)–ln(357–Deposition))/0.0021) and M4 (LysIntake = 117+(ln(444)-ln(444–Deposition))/0.0027) presented no limitation when calculating LysIntake, but residue assessment statistics indicated a better predictive capacity for the factorial model with the M4 parameters. This result revealed that the biological interpretation and predictive capacity were not reconciled in the same model. M3 has parameters that assist the biological interpretation, but its application in the factorial model resulted in 10% less predictive capacity compared to M4 (Figure 2).

Table 6. Observed and estimated values of the lysine deposition by linear and nonlinear factorial models

Observed and predicted variables	Lysine in diets, g/kg						
	1.7	3.4	5.0	6.7	8.4	11.8	16.8
Observed variables							
Lysine Intake	190.1	333.8	484.0	586.4	889.3	955.3	1155.7
Lysine Deposition in Egg	62.8	126.8	190.5	281.8	297.1	284.1	286.7
Predicted variables							
Model 1: LysIntake = 3.69×Deposition+36×$BW^{0.67}$							
Predicted lysine Intake	241.0	477.7	713.5	1050.7	1106.9	1059.3	1069.2
Predicted lysine Deposition in egg	53.8	96.4	140.9	171.3	261.5	281.0	340.6
Erro	10.4	30.4	45.8	105.6	36.5	2.8	-49.9
Model 2: LysIntake = 2.15×Deposition+52×$BW^{0.67}$							
Predicted lysine Intake	148.5	286.8	424.8	621.4	654.2	626.6	632.7
Predicted lysine Deposition in egg	82.1	148.6	218.1	265.5	406.4	436.9	530.1
Erro	-17.2	-21.8	-33.4	8.6	-107.9	-153.3	-237.1
Model 3: LysIntake = 133+(*ln*(357)-*ln*(357-Deposition))/0.0021							
Predicted lysine Intake	225.4	342.4	503.4	879.2	1043.3	911.7	945.3
Predicted lysine Deposition in egg	39.8	120.4	183.9	216.4	280.8	289.9	311.4
Erro	26.0	6.4	2.0	60.6	16.8	-5.8	-23.5
Model 4: LysIntake = 117+(*ln*(444)-*ln*(444-Deposition))/0.0027							
Predicted lysine Intake	173.6	241.7	327.0	490.7	533.7	498.6	507.2
Predicted lysine Deposition in egg	61.1	145.4	206.5	235.5	286.7	293.0	307.1
Erro	4.9	-18.6	-20.3	42.3	10.7	-8.9	-19.7
Model 5: LysIntake = 114+(*ln*(327)-*ln*(327-Deposition))/0.0027							
Predicted lysine Intake	193.3	296.1	446.0	857.2	994.1	924.3	997.3
Predicted lysine Deposition in egg	60.6	145.0	206.3	235.3	286.6	292.9	307.1
Erro	5.4	-18.3	-20.1	42.4	10.8	-8.9	-19.6
Model 6: LysIntake = 511+(*ln*(288)-*ln*(288-Deposition))/1.025							
Predicted lysine Intake	511.2	511.6	512.1	514.1	513.6	513.8	513.1
Predicted lysine Deposition in egg	288.0	288.0	287.5	118.0	118.0	118.0	118.0
Erro	-225.2	-161.2	-96.6	163.8	179.1	166.1	168.7
Model 7: LysIntake = 374+(*ln*(269)-*ln*(269-Deposition))/0.0041							
Predicted lysine Intake	439.2	530.0	692.4	.	1044.6	1071.8	1070.1
Predicted lysine Deposition in egg	236.8	186.0	126.9	92.4	30.8	24.2	11.0
Erro	-175.3	-59.2	68.4	194.5	265.9	259.9	275.1
Model 8: LysIntake = 139+(*ln*(314)-*ln*(314-Deposition))/0.0028							
Predicted lysine Intake	219.0	324.2	482.3	974.3	1382.5	1161.4	1096.2
Predicted lysine Deposition in egg	56.4	140.5	200.7	228.8	277.6	283.5	296.5
Erro	9.6	-13.7	-14.3	49.1	19.8	0.6	-9.1

Input variable: observed lysine intake, mg/kg of $BW^{0.67}$.
Output variable: deposition of lysine in the egg, mg/kg of $BW^{0.67}$.
Error: difference between observed and estimated for deposition of lysine in the egg.

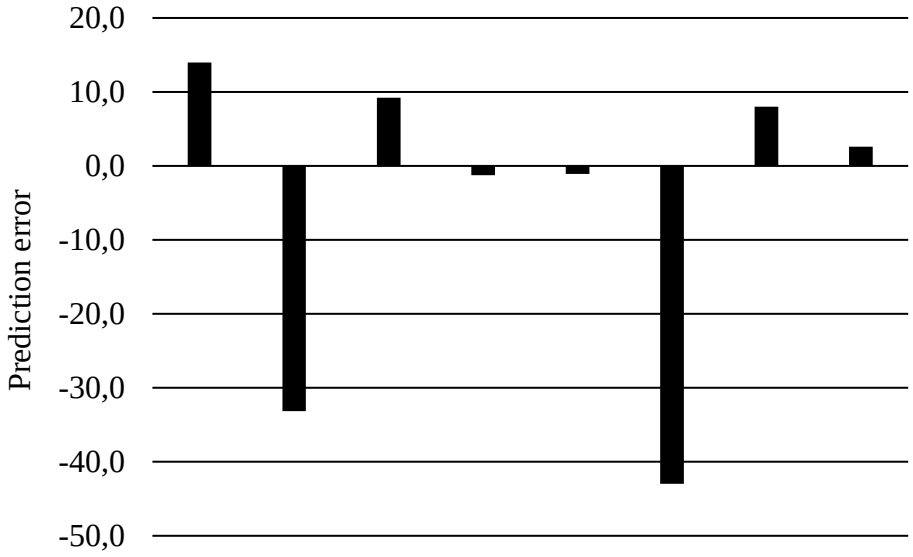
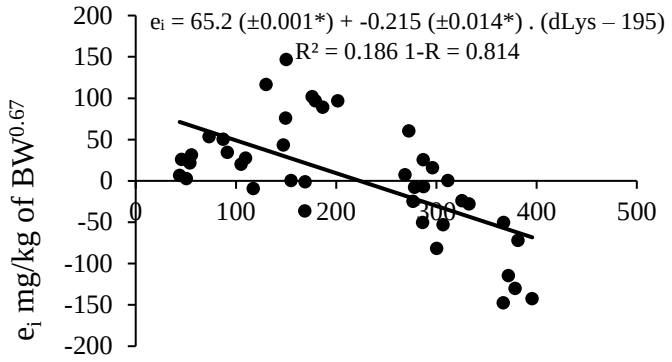
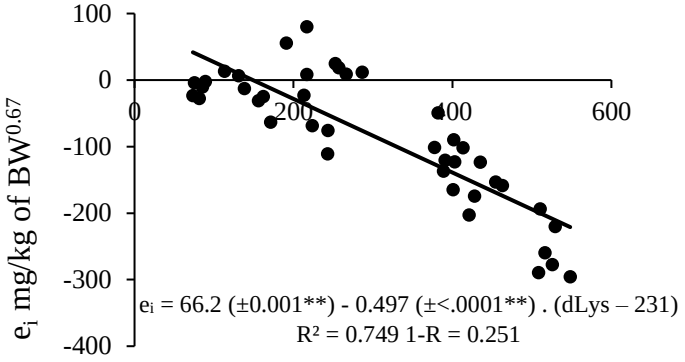


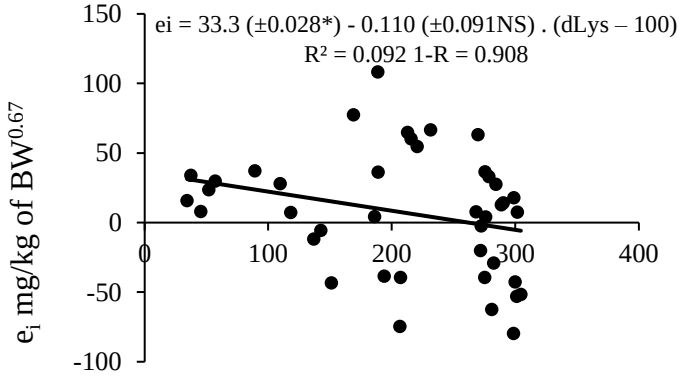
Figure 1. Mean predicted of percentage lysine deposition errors for models (M).



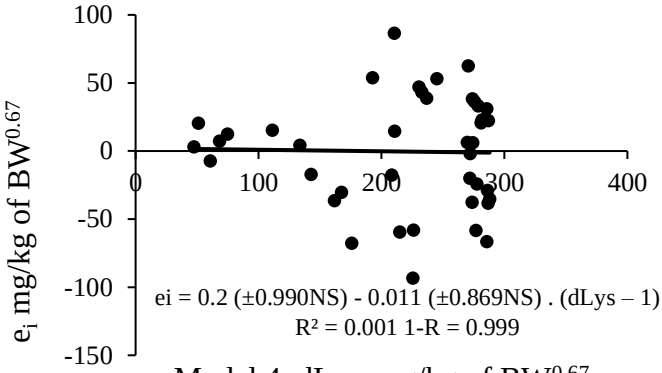
Model 1. dLys, mg/kg of $BW^{0.67}$



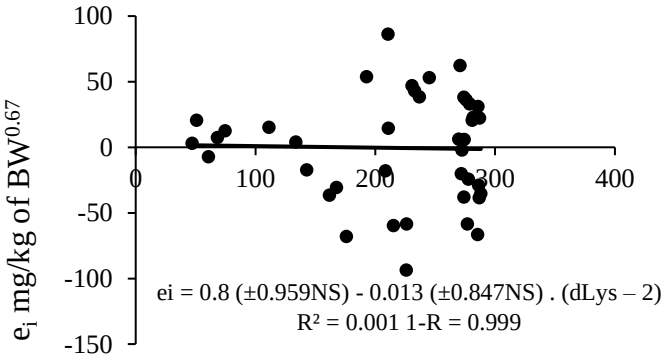
Model 2. dLys, mg/kg of $BW^{0.67}$



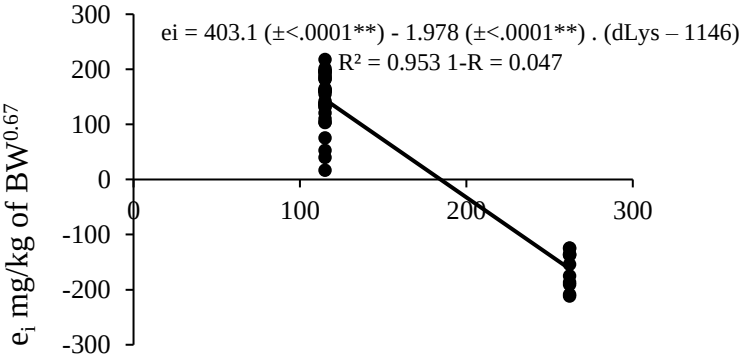
Model 3. dLys, mg/kg of $BW^{0.67}$



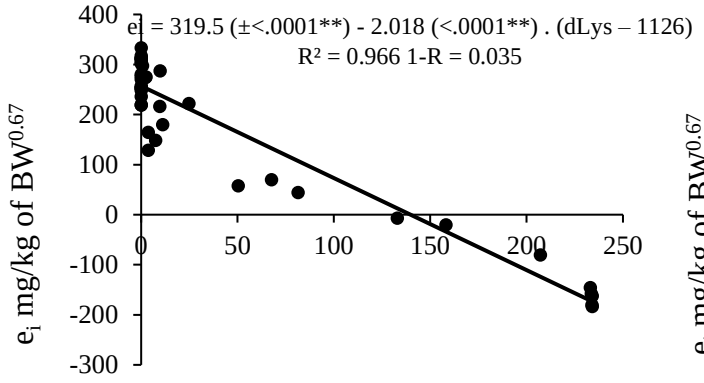
Model 4. dLys, mg/kg of $BW^{0.67}$



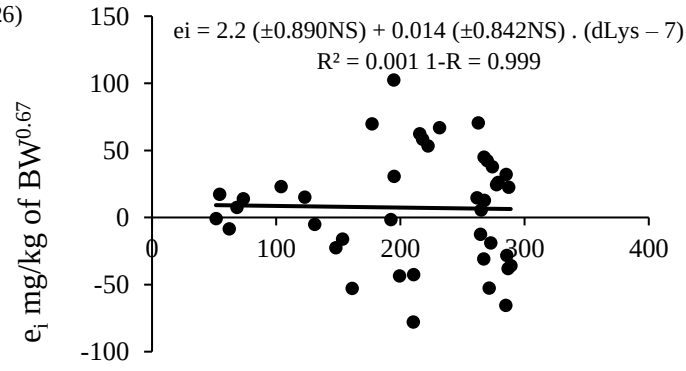
Model 5. dLys, mg/kg of $BW^{0.67}$



Model 6. . dLys, mg/kg of $BW^{0.67}$



Model 7. dLys, mg/kg of $BW^{0.67}$



Model 8. dLys, mg/kg of $BW^{0.67}$

Figure 2. Relationship between prediction residual (e_i) and predicted values for deposited lysine (dLys) by different models. Model 1 and 2: linear and Model 4,5,6,7 and 8: non-linear. NS $p>0.05$; ** $p<0.01$

3.4 Discussion

To our knowledge, this is the first study to investigate the relationship between Lys levels and the response by Japanese quail breeders. The experimental period used here was 21 days, conforming with methodologies by Silva et al. (2019). The experimental period could be reduced if there was greater differences in the diet levels (amplitude) of Lys. Both the present study and that of Silva et al. (2019) show that the definition of the treatments and their amplitude should also be considered, along with the experimental period. The breadth of Lys levels and responses, especially in the egg production, is indispensable to support the findings independent of the statistical tool. Previous studies with Japanese quails obtained an amplitude of 4% (Pinto et al., 2003; Costa et al., 2008) and 8% (Oliveira et al., 1999), whereas this survey returned 77%. The results showed that the levels of Lys in the diet were limiting for Japanese quails breeders. The amplitude Lys deposition in the egg was close to 470%, supporting the findings of this study regarding the dietary limitation of Lys. In addition to experimental period reduced, was followed this study the "reduction", formulated by Willian Russel and Rex Burch, which allowed the reduction in the number of animals used to maintain the precision of results (Hubrecht & Carter).

