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

SISTEMA DE ENERGIA LÍQUIDA PARA GALINHAS DE POSTURA

Raully Lucas Silva
Zootecnista

2024

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SISTEMA DE ENERGIA LÍQUIDA PARA GALINHAS DE POSTURA

Raully Lucas Silva

Orientadora: Profa. Dra. Nilva Kazue Sakomura

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

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IMPACTO POTENCIAL DESTA PESQUISA

Os modelos desenvolvidos neste estudo permitem que nutricionistas incorporem o sistema de energia líquida na formulação de dietas para galinhas de postura, reduzindo os custos totais com alimentação e promovendo uma maior eficiência na produção de ovos. Além disso, o estudo oferece uma compreensão aprofundada sobre a utilização de energia pelas aves, facilitando a implementação do sistema de EL pela indústria avícola, o que garante uma nutrição de maior precisão e maior rentabilidade do setor.

POTENTIAL IMPACT OF THIS RESEARCH

The models developed in this study allow nutritionists to incorporate the net energy system into diet formulation, reducing overall feed costs and promoting greater efficiency in egg production. Additionally, the study provides a deep understanding of energy utilization by laying, facilitating the implementation of the NE system by the poultry industry, ensuring improvements in precision nutrition and higher profitability.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: SISTEMA DE ENERGIA LÍQUIDA PARA GALINHAS DE POSTURA

AUTOR: RAULLY LUCAS SILVA
ORIENTADORA: NILVA KAZUE SAKOMURA

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

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Jaboticabal, 11 de abril de 2024

DADOS CURRICULARES DO AUTOR

Raully Lucas Silva, filho de João Batista Pereira da Silva e Mairelane Isabel de Souza, nasceu em 13 de maio de 1991, na cidade de Manhumirim, Minas Gerais. Ingressou no curso de Zootecnia na Universidade Federal de Viçosa, Minas Gerais, em março de 2011, concluindo a graduação em 2017. Durante esse período, foi bolsista de iniciação científica pela Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) entre 2011 e 2013. De agosto de 2013 a dezembro de 2014, foi bolsista do programa Ciências Sem Fronteiras, cursando parte da graduação na Montana State University, Bozeman, EUA. Em março de 2018 iniciou o mestrado em Zootecnia pela Universidade Federal de Viçosa, sob orientação do Prof. Dr. Luiz Fernando Teixeira Albino, realizando pesquisas na área de nutrição e produção de monogástricos. Submeteu-se à defesa da dissertação em 19 de fevereiro de 2020. Em março do mesmo ano iniciou o doutorado na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista “Júlio de Mesquita Filho”, sob orientação da Profa. Dra. Nilva Kazue Sakomura. Sua pesquisa concentrou-se na área de respirometria e calorimetria de galinhas de postura, visando desenvolver equações de predição de energia líquida. De março a setembro de 2023 foi bolsista do programa Capes Print de Doutorado Sanduíche na University of New England, em Armidale, Austrália, onde realizou parte de sua pesquisa. A tese foi submetida à defesa em abril de 2024.

DEDICATÓRIA

Para a mulher mais forte, determinada e autêntica que já tive o privilégio de conhecer.

Esse era o nosso sonho, e estou certo que você estará me acompanhando lá de cima. Você me ensinou muito mais do que simplesmente trilhar o meu próprio caminho e a lutar pelo que se deseja, você me ensinou a acreditar no poder transformador e libertador da educação. Por todas essas lições e tantas outras lembranças que estão gravadas eternamente na minha memória, dedico este trabalho a você, aos seus ensinamentos, à sua vida, à sua memória, e acima de tudo, ao amor que sinto por você.

Mairelane Isabel de Souza, minha mãe, a você dedico esse trabalho.

Sonhamos o voo, mas tememos as alturas. Para voar é preciso amar o vazio. Porque o voo só acontece se houver o vazio. O vazio é o espaço da liberdade, a ausência de certeza. Os homens querem voar, mas temem o vazio. Não podem viver sem certezas. Por isso trocam o voo por gaiolas. As gaiolas são o lugar onde as certezas moram.

Rubem Alves

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A conclusão desse trabalho representa a realização de um sonho. Ao longo dos últimos treze anos dedicados à Zootecnia e à nutrição animal, enfrentei diveros desafios e, em muitos momentos considerei desistir. So não o fiz graças a uma grande rede de apoio ao qual gostaria de expressar minha sincera gratidão.

A minha mãe Mairelane de Souza (em memória), pelo amor incondicional e apoio sem limites. Obrigado por acreditar em mim e por me mostrar o caminho. Apesar da saudade imensa sei que você está sempre comigo, e por isso você sempre será lembrada.

Ao meu pai, João Batista, por acreditar em mim, por todo esforço em prol da minha educação e por me ensinar valores fundamentais para ser um homem ético e de caráter. Você foi o meu alicerce em toda essa jornada, e não teria conseguido sem você.

Ao meu irmão Silvestre Neto, pela parceria, amizade e cumplicidade em todos esses anos. Obrigado pelo apoio constante e por todo o amor concedido a mim.

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Aos animais, que são os protagonistas desse projeto e a razão para que tudo isso acontecesse, o meu imenso respeito, carinho e consideração.

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"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Determinação da energia líquida de ingredientes para galinhas em produção"**, protocolo nº 5402/20, sob a responsabilidade da Profa. Dra. Nilva Kazue Sakomura, 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 10 de dezembro de 2020.

Vigência do Projeto	25/02/2021 a 15/06/2021
Espécie / Linhagem	*Gallus gallus domesticus* / Lohmann
Nº de animais	324
Peso / Idade	1500 g / 24 semanas
Sexo	Fêmeas
Origem	Granja Mayra, Pedralva / MG

Jaboticabal, 10 de dezembro de 2020.

Fabiana Pilarski
Profa. Dra. Fabiana Pilarski
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
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SISTEMA DE ENERGIA LÍQUIDA PARA GALINHAS DE POSTURA

RESUMO - O sistema de energia líquida (EL) representa uma abordagem inovadora e eficiente para otimizar a formulação de dietas, visando máxima produção. Este conceito já foi amplamente estudado na nutrição de suínos e bovinos onde é bem difundido e aceito entre nutricionistas. Entretanto, essa abordagem ainda não é amplamente utilizada na nutrição de aves. Diversos autores têm trabalhado para desenvolver equações de predição de EL e implementar esse sistema no meio avícola. Porém, a maior parte dessas pesquisas é realizada em frangos de corte, sendo que a maioria das equações obtidas nesses estudos é extrapolada para galinhas de postura. Entretanto, recentemente foi demonstrado que as galinhas poedeiras são mais responsivas aos nutrientes da dieta do que os frangos de corte, especialmente em relação ao extrato etéreo e à proteína bruta. Deste modo, o presente estudo visa desenvolver equações de predição das exigências de EL para galinhas poedeiras em fase de postura, bem como equações de predição de EL dos ingredientes a partir da composição nutricional das dietas. Os ensaios foram realizados utilizando um sistema equipado com câmaras respirométricas de circuito aberto e analisadores específicos para determinar o consumo de oxigênio (VO_2) e a produção de CO_2 (VCO_2). A partir da equação de Brouwer (1965), a produção de calor total (PCT) e a produção de calor em jejum (PCJ) foram determinadas. As aves utilizadas neste estudo foram alimentadas com diferentes dietas variando a composição nutricional, visando obter uma ampla variação entre os nutrientes para gerar equações de predição robustas. Após os cálculos de retenção energética no corpo e no ovo e dos valores de energia líquida, modelos não lineares de efeito misto foram ajustados com o intuito de determinar a exigência de energia líquida das aves, e uma regressão linear múltipla foi ajustada para determinar a NE dos alimentos em função dos componentes químicos das dietas. Após o ajuste, os modelos foram avaliados quanto à confiabilidade da predição utilizando os resíduos médios entre os valores observados e preditos. Os modelos de efeito misto determinaram uma exigência de 81.172 kcal/kg$^{0.75}$ e 94.16 kcal/kg$^{0.75}$, para energia líquida e metabolizável, respectivamente. Os modelos ajustados a partir de regressão múltipla para predizer a energia líquida dos ingredientes foram: NE = 0.765×AME - 8.95×CP + 18.24×EE, e NE = 0.779×AME - 6.35×CP + 18.65×EE, para predição dos valores de NE a partir de AME e AMEn, respectivamente. Tais resultados contribuem para a implementação do sistema de energia líquida na nutrição de galinhas de postura, uma vez que os modelos propostos fornecem informações acuradas sobre a exigência da ave e a composição de NE dos ingredientes, possibilitando a formualção de dietas práticas utilizando esse sistema.

Palavras-chave: Calorimetria indireta, respirometria, produção de calor, análise multivariada, energia líquida.

NET ENERGY SYSTEM FOR LAYING HENS

ABSTRACT - The net energy (NE) system represents an innovative and efficient approach to optimize diet formulation for maximum production. This concept has been extensively studied in swine and cattle nutrition, where it is well-established and accepted among nutritionists. However, this approach is not widely utilized in poultry nutrition. Several authors have worked on developing NE prediction equations and implementing this system in the poultry industry. However, most of this research is conducted in broiler chickens, and the equations derived from these studies are often extrapolated to laying hens. However, it has recently been demonstrated that laying hens are more responsive to dietary nutrients than broiler chickens, especially regarding ether extract and crude protein. Thus, the present study aims to develop NE requirement prediction equations for laying hens in the laying phase, as well as NE prediction equations for ingredients based on the nutritional composition of diets. The trials were conducted using a system equipped with open circuit respirometric chambers and specific analyzers to determine oxygen consumption (VO_2) and carbon dioxide production (VCO_2). Using the Brouwer equation (1965), total heat production (THP) and fasting heat production (FHP) were determined. The birds used in this study were fed with different diets varying the nutritional composition. The idea is to obtain a wide variation of nutrients to generate robust prediction equations. After calculating the energy retention in body and eggs, as well as the values of net energy, nonlinear mixed-effects models were fitted to determine the requirement of NE for the birds. Also, a multiple linear regression was fitted to determine the NE of the ingredients based on the chemical components of the diets. After fitted, the models were evaluated for prediction reliability using the mean residuals between observed and predicted values. The mixed-effects models determined a requirement of 81.172 kcal/kg$^{0.75}$ and 94.16 kcal/kg$^{0.75}$ for net energy and metabolizable energy, respectively. The models adjusted from multiple regressions to predict the net energy of the ingredients were: $NE=0.765\times AME-8.95\times CP+18.24\times EE$, and $NE=0.779\times AME-6.35\times CP+18.65\times EE$, for predicting NE values from AME and AMEn, respectively. These results contribute to the implementation of the net energy system in laying hen nutrition, as the proposed models provide accurate information on the bird's requirement and the NE composition of ingredients, enabling the formulation of practical diets using this system.

Keywords: Indirect calorimetry, respirometry, heat production, multivariate analysis, net energy.

LISTA DE ABREVIATURAS

ADE: Ação dinâmica especifíca
AME: Apparent metabolizable energy
AME_i: Apparent metabolizable energy intake
AME_n: Metabolizable energy corrected for nitrogen
Avp: Available phosphorus
BW: Body weight
BWG: Body weight gain
CF: Crude fiber
CP: Crude protein
dCHO: Digestible carbohydrate
dCP: Apparent digestible crude protein
dEE: Digestible ether extract
DM: Dry matter
EB: Energia bruta
EE: Extrato etéreo
EL: Energia líquida
EM: Egg mass
EM: Energia metabolizável
EMV: Energia metabolizável verdadeira
ER: Energia retida
FDN: Fibra em detergente neutro
FHP: Fasting heat production
FHP: Fasting heat production
FI: Feed intake
F_{in}: Air ingoing flow
FL: Feed level
F_{out}: Air outgoing flow
GE: Gross energy
GE_{egg}: Gross energy in eggs
HI: Heat increment
IC: Incremento calórico

k: Efficiency of energy utilization

k_{body}: Efficiency of energy utilization for tissue deposition in body

k_{egg}: Efficiency of energy utilization for tissue deposition in eggs

k_m: Efficiency of energy utilization for maintenance

ME: Metabolizable energy

ME_i: Metabolizable energy intake

ME_m: Metabolizable energy for maintenance

ME_p: Metabolizable energy for production

MUX: Multiplexer

NEi: Net energy intake

NE_m: Net energy for maintenance

NR: Nitrogen retention

PB: Proteína bruta

PCJ: Produção de calor de jejum

PCT: Produção de calor total

PV: Peso vivo

QR: Quociente respiratório

RE: Retained energy

RE_{body}: Retained energy in body

$RE_{body\text{-}fat}$: Retained energy in body as fat

$RE_{body\text{-}pt}$: Retained energy in body as protein

RE_{egg}: Retained energy in eggs

$RE_{egg\text{-}fat}$: Retained energy in eggs as fat

$RE_{egg\text{-}pt}$: Retained energy in eggs as protein

RE_{fat}: Retained energy as fat

RE_{pt}: Retained energy as protein

RES: Residual

RMSE: Root mean square errors

RQ: Respiratory quotient

RSD: Residual standard deviation

SD: Standard deviation

SEM: Standard error of means

THP: Total heat production
TMB: Taxa metabólica basal
VCO$_2$: Volume de gás carbônico
VO$_2$: Volume de oxigênio

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CAPÍTULO 3

Figure 1. Validation of net energy prediction models through comparisons between predicted and observed NE values of 16 diets from Barzegar et al. (2019).

CAPÍTULO 4

Figure 1. Determination of net energy (NE) and metabolizable energy (ME) requirements in different strains of light and heavy hens, using models 4 and 5 presented in Chapter 2.

Figure 2. Relationship between efficiency of energy utilization (NE/AME) and nitrogen excretion (g/kg) of laying hens fed with different levels of net energy and metabolizable energy (n=32; each point represents the average of four experimental units).

CAPÍTULO 1: CONSIDERAÇÕES INICIAIS

1. Introdução

Embora a energia não seja considerada um nutriente *per se,* ela desempenha um papel crucial no desenvolvimento e na produção adequada das aves, sendo essencial garantir quantidades adequadas de energia nas dietas (Hilton, 2020). Além disso, os custos associados a ingredientes e alimentação representam a maior fração dos custos na produção animal, com a energia representando até 60% dos gastos totais atribuídos à dieta (Wu et al., 2018, Barzegar et al., 2019a). Isso se deve ao fato das aves consumirem prioritariamente para atender as suas demandas energéticas. Por isso, estimar de maneira acurada os valores de energia disponível nos ingredientes é determinante para melhorar o desempenho dos animais e reduzir os custos com alimentação (De Groote, 1974; Carré et al., 2014).