The feed of quails used in a breeding program should provide nutritional levels that support the expression of the maximum genetic potential. Therefore, linear procedures are limited to generating recommendations that approach the population average (Baker, 1986; Basaglia et al., 2005; Hauschild, Pomar & Lovatto, 2010; Silva et al., 2019) as opposed to the maximum. Among the variables analyzed here, the relationship between ingestion and deposition of Lys in the egg was selected to interpret the responses of the birds using exponential models. The results obtained in this study support the hypothesis that the method used can influence the Lys intake calculated and the interpretation of the animal response, especially the genetic potential of the bird, using six different exponential functions.

M2 was included in this analysis as a benchmark, especially for the interpretation of its parameters. The R_{max} parameter of the broken-line model is associated with the average population potential (Cosse & Baker, 1996; Hauschild, Pomar & Lovatto, 2010) however, the R_{max} values of the dual-exponential models (M6 and M7) were similar to that of the broken-line model. As both M6 and M7 models approximate the nutritional requirements of the average bird, the R_{max} parameter population is forced to underperform and conform to the average bird population as their requirements are not being fully met. The parameterization of the exponential double was defined to (Strathe et al., 2011) approximate the asymptotic response of the model to the observed values, thereby avoiding the use of an asymptotic response ratio to establish optimal performance and necessary intake (Strathe et al., 2011). In dose-response studies, the use of proportions to establish optimal performance and the respective nutrient intake may vary from 50% to 95% of R_{max} (Halle, Jeroch & Gebhardt, 1984; Cosse & Baker, 1996; Samadi & Liebert, 2008; Strathe et al., 2011). Therefore, it represents a criterion that confuses the lack of model adjustment and the proportion of optimal performance. In an attempt to approximate the adjustment of the model to the data and the parameterization, the double exponential used could have limited the adjustment of the functions to studies with smaller amplitudes in the responses of nutrient deposition intake. In this study, the treatments vastly

modified the responses of the birds by close to 476%, and the double exponential functions presented the poorest performance.

Two other objectives investigated in this research were the evaluation of the ability to interpret the response through the parameters of the model, and the predictive capacity when applied in a factorial approach. The results showed that it was not possible to reconcile the two objectives by the same mathematical model. The model that presented consistent estimation of the parameters and that aided in the interpretation of the response was the monomolecular with four parameters (M3). However, this model presented less predictive capacity when compared to the monomolecular with three parameters (M4). In a detailed analysis, the difference in the accuracy of these models (M3: 0.908 vs. M4: 0.999) is related to the scalar error, mainly the error of 26 mg/kg $BW^{0.67}$ in the M3 relative to the observed value at the first level of Lys in the diet, since the prediction bias value could only scarcely justify some differences between the models (Figure 2).

The estimated R_{min} parameter of 3.1 was close to zero (2–6 mg/kg $BW^{0.67}$), and this value has biological support. The lower level diet (1.7 g/kg) does not provide sufficient Lys for egg formation. Therefore, a significant body weight reduction was observed, equivalent to approximately 4 mg/kg $BW^{0.67}$ of Lys mobilized daily (Table 4). Subtracting the maintenance of 133 mg/kg $BW^{0.67}$, from the intake of 190 mg/kg $BW^{0.67}$ at the lowest level of Lys (1.7 g/kg), only 57 mg/kg $BW^{0.67}$ synthesis and deposition in the egg would be available. The prediction of the M4 of dLys and LysIntake at the lowest level was 61 and 174 mg/kg $BW^{0.67}$, respectively, resulting in 108% utilization efficiency, which indicates body reserve mobilization to sustain the minimal deposition of Lys in the egg. Based on this, the R_{min} parameter estimated by M4 of 117 mg/kg $BW^{0.67}$ has no biological support, as it represents close to double the value seen in the diet with a lower level of Lys (63 mg/kg $BW^{0.67}$). Some authors attribute the interpretation of maintenance requirement to the R_{min} of M4 considering that the value of R_{min} represents an inevitable loss and must be provided in equal quantity by diet to avoid the animal undergoing a negative nitrogen balance. This finding reinforces the initial hypothesis that some factorial models use the value of the maintenance requirement extracted on the axis of the ordinate (Strathe et al., 2011; Dorigam et al., 2014; Dorigam et al., 2017), when the ideal is on the axis of the abscissa (Kebreab et al., 2008; Silva et al., 2019), to avoid confusion between minimal response, R_{min}, axis of the ordinate (Silva et al., 2013; Dorigam et al., 2017), and requirement of maintenance, Lysm, on the axis of the abscissa.

With the four-parameter monomolecular function, it was possible to estimate the maintenance requirement for Lys based on production responses close to zero. The use of curvilinear models for this purpose can be considered as a reasonable option, since for parameter estimation, all observations were used from the lowest to the highest level of Lys in the diet. When compared to the estimate of Silva et al. (2019) of 156.8 mg/kg $BW^{0.75}$, the figures appeared to differ, but in this research the metabolic weight was calculated using the $BW^{0.67}$, and Silva et al. (2019) used $BW^{0.75}$. When standardized the value of Silva et al. (2019) to the same basis used here: in the result is 136 mg/kg $BW^{0.67}$, considering a mean BW of 0.16 kg (Table 4), and this value is in the confidence interval of 128–137 mg/kg $BW^{0.67}$ estimated for Lysm in this survey.

The requirement for retention of quail breeders was 2.6 times greater than that of cut breeders (51 mg/kg $BW^{0.67}$) (Silva et al., 2015b) and 2.2 times greater than commercial dusts (61 mg/kg $BW^{0.67}$) (Silva et al., 2015b), demonstrating the difference between genotypes for egg production function, and thereby justifying this research.

Based on the factorial calculation of LysIntake and dLys (Table 6), it was possible to obtain the utilization efficiency of each level of Lys in the diet, with an average of 41% obtained with Model 3, and 87% for Model 4. The requirement of Lys per g egg mass calculated on the basis of these models was 23 mg/g for Model 3 and 11 mg/g for Model 4 considering the relationship between Lys deposition and use efficiency: 8.853/0.41 = 23 mg/g for Model 3 and 8.853/0.87 = 11 mg/g for Model 4. In previous studies, the efficiency of Lys was 47% (Silva et al., 2019), and Met + Cys, Thr, and Trp, returned values of 59%, 42%, and 26%, respectively (Sarcinelli et al., 2014). The mean of these results is 43%, which is similar to the average efficiency, considering all treatments, obtained with models M3 and M4, verifying the importance in the selection of the function to interpret and predict the animal response. Despite the similarity between the values found in this search (41%) and with the average (43%) obtained from previous studies (Sarcinelli et al., 2014; Silva et al., 2019), it is important to highlight the limitation of information on the concentration of amino acids contained in the quail egg, especially for tryptophan which was found in only one publication (Ali, 2019) and tritonin, whose concentration varied from 5.3 (Ali, 2019) to 7.3 mg/egg (Genchev, 2012). Therefore, establishing the amino acid profile of the quail egg will help to consolidate the understanding of the efficiency of amino acid use, since recent studies have reported that this efficiency by quails is half that of other layers (Sakomura et al., 2015; Silva et al., 2015b).

The daily Lys intake calculated by the non-linear factorial model was 284 mg/bird for a bird of 0.170 kg BW and daily production of 10 g/bird EO. To use the model, the first step is to change the values of BW ($0.305 = 0.170^{0.67}$) and EO ($32.8 = 10 \times 0.305$) to metabolic body weight (MBW). EO is then transformed to dLys (290 mg/kg $BW^{0.67}$), multiplying 32.8 by 8.853 (8.853 is the relationship between dLys and EO). To calculate LysIntake initially, only dLys (290 mg/kg $BW^{0.67}$) was used to obtain LysIntake in mg/kg $BW^{0.67}$: 931 mg/kg $BW^{0.67}$ = (133 + (ln (357) -ln (357-290))/0.0021), then multiplying by MBW (0.30) This model assumes solutions for dLys < 357 mg/kg $BW^{0.67}$, equivalent to 12.3 EO, which is the maximum egg mass production. Another limitation of this model relates to the diet, with a value of 0.0021 representing the rate of use of the dietary protein, based on the ingredients maize, soybean, and corn gluten, with 60%, necessitating the use of the proposed model with other ingredients.

The factorial model prediction was positioned based on the equation parameters in relation to the values found in the literature, which used studies with Japanese quail eggs, due to the absence of studies with breeders. The value of LysIntake for a bird with 0.170 kg BW and with daily production of 10 g/bird EO was 284 mg/bird. By the linear factorial model of Rostagno et al. (2017), LysIntake was 267 mg/bird daily. The model of Rostagno et al. (2017) has been accepted by technicians and researchers in the area, and the difference shown here of 18 mg/bird may be a limiting factor for animals that are in genetic selection programs, especially considering the cumulative effect of the subdosage. Using the responses of 9.04 EO and 0.154 BW from the previous (Pinto et al., 2003) survey, LysIntake was calculated as 247 mg/bird

using the non-linear factorial model proposed here, differing by 7 mg/bird from the value of 254 mg/bird (Pinto et al., 2003).

Therefore, the four-parameter monomolecular function proposed in this study is adequate for interpreting the animal response. The parameters of this function when used for non-linear factorial calculations were suitable for calculating lysine intake for Japanese quail breeders.

3.5 Conclusions

The methodology used limited the supply of lysine and the birds responded to the degree of limitation, and the lysine response curve could be studied carefully. Considering the ability to interpret to predict the animal response the monomolecular function with four parameters was balanced against the statistics of adjustment and selection of models, being a reasonable option. It was possible to estimate the requirement of lysine for maintenance 133 ± 2 mg/kg $BW^{0.67}$ and based on average 41% efficiency the requirement of 22 mg Lys was obtained to produce 1 g egg output. The daily intake Lys calculated by the non-linear factorial model was 284 mg/bird for a bird with 0.170 kg BW and with daily production of 10 g/bird EO.

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Data availability statement

The following information was supplied regarding data availability: The raw measurements are available in the Supplementary Files.

Disclosure statement

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CAPÍTULO 4 - DETERMINATION OF THE OPTIMAL DIETARY AMINO ACID RATIO BASED ON EGG QUALITY FOR JAPANESE QUAIL BREEDER

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Determination of the optimal dietary amino acid ratio based on egg quality for Japanese quail breeder

Lizia C Carvalho[1], Dimitri Malheiros[2], Michele B Lima[1], Tatyany S A Mani[1], Jaqueline A Pavanini[1], Ramon D Malheiros[2], Edney P Silva[1]

[1]Department of Animal Sciences, UNESP – Univ Estadual Paulista, Via de Acesso Professor Paulo Donato Castellane, S/N, 14883900, Jaboticabal, São Paulo, Brazil.

[2]Prestage Department of Poultry Science, North Carolina State University, Raleigh, North Caroline, United State

* Corresponding author. edney.silva@unesp.br

Abstract

The objective of this study was to determine the ideal amino acid ratio for Japanese quail based on egg quality. In total, 120 Japanese quail were used. A completely randomized design was used with 12 treatments and 10 replicates per treatment. The treatments consisted of a balanced protein (BP) and the subsequent 11 diets were obtained by the 40% deletion of the BP a specific test for Lys, Met + Cys, Thr, Trp, Arg, Gly + Ser, Val, Ile, Leu, His, and Phr + Tyr. The trial lasted for 25 days. At the end of the trial, egg weight (EW), albumen height, albumen diameter, albumen index, yolk height, yolk diameter, yolk index, Haugh unit, eggshell weight (ESW), and eggshell percentage were measured. The ideal ratio was calculated when a statistical difference was detected using Dunnett's test. Only the EW and ESW variables differed from those of BP. The ideal amino acid ratios considering Lys as 100 for EW and ESW were Met + Cys 82 and 83, Thr 60 and 68, Trp 18 and 21, Arg 109 and 112, Gly + Ser 99 and 102, Val 77 and 87, Ile 61 and 67, Leu 155 and 141, His 34 and 37, Phe + Try 134 and 133, respectively.

Key words

egg weight; shell weight; fractional reduction; deletion method; reproduction

4.1 Introduction

Egg formation depends on maternal nutrition [1,2]. Some studies have shown that maternal nutrition can modify the composition and characteristics of eggs and consequently affect embryonic development [1,3,4]. Among nutrients, amino acids are essential for egg formation. The ideal profile of dietary amino acids must support the protein synthesis of target tissues, which in this research consists of proteins that make up eggs, such as low- and high-density lipoproteins, phosvitain and livetin, which make up the yolk, ovoalbumin, ovotransferin, ovomucoid, ovoglubin and ovomucin that form the albumen [5,6]. Glucosamine,

glycosaminoglycans, elastin, collagen (I, V, X), osteopontin, and clusterin are present in the eggshell membrane [5,7].

These proteins are synthesized in the liver, magnum, and uterus (or eggshell gland) to form the yolk, albumen, and eggshell, respectively [8], and are related to the dietary supply of amino acids [9,10]. The essential amino acids arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val) are vital for the constitution of embryonic tissues [11]. The main effects of amino acids are related to production aspects [12], such as the effect of Lys on egg production [12]. Research has linked the effects of Ile and Met on bird fertility [13,14]. Kim et al. [15] and Ullah et al. [16] described the outcomes of imbalances involving Leu, Ile, and Val on egg quality, especially the albumen and eggshell, which are able to regulate protein synthesis [17] and alter the integrity of egg membranes. These findings were corroborated by other studies [18–20], which evaluated the effects of Arg, Trp, and Thr.