A energia metabolizável (EM) vem sendo utilizada como o sistema mais difundido que integra tanto o conteúdo dos ingredientes e da dieta, assim como a expressão das exigências de energia das aves, ambos na mesma base. Entretanto, apesar de ser considerado um sistema prático, ele não representa de forma acurada a utilização energética da ave, pois não abrange o particionamento da energia no corpo para manutenção, retenção de energia como tecido, e o incremento calórico (IC). Para uma descrição acurada do destino metabólico da energia, considerando tal particionamento, só seria possível utilizando o sistema de energia líquida (EL), que vem sendo proposto como um sistema que supre as limitações do atual modelo de EM (De Groote, 1974; Carré et al., 2002; Swick et al., 2013; Barzegar et al. 2019a).

A vantagem do sistema de energia líquida (EL) é devido à avaliação aprimorada da energia dos alimentos e da exigência da ave quando em comparação com o sistema de EM. Isto se deve ao fato do sistema de EM superestimar os valores de energia retida (ER) em ingredientes com alto teor de proteína e subestimar em ingredientes com alto teor de gordura. Dados obtidos por Barzegar et al. (2020) mostraram que aves de postura são mais suscetíveis as variações nos níveis de gordura e proteína da dieta, sendo que a formulação de ração na base de EL traria mais benefícios para galinhas poedeiras do que para frangos de corte. Porém, os dados para a implementação do sistema de energia líquida para poedeiras ainda é limitado (Chudy et al., 2003; Sakomura, 2004; Sakomura et al., 2005) sendo que a utilização dos valores de exigência de energia de frangos de corte em crescimento é adotado também para poedeiras pela maioria dos nutricionistas (Janssen, 1989; Bourdillon et al., 1990). Frangos de corte apresentam taxa de crescimento maior do que galinhas poedeiras, o que gera diferenças ao digerir, metabolizar e utilizar componentes alimentares entre eles, condicionando grandes variações na composição corporal. Por isso a utilização de valores de EL de dietas para frangos em poedeiras não é uma estratégia viável (Ravindran et al., 2004; Adeola et al., 2018; Barzegar et al., 2019b).

Embora existam diversas equações de energia líquida propostas na literatura (Carré et al., 2014; De Groote, 1974; Pirgozliev and Rose, 1999; Swick et al., 2013; Wu et al., 2018, Cerrate et al., 2019), a maior parte das pesquisas é direcionada para frangos de corte, dada a significativa importância econômica dessa linhagem nos custos de alimentação na produção avícola. Por isso, há ainda muita variabilidade entre os valores de EL obtidos nesses experimentos, o que dificulta a sua utilização futura na nutrição de poedeiras. Portanto, torna-se necessário desenvolver pesquisas visando construir equações de predição de EL para poedeiras.

O presente estudo foi desenvolvido na Universidade Estadual Paulista (Unesp/FCAV) e determinou as exigências de EL de galinhas poedeiras, quanto o valor de EL dos ingredientes utilizados na alimentação das aves com o objetivo de aprimorar esse sistema e tornar viável à sua utilização na nutrição de aves de postura.

2. Revisão de literatura

2.1. Metabolismo energético em aves

A energia pode ser definida como a capacidade de realizar trabalho e produzir calor, sendo mensurada somente quando ocorre a transformação de uma forma energética para outra. No âmbito da nutrição animal, o foco principal está na energia química e térmica. A primeira está relacionada às ligações entre compostos químicos presentes nos nutrientes ingeridos pelos animais. A quebra ou formação dessas ligações gera energia que o organismo utilizará para realizar atividades. A energia térmica, por outro lado, está associada à temperatura de um sistema e à produção e transferência de calor (Kleiber, 1961).

Os principais nutrientes na nutrição animal, como carboidratos, proteínas e lipídios são utilizados como fontes de energia e desempenham um papel crucial para o crescimento e desenvolvimento dos animais. Além disso, a energia contida nesses nutrientes pode ser transferida de um animal para outro durante a gestação, lactação e postura de ovos, por exemplo. A energia dos nutrientes também pode ser convertida em calor por meios dos processos químicos resultantes do metabolismo, sendo o calor o principal subproduto que permite aos animais homeotérmicos manterem uma temperatura corporal adequada para sustentar os processos vitais (Armsby, 1917; Van Es and Boekholt, 1987).

Carboidratos, proteínas e lipídeos passam por transformações químicas que envolvem a liberação, transferência ou utilização da energia contida nesses compostos. Esses processos, conhecidos como metabolismo energético, podem ser analisados em diferentes níveis, desde os eventos celulares relacionados à transferência de energia até as demandas energéticas de todo o organismo animal. O entendimento destes processos, que abrange aspectos bioquímicos, fisiológicos e nutricionais, é crucial para obter uma compreensão mais abrangente sobre como os animais utilizam a energia proveniente dos alimentos (Dauncey, 1979).

A energia derivada dos alimentos é prioritariamente destinada a manutenção de processos vitais, como respiração, regulação da temperatura corporal e circulação sanguínea. Essas atividades estão inclusas no conceito de calor metabólico basal dentro do sistema de particionamento de energia. O excedente de energia consumida pelos animais pode ser armazenado como tecido corporal ou liberado na forma de calor. Este calor produzido será utilizado para aquecer o corpo se necessário ou simplesmente dissipado para o ambiente (Oliveira Neto et al., 2000). A soma de todo calor metabolicamente produzido pelo animal é chamada de produção de calor total (PCT), uma medida valiosa para estimar o gasto energético e a eficiência de produção dos animais (Yamamoto et al., 1979).

A eficiência na conversão de nutrientes ingeridos pelos animais em energia térmica pode ser mensurada através do particionamento da produção de calor metabólico. Contudo, a realização desse particionamento em componentes fisiológicos é extremamente complexo e, por vezes controverso. Isto ocorre, em partes, devido à falta de conhecimento e quantificação de todos os processos fisiológicos e metabólicos que contribuem para a produção de calor (Baldwin and Bywater, 1984). Segundo van Milgen et al. (1997), a PTC é composta pelo calor produzido para manutenção ou produção de calor durante o jejum (PCJ) juntamente com o incremento calórico. Este último abrange o efeito térmico da dieta e a produção de calor associada à atividade física em um estado normal do animal. A figura 1, elaborada por Noblet e van Milgen (1999), ilustra o conceito do particionamento de energia. A produção de calor para mantença também pode ser definida como taxa metabólica basal (TMB) e é frequentemente associada a PCJ. Por ser uma característica intrínseca do animal, ela não sofre alteração e representa a maior fração do gasto calórico (60%). Os outros fatores incluem os efeitos térmicos do alimento a curto e longo prazo, enquanto a atividade física é a principal responsável pela variação na produção de calor dos animais ao longo do dia.