Several methods are available to determine the daily intake of each essential amino acid based on egg quality assessment to ensure proper embryo formation [14,20–23]. The ideal amino acid profile is used to establish essential amino acid requirements in proportion to Lys requirements. Nitrogen balance has been established as a criterion commonly used for growing animals [24–26], commercial layers [27], and broiler breeders [28]. The deletion technique has been preferred to establish ideal amino acid profiles due to the possibility of studying all essential amino acids concurrently. The challenge in this research was to apply the method to variables related to egg quality, such as egg weight (EW), format index, and yolk and albumen content. All of these variables are sensitive in detecting the effects of limiting a specific amino acid in the diet [2,4,29]. However, no research has been conducted using this information to establish an ideal amino acid profile. The present methodology allows for the establishment of an optimal

ratio of all essential amino acids simultaneously, with the same group of animals and employing the same control diet, which attenuates environmental effects [28,30]. Therefore, this study aimed to establish the ideal ratio of essential amino acids (Lys, Met + Cys, Thr, Trp, Arg, Gly + Ser, Val, Ile, Leu, His, and Phe + Tyr) for Japanese quail breeding based on egg quality using the deletion method.

4.2 Materials and Methods

Location and ethics approval. This study was conducted in the Poultry Sector of the Animal Science Department of the Universidade Estadual Paulista (UNESP/FCAV) in accordance with ethical standards and approved by the Ethics Committee for the Use of Animals under protocol 012203/17.

4.2.1 Housing, animals, and experimental design

Experiments were conducted in a climatic chamber composed of refrigerators and exhausters that maintained the temperature at 24 ºC. The birds were housed individually in galvanized wire cages measuring 0.26 m × 0.37 m × 0.36 m, equipped with a linear feeder and nipple drinkers throughout the experimental period. The light program maintained throughout the experimental period consisted of 16 h of light and 8 h of darkness. A total of 120 Japanese quail breeding at 16 weeks of age, during the peak laying period, were used. The birds were standardized by weight and egg production and distributed by experimental units. A completely randomized design was used with 12 treatments and 10 replicates per treatments.

4.2.2 Experimental diets

In this study, a control diet was formulated to form a balanced protein (BP) with all of the nutritional requirements for Japanese quail as estimated by Rostagno et al. [31] for commercial Japanese quail since it does not provide nutritional requirements for breeders. Nitrogen and essential amino acids were provided by corn, soybean meal, corn gluten meal, and crystalline amino acids (Table 1).

The other experimental diets, total of 11 diets with different limiting amino acids, were obtained by deletion BP using corn starch (Tables 2 and 3). The total amino acid contents of the ingredients used in the formulation were analyzed by Evonik Industries AG, São Paulo, Brazil using a near-infrared spectrometer (NIRs) before formulating diets. The values were converted into digestible basis using digestibility coefficients from Rostagno et al. [31]. The deletion was 40% of the amino acid requirement to be evaluated in each treatment, and the other nutrients and energy were recomposed to meet the same BP level, except for the test amino acid, which was depleted by 40%, according to the methodologies described by Dorigam et al. [28]. The nutritional levels of amino acids in experimental diets were described in Table 3.

Table 1. Composition of the control diet (balanced protein).

Item	Content, g/kg
Corn	647.6
Soyabean meal (47%)	120.8
Corn Gluten (60%)	52.1
Dicalcium phosphate	11.5
Limestone	70.6
Sodium chloride	3.4
Potassium chloride	3.4
L-lysine (55%)	3.8
DL-methionine (99%)	9.5
L-threonine (98%)	2.7
L-tryptophan	1.0
L-arginine	4.8
L-glycine	1.3
L-valine	1.3
L-histidine	1.5
L-phenylalanine	0.8
L-glutamate	10.0
Choline chloride (60%)	1.6
Premix – Vitaminic[1]	0.2
Premix – Mineral[1]	0.2

[1]Content per kg of the diet- vit A, 6.668 IU; vit D3, 1.668 IU; vit E, 8 IU; vit K3, 2 mg; vit B1, 1 mg, vit B2, 3.34 mg; vit B6, 2 mg; vit B12, 9 mcg/kg; niacin, 21 mg; chlorine, 0.13 g; pantothenate acid, 8 mg; folic acid, 0.46 mg/kg, biotin, 0.05 mg/kg; 0.46; copper, 8 mg/kg; iron, 6,25 mg/kg; manganese, 70 g; zinc, 25 g; iodine, 6.25 mg; selenium 1.25 mg.

Table 2. Composition of the diet for all tested amino acids.

Item	Diets, g/kg										
	Lys	**Met+Cys**	**Thr**	**Try**	**Arg**	**Gly+Ser**	**Val**	**Ile**	**Leu**	**His**	**Phe+try**
Balanced protein	600.0	600.0	596.9	598.6	596.4	590.0	600.0	596.6	597.6	600.0	600.0
Soy oil (47%)	11.5	17.7	11.6	11.5	11.6	15.1	11.5	11.6	11.5	11.5	20.6
Dicalcium phosphate	6.0	6.0	6.0	6.0	6.0	6.2	6.0	6.0	6.0	6.0	6.0
Limestone	27.9	28.0	28.2	28.0	28.2	28.6	27.9	28.2	28.1	27.9	27.9
Sodium chloride	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Potassium chloride	4.8	4.8	4.8	4.8	4.8	4.9	4.8	4.8	4.8	4.8	4.8
DL-methionine (99%)	3.6	0.0	3.7	3.6	3.7	3.7	3.6	3.7	3.6	3.6	3.6
L-lysine (55%)	0.0	8.0	8.0	8.0	8.0	8.2	8.0	8.0	8.0	8.0	8.0
L-threonine (98%)	2.7	2.7	0.0	2.7	2.7	2.7	2.6	2.7	2.7	2.7	2.7
L-tryptophan	0.9	0.9	0.9	0.0	0.9	1.0	0.9	0.9	0.9	0.9	0.9
L-arginine	5.2	5.2	5.2	5.2	0.0	5.2	5.2	5.2	5.2	5.1	5.1
L-valine	3.4	3.3	3.4	3.3	3.4	3.4	0.0	3.4	3.3	3.3	3.3
L-isoleucine	2.9	2.9	2.9	2.9	2.9	3.0	2.9	0.0	2.9	2.9	2.9
L-leucine	6.7	6.7	6.7	6.7	6.7	6.8	6.7	6.7	0.0	6.7	6.7
L-glycine	5.1	5.1	5.1	5.1	5.2	0.0	5.1	5.1	5.1	5.1	5.1
L-phenylalanine	6.0	6.1	6.1	6.1	6.1	6.2	6.0	6.1	6.1	6.0	0.0
L-histidine	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	0.0	1.9
L-Glutamate	54.1	47.8	54.0	45.3	61.7	55.1	54.0	47.4	51.8	48.5	49.1
Choline chloride (60%)	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Corn starch	100.0	51.3	100.0	100.0	100.0	54.4	99.1	100.0	100.0	99.1	48.0
Sugar	65.1	100.0	65.0	68.3	58.2	100.0	62.6	70.7	72.7	65.7	100.0
Inert (cellulose)	88.8	100.0	86.2	88.7	88.2	100.0	89.7	87.6	84.3	88.8	100.0
Premix – Vitaminic[2]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Premix – Mineral[2]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

[1]Table 2; [2]Content per kg of the diet- vit A, 6.668 IU; vit D3, 1.668 IU; vit E, 8 IU; vit K, 3, 2 mg; vit B1, 1 mg. vit B2, 3.34 mg; vit B6, 2 mg; vit B12, 5 mcg/kg; niacin, 21 mg; chlorine, 0.13 g; pantothenate acid, 8 mg; folic acid, 0.46 mg/kg; biotin, 0.05 mg/kg; copper, 8 mg/kg; iron, 60 g; manganese, 70 g; zinc, 25 g; iodine, 6.25 mg; selenium, 0,12 mg.

Table 3. Nutritional levels of amino acids in experimental diets.

Itens	**Diets**											
	BP	**Lys**	**Met+Cys**	**Thr**	**Trp**	**Arg**	**Gly+Ser**	**Val**	**Ile**	**Leu**	**His**	**Phe+Tyr**
Metabolizable energy (MJ/kg)	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7
Calcium (g/kg)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Avaliable phosphorus (g/kg)	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Crude protein (g/kg)	180.1	180.1	180.1	180.1	180.1	180.1	180.1	180.1	180.1	180.1	180.1	180.1
Crude fiber (g/kg)	18.8	11.3	11.3	11.2	11.3	11.2	11.3	11.1	11.2	11.2	11.3	11.3
Starch (g/kg)	423.3	341.7	298.3	340.4	341.1	340.2	340.9	299.3	340.3	340.7	340.9	296.1
Crude fat (g/kg)	33.9	31.8	37.9	31.7	31.7	31.7	31.8	37.2	31.7	31.7	31.8	40.8
NFE (g/kg)	663.1	675.0	668.8	675.1	675.1	675.2	675.0	679.7	675.1	675.1	675.0	665.9
Lysine (g/kg)	10.9	6.6	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9
Metionine+Cystine (g/kg)	9.0	9.0	5.4	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Threonine (g/kg)	6.6	6.6	6.6	3.9	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
Tryptophan (g/kg)	2.3	2.3	2.3	2.3	1.4	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Arginine (g/kg)	12.7	12.7	12.7	12.7	12.7	7.6	12.7	12.7	12.7	12.7	12.7	12.7
Glycine+serine (g/kg)	12.5	12.5	12.5	12.5	12.5	12.5	7.5	12.5	12.5	12.5	12.5	12.5
Valine (g/kg)	8.2	8.2	8.2	8.2	8.2	8.2	8.2	4.9	8.2	8.2	8.2	8.2
Isoleucine (g/kg)	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	4.2	7.1	7.1	7.1
Leucine (g/kg)	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	9.8	16.5	16.5
Histidine (g/kg)	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	2.7	4.6
Phenylalanine+Tyrosine (g/kg)	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	14.8	8.9

BP, balanced protein.

4.2.3 Data collection

The trial lasted 25 days and was divided into adaptation and egg collection, with 20 days of adaptation and 5 days of egg collection or up to a total of 15 eggs per treatment. The daily amount of feed provided was 24 g per bird and water was ad libitum. Birds were weighed at the beginning and end of experiments to determine their body weights. Feed leftovers were measured at the end of the trial to determine consumption. Egg production was measured daily. The variables evaluated were feed intake (FI. g/bird/day), crude protein intake (CPIntake. g/ave/day), body weight (g), EP (%/bird/day), egg weight (EW. g), egg mass (g/bird/day), and feed conversion by egg mass (FCR. Feed intake g/Egg weight g and Protein intake g/Protein deposition in egg g). During the data collection period and tested were immediately, eggs were collected, weighed, and broken to measure the height, diameter, albumen, and yolk index and Haugh unit. Additionally, the eggshells were evaluated for weight and percentage.

4.2.4 Egg quality analysis

At the end of the trial, egg weight (EW), albumen height, albumen diameter, albumen index, yolk height, yolk diameter, yolk index, Haugh unit, eggshell weight (ESW), and eggshell percentage were measured. Heights of albumen and yolk were measured using a digital micrometer coupled to a tripod base, and diameters were measured using digital calipers. The HU values were calculated from the logarithmic relationship between the height of the dense albumen and the EW. This formula [32] was applied to each egg collected, as described in Equation (1):

$$HU = 100 \log (H + 7.57 - 1.7 W^{0.37}) \quad (1)$$

where: H = albumen height in mm and W = EW. g

The index yolk (YI) and albumen (AI) were determined by considering the relationship between the height (H) and diameter (D) of the respective components (as described by Funk) [33]. Eggshells were washed with water and dried by forced air circulation at 55 ºC for 72 h. After drying. the shell was weighed using a digital scale accurate to 0.01 g. Shell percentage was obtained considering the relation between shell weight (ESW) and EW.

4.2.5 Statistical analysis

Data were subjected to homoscedasticity of variance and error normality tests. Upon satisfying the premises, analysis of variance, declared as significant at 0.05, was performed and when treatment effects were detected. Dunnett's test was applied for all egg quality variables. All data were analyzed using SAS software (v.9.4; SAS Institute Inc., Cary, CA, USA, 2014).

4.2.6 Determination of ideal amino acid:Lys ratios

The ideal proportion of amino acids were determined following the principles of Green and Hardy [34], modified in this study to use egg quality variables. The calculation consists of four steps: Step 1: Calculate the proportion of reduction (Yr) of the analyzed variables of each experimental unit in relation to BP as follows:

$$Yr = 100 \times (1 - Yi/\bar{Y}\ BP)$$

where Yr is the percentage of reduction in the response of the analyzed variable of each treatment; Yi is the response of each treatment and $\bar{Y}$ BP is the mean value of the response of the control treatment, which received the BP.

Step 2: The Yr values were standardized considering the percentage of deletion applied in the treatments (40%) as follows: PYr = Yr/40. where PYr is the standardized Yr value for the applied deletion.

Step 3: The treatment's actual deletion ratio (RDP) was calculated as follows:

$$RDP = 40 \times [1 - (PYri/PYrmax)]$$

where 40% is the initial deletion value. PYrmax is the maximum PYr of the analyzed variable and PYri is the PYr associated with a deficient diet resulting from the second step.

Step 4: The optimal in-feed amino acid (AAI) was calculated as follows:

$$AAI = AABP - [AA\ BP \times (RDP/100)]$$

where AABP represents the concentration of the amino acid in the BP (g/kg) and RDP is the actual deletion ratio resulting from the third step.

Step 5: The ideal ratio of amino acids to Lys (IAAR) is calculated as follows:

$$IAAR = [AAI/Lys] \times 100$$

where AAI is the value found for each amino acid (Met + Cys. Thr. Trp. Arg. Gly + Ser. Val. Ile. Leu. His. and Phe + Tyr) and Lys is the value found from AAI to Lys.

4.3 Results

The responses obtained for the productive performance responses were significantly affected by limiting dietary and treatment BP ($p < 0.05$; Table 4). Based on the results of the Dunnett test for limitation in Lys, Thr, Try, Arg, and Val FI was affected. However, only for Val was there a difference in the intake relative to BP. The lower IF contributed to the lower ME in the Lys, Thr, Try, and Val limiting treatments. Lys limitation still affected FCR and CPCR, by 30% and 34%, respectively, when compared to BP.

Table 4. Average responses to dietary limited in amino acids.

Amino Acid	Feed intake (g/bird day^{-1})	Protein intake (g/bird day^{-1})	Egg mass (g day^{-1})	FCR[1] (g/g)	FCR[2] (g/g)
Lysine	18.62b	2.46a	4.15b	4.90a	1.87b
Met+Cys	21.14a	2.58a	6.94a	3.13a	1.05a
Threonine	18.83b	2.40a	5.09b	4.35a	1.62a
Tryptophan	19.15b	2.56a	4.78b	4.41a	1.78b
Arginine	19.05b	2.42a	5.79a	3.58a	1.17a
Gly+Ser	22.43a	2.84a	6.71a	3.86a	1.28a
Valine	14.78b	1.91b	3.81b	3.92a	2.08b
Isoleucine	20.18a	2.53a	5.29a	3.83a	1.30a
Leucine	21.39a	2.71a	6.66a	3.54a	1.31a
Histidine	22.55a	2.77a	7.18a	3.36a	1.12a
Phe+Try	20.39a	2.51a	6.63a	3.16a	1.24a
BP	21.27a	2.61a	7.14a	3.09a	1.09a
Mean ± SE	19.98 ± 1.82	2.53 ± 0.25	5.83 ± 1.79	3.77 ± 1.30	1.39 ± 0.42
P value	<0.0001	<0.0001	<0.0001	<0.0530	<0.0001

FCR 1, feed conversion ratio; FCR 2, crude protein conversion ratio; BW, body weight; BP, balanced protein. a, b, mean values with b within the line were significantly different ($p < 0.05$) compared with balanced protein, by the Dunnett test.

Table 5. Effects of the dietary amino acid limitation on the egg quality of Japanese quail breeders.

Amino Acid	Variables									
	EW	**ESW**	**ESP**	**AH**	**AD**	**AI**	**HU**	**YH**	**YD**	**YI**
Lysine	8.36b	0.64b	8.08a	3.53a	8.27a	0.42a	86.57a	7.40a	20.61b	0.36a
Met+Cys	8.43b	0.64b	7.60a	4.39a	7.28a	0.59a	91.53a	7.97a	22.20b	0.36a
Threonine	8.37b	0.55b	7.27a	4.49a	6.99a	0.62a	92.03a	7.89a	21.13b	0.37a
Tryptophan	9.43b	0.65b	6.83a	4.17a	6.67a	0.54a	89.19a	7.38a	22.49a	0.33a
Arginine	8.71b	0.65b	7.54a	4.00a	7.60a	0.53a	90.56a	7.71a	21.58b	0.35a
Gly+Ser	9.22b	0.71b	7.76a	4.37a	7.59a	0.62a	90.52a	7.64a	22.39a	0.33a
Valine	8.22b	0.54b	6.64a	3.81a	5.53b	0.70a	88.32a	7.76a	21.64b	0.35a
Isoleucine	8.74b	0.62b	6.99a	4.10a	7.92a	0.53a	89.36a	7.85a	23.16a	0.34a
Leucine	8.16b	0.68b	8.38b	4.50a	5.68a	0.79b	92.23a	8.20a	22.73a	0.36a
Histidine	9.59b	0.73a	7.00a	4.05a	7.44a	0.49a	88.36a	7.44a	23.08a	0.32a
Phe+Try	8.46b	0.66b	7.84a	4.29a	6.48a	0.61a	90.78a	7.98a	22.81a	0.36a
Balanced protein	10.76a	0.84a	7.79a	4.07a	7.87a	0.52a	87.69a	8.22a	24.24a	0.34a
Mean ± SE	8.86 ± 1.05	0.67 ± 0.10	7.53 ± 1.12	4.15 ± 0.69	7.09 ± 1.67	0.58 ± 0.19	89.62 ± 3.94	7.79 ± 0.62	22.34 ± 1.60	0.35 ± 0.03
P value	<0.0001	<0.0001	0.0144	0.0530	<0.0001	0.0009	0.0148	0.5218	<0.0001	0.0176

EW—Egg weight (g); ESW—Egg shell weight (g); ESP—Eggshell proportion (%); AH—Albumen height (mm); AD—Albumen diameter (mm); AI—Albumen index (%); HU—Haugh unit; YH—Yolk height (mm); YD—Yolk Diameter (mm); YI—Yolk index (%). a, b. mean values with b within the line were significantly different ($p < 0.05$) compared with balanced protein. by the Dunnett test.

Limiting dietary treatments of His, Phe + Tyr, and Leu affected EW and ESW. When the means were compared using Dunnett's test, considering BP as a reference, only the EW and ESW variables were found to have significant effects on the dietary treatments ($p < 0.05$) except for the difference between BP and the His-limited dietary treatment (Table 5) which was not significant for ESW ($p > 0.05$). Therefore, only EW and ESW variables were used to establish the ideal amino acid ratio.

The EW for BP was 10.76 g, while the EW of the other dietary treatments ranged from 9.59 to 8.16 g for the limiting dietary treatments involving His and Leu, respectively. Thus, the minimum reduction was approximately 11% for His and 24% for Leu. For ESW, the mean value for BP was 0.84 g, ranging from 0.73 and 0.54 g for the His and Val limited dietary treatments, respectively, which were equivalent to a reduction of 13% and 36%. when compared to BP.

The His exhibited the least limitation in terms of the two response variables EW and ESW, compared to BP. On the other hand, Leu and Val were amino acids that presented the greatest limitation, with a visible deterioration in egg quality, especially EW and ESW ($p < 0.05$).

These results were used to calculate the optimal concentration of the respective amino acids using Leu and Val as control standards to calculate the actual deletion. Table 6 presents AAI and IAAR values of the evaluated amino acids. AAI and IAAR values differed between EW and ESW variables. The distance quantified by the standard deviation was 8% for AAI between EW and ESW and 7% for IAAR between EW and ESW.

Table 6. Summarized results of the individual amino acid deletions for egg weight (EW) and eggshell weight (ESW) of Japanese quail's breeders

Variables	EW				ESW			
	Yr	RDP	IAA	IAAR	Yr	RDP	IAA	IAAR
Lys	22.30	3.15	1.06	100	23.32	13.82	0.94	100
Met+Cys	21.69	4.16	0.86	82	23.45	13.68	0.78	83
Thr	22.22	3.28	0.63	60	33.45	2.45	0.63	68
Trp	12.36	19.57	0.18	18	22.53	14.72	0.2	21
Arg	18.60	9.26	1.15	109	20.14	17.39	1.05	112
Gly+Ser	14.39	16.22	1.05	99	14.90	23.28	0.96	102
Val	23.61	0.98	0.81	77	35.64	0	0.82	87
Ile	18.76	9	0.65	61	25.51	11.37	0.63	67
Leu	24.21	0	1.64	155	18.59	19.13	1.33	141
His	10.84	22.09	0.36	34	14.48	23.75	0.35	37
Phe+Tyr	21.37	4.68	1.41	134	21.57	15.79	1.25	133

Yr = per cent reduction in EW and ESW (%); RDP = real deleted proportion; IAA = amino acid requirement; IAAR=optimal in-feed amino acid ratio (%).

4.4 Discussion

Maternal amino acid nutrition is essential for egg formation. which later supports embryonic development [3]. This study aimed to apply the deletion method to establish an ideal amino acid profile using egg quality variables. The results obtained in the present study indicated that the applied deletion of 40% of the studied amino acids (Lys, Met + Cys, Thr, Trp, Arg, Gly + Ser, Val, Ile, Leu, His, and Phe + Tyr) limited the responses of the female breeders of the Japanese quail, verifying the worsening of performance and egg quality variables (Tables 4 and 5), especially for EW and ESW. These results support the objective proposed in this research, which assumed the existence of a dose-response relationship (Table 5). The only exception was for the amino acid His; although the dose affected the EW response, the reduction found for ESW was not significantly different according to the BP Dunnett's test, which had no dietary limitation (Table 5). Previously published results [29] validated the limitation of the test amino acid Arg based on its response to EW. According to these authors, EW is the most sensitive response in Japanese quails. The convention proposed by Morris and Gous [35] has prevailed for commercial laying hens, and dietary amino acid limitations primarily affect bird egg production, with little change in EW. This understanding was corroborated by other studies carried out with commercial laying hens [36,37] and broiler breeders [19,38,39]. However, recent Japanese quail results support the fact that these birds are more sensitive to reduced levels of amino acids in their diet, and that a significant reduction in EW occurs [29,40–42].

The results of this study indicated that nutritional limitation involving the tested amino acids was able to modify EW. This effect is related to nutritional deficiency imposed on the breeder birds, which decreases embryo birth weights [1]. One hypothesis to explain the reduction in EW is that the weight of the embryo is related to maternal protein loss. causing a decrease in protein synthesis under dietary conditions deficient in essential amino acids [1,4]. The 40%

limitation imposed on the diets decreased the supply of amino acids to meet the physiological processes related to the maintenance of body weight, where a small part would be available for processes related to protein deposition in the egg. Therefore. To compensate for the loss of amino acids and to maintain plasma levels [43,44], only muscle protein mobilization remains. In addition to EW, ESW was also significantly affected given the greater thickness of the eggshell membrane protein constitution. which was considered in the ESW computation.

The eggshell is a structure whose function is to protect the interior of the egg from physical and microbial agents, regulate gas, water, and metabolite exchanges, and provide mineralized components for embryo development [45]. The effects of dietary amino acid limitation on ESW have been reported, in particular regarding the protein that composes the protein matrix and influences eggshell texture [45,46]. Mann and Mann [47] identified two proteins from the ovocleidin-17 family as the main components of eggshell protein matrix. In quails, the eggshell membrane is thicker than that found in chicken eggs. This difference lies in the number of protein families in the membrane constitution; in quails, there are two families, while in eggs from chickens, there is only one family Mann and Mann [47].

The YD verified in the limited dietary treatments involving Lys, Met + Cys, Thr, Arg, and Val was significantly lower than the value obtained for BP. El-Tarabany [48] reported that yolk diameter is positively correlated with EW, corroborating the results obtained in this study.

His was the only amino acid that exhibited a difference in EW. His is an essential amino acid [49,50] which does not present immediate signs of deficiency. but a function of protein metabolism that compensates for such deficiency through hemoglobin and carnosine catabolism [51]. Robbins et al. [52] demonstrated that the growth rate of broiler chicks fed His-deficient diets was recovered by intravenous administration of L-carnosine. However, there was no increase in the plasma concentration of His, supporting the hypothesis that muscle carnosine

is rapidly metabolized to His and used in priority demands such as the synthesis of regulatory proteins.

The results obtained for AAI revealed a difference of 8% when calculated based on EW and ESW. For IAAR, the difference between EW and ESW variables was approximately 8.4%. The amino acid Leu showed a greater degree of limitation. and one hypothesis may be related to its concentration in egg protein composition [1,53]. In addition, there exists potential antagonism involving Val and Ile [50,54,55]. The amino acid Leu has been considered the most efficient in protein synthesis among BCAAs (Lynch et al. 2006), since Leu induces the activation of the mTOR complex, which stimulates protein synthesis [56]. Our results corroborate the suggestion of Macelline et al. [6], who indicate that farmers should pay attention to dietary Leu levels.

The amino acid profile of the target tissue is usually used when there is no information regarding the ideal amino acid ratio. The ideal relationship obtained considering the composition of the egg presented by Bayomy et al. [57] was as follows: Met + Cys 118%, Thr 52%, Arg 35%, Gly + Ser 75%, Val 69%, Ile 57%, Leu 108%, His 35%, and Phe + Tyr 97%.

The ideal ratios obtained based on the composition of meat presented by Bayomy et al. [57], was (Lys 100%) Met + Cys 37%, Thr 29%, Arg 31%, Gly + Ser 33%, Val 48%, Ile 60%, Leu 55%, His 38%, and Phe + Tyr 84%. These values are different when compared to the results obtained in this study. Suggesting that the composition of the target tissue alone may not represent the best option for establishing ideal ratios of amino acids [57,58], since the proportion of dietary amino acids is modified during digestion and absorption processes, which precede deposition in the target tissue [55].

The IAA obtained for EW and ESW estimated by the deletion method (Table 6) showed considerable variation from 0.86 to 19.13% for the same amino acid. The greatest variations were observed for Leu, Phe + Tyr, Lys, Met + Cys, Arg, Gly + Ser, Trp, Ile, His, Val, and Thr,

with values of 19.13, 11.65, 11.02, 9.93, 8.97, 8.42, 6.04, 2.60, 2.13, 0.86, and 0.99%. respectively. In addition, only the IAAs of Thr and Val were higher in terms of eggshell weight; however. with a difference of 11.65% in the IAA for Lys between the variables (EW:1.06; ESW:0.94), it was possible to reduce the proportional distance between Lys and the other amino acids. Thus, the amino acid requirements increased for all when the eggshell weight was met, except for Leu and Phe + Thr.

The NRC [59] and Rostagno et al. [60], based their recommendations on compilations of studies. Due to the lack of research for breeders the requirements for breeders and commercial quails were not differentiated. However, it is expected that due to the genetic differences between these birds a modification in IAA and consequently IAAR is likely. The results of Rostagno et al. [60], indicate that IAA for all amino acids studied are higher for commercial quails, when an average was made between the IAA of EW and ESW, with difference ranging from 5.40 (Val) to 35.37% (His). When evaluations between the AAIs are specific for each variable the highest variation was for AAI for ESW, with a difference of up to 37% for the amino acids Gly + Ser and His and the lowest difference was for Val (5%). The observed for IAA of EW with highest difference for His (34%) and lowest for Leu (5%). To compare the results with NRC [59], the IAAs of NRC [59] were transformed considering the digestibility of lysine with an average of 89% [31]. When compared. Less variation was observed with proximity of the estimated results for Arg. Gly + Ser and Val (1.12, 1.04, and 0.82% in feed, respectively) for IAA from EW and Thr. Val and Phe + Try (0.66, 0.82, and 1.25% in feed, respectively) for IAA from ESW. However, for the other amino acids IAA are higher for NRC [59] results, being only for ESW results for Arg. Gly + Ser. Ile and His (1.12, 1.42, 0.80, 0.37% in feed, respectively) IAA were higher.