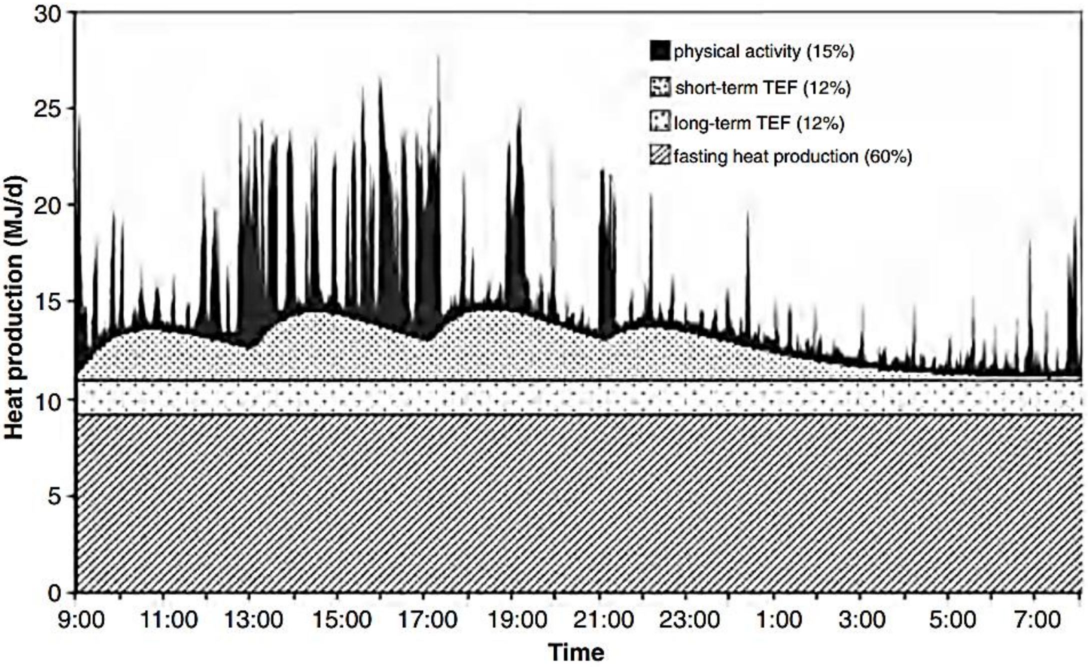

Figura 1. Componentes da produção de calor em um suíno em crescimento (60kg) oferecido 2,4 MJ de EM/kg $PV^{0.60}$×d em quatro refeições às 09:00, 13:00, 17:00 e 21:00 horas. Figura adaptada de Noblet e van Milgen (1999).

Assim, é fundamental realizar uma mensuração precisa da PCJ para compreender o metabolismo energético animal. O procedimento mais acurado e amplamente aceito pelos pesquisadores para obter uma estimativa confiável dos valores de manutenção é a mensuração desse componente em condições padrão de jejum, repouso físico e temepratura. (Bursztein et al., 1989; Garrow, 1974; Harris & Benedict, 1919; Noblet et al., 1994).

2.1.1. Produção de calor em jejum

A produção de calor em jejum (PCJ) pode ser determinada por meio de análise de regressão linear simples ou através da mensuração direta em câmaras respirométricas. No primeiro método, a PCJ é obtida ao calcular uma regressão entre a produção total de calor e o consumo diário de energia metabolizável de aves alimentas com diferentes níveis de ingestão. A regressão é então extrapolada para ingestão de AME igual a zero sendo o valor obtido da produção de calor nesse ponto definido como a produção de calor em jejum. Neste método é necessário obter os valores de PTC para cada um dos níveis de ingestão de AME estudados, sendo utilizado para tal, as análises de respirometria e calorimetria, ou a técnica do abate comparativo (ambas as técnicas serão discutidas no item 2.2.1 e 2.2.2 deste trabalho).

A determinação da produção de calor em jejum pelo ajuste de equações lineares tem sido amplamente utilizada devido a facilidade que esse modelo apresenta em predizer esse parâmetro. Entretanto, diversos autores têm criticado esse método, pois ele apresenta limitações importantes, além de apresentar valores de PCJ que muitas vezes são considerados inconsistentes (Blaxter, 1989; Koong et al., 1983; de Lange et al., 2006). O fato de a produção de calor ser mensurada em uma determinada faixa de ingestão de AME (geralmente entre 60% e 100% do consumo *ad libitum*) e posteriormente ser extrapolada para um nível de ingestão de AME igual a zero, acaba resultando em imprecisões na inclinação e interceptação do modelo. Ou seja, os valores obtidos de PCJ ajustando uma equação linear serão significativamente menores do que os valores obtidos por meio do sistema de calorimetria. Além disso, Renaudeau et al., 2007 observaram que os animais conseguem adaptar o seu gasto energético basal ao nível de ingestão de ração e/ou intensidade de crescimento, resultando em valores subestimados de produção de calor. A figura 2, representa o efeito que o nível de alimentação apresenta sobre a mensuração da PCJ. O modelo linear emprega um valor de PCJ para cada nível de alimentação utilizado, quando quatro níveis, ou mais, são utilizados no modelo o valor obtido para mantença (PCJ) acaba sendo subestimado, afetando os valores de energia líquida (EL) e eficiência (k) de utilização da dieta. Deste modo, os valores de PCJ obtidos por meio de regressão entre PCT e AME não seriam adequados para determinar a taxa metabólica basal. Em vez disso, preferencialmente, deveria se utilizar a PCJ mensurada em animais que foram alimentados *ad libitum,* mas que estão em jejum por um determinado período (Labussiere et al., 2011).

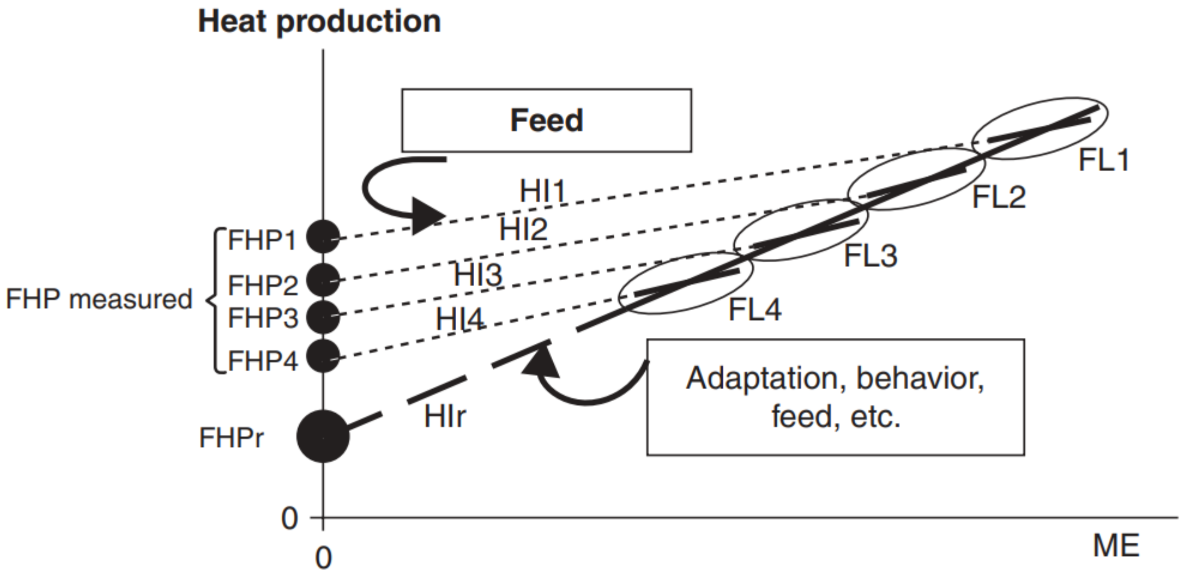

Figura 2. Representação esquemática do efeito do nível de alimentação (FL, sigla em inglês) sob a produção de calor e na produção de calor em jejum (FHP, sigla em inglês) em animais monogástricos. Cada FHP corresponde a produção de calor extrapolada ao nível de alimentação igual a zero para cada FL, durante o período imediatamente anterior. O FHPr (r de regressão) é obtido a partir da regressão entre produção total de calor e todos os níveis de alimentação (energia metabolizável – ME, sigla em inglês). A inclinação é o incremento calórico (HI, sigla em ingês). Figura adaptada de Labussiére et al., (2009).