However, the AAI was estimated based on egg quality to determine the AAI related to Lys. The intake of amino acids by the proportion between them can be modified in relation to the AAI. When comparing the AARI for commercial quails based on the recommendations of Rostagno et al. [60]. NRC [59] and Silva and Costa [61] for Lys at 100%: 82, 70, and 74; 61, 74, and 71; 21, 19, and 19; 115, 126, and 133; 115 and 117; 75, 92, and 92; 65, 90, and 92; 150, 142, and 151; 42, 42, and 44; 135, 140, and 149 for Met + Cys, Thr, Trp, Arg, Gly + Ser, Val, Ile, Leu, His and Phr + Tyr, respectively, except for Gly + Ser from Silva and Costa [61] which was not determined. Lower ratios were observed for the recommendations of NRC [59] and Silva and Costa [61]. Rostagno et al. [60] recommended higher ratios between amino acids and Lys, approaching the IAARs determined in this study. However. the slight increase did not provide ratios close to those found for EW in Trp. Arg. Gly + Ser. Ile and His. with an increase in 19.84, 5.35, 15.93, 6.21, and 23.71% difference in the AA:Lys ratio, respectively. Corroborating with that found for ESW, where differences of 9.63, 12.64, 14.09, 6.24, and 10.69% differed for Thr. Gly + Ser. Val. Leu and His in AA:Lys ratios, respectively, His and Val being the amino acids that differed most when compared with the reference used today for formulation of rations.

The ratios for Met + Cys:Lys (82:100) and Phe + Try:Lys (135:100), did not differ with that recommended by Rostagno et al. [60] for the two IAAR determined in that study (EW and ESW = 82 and 83 for Met + Cys and 134 and 133 for Phe + Try, in percentage, relative to Lys), minimal differences were found for Thr:Lys (61:100) and Val:Lys (75:100) in EW and between Trp:Lys (21:100), Arg:Lys (115:Lys) and Ile:Lys (65:100). However, differences in ratios were found for most amino acids. Previous recommendations used for broilers may have overestimated the IAAR for all amino acids except for Val for birds production. The current IAAR recommendations for broilers in contrast to all previously used techniques apply a target

tissue to ensure quality of protein synthesis and minimize lighter EW and ESW during production, thus modifications were noticed when a target trait is selected as a basis.

Understanding how the limitation of essential amino acids influences egg quality is essential for the animal category studied in this work, as the physical quality of eggs can be used as a selection trait directly and indirectly in genetic improvement [62]. Among the characteristics studied (EW and ESW), determining the optimal relationship between essential amino acids presents high heritability [63,64].

In a study by Hegab and Hanafy [65] with Japanese quail breeders, it was observed that EW influenced the hatchability of eggs and increased the weight of chicks at hatch. Regarding the characteristics of ESW, the study also found that larger ESWs have a higher hatchability rate, resulting in greater eggshell volume, pore count, and surface area [65]. Therefore, the contribution of nutrition to maintaining the desired quality of these parameters is extremely important for genetic companies and nutritionally enables animals to express their genetic potential.

4.5 Conclusions

In conclusions, the application of the deletion method with a limitation of 40% of dietary amino acid deletion, allowed the EW and ESW variables to be sensitive for all tested amino acids. Thus, it was possible to establish the ideal profile of essential amino acids in the diet simultaneously, focusing on a target tissue, (which in this research was EW and ESW).

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CAPÍTULO 5 - DETERMINATION OF THE OPTIMAL IN-FEED AMINO ACID RATIO FOR JAPANESE QUAIL BREEDERS BASED ON UTILIZATION EFFICIENCY

Este capítulo corresponde ao artigo científico publicado na revista Animals, 12: 2953, 2022.

Determination of the optimal in-feed amino acid ratio for Japanese quail breeders based on utilization efficiency

Lizia C Carvalho[1], Tatyany S A Mani[1], Michele B Lima[1], Jaqueline A Pavanini[1], Rita B Vieira[1], Lizandra Amoroso[1], Edney P Silva[1]

[1] Department of Animal Sciences, UNESP – Univ Estadual Paulista, Via de Acesso Professor Paulo Donato Castellane, S/N, 14883900, São Paulo/Jaboticabal, Brazil.

Corresponding author: Edney P Silva, edney.silva@unesp.br

Simple Summary

Breeder reproductive responses are optimized if nutritional, environmental, and health requirements are adequately met. Thus, the ideal concentration of amino acids in the diet must be obtained to prevent excess or deficiency to the animal. This may occur due to the inefficiency in the production or excessive excretion of nitrogen. Therefore, it is necessary to determine the optimal relationship for this nutrient category. These results contribute to ensuring optimal ratios of essential amino acids in the diets of Japanese quail breeders based on amino acid efficiency.

Abstract

The description of the genetic potential is the first step to estimating amino acid requirements and the ideal amino acid relation (IAAR). The aim of this study was to estimate the parameters that describe the daily maximum theoretical nitrogen retention (NRmaxT, mg/$BW_{kg}^{0.67}$), daily nitrogen maintenance requirement (NMR, mg/ $BW_{kg}^{0.67}$), protein quality (b), dietary efficiency of the limiting amino acid (bc^{-1}) and determine the lysine requirement and the IAAR for Japanese quail breeders. Two nitrogen balance assays were performed, one assay using 49 quails distributed in seven treatments (protein levels between 70.1 and 350.3 g/kg) and seven replicates and other assay to determine the IAAR by the use of bc^{-1}, 12 treatments and 10 replicate, with a control diet (CD) and 11 treatments that had limited essential amino acids by providing only 60% of the CD. The values obtained for $NR_{max}T$, NMR, b and bc^{-1} were 3386.61, 0.000486 and 0.000101, respectively. The daily intake of Lys was 291 mg/bird day. Lys was set at 100% for determining the IAAR: 87, 67, 21, 117, 96, 66, 142, 39, and 133 for Met + Cys, Thr, Trp, Arg, Val, Ile, Leu, His, and Phr + Tyr, respectively, for Japanese quail breeders.

Keywords: dilution technique; exponential model; Goettingen approach; lysine requirement

5.1 Introduction

Laying birds need amino acids for maintenance, tissue growth, and fertile egg production [1]. Deficiencies or excess of nutrients in hens can cause deficiency and toxicity in offspring, respectively [2,3]. For breeders, the evaluation of maximum productive performance responses is also highlighted, which can only be achieved if the nutritional and environmental requirements are met with precision. Therefore, commercial feeds for breeder quail are formulated in accordance with the recommendations for commercial quails [4,5], which results in excessive feeding of amino acids to these breeder quail. Breeder quail can show changes in production, egg weight, and egg mass when compared to commercial quail, which drives the need for further research in the amino acid requirements for these birds [6].

The ideal relationship between amino acids is expressed in relation to lysine (Lys) [7]. In addition, Lys is considered the second limiting amino acid in diets based on corn and soybean meal for poultry [8]. In laying birds, Lys is used for egg formation, and its requirements change according to the number of eggs produced [9]. Lower levels (0.59%) of Lys are responsible for a decrease in the number of ovarian follicles and immature reproductive organs in broiler breeders.

According to Emmans and Fisher [10], determining the description of the genetic potential of an animal is the first step in estimating amino acid requirements. Some researchers [11–14] have used the “Goettingen approach”, which uses the nitrogen balance assay and Mitscherlich function to interpret the genetic potential for protein deposition, as well as to calculate the required amino acid intake to support protein growth. The method of individual deletion of the test amino acid in the nitrogen balance has been used to determine the ideal amino acid ratio (IAAR) [11,12,14,15]. Samadi and Liebert [11] used the observed slope of the responses between diets to determine the ideal proportions of amino acids. Therefore, this study aimed to

estimate the parameters that describe the maximum theoretical daily nitrogen retention ($NR_{max}T$) and the daily nitrogen maintenance requirement (NMR) using the Goettingen model. These were applied to calculate the protein quality (b) and dietary efficiency of the limiting amino acid (bc^{-1}), to determine the daily requirement of lysine (Lys), and to establish the ideal profile of essential amino acids (Lys, Met + Cys, Thr, Trp, Arg, Gly + Ser, Val, Ile, Leu, His, and Phe + Tyr) for Japanese quail breeds, based on the efficiency of dietary protein utilization using the deletion method.

5.2 Materials and Methods

5.2.1 Location and ethics approval

Two nitrogen balance trials were conducted in the Poultry Sector of the Animal Science Department of the Universidade Estadual Paulista (UNESP/FCAV) in accordance with ethical standards and approved by the Ethics Committee for the Use of Animals under protocol 012203/17. In assay 1 was the determination of NMR, NRmaxT, and Lys requirement using utilization efficiency, and in assay 2 was the determination of amino acid efficiency of utilization, requirements, and optimal amino acid ratio.

Assay 1

5.2.2 Housing, Animals and Experimental Design

A total of 49 Japanese quail breeders at 14 weeks of age, during the peak laying period, were used. The birds were standardised by weight and egg production and distributed by experimental units. A completely randomised design was used with seven treatments and seven replicates with a bird in each experimental unit. Experiments were conducted in a climatic

chamber composed of air conditioning and exhausters that maintained the temperature at 24 ºC. The birds were housed in galvanized wire cages measuring 0.26 m × 0.37 m × 0.36 m, equipped with a linear feeder and nipple drinkers throughout the experimental period. The light program maintained throughout the experimental period consisted of 16 h of light and eight hours of darkness.

5.2.3 Experimental diets

Initially, two feeds were formulated: a formulation with a high crude protein content (HPD) and a relative deficiency in Lys compared to the other amino acids, and a second formulation that was free of protein and amino acids (NFD) were prepared (Tables 1 and 2). The intermediate experimental levels of Lys were obtained by diluting the HPD with NFD. The HPD and NFD diets were named N7 and N0, respectively. The N0 and N7 diets were formulated to contain 0% and 1.68% of Lys, respectively, and were diluted in adequate proportions to obtain the increasing levels of Lys, meeting the recommendations of the other nutrients following the methodology described by Rostagno et al. [16]. The treatments consisted of seven increasing levels of Lys: N1: 3.4 g/kg; N2: 5.0 g/kg, N3: 6.7 g/kg, N3: 8.4 g/kg, N5: 11.8 g/kg, N6: 13.4 g/kg and, N7: 16.8 g/kg.

Table 1. Composition (g/kg) of the diets used in the lysine assay.

Ingredient (g/kg)	HPD[a]	NFD[b]
Corn	356.97	-
Soybean meal	315.97	-
Corn gluten meal (60% CP)	181.22	-
Soybean oil	20.00	24.84
Dicalcium phosphate	10.13	15.02
Limestone	69.81	69.81
Salt	3.34	3.67
Choline chloride (60%)	0.84	3.40
Mineral premix[c]	0.25	0.25
Vitamin premix[c]	0.25	0.25
DL-Met (99%)	4.88	-
L-Lys HCl (78%)	5.72	-
L-Thr	2.71	-
L-Val	3.21	-
L-Ile	2.00	-
L-Arg	10.72	-
LTrp	1.81	-
Potassium chloride	-	11.95
Corn starch	-	249.03
Sugar	-	496.74
Rice husks	-	125.00

[a] HPD, high protein diet. [b] NFD, nitrogen free diet. c Content per kg of the diet-vit A, 6.668 IU; vit D3, 1.668 IU; vit E, 8 IU; vit K3, 2 mg; vit B1, 1 mg, vit B2, 3.34 mg; vit B6, 2 mg; vit B12, 9 mcg/kg; niacin, 21 mg; chlorine, 0.13 g; pantothenate acid, 8 mg; folic acid, 0.46 mg/kg, biotin, 0.05 mg/kg; 0.46; copper, 8 mg/kg; iron, 6.25 mg/kg; manganese, 70 g; zinc, 25 g; iodine, 6.25 mg; selenium 1.25 mg.

Table 2. Nutritional levels of experimental diets.

Itens	HPD[a]	NFD[b]
Calculated composition (g/kg)[c]		
Metabolizable energy (MJ/kg)	12.5	12.5
Calcium (g/kg)	30.0	30.0
Avaliable phosphorus (g/kg)	3.0	3.0
Analyzed composition (g/kg)		
Crude protein	350.0	NI[e]
Digestible Lys[d]	16.8	NI
Digestible Met + Cys	17.1	NI
Digestible Met	11.0	NI
Digestible Trp	3.0	NI
Digestible Thr	15.0	NI
Digestible Arg	25.0	NI
Digestible Val	17.0	NI
Digestible Ile	15.0	NI
Digestible Phe	19.0	NI

[a] HPD, high protein diet. [b] NFD, nitrogen free diet. c The nutrient content of the ingredients used in the formulation was analysed using a near-infrared spectrometer (NIR). [d] The total amino acid content of the diets were analysed using HPLC and digestible content calculated using coefficients from Rostagno et al. (2011). [e] NI, not identified.

5.2.4 Measurements and variables analyzed.

The experiment occurred over 22 days, with 7 days of adaptation and 15 days of data collection. The maximum consumption per kilogram of metabolic weight ($BW^{0.67}$) was determined during the adaptation period, when the birds were fed ad libitum. In the subsequent period, the diets were supplied based on the kg of BW0.67 of each bird. The supply of feed was adjusted after each weekly weighing of the birds. To avoid waste, the feed for each experimental unit was divided into two meals per day. Subsequently, the supply was controlled in the following days of adaptation and collection period. To obtain Animals 2022, 12, 2953 4 of 15 daily feed intake, leftovers were weighed daily. Birds were weighed at the beginning and end of the experimental period to measure body weight.

Egg production and weight was measured daily to quantify the daily egg output throughout the experimental period. Eggs were collected, identified and frozen at 20 ºC. The total excreta were collected in trays adapted under the cages, twice a day, during the 15 days, placed in plastic bags and stored in a freezer (20 ºC) until the end of the period.

5.2.5 Chemical analyses

The eggs and excreta were homogenized, and a sample was taken for drying in a forced air ventilation oven at 55 ºC for 72 h. Subsequently, the eggs and excreta were weighed, and ground in a Thomas-Wiley mill with a 1 mm sieve. Samples of the collected material were used to determine dry matter and nitrogen content by the Kjeldahl method, according to AOAC [17].

5.2.6 Statistical analysis

The variables analysed were nitrogen intake (NI), nitrogen excretion (NEX), nitrogen in egg output (NMO) and nitrogen deposition (ND). The nitrogen retention (NR, NR = ND + NMO + NMR) represents the total nitrogen retained by the bird. NMR is the minimum daily nitrogen value for maintenance and was obtained by the relation between NEX and NI adjusted by the exponential function:

$$NEX = NMRe^{b \times NI} \quad (1)$$

where b is the slope of the exponential function, and e is the Euler's number (ln). The daily theoretical maximum nitrogen retention ($NR_{max}T$, $mg/BW_{kg}^{0.67}$) was estimated by the relationship between NI and ND adjusted by the exponential function:

$$NR = NR_{max}T\ (1\text{-}e^{-b \times NI}) \quad (2)$$

where NR is the daily nitrogen retention ($mg/BW_{kg}^{0.67}$), and b is the slope of the nitrogen retention curve.

With the transformation of Equation (2), the calculation of the parameter b is performed, determining the quality of the protein:

$b = \ln [NR_{max}T - \ln (NR_{max}T\text{-}NR)] / NI$ (3)

The Lys requirement was calculated using the equation:

$LAAI=(\ln NR_{max}T - \ln(NR_{max}T\text{-}NR))/(16\times bc^{-1})$ (4)

where LAAI is the daily intake of the limiting amino acid (Lys) ($mg/BW_{kg}^{0.67}$), c is the concentration of the first limiting amino acid in the protein (g/16 g nitrogen), and bc^{-1} is the linear relationship between b and c, which expresses the efficiency of the amino acid studied (Lys).

The assumptions of homoscedasticity and residual normality were tested. Subsequently, the data were fitted to exponential models using PROC NLMIXED using SAS software (SAS Institute Inc., Cary, NC, USA, 2014, version 9.4), considering a significance of 0.05%.

Assay 2

5.2.7 Housing, animals and experimental design

Experiments were conducted in a climatic chamber composed of air conditioning and exhausters that maintained the temperature at 24 ºC. The birds were housed in galvanized wire cages measuring 0.26 m × 0.37 m × 0.36 m, equipped with a linear feeder and nipple drinkers throughout the experimental period. The light program maintained throughout the experimental period consisted of 16 h of light and eight hours of darkness. A total of 120 Japanese quail breeders at 16 weeks of age, during the peak laying period, were used. The birds were standardised by weight and egg production and distributed by experimental units. A completely randomised design was used with 12 treatments and 10 replicates.

5.2.8 Experimental diets

In this study, a control diet (CD) diet was formulated with all the nutritional requirements for Japanese quail as estimated by Rostagno et al. [16] for commercial Japanese quail because it does not provide nutritional requirements for breeders. Nitrogen and essential amino acids were provided by corn, soybean meal, corn gluten meal, and crystalline amino acids (Table 3).

Table 3. Composition of the control diet (balanced protein).

Itens	**Content, %**
Corn	64.76
Soyabean meal (47%)	12.08
Corn Gluten (60%)	5.21
Dicalcium phosphate	1.15
Limestone	7.06
Sodium chloride	0.34
Potassium chloride	0.34
L-lysine (55%)	0.38
DL-methionine (99%)	0.95
L-threonine (98%)	0.27
L-tryptophan	0.10
L-arginine	0.48
L-glycine	0.13
L-valine	0.13
L-histidine	0.15
L-phenylalanine	0.08
L-glutamate	1.00
Choline chloride (60%)	0.16
Premix – Vitaminic[1]	0.02
Premix – Mineral[1]	0.02

[1] Content per kg of the diet-vit A 6.668 IU; vit D3 1.668 IU; vit E 8 IU; vit K 3.2 mg; vit B1 1 mg; vit B2 3.34 mg; vit B6 2 mg; vit B12 5 mcg/kg; niacin 21 mg; chlorine 0.13 g; pantothenate acid 8 mg; folic acid 0.46 mg/kg; biotin 0.05 mg/kg; copper 8 mg/kg; iron 60 g; manganese 70 g; zinc 25 g; iodine 6.25 mg; selenium 0.12 mg.

The other experimental diets, of a total of 11 diets with different limiting amino acids, were obtained by diluting CD using corn starch (Tables 4 and 5). The dilution was 40% of the amino acid requirement to be evaluated in each treatment, and the other nutrients and energy were recomposed to meet the same CD level, except for the test amino acid, which was depleted by 40%, according to Dorigam et al. [14].

5.2.9 Data collection

The trial lasted 20 days, with feed supply and data collection, the first seven days being adaptation and 13 days total collection of excreta and eggs. To obtain daily feed intake, feed leftovers were weighed daily. Birds were weighed at the beginning and end of the experimental period to measure body weight. Egg production was measured daily, as well as egg weight, to obtain daily egg output (egg production egg weight) throughout the experimental period.

5.2.10 Chemical analyses

The methodology used was the same as assay 1.

Table 4. Composition of the diet for all tested amino acids.

Itens	Diets, %										
	Lys	**Met+Cys**	**Thr**	**Try**	**Arg**	**Gly+Ser**	**Val**	**Ile**	**Leu**	**His**	**Phe+try**
Balanced diet[1]	60.00	60.00	59.69	59.86	59.64	59.00	60.00	59.66	59.76	60.00	60.00
Soy oil (47%)	1.15	1.77	1.16	1.15	1.16	1.51	1.15	1.16	1.15	1.15	2.06
Dicalcium phosphate	0.60	0.60	0.60	0.60	0.60	0.62	0.60	0.60	0.60	0.60	0.60
Limestone	2.79	2.80	2.82	2.80	2.82	2.86	2.79	2.82	2.81	2.79	2.79
Sodium chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Potassium chloride	0.48	0.48	0.48	0.48	0.48	0.49	0.48	0.48	0.48	0.48	0.48
DL-methionine (99%)	0.36	0.00	0.37	0.36	0.37	0.37	0.36	0.37	0.36	0.36	0.36
L-lysine (55%)	0.00	0.80	0.80	0.80	0.80	0.82	0.80	0.80	0.80	0.80	0.80
L-threonine (98%)	0.27	0.27	0.00	0.27	0.27	0.27	0.26	0.27	0.27	0.26	0.26
L-tryptophan	0.09	0.09	0.09	0.00	0.09	0.10	0.09	0.09	0.09	0.09	0.09
L-arginine	0.52	0.52	0.52	0.52	0.00	0.53	0.51	0.52	0.52	0.51	0.51
L-valine	0.34	0.33	0.34	0.33	0.34	0.34	0.00	0.34	0.33	0.33	0.33
L-isoleucine	0.29	0.29	0.29	0.29	0.29	0.30	0.29	0.00	0.29	0.29	0.29
L-leucine	0.67	0.67	0.67	0.67	0.67	0.68	0.67	0.67	0.00	0.67	0.67
L-glycine	0.51	0.51	0.51	0.51	0.52	0.00	0,51	0.51	0.51	0.51	0.51
L-phenylalanine	0.60	0.61	0.61	0.61	0.61	0.62	0.60	0.61	0.61	0.60	0.00
L-histidine	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.00	0.19
L-Glutamate	5.41	4.78	5.40	4.53	6.17	5.51	5.40	4.74	5.18	4.85	4.91
Choline chloride (60%)	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Maize starch	10.00	5.13	10.00	10.00	10.00	5.44	9.91	10.00	10.00	9.91	4.80
Sugar	6.51	10.00	6.50	6.83	5.82	10.00	6.26	7.07	7.27	6.57	10.00
Inert	8.88	10.00	8.62	8.87	8.82	10.00	8.97	8.76	8.43	8.88	10.00
Premix – [2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

[1] Table 4; [2] Content per kg of the diet-vit A 6.668 IU; vit D3 1.668 IU; vit E 8 IU; vit K 3.2 mg; vit B1 1 mg; vit B2 3.34 mg; vit B6 2 mg; vit B12 5 mcg/kg; niacin 21 mg; chlorine 0.13 g; pantothenate acid 8 mg; folic acid 0.46 mg/kg; biotin 0.05 mg/kg; copper 8 mg/kg; iron 60 g; manganese 70 g; zinc 25 g; iodine 6.25 mg; selenium 0.12 mg.

Table 5. Nutritional levels of experimental diets.

Itens	Diets											
	CD	**Lys**	**Met+Cys**	**Thr**	**Trp**	**Arg**	**Gly+Ser**	**Val**	**Ile**	**Leu**	**His**	**Phe+Try**
Metabolizable energy (MJ/kg)	11,7	11,7	11,7	11,7	11,7	11,7	11,7	11,7	11,7	11,7	11,7	11,7
Calcium (g/kg)	30,0	30,0	30,0	30,0	30,0	30,0	30,0	30,0	30,0	30,0	30,0	30,0
Avaliable phosphorus (g/kg)	2,8	2,8	2,8	2,8	2,8	2,8	2,8	2,8	2,8	2,8	2,8	2,8
Crude protein (g/kg)	180,1	180,1	180,1	180,1	180,1	180,1	180,1	180,1	180,1	180,1	180,1	180,1
Lysine (g/kg)	10,9	6,6	10,9	10,9	10,9	10,9	10,9	10,9	10,9	10,9	10,9	10,9
Metionine+Cystine (g/kg)	9,0	9,0	5,4	9,0	9,0	9,0	9,0	9,0	9,0	9,0	9,0	9,0
Threonine (g/kg)	6,6	6,6	6,6	3,9	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6
Tryptophan (g/kg)	2,3	2,3	2,3	2,3	1,4	2,3	2,3	2,3	2,3	2,3	2,3	2,3
Arginine (g/kg)	12,7	12,7	12,7	12,7	12,7	7,6	12,7	12,7	12,7	12,7	12,7	12,7
Glycine+serine (g/kg)	12,5	12,5	12,5	12,5	12,5	12,5	7,5	12,5	12,5	12,5	12,5	12,5
Valine (g/kg)	8,2	8,2	8,2	8,2	8,2	8,2	8,2	4,9	8,2	8,2	8,2	8,2
Isoleucine (g/kg)	7,1	7,1	7,1	7,1	7,1	7,1	7,1	7,1	4,2	7,1	7,1	7,1
Leucine (g/kg)	16,5	16,5	16,5	16,5	16,5	16,5	16,5	16,5	16,5	9,8	16,5	16,5
Histidine (g/kg)	4,6	4,6	4,6	4,6	4,6	4,6	4,6	4,6	4,6	4,6	2,7	4,6
Phenylalanine+Tyrosine (g/kg)	14,8	14,8	14,8	14,8	14,8	14,8	14,8	14,8	14,8	14,8	14,8	8,9

CD, Control diet

5.2.11 Statistical analysis

The variables analysed were NI, NEX, NMO and ND, with subsequent calculation of NR. Data were subjected to homoscedasticity of variance and error normality tests. Upon satisfying the premises, analysis of variance, declared as significant at 0.05, was performed, and when treatment effects were detected, Dunnett's test was applied for all variables. All data were analysed using SAS software (v.9.4; SAS Institute Inc., 2014).

The ideal proportion of amino acids (IAAR) was determined from the limiting diets in which the tested amino acid was reduced by 40%. The determination of the IAAR by the slope of the linear function (bc^{-1}), expressed as feed efficiency of the limiting amino acid under study, where protein quality (b) in each treatment was obtained by Equation (3) by Samidi e Liebert [13]:

$$b = (\ln NR_{max}T - \ln (NR_{max}T\text{-}NR)) / (NI) \quad (5)$$

where NRmaxT is the maximum theoretical daily nitrogen retention ($mg/BW_{kg}^{0.67}$), which was determined in experiment 1 (3386.61 $mg/BW_{kg}^{0.67}$), NR is the daily nitrogen retention ($mg/BW_{kg}^{0.67}$) and NI is the daily nitrogen intake ($mg/BW_{kg}^{0.67}$).

The concentration of the first limiting amino acid in the dietary protein (c) was calculated for each of the diets tested, by the following equation:

$$c = 16 \times LAAI/NI \quad (6)$$

where LAAI is the intake of the test amino acid and NI is the nitrogen intake.

The relationship between the efficiency of lysine (reference) and the efficiency of the limiting amino acid under study was used to derive the optimal intake of the amino acid (AAI) (AAI = bc□1Lys/bc□1AA), according to Dorigam et al. [14] and Wecke and Liebert [12]. The IAAR was calculated for each amino acid: Met + Cys, Thr, Trp, Arg, Gly + Ser, Val, Ile, Leu, His e Phe + Tyr.

5.3 Results

Assay 1: determination of NMR $NR_{max}T$, and Lys requirement using utilization efficiency.

The results of the nitrogen balance assays are shown in Table 6. The increase in dietary protein levels influenced all the variables studied ($p < 0.0001$). Furthermore, a gradual increase in NI and NEX was observed between the levels of Lys and protein in the diet. However, the ND was gradual up to level five, with a daily difference of only 48.58 mg/ $BW_{kg}^{0.67}$ between levels five and six, and 35.93 mg/$BW_{kg}^{0.67}$ between levels six and seven. The same effect was observed for NMO and NR, with a slight decrease from level five to six (55.22 mg/ $BW_{kg}^{0.67}$ and 103.80 mg/ $BW_{kg}^{0.67}$, respectively) and an increase up to level seven (68.12 mg/ $BW_{kg}^{0.67}$ and 177.94 mg/ $BW_{kg}^{0.67}$, respectively). The difference in dietary Lys levels provided a range of 78.06% in NMO, which influenced the difference of 68.35% from level one to level seven of Lys in NR.