Uma outra maneira para determinar a PCJ é com a utilização da câmaras respirométricas, podendo ser utilizado a calorimetria indireta de circuito aberto ou fechado (Close e Mount, 1975). Neste método, a produção de calor e mensurada após a retirada do alimento por no mínimo 15 horas. Espera-se que a produção de calor do animal reduza regularmente até atingir um platô, o que corresponde a digestão completa e ao início do metabolismo dos nutrientes consumidos. De acordo com Close e Mount (1975), após a retirada do alimento o animal pode expressar uma atividade física excessiva, o que pode levar a uma variabilidade dos dados de PCJ obtidos durante esse período. Portando, a maioria das mensurações de PCJ em aves são provenientes de dados de produção de calor obtidos ao longo de 18 a 24 horas de jejum. Além disso, os animais podem ser mantidos no escuro para reduzir a atividade física e assim obter uma produção de calor fidedigna a taxa metabólica basal. (Li et al., 2017). Van Milgen et al. (1998) propôs um método mais sofisticado para estimar a PCJ. Milgen sugeriu que a PCJ fosse padronizada e derivada matematicamente da PCT noturno em estado livre de atividade física, em associação com o platô da produção de calor de animais em jejum de pelo menos 24 horas. De acordo com van Milgen & Noblet (2000) essa estimativa de PCJ embora seja medida durante um período em que o animal está em estado catabólico, ainda mantém uma relação com a fase anabólica da produção de calor, não sendo tão influenciado pelo nível de alimentação em comparação com as estimavas de PCJ que utilizam o método da regressão.

O PCJ também pode ser assumido como uma função alométrica em relação ao peso corporal do animal, sendo $PCJ=a*PV^{b}$, onde a e um coeficiente constante em kcal e PV^{b} e o peso vivo metabólico (kg) que correlaciona o peso corporal a área de superfície do corpo do animal (Vohra et al., 1975). A utilização do valor de 0,75 para o coeficiente alométrico vem do compilado de vários trabalhos sobre o valor de PCJ para várias espécies de animais adultos. A utilização do chamado peso metabólico calculado como PV^0,75 permitiu uma constância nos valores de PCJ entre diferentes animais adultos com diferentes pesos corporais (Bowes et al., 2021; Brody, 1945; Kleiber, 1961). Entretanto, a utilização deste conceito para animais em crescimento foi questionada por Koong et al., (1982) que sugeriu que o PCJ não era proporcional ao peso metabólico no caso de animais em crescimento. Estes dados foram posteriormente confirmados por Noblet et al., (1999). Além disso, de acordo com van Milgen et al., (1998) as características do animal como o genótipo no caso de suínos e as condições alimentares anteriores a mensuração deveriam ser consideradas na estimativa da PCJ. Noblet et al., (2015) sugeriu os expoentes de 0,60 e 0,70 seriam mais apropriados para suínos em crescimento e frangos de corte, respectivamente. De acordo com Labussière et al., (2011) os valores apresentados em literaturas mais antigas sobre animais em crescimento expressos em kg de $PV^{0.75}$ cujo resultados foram obtidos por meio do método de regressão aplicado a animais alimentados com níveis diferentes de alimentação, deveriam ser abandonados e substituídos por valores mais recentes de PCJ obtidos por meio de mensurações diretas, onde o expoente é específico a cada espécie.

2.1.2. Incremento calórico

O aumento na taxa metabólica após a ingestão de alimento é definido como incremento calórico (IC), também denominado de ação dinâmica específica (ADE) ou termogênese induzida pela dieta (Rubner 1902; Maynard e Loosli 1969). Este aumento da produção de calor após a alimentação está relacionado aos processos químicos e físicos envolvidos na mastigação, digestão, absorção e metabolismo dos nutrientes (Smith et al., 1978). Durante os anos o IC tem sido considerado como uma perda inevitável ou um grande desperdício de energia ingerida, entretanto, Simek (1975) sugeriu que o calor advindo do IC era utilizado para manutenção da temperatura corporal dos animais endotérmicos, reduzindo os custos associados a energia de mantença.

Diferentes nutrientes requerem diferentes quantidades de energia para serem digeridos e metabolizados, desse modo a produção de calor resultante dos ingredientes é diferente entre eles. Em aves e mamíferos o IC resultante do metabolismo de proteínas e muito maior que o IC advindo dos carboidratos e gorduras. Assim, podemos particionar o IC advindo dos nutrientes, como por exemplo, o IC resultante da gordura quando expresso como uma porcentagem da energia metabolizável varia de 4% a 10%, o carboidrato de 6% a 15%, já a proteína representa em torno de 30% do IC (Brody, 1945; Smith et al., 1978). De acordo com Blaxter (1989), a proteína é uma fonte de energia menos eficiente quando comparada com o carboidrato e a gordura. Uma explicação para isso é que quando a proteína é utilizada como fonte de energia os resíduos nitrogenados decorrentes do metabolismo requerem energia para serem excretados. Além disso, a proteína dietética estimula a deposição e renovação proteica que requerem um gasto de aproximadamente 71 kcal EM/g para alimentar as vias metabólicas. (Reeds et al., 1982; Reeds and Fuller, 1983; Musharaf e Latshaw, 1999).

A correta determinação do incremento calórico traz informações importantes sobre a eficiência de utilização da energia pelos animais. Quanto maior for a relação EL/EM maior será a conversão da energia metabolizável em energia líquida, o que resultará em um menor incremento calórico. Neste aspecto podemos determinar a eficiência de uma dieta baseado em sua ineficiência, ou seja, nos valores de IC. Um exemplo disso, Musharaf e Latshaw (1999), apresentaram dietas mais eficientes (EL/EM) quando houve redução no teor de proteína bruta da dieta, já que esse nutriente apresenta um alto valor de IC, como demonstrado anteriormente. Além disso, Liu et al. (2017) determinaram que a ingestão de energia metabolizável também pode alterar a eficiência EL/EM e IC. Esses autores afirmaram que o aumento na ingestão de alimentos em frangos de corte reduziu a eficiência de utilização de energia, pois com o aumento do consumo uma maior quantidade de IC é produzida, resultando proporcionalmente em uma menor EL. MacLeod et al. (1979), mostraram em seus estudos que galinhas poedeiras em restrição de consumo apresentavam menor IC e maior EL/EM do que galinhas alimentadas de forma *ad libitum*.

Deve ser enfatizado que a produção de calor de manutenção inclui uma fração do IC (Musharaf e Latshaw, 1999). Blaxter (1989) mostrou que proteínas, carboidratos e gorduras quando usadas pelo animal para manutenção apresentam uma eficiência 20% maior do que quando utilizadas para produção. Ou seja, o IC é menor quando os nutrientes são usados para manutenção do que para crescimento e produção. Em resumo, o IC é menor quando os animais são alimentados até o nível de mantença, o que aumenta a eficiência de utilização da energia dietética para ser usado para PCJ. Entretanto, o excedente de energia as necessidades basais do animal leva a um aumento do IC, que será liberado ao ambiente diminuindo a eficiência energética (Musharaf e Latshaw, 1999).

2.2. Metodologias para avaliar o metabolismo energético em aves

2.2.1. Calorimetria indireta

A calorimetria indireta é um sistema que tem sido amplamente utilizado para investigar e compreender o metabolismo energético em animais e seres humanos (Riveros et al., 2023). Esse método parte do pressuposto de que a energia liberada pelos processos de oxidação dos nutrientes pode ser quantificada através da relação estequiométrica entre o consumo de oxigênio (O_2) e a produção de dióxido de carbono (CO_2) (Brouwer, 1965). O sistema de calorimetria indireta apresenta dois tipos de design: o circuito fechado e o circuito aberto. Os primeiros pesquisadores dessa área utilizaram o circuito fechado por um longo período de tempo, e ainda hoje ele é considerado um excelente sistema para mensurar trocas gasosas. Neste circuito, as taxas de consumo de oxigênio e produção de CO_2 são medidas através de mudanças no volume e pressão. Isso significa que, conhecendo o fluxo de entrada, o volume inicial e o volume final dos cilindros que alimentam o sistema, é possível mensurar a utilização dos gases pelos animais. Por outro lado, no circuito aberto, o sistema possui duas aberturas em suas extremidades para capturar o ar atmosférico por meio de uma bomba de fluxo que cria pressão negativa. O ar que entra no sistema é analisado quanto a parâmetros como pressão atmosférica, temperatura ambiente e pressão parcial de vapor de água. Em seguida, uma amostra é seca e analisada com equipamentos específicos para quantificar a quantidade de oxigênio e CO_2 consumidos. Sabendo-se as concentrações dos gases na atmosfera, após a expiração dos animais, é possível mensurar a utilização de oxigênio e a produção de CO_2 (Simonson 1990).

Tanto o sistema fechado quanto o aberto são eficientes para mensurar a produção de calor, uma vez que ambos os sistemas levam em consideração que a oxidação completa de carboidratos, lipídeos e proteínas é um processo exotérmico que resulta na produção de CO_2 e H_2O como produtos metabólicos. Dessa forma, por meio do consumo de O_2 e produção de CO_2 é possível ter uma estimativa acurada da quantidade de calor gerado no metabolismo dos animais. No caso das proteínas, além da produção de CO_2 e H2O, também ocorre a liberação de nitrogênio, que é excretado na urina e fezes. Desse modo, a correta mensuração da excreção de nitrogênio nas excretas das aves é essencial para obter estimativas precisas da produção de calor por meio da calorimetria indireta. (McLean e Tobin, 1987; Ferrannini, 1988). (Jequier, 1987.

A determinação do volume de oxigênio (VO_2) e CO_2 (VCO_2) pelo sistema de calorimetria indireta permite a determinação do calor gerado pela oxidação de diferentes substratos por meio de equações de estequiometria. O oxigênio gasto e o CO_2 produzido nos processos de oxidação varia entre cada nutriente. Um conceito fundamental nesse sentido é o quociente respiratório (QR) que expressa a relação entre a produção de CO_2 e o consumo de O_2. O QR fornece informações sobre qual substrato está sendo predominantemente utilizado pelo metabolismo na formação de energia, em um determinado momento. Para a glicose, o QR é igual a 1, o que indica que o oxigênio utilizado na oxidação é equivalente ao CO_2 produzido. Diferentemente, proteínas e lipídeos demandam uma maior quantidade de oxigênio para serem completamente oxidados e produzem uma menor quantidade de CO_2, resultando em um QR de 0,80 e 0,70 respectivamente. As principais equações estequiométricas foram desenvolvidas por Ferrannini (1988) e Simonson (1990) e podem ser observadas abaixo:

$$1\text{g glicose} + 0{,}746\text{L O2} \rightarrow 0{,}746\text{L CO2} + 0{,}60\text{g H2O (Eq. 1)}$$

$$1\text{g lipídeo} + 2{,}019\text{L O2} \rightarrow 1{,}427\text{L CO2} + 1{,}09\text{g H2O (Eq. 2)}$$

$$1\text{g proteína} + 0{,}966\text{L O2} \rightarrow 0{,}774\text{L CO2} + 0{,}45\text{g H2O (Eq. 3)}$$

A partir dos cálculos acima é possível demonstrar um conceito fisiológico importante. A maneira mais eficiente de utilizar oxigênio para produzir energia é através da oxidação da glicose, sendo a oxidação de gordura e proteína mais dispendiosas em termos de "moeda de troca" de O_2.

A partir dos trocas gasosas utilizadas na oxidação dos nutrientes, e desconsiderando as perdas na forma de metano (CH_4) e nitrogênio é possível determinar a produção de calor utilizando a equação de Brouwer (1965), onde:

$$\text{PCT (kcal)} = 3{,}87\text{ litros de O2 consumidos} + 1{,}23\text{ litros de CO2 produzidos (Eq. 4)}$$

Uma vez que os valores de PCT são obtidos, a energia líquida pode ser determinada pela diferença entre o incremento calórico e a ingestão de energia metabolizável diária (McDonald et al., 2011).

2.2.2. Técnica do abate comparativo

A técnica do abate comparativo é um método clássico utilizado para avaliar mudanças na composição corporal em um determinado período de tempo. Desenvolvido por Sibbald e Fortin (1982), esse método envolve o abate de uma quantidade representativa de aves no início e ao final do período experimental. As aves são depenadas, congeladas, serradas, moídas, homogeneizadas e, por fim liofilizadas para análises proximais. Após a secagem, as amostras são submetidas a análises de matéria seca, nitrogênio, extrato etéreo, e cinzas. Os dados resultantes são combinados com as medições de peso vivo para prever a composição da carcaça (Wolynetz e Sibbald, 1984).

Embora seja um método eficiente para determinação da energia retida, o abate comparativo não apresenta resultados precisos como a calorimetria indireta. Isso ocorre porque aves da mesma idade e peso podem apresentar composições diferentes, gerando imprecisões nos valores obtidos por este método (Just et al., 1982). Além disso, o abate comparativo é muitas vezes evitado devido à dificuldade de preparar e homogeneizar as amostras, principalmente no que diz respeito a resistência das penas ao processo de maceração, além da necessidade de abater as aves e não ser possível acompanhar as mudanças corporais ao longo do tempo no mesmo animal (Sibbald and Fortin, 1982 Salas, et. al., 2012).

2.3. Sistemas de energia

2.3.1. Energia bruta

A energia contida nos ingredientes pode ser determinada pela quantidade de calor liberado durante uma reação de combustão. Embora diferentes unidades de medidas possam ser utilizadas, a quilocaloria (kcal) é a mais utilizada no contexto da nutrição. Essa unidade corresponde a quantidade de calor necessária para elevar a temperatura de 1000g de água em 1°C. Assim, utilizando uma bomba calorimétrica, é possível determinar as quilocalorias associada aos ingredientes. Através de uma pequena quantidade de amostra que é completamente oxidada a liberação de calor é medida e quantificada pela bomba calorimétrica adiabática (Buskirk & Mendez, 1980).

O conteúdo de energia bruta (EB) presente nos ingredientes varia consideravelmente, sendo essas diferenças exclusivamente atribuídas à composição química. A partir dessas mensurações foi possível determinar que entre todos os componentes orgânicos, os carboidratos (amido, açúcares e fibra) apresentam menor conteúdo de EB, enquanto a gordura está associada a valores altos de EB (Sauvant et al., 2004).