Table 6. Mean body weight (BW, kg), feed intake (FI, g/d), daily nitrogen intake (NI, mg/$BW_{kg}^{0.67}$), daily nitrogen excretion (NEX, mg/$BW_{kg}^{0.67}$), daily nitrogen deposition (ND, mg/$BW_{kg}^{0.67}$), nitrogen deposited in egg mass (NMO, mg/$BW_{kg}^{0.67}$), and daily nitrogen retention (NR, mg/$BW_{kg}^{0.67}$) obtained in nitrogen balance trials with Japanese quail breeders receiving graded levels of protein limitation in lysine.

Diets	**N1**	**N2**	**N3**	**N4**	**N5**	**N6**	**N7**	**DP**	**P-valor**
BW (kg)	0.13	0.13	0.15	0.17	0.16	0.17	0.18	0.02	<.0001
FI (g/d)	15.64	17.76	22.34	22.60	24.36	23.89	23.51	3.60	<.0001
NI (mg/$BW_{kg}^{0.67}$)	611.37	1039.34	1610.75	1610.85	2780.78	3023.17	3645.79	1059.28	<.0001
NEX (mg/$BW_{kg}^{0.67}$)	468.78	625.75	819,56	1094.95	1685.89	1976.86	2563.54	749.89	<.0001
ND (mg/$BW_{kg}^{0.67}$)	178.81	382.25	515.91	791.19	1094.89	1046.31	1082.24	359.69	<.0001
NMO (mg/$BW_{kg}^{0.67}$)	234.29	450.75	740.95	919.71	1055.08	999.86	1067.98	348.92	<.0001
NR (mg/$BW_{kg}^{0.67}$)	838.48	1254.06	1878.61	1957.41	2575.24	2471.44	2649.38	657.35	<.0001

N1 3.4 g lysine/kg; N2 5.0 g lysine/kg; N3 6.7 g lysine/kg; N3 8.4 g lysine/kg; N5 11.8 g lysine/kg; N6 13.4 g lysine/kg; N7 16.8 g lysine/kg.

The exponential function between NI and NEX was used to estimate the NMR (Figure 1). The NMR value obtained was 425.27 mg/ $BW_{kg}^{0.67}$ per day for quail in the laying period, where NI was equal to zero. With the adjustment of the non-linear regression between NI and NR, it was possible to estimate the value of 3386.61 mg/ $BW_{kg}^{0.67}$ of daily NRmaxT (Figure 2).

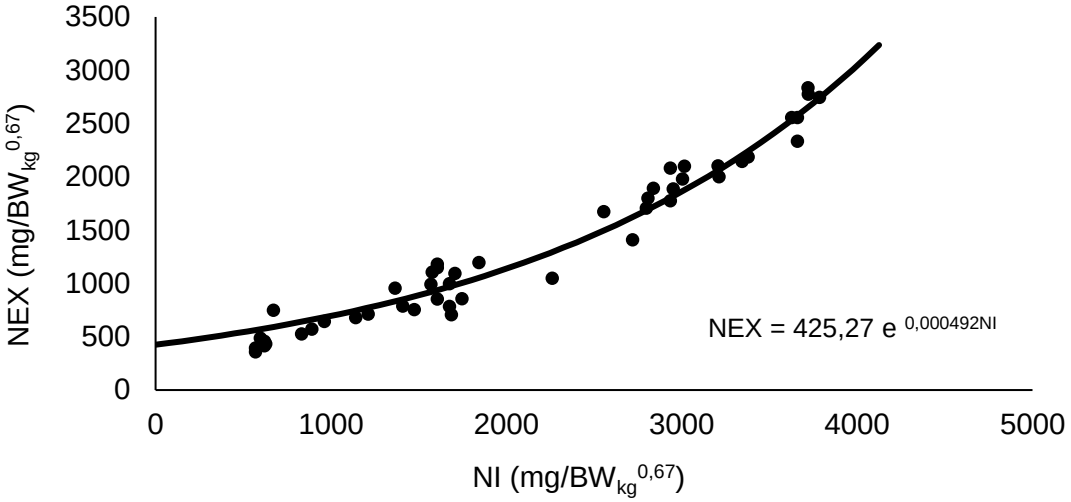

Figure 1. Estimation of the nitrogen requirements for maintenance by fitting an exponential function between the ni-trogen intake (NI) and nitrogen excretion (NEX) during a gradual increase in supplied protein limited in lysine for Japanese quail breeders. Values observed (●) and predicted (**-**).

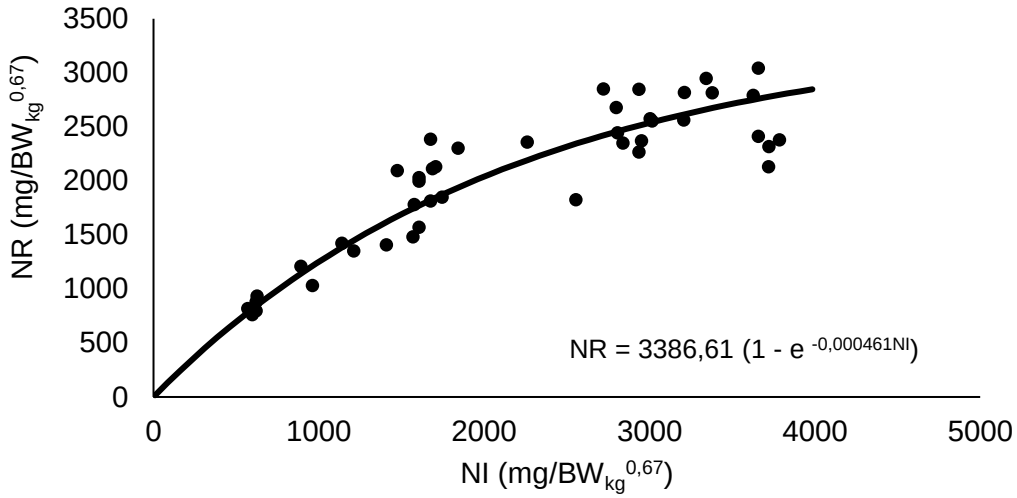

Figure 2. Estimation of the theoretical potential for nitrogen retention in Japanese quail breeders based on the expo-nential fitting between the daily nitrogen intake (NI) and the daily nitrogen retention (NR). Values observed (●) and predicted (**-**).

Based on the models used to calculate nitrogen utilization efficiency, that is, the dietary protein quality ($b = [\ln(NR_{max}T) - \ln(NR_{max}T - NR)]/NI$), it was possible to estimate the value of b = 0.000486. Using the ratio of b concentration of the limiting amino acid in the protein (c), it was possible to estimate the Lys efficiency of utilization Lys bc^{-1} = 0.000101. Based on the parameters derived from the models and on the response of Japanese quail breeders in this study, the Lys intake was required to reach 80% of the $NR_{max}T$ value, which was 961.90 $mg/BW_{kg}^{0.67}$ per day for a bird weighing 0.16 kg Moreover, the daily intake of Lys is 291 mg/bird/day or 1.164% Lys in the diet, considering a feed intake of 25 g/bird.

Assay 2: Determination of amino acid utilization efficiency, requirements, and optimal amino acid ratio.

The results of the nitrogen balance test for Japanese quail breeders are presented in Table 7. From these data, the values of b and bc^{-1} were derived, which enabled the deter-mination of the IAAR. The birds that consumed diets with amino acid deletion showed, a reduction in nitrogen retention ranging from 1.47% to 45.69%, in the nitrogen balance, with the addition of NMO. Within this range glycine+serine was the amino acid treatment with the smallest reduction (18.60 $mg/BW_{kg}^{0.67}$) and valine was the treatment with the highest (579.98 mg/ $BW_{kg}^{0.67}$) when compared with CD. These results were influenced by the NI, which differed for the birds that consumed a diet limited in a valine (p = 0.0165) and glycine+serine (p = 0.0022) compared with to that of the CD treatment group. All birds showed an increase in NEX after the treatments with individually limited amino acids, with an overall average that was 31.90% higher than that of the CD treatment group. In particular, birds that consumed a leucine-limited diet had an increased NEX of 42.23% when compared with that of the CD group.

Table 7. Mean values for the effect of exclusion of an amino acid from the diet of Japanese quail breeders in a nitrogen balance trial.

Variables	**BW**	**FI**	**NI**	**NEX**	**NMO**	**ND**	**NR**	***b* (10^{-4})**
Lys	0.15^{a}	18.62^{b}	1390.03^{a}	782.30^{a}	219.19^{b}	590.22^{a}	920.70^{b}	230^{b}
Met+Cys	0.15^{a}	21.14^{a}	1443.38^{a}	804.75^{a}	320.60^{a}	618.18^{a}	1054.89^{a}	258^{b}
Thr	0.14^{b}	18.83^{b}	1412.50^{a}	866.79^{b}	249.33^{a}	572.64^{a}	934.33^{b}	225^{b}
Trp	0.15^{a}	19.15^{a}	1443.75^{a}	840.79^{b}	239.16^{a}	602.96^{a}	965.81^{b}	236^{b}
Arg	0.15^{a}	19.05^{a}	1411.32^{a}	853.52^{b}	332.47^{a}	557.79^{b}	1007.84^{b}	252^{b}
Gly+Ser	0.15^{a}	22.43^{a}	1633.31^{b}	878.27^{b}	366.55^{a}	755.04^{a}	1241.36^{a}	280^{a}
Val	0.14^{b}	14.78^{b}	1185.58^{b}	777.85^{a}	164.52^{b}	368.44^{b}	682.78^{b}	191^{b}
Ile	0.14^{a}	20.18^{a}	1479.50^{a}	840.33^{b}	281.48^{a}	586.89^{a}	979.21^{b}	240^{b}
Leu	0.15^{a}	21.39^{a}	1538.34^{a}	913.06^{b}	349.05^{a}	625.29^{a}	1094.62^{a}	254^{b}
His	0.16^{a}	22.55^{a}	1546.02^{a}	864.84^{b}	357.82^{a}	681.19^{a}	1161.40^{a}	273^{b}
Phe+Tyr	0.15^{a}	19.66^{a}	1458.94^{a}	843.05^{b}	315.82^{a}	600.69^{a}	1038.13^{a}	253^{b}
DC	0.16^{a}	21.27^{a}	1374.43^{a}	654.44^{a}	376.56^{a}	744.63^{a}	1258.61^{a}	336^{a}
Mean	0.15	19.92	1444.00	826.41	301.29	611.84	1040.15	255
SEM	0.001	0.25	15.37	14.10	11.95	15.11	23.17	0.05
p- Value	0.0213	<.0001	<.0001	0.0195	0.0019	<.0001	<.0001	<.0001

BW, body weight; FI, feed intake (g/day); NI, daily nitrogen intake (mg/$BW_{kg}^{0.67}$); NEX, daily nitrogen excretion (mg/ $BW_{kg}^{0.67}$); NMO, daily nitrogen in egg mass (mg/ $BW_{kg}^{0.67}$); ND, daily deposited nitrogen (mg/ $BW_{kg}^{0.67}$); NR, daily nitrogen retention (mg/ $BW_{kg}^{0.67}$); b, slope of the exponential function (protein efficiency); a, b, mean values with b superscript within the line were significantly different ($p < 0.05$) compared with control diet, by the Dunnett test.

Body weight was compromised in birds that received diets limited in threonine and valine, whereas the others showed no difference compared with that in the CD group ($p > 0.05$). In contrast, the NMO was lower than 376.56 mg/$BW_{kg}^{0.67}$ (the mean CD) for all treatments with one limiting amino acid, but only diets limited in lysine and valine differed significantly from CD ($p < 0.05$), with 168.85 and 223.52 mg/ $BW_{kg}^{0.67}$, respectively.

After a more specific evaluation of the responses using the estimation of parameter b, the limiting diets presented lower protein quality when compared with that of CD ($p < 0.0001$). An exception to this was the diet limited in glycine + serine ($p = 0.1064$). When evaluating the values of bc^{-1}, the highest use efficiency of an amino acid was for tryptophan (0.000226), and the lowest was for leucine, phenylalanine + tyrosine, arginine, and lysine (Table 8).

Table 8. Amino acid utilization efficiency (bc^{-1}), optimal amino acid intake (AAI) and optimal amino acid ratio (IAAR) for Japanese quail breeders in a nitrogen balance trial.

Variables	***bc*$^{-1}$**	**AAI**	**IAAR**
Lys	0.000046	1	100
Met+Cys	0.000056	0.87	87
Thr	0.000074	0.67	67
Trp	0.000226	0.21	21
Arg	0.000042	1.17	117
Gly+Ser	0.000048	-	-
Val	0.000051	0.96	96
Ile	0.000072	0.66	66
Leu	0.000033	1.42	142
His	0.000123	0.39	39
Phe+Tyr	0.000036	1.33	133

The ideal ratio derived from the efficiency of the individual amino acids (bc^{-1}) in this study, using the Goettingen approach, is presented in Table 8. A 40% reduction was sufficient to estimate the ideal ratio for all essential amino acids tested, except for glycine + serine, owing to the efficiency of use (bc^{-1}).

5.4 Discussion

To the best of our knowledge, this study is the first to determine the Lys requirement for Japanese quail breeders. We used the nitrogen balance and estimated the $NR_{max}T$, NMR, b, and bc^{-1} [11,13,14]. Moreover, using dietary protein utilization efficiency, we determined the ideal ratio of essential amino acids based on amino acid deletion.

Birds had lower feed intake amounts at lower concentrations of limiting amino acids (N1 and N2, Table 3), an effect related to the Lys content [18]. Dietary Lys deficiency renders the rate of protein synthesis unfeasible for cell cycle proliferation, leading to cell apoptosis [19]. This can lead to lower ovary and oviduct weight and a consequent decrease in broiler breeders' production [9]. The reduction in egg production decreases energy requirements, which induces intake regulation [20]. Considering that the energy content at all levels of Lys were the same, the amount of energy required for production would become smaller, and, consequently, the feed intake would be lower. Thus, the lowest concentration of Lys (N1 = 3.4 g/kg and N2 = 5.0 g/kg) made it impossible to maintain production [20]. In this study, a significant increase in egg output was observed up to N5.