Ao contrário de outros sistemas de energia, a EB não está diretamente associada ao metabolismo animal, o que a torna facilmente mensurável e de fácil predição por meio de modelos lineares. A partir de dados de diferentes estudos, Noblet et al., (2004) propuseram a seguinte equação para predição da EB a partir da composição química:

$$EB = 54{,}97\ \mathrm{PB} + 92{,}97\ \mathrm{EE} + 41{,}58\ \mathrm{Amido} + 39{,}43\ \mathrm{Açucar} + 44{,}93\ \mathrm{FDN} + 42{,}30\ \mathrm{Residuo}\ (\mathrm{Eq.}\ 5)$$

Onde, PB é proteína bruta, EE é extrato etéreo, FDN é fibra em detergente neutro, e resíduo é a diferença entre a matéria orgânica e as outras frações identificadas na equação. Como demonstrado, os coeficientes associados aos carboidratos são menores, intermediários para proteína e mais alto para lipídeos, o que reflete bem o valor energético individual desses nutrientes. Alguma variação nos valores de energia pode existir, por exemplo, a diferença nos valores de energia entre amido e açúcares está relacionada principalmente ao grau de polimerização dos carboidratos. Além disso, os valores associados a PB e EE estão sujeitos a variações dependendo da composição de aminoácidos e, em menor grau, da composição de ácidos graxos, respectivamente (van Milgen et al., 2003).

2.3.2. Energia metabolizável

O sistema de energia metabolizável (EM) tem sido utilizado a décadas para determinar os valores energéticos dos alimentos e as exigências das aves, além de ser considerado o modelo mais prático e simples de ser utilizado no processo de formulação de dietas (Sauvant et al., 2004; Rostagno et al., 2017; Barzegar et al., 2020). Esse sistema foi inicialmente desenvolvido por Hill & Anderson na Universidade Cornell em 1958, utilizando dois grupos de aves, em diferentes idades (14 e 28 dias) e duas dietas com concentrações diferentes de glicose (5% e 45%). Para padronizar o método, ainda foi utilizado óxido crômico (Cr_2O_3) como indicador indigestível. Durante os quatro últimos dias de ensaio, as excretas foram coletadas, e posteriormente homogeneizadas, secas e moídas. O teor de umidade, nitrogênio, energia bruta e a recuperação do óxido crômico tanto da dieta quanto das excretas foram determinados. Com base nos resultados, equações foram propostas para calcular os valores de energia metabolizável (EM) e energia metabolizável corrigida pelo balanço de nitrogênio (EMn), conforme as seguintes fórmulas:

$$\mathrm{EM} = \frac{\mathrm{EB\ ingerida} - \mathrm{EB\ excretada}}{\mathrm{Matéria\ seca\ ingerida}} \qquad (\mathrm{Eq.}\ 6)$$

$$\mathrm{EMn} = \frac{\mathrm{EB\ ingerida} - (\mathrm{EB\ excretada} + 8{,}22 \times \mathrm{Balanço\ de\ nitrogênio})}{\mathrm{Matéria\ seca\ ingerida}} \quad (\mathrm{Eq.}\,7)$$

Sendo o balaço de nitrogênio determinado pela diferença entre nitrogênio ingerido e nitrogênio excretado. Hill e Anderson (1958) assumiram que, se o nitrogênio não for retido no corpo ele será excretado como ácido úrico, propondo o fator de correção de nitrogênio de 8,22 kcal/g. A correção para retenção zero de nitrogênio simplifica os cálculos, considerando que os componentes da dieta são totalmente utilizados como fonte de energia (McNab e Boorman, 2002). Além disso, esse fator de correção foi justificado pela capacidade de comparar dados de aves em diferentes estágios de desenvolvimento (Lopez e Leeson, 2008).

Farrell (1978) atribuiu o conceito “aparente” ao sistema de EM, assumindo que parte da energia obtida nas excretas é advinda da renovação celular que ocorre normalmente no organismo, sendo, portanto, energia endógena das aves e não apenas dos nutrientes não digeridos. Diante disso, o sistema de energia metabolizável verdadeira (EMV) foi proposto para superar as limitações do sistema anterior. Aves adultas, geralmente galos, eram submetidas a 24 horas de jejum prévio para esvaziar o tubo digestivo e, em seguida, alimentadas forçadamente. Após a ingestão de ração, os animais eram submetidos novamente ao jejum, e as excretas eram coletadas quantitativamente durante esse período. Além disso, uma ave era mantida em jejum constante, e dessa forma, as perdas endógenas e metabólicas eram determinadas (Sibbald, 1976). No entanto, o sistema de EMV foi rapidamente criticado, pois a alimentação forçada não representa a sinergia que ocorre em condições fisiológicas normais em aves alimentadas *ad libitum* (Leeson e Summers, 2001).

Apesar de ser um método confiável e amplamente utilizado, o sistema de EM apresenta limitações que devem ser consideradas. Por exemplo, o valor de EM atribuído aos alimentos irá diferir de acordo com o estágio de desenvolvimento, aptidão (carne ou ovos) e idade das aves (Garnsworthy et al., 2000; Svihus e Gullord, 2002). Portanto, diferentes valores de energia podem ser obtidos dos ingredientes devido ao estado fisiológico e aos processos digestivos dos animais. (Begin, 1967; Pym e Farrell, 1977; Lopez e Leeson, 2005; Cozannet et al., 2010). Sakomura (2004) observou um incremento na energia corporal devido ao aumento da deposição de gordura à medida que a idade das aves avançava. Além disso, a forma e composição da dieta também podem alterar os valores de energia metabolizável. Wu et al. (2018) observaram diferentes valores de utilização de energia em ingredientes como farelo de soja, óleo de soja e milho em rações para frangos de corte quando comparado com aves de postura. Por fim, o sistema de EM não explica a partição da energia no corpo da ave para as diferentes funções e atividades, não sendo possível determinar a eficiência de utilização dos nutrientes para produção, ou retenção corporal (Barzegar et al., 2019a).

2.3.3. Energia líquida

Considera-se que o sistema de energia líquida foi desenvolvido quando Armsby e Fries (1915) introduziram a perda de energia como incremento calórico ao sistema de energia metabolizável, definindo-o como: EL=EM-IC. Este sistema foi então sugerido como mais acurado para mensurar o valor energético dos ingredientes, uma vez que leva em consideração o efeito térmico da dieta na produção de calor associada aos animais (van Milgen et al., 1997). Considerar o IC na avaliação da energia dos alimentos aprimoraria os valores obtidos na utilização dos nutrientes. Portanto, ao comparar os sistemas de EL e EM para avaliar a energia dos alimentos, o sistema de EL apresenta valores mais acurados (De Groote, 1974). Pirgozliev e Rose (1999) estudaram 40 diferentes ingredientes frequentemente utilizados na formulação de dietas para aves e obtiveram valores mais precisos da utilização de energia quando utilizaram o sistema de EL no lugar do sistema de EM.

O sistema de EL é considerado economicamente mais vantajoso, uma vez que rações baseadas nesse sistema apresentam redução no conteúdo energético e proteico da dieta e garantem o mesmo desempenho dos animais. De acordo com De Groote (1974), rações formuladas no sistema de EL são, em média, 2,43% mais baratas do que aquelas baseadas no sistema de EM. Isso ocorre porque o sistema de EL leva em consideração as diferenças na eficiência da utilização metabólica da energia.

Carré et al. (2014) determinaram que a relação EL/EM para proteína bruta, lipídeos, e amido são de 76, 86 e 81% em frangos de corte, elucidando que a eficiência de utilização de energia (EL/EM) das proteínas é menor que a das gorduras, o que comprova que o modelo de EM subestima o valor energético da gordura e superestima o valor energético dos ingredientes proteicos. Isso permite uma avaliação energética mais precisa dos ingredientes da dieta, podendo ser explorado na formulação de menor custo, resultando em uma melhoria substancial na eficiência nutricional e econômica da produção de aves (De Groote, 1974). Dessa forma, a adoção do sistema de EL possibilitaria que nutricionistas formulassem dietas mais eficazes e com custos reduzidos (Barzegar et al., 2019).

Enquanto as medições de EB e EM são relativamente fáceis e podem ser realizadas em um grande número de alimentos a um custo razoável, a medição de EL é muito mais complexa, cara e envolve teorias complicadas que devem ser levadas em consideração (Noblet et al., 1994). Por exemplo, como descrito anteriormente, a PCJ deve ser mensurada de maneira adequada para obter valores fidedignos de EL, não sendo recomendado a mensuração do PCJ através de níveis de alimentação e ajustes de modelos lineares, uma vez estes subestimam o valor de PCJ (Figura 2) (van Milgen et al., 1997). Além disso, para evitar o viés no cálculo da NE em diferentes alimentos é necessário realizar medições em animais semelhantes (ou seja, mesmo sexo, mesma linhagem, e na mesma faixa de peso corporal), mantendo esses animais dentro da zona termoneutra e minimizando a variação no comportamento alimentar, mantendo o mesmo nível de alimentação entre as aves. Nessas circunstâncias, a melhor alternativa é utilizar de equações confiáveis de predição de NE estabelecidas a partir de medições padronizadas (Noblet et al., 2022).

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CAPÍTULO 2: ARE MIXED MODELS MORE EFFECTIVE THAN LINEAR MODELS IN PREDICTING ENERGY UTILIZATION FOR LAYING HENS?

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METABOLISM AND NUTRITION

Are mixed models more effective than linear models in predicting energy utilization for laying hens?

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Abstract

This study aimed to develop mathematical models that can overcome the limitations of linear energy partitioning models in laying hens. Three linear models and two nonlinear mixed-effects models were fitted to predict the energy utilization efficiency for body and egg deposition, as well as the maintenance requirements for metabolizable energy (ME_m) and net energy (NE_m). Thirty diets were individually formulated to achieve values below and above the nutritional recommendation of Hy-line guidelines. Heat production and energy metabolism were measured in laying hens during the production phase using six open-circuit respirometric chambers. Diets were randomly divided into five groups; each diet was replicated four times with 6 hens per replication. The experimental protocol included a five-day adaptation to the experimental diets, two days of chamber adaptation, four days of data collection under feeding conditions, and one day of measurement under fasting conditions. Feed intake, bird weight, egg production, egg mass and excreta production were measured daily. The variables for total heat production (THP) and fasting heat production (FHP) were obtained through the Brouwer equation using VO_2 and VCO_2 measurements. Apparent metabolizable energy (AME) was determined by measuring ingested and excreted gross energy (GE) content. Retained energy (RE) was determined as the difference between metabolizable energy intake (ME_i) and THP, and subsequently partitioned between retained energy in body and egg. RE in eggs and body were partitioned into energy retained as protein and fat. Statistical analyses involved linear regressions and nonlinear mixed-effects regressions of the main variables described. The values obtained for ME_m, NE_m and the efficiency of energy retention in body (k_{body}) and egg (k_{egg}) in the linear models were 106.41 kcal/kg$^{0.75}$×d, 90.36 kcal/kg$^{0.75}$×d, 0.843 and 0,779, respectively. For nonlinear models the values were 94.15 kcal/kg$^{0.75}$×d, 81.72 kcal/kg$^{0.75}$×d, 0.463 and 0.638 for ME_m NE_m, k_{body} and k_{egg} respectively. Nonlinear models were considered the ideal choice to determine ME_m and NE_m requirements due to the lowest error (RMSE). The NE system showed lower RMSE compared to the ME system and was therefore more accurate in expressing the energy requirements of laying hens.

Keywords: Energy metabolism, net energy, heat production, fasting heat production, indirect respirometry.

Introduction

Over the last few years, significant progress in poultry genetics has led to the development of highly efficient laying hen strains improving egg production. These birds were selected to be smaller-sized, with lower feed intake, and have demonstrated a remarkable persistence in egg production rate. However, as these laying hen strains advanced genetically, their nutritional requirements must be adjusted, especially regarding energy intake, which represents the largest fraction of feed cost (Luiting, 1990; Gous, 2007; Sakomura et al., 2019). Hence, understanding energy utilization is essential to accurately determine energy requirements for maximum efficiency (Barzegar et al., 2019). Several studies have been conducted to determine the requirements of energy based on the metabolizable (ME) and net energy (NE) system, following the factorial approach to estimate the coefficients of maintenance (k_m) and production for both systems (Waring and Brown, 1965; Grimbergen, 1970; Burlacu and Baltac, 1971; Davis et al., 1972, 1973, Sakomura, 2004). However, the estimation of these parameters has been based on simple and multiple linear regression models, and statistical issues have been associated with this approach. These models assume a constant ratio relationship between energy intake and energy expenditure, regardless of changes in dietary composition or the animal metabolic status (Koong, 1977; Moraes, 2019).

A more detailed and flexible approach for modeling energy utilization, avoiding the limitations of linear equations, is provided by mixed models (Romero et al., 2009; Strathe et al., 2010). Mixed models incorporate fixed and random effects and have been considerably extended in the past two decades with the complete characterization of the biological interpretation under different feeding conditions, maintenance requirements, and efficiencies of energy utilization for production (Feng et al., 2009; Moraes, 2019). Moreover, this model has been successfully applied to determine energy deposition and partitioning for pigs (van Milgen and Noblet, 1999; Strathe et al., 2010), dairy cows (Moraes et al., 2015), fish (Azevedo et al., 2005), and broiler breeders (Romero et al., 2009; Teofilo et al., 2023). However, there is limited information regarding the utilization of this approach for laying hens.

Additionally, coefficients of energy efficiency (k) and the estimates of metabolizable (ME) and net energy (NE) for maintenance have been determined by subjecting birds to various levels of ME intake through feed intake restriction. However, despite being a widely used technique, feed intake restriction induces metabolic and physiological changes in the birds and does not fully express the necessary biological aspects for accurately determining these coefficients (De Lange et al., 2006; Labussière et al., 2011). Consequently, this approach influences the values of energy retention, which are typically partitioned between retained energy in the body tissue and for egg formation. As a result, the efficiency of energy deposition in the egg (k_{egg}) exhibits a wide variation in coefficients reported in the literature, such as, 81% (Titus, 1928), 63% (Bird and Sinclair, 1939), 74% (Bolton, 1959), and 78% (Barzegar, 2019).

Therefore, we hypostatized that the estimation of ME and NE requirements through nonlinear mixed models in birds fed *ad libitum* is a feasible alternative, providing more reliable values. Thus, this study aimed to compare linear and nonlinear mixed-effects models in determining ME and NE requirements for maintenance, as well as coefficients of energy deposition efficiency in the body and egg of laying hens fed with different diets, varying the chemical composition.

Material and methods

Birds and experimental design

ForC:\for this experiment was used Hy-line W80 laying hens obtained at 19-wks-old from the commercial farm (Granja Mayra, MG, Brazil). The birds were allocated in individual cages equipped with feeders and nipple drinkers in a climate-controlled poultry house. During the pre-experimental period, birds were fed a standard commercial diet based on corn and soybean meal formulated to meet the nutritional recommendations of the genetic guidelines (Hy-Line