NMR is the minimum nitrogen retention, which was estimated considering the intercept of the exponential function when NI = 0 [21]. According to Liebert [21], the NMR value does not consider nitrogen loss from feathers and skin desquamation. Therefore, the NMR results were reported as the approximate average amount of nitrogen [14]. The daily NMR of 425 $mg/BW_{kg}^{0.67}$ for Japanese quail breeders was 1.7 times higher than that found for broiler breeders [22]. Using the comparative slaughter technique, Silva et al. [22], found that the NMR is 760 $mg/ BW_{kg}^{0.67}$ per day for commercial Japanese quails, a value 1.8 times higher than that found in this study for adult birds.

Another important factor causing the difference between the NMR values is the methodology used. NEX determined using the comparative slaughter technique accounts for the nitrogen lost via feathers, which is not included in the nitrogen balance technique [23]. The food restriction imposed in the study conducted by Silva et al. [22], i.e., a lower feed supply (80, 60, and 40%) as the form of limitation, makes homeostasis unfeasible and modifies the anabolic and catabolic responses of the animal. Body proteins are targeted for oxidation and are converted into glucose or ketone bodies for energy generation [24]. Due to the lack of scientific studies determining NMR for Japanese quail breeders, the value found is a reference for other studies (425.27 $mg/BW_{kg}^{0.67}$ per day).

The value of 3386.61 $mg/BW_{kg}^{0.67}$ is the maximum daily retained nitrogen, under nonlimiting conditions, for Japanese quail breeders. This value is expressed as the theoretical limit of the exponential function [13,21]. One application of this constant for nutritional programs is to determine the maximum potential of the strain and allow the estimation of demand according to the production objective. In addition, it is fundamental for practical modelling applications [21].

The estimated $NR_{max}T$ values for laying breeders were 1639.9 $mg/BW_{kg}^{0.67}$ and 1554.2 $mg/BW_{kg}^{0.67}$ for 31–35 and 46–50 weeks, respectively [14], and 1883 $mg/BW_{kg}^{0.67}$ for commercial laying hens [15]; both studies used the nitrogen balance methodology. The values approached a 51% difference between the values found for Japanese quail breeders and that for heavy breeders, and the difference decreased slightly to 55% when compared with commercial layers. Comparing $NR_{max}T$ results is impossible due to variations in strains, age, feed consumption, and diet characteristics [15,21].

Parameter b was estimated as 0.000486, representing the function's growth rate. The interpretation of b depends on protein quality and is independent of nitrogen intake [21]; the

amino acid variation in the protein reduces or increases the amount of nitrogen to its maximum potential [23]. Likewise, calculating the daily requirement of Lys depends only on the efficiency of the dietary amino acid; thus, the parameter bc^{-1} was established [25].

The daily value of Lys intake for a bird of 0.16 kg (291 mg/bird) was defined by the efficiency of Lys utilization for the studied diet. This estimated value for the daily intake of Lys reached 80% of the $NR_{max}T$, which was observed in birds of treatment 7 (N7 = 16.8 g/kg of feed). These results characterize the maximum genetic potential of the animal [23,26].

Lys has physiological functions in all cells and tissues during the synthesis of various indispensable compounds [19]. In peak-laying birds, in addition to vital functions, the Lys metabolic pathway, in addition to vital functions, is directed towards yolk and albumen formation, where 87% and 67% of the Lys in the yolk and albumen at peak production, respectively, are from dietary sources [6]. Therefore, Lys intake in the diet is directly related to egg production [9], and insufficient Lys intake makes egg production unfeasible. Establishing Lys requirements is extremely important, as it is considered the reference amino acid to establish the proportions of other essential amino acids [6].

Such proportions were determined from the IAAR based on the deletion of amino acids by the use efficiency of use of dietary protein from the nitrogen balance compared to a CD. Among all the amino acids studied, reduction the of valine in the diet had the biggest influence, reducing feed consumption (30.51%), body weight (12.50%), and nitrogen excreted in the egg output (57.60%), compared with those in birds from the CD group. However, some studies have shown that attention must be paid to the levels of leucine and isoleucine when considering the dietary levels of valine [27,28]. This is because they have a branched chain (BCAA), and their excesses or deficiencies can result in antagonism [29]. Proportional differences were observed in the CD for Leu:Val and Ile:Val at 100:50 and 100:115, respectively, as well in the valine-limited diet

where the ratio for Leu:Val and Ile:Val was 100:30 and 100:69, respectively. Therefore, leucine and isoleucine were proportionately higher in the limiting diet. Metabolically, excess leucine induces branched-chain aminotransferase activity, leading to the catabolism of other BCAAs [30]. Valine is the amino acid most susceptible to antagonism and enzymatic degradation [31]. Furthermore, excess leucine stimulates the synthesis of protein and inhibits protein degradation. However, with a deficiency of this amino acid, the stimulation of synthesis further exacerbates the amino acid imbalance in the plasma pool [32]. This corroborates the weight loss observed among the birds, which may be justified by the breakdown of muscle proteins to maintain the plasma balance of amino acids. The detection of an amino acid deficiency in the anterior piriform cortex induces the animal to reduced feed intake [33] as a preventive mechanism. In other studies, the animal reduced the intake of a limiting diet [34,35], which corroborates with the results of this study.

The requirements for the use of an amino acid were divided into the need for maintenance and the efficiency of protein retention, which for birds in production is related to egg output. This was achieved using a factorial approach. This is affected by the deletion of lysine and valine in the diet, via nitrogen deposition, and the logical approach that the 40% limitation made amino acids unavailable for protein synthesis in egg production. In a study by Azzam et al. [36], the non-addition and lower level (1 g/kg) of L-val in the diet significantly affected the serum albumin level. Notably, hepatic production of yolk lipoproteins is regulated by BCAAs [37]. The lysine-related NMO reduction is directly related to egg weight and can be explained by the reduction in egg protein when birds are fed low-lysine diets [38,39]. In lysine metabolism, muscle tissue is manipulated by the rate of egg [6]. In addition, broiler breeders subjected to a dietary lysine deficiency of 44.75% showed a significant difference in egg and chick weights [39]. Kim et al. [9] demonstrated broiler breeder hens fed a 30% lysine reduced diet (0.55%

Lys in the diet) had lower oviduct and ovary weights and follicular recruitment. This was accompanied by a delay in ovulation due to apoptosis and necrosis the ovarian follicles, which culminated in a drop in egg production. Therefore, lysine deficiency in the diet makes optimal production impossible because excess lysine is destined for producing eggs only after meeting the muscular requirement [6].

To determine the IAAR, the statistical difference between the individual amino acids and CD must be confirmed in the evaluation of parameter b [11]. However, the Gly + Ser-limiting diet did not produce a significant decrease in protein quality when compared to that of CD, indicating an excess of this amino acid in the CD.

The other limiting diets, in terms of individual amino acids, reduced protein quality, which made it possible to estimate the IAA value. Among the studies that recommended amino acid requirements in the literature, only the study by Hanafy and Attia [40] used Japanese quail breeders. However, the recommendation was not estimated (0.2% in the diet) but based on the treatment that resulted in better productivity and reproductive performance. Thus, one can observe the fragility of amino acid nutrition for hens that are fed diets formulated according to recommendations set for commercial laying hens [4,5]. We expect that the dissimilarity in the amino acid requirements is likely due to the difference in genetic potential. When we analysed the recommendation by Rostagno et al. [5], the IAA for all amino acids, except for Val, which was above the recommended value in this study, ranged from 4 (Thr = 0.70%) to 19% (His = 0.48%) among all amino acids studied, except Gly + Ser. According to the NRC [4], the recommended value was transformed into a digestible amino acid considering 89% [16]; therefore, the values for Lys,Met + Cys, Thr, Trp, Arg, Val, Leu, His, and Phe + Try were below those determined in this study (0.79, 0.55, 0.59, 0.15, 0.93, 0.73, 1.12, 0.33, and 1.11% in the diet), with differences reaching 56, 38, and 32% for Met + Cys, Trp, and Val, respectively.

Only the IAA for Ile was higher than that for commercial quail (1.12% in the diet). Thus, according to the tables currently used as a basis for formulating rations for breeders, no amino acids presented IAA values close to the results of this study, thereby confirming the modification of the requirement by the genotype.

Furthermore, Lima et al. [41] and Sarcinelli et al. [42] recommended 0.78% and 0.70% of Thr in the diet of Japanese quail, respectively, which corresponds to values 14% and 5% higher than that found in this study (0.67%). Moreover, AAI values were determined in a study by Lima et al. [43], where the estimate for Arg was 17% higher (1.14%) than that found in this study (1.17%). For Trp, they estimated 0.22% in their diet [42], which was 6% higher than the determined value (0.21%). However, the amount of Val in the diet was 62% lower (0.59%) in a study by Martinez et al. [44], in which the authors considered an 11 g/day of egg output and 0.17 kg of body weight. Lower values were also observed for Met + Cys, with a difference of 12% from the estimate by Sarcinelli et al. [42] (0.76% Met + Cys in the diet). No studies have been found in the literature on the IAA for other amino acids.

However, based on the results found in the IAAR of essential amino acids proportional to Lys, breeders need to intake higher proportion of the amino acid intake of Met + Cys, Thr, Trp, Arg, Val, and Ile. For Leu, His, and Phe + Tyr, the recommendations for commercial quails are proportionately higher than those of Rostagno et al. [5]. Thus, when we used the Lys requirement estimated in this study for a 0.16 kg bird (291 mg/bird/day) to determine the IAAR intake of Met + Cys, Thr, Trp, Arg, Val, Ile, Leu, His, and Phe + Tyr as 252, 195, 60, 340, 279, 192, 413, 113, and 387 mg/bird/day, respectively, all amino acids except for His had a higher intake requirement for breeders when compared to that recommended by Rostagno et al. [5] for commercial quails.

Several factors may explain these observations. First, with genetic improvements made in these birds through crosses and specific selections, more efficient commercial birds that need a lower amino acid intake were developed. According to differences related to the methods used to derive the IAAR, studies by Rostagno et al. [5], compiling previous studies, used different diets, ages, and methods to estimate the IAAR with different response criteria. Most of the studies cited in this discussion for IAA are dose-response studies [43,44], unlike the present study, which used a nitrogen utilization model to determine individual efficiency with only an experimental diet.

5.5 Conclusions

Breeders of the Japanese quail have lower dietary efficiency than commercial birds; therefore, the consumption thus far may have been underestimated for these birds. theless, the current results should not be generalised, and further studies investigating the recommendations for amino acid intake should be conducted to validate these results.

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CAPÍTULO 6 – IMPLICAÇÕES

Respostas reprodutivas de reprodutoras são expressas caso nutrição, ambiente e sanidade sejam atendidos com precisão. Assim, a concentração de aminoácidos na dieta deve ser atendida, impedido que excesso e deficiência prejudique a utilização de aminoácidos pela ineficiência na produção ou excreção excessiva de nitrogênio, respectivamente. Portanto, é necessário determinar a relação ideal para reprodutoras e ter um profundo entendimento da necessidade de lisina da dieta. Como solução, duas etapas de pesquisas foram seguidas e descritas no capítulo 1, onde a primeira, foi conhecer a exigência de lisina e a segunda, determinar a relação da lisina com os demais aminoácidos essenciais.

O primeiro passo, foi resolvido e determinado por meio de duas metodologias descritas nos capítulos 3 e 5. No capítulo 3, a ingestão diária de lisina foi determinada por um modelo monomolecular de quatro parâmetros, com base na resposta de deposição de lisina na massa de ovo. O modelo foi proposto na pesquisa com a inclusão do parâmetro de mínima resposta no eixo da ordenada. Assim, foi possível com esse modelo determinar a ingestão diária de lisina e estimar a exigência de mantença diária para lisina baseada em respostas de produção próximo a zero, além de ser possível ter uma interpretação biológica para as respostas dos parâmetros.

Posteriormente, outra metodologia foi utilizada para determinar a ingestão diária de lisina, no capítulo 5. Utilizando os princípios de “Goettingen”, foi estimado os parâmetros que caracteriza a máxima retenção proteica (NRmaxT) e o nitrogênio para mantença (NMR), através de um ensaio de balanço de nitrogênio. Esses, foram

aplicados para calcular a qualidade da proteína e eficiência dietética do aminoácido limitante e assim, determinar a necessidade diária de lisina.

No segundo passo, foi determinado as proporções ideais de aminoácidos dietéticos, descrito nos capítulos 4 e 5 ambas têm como princípio o método da deleção para determinar as variáveis analisadas, no entanto foram empregadas diferentes metodologias para determinar a relação ideal. Com os resultados do quarto capítulo, foi possível estimar a relação ideal dos aminoácidos com base em um tecido alvo, que nessa pesquisa foi o ovo, com diferença entre o peso total do ovo e peso da casca, utilizando passos simples que tem como base o modelo Broken-line. Os resultados de relação ideal dos aminoácidos no capítulo 5, teve como princípio a eficiência de utilização dos aminoácidos para estimar a relação ideal, onde é considerado o genótipo e a idade do animal, utilizando os valores estimados dos parâmetros de NRmaxT e NMR.

Com os resultados desses estudos, foi observado coerência com a literatura, no entanto, inconsistência para as exigências de aminoácidos para codornas comerciais e reprodutoras, sendo as codornas reprodutoras menos eficientes na utilização dos aminoácidos que as aves desenvolvidas para postura comercial. Diante disso, os resultados gerados nessa tese contribuem para garantir ingestão ideal de aminoácidos essenciais em dietas para codornas Japonesa reprodutora baseado na eficiência de utilização dos aminoácidos ou na qualidade do ovo, com objetivo de melhorar o peso do ovo ou a qualidade casca. Essa descoberta, torna possível orientar nutricionistas a escolher a melhor estratégia nutricional para essa categoria animal. No futuro, recomendamos associar as exigências estimadas aqui em curvas de produção para determinar estratégias alimentares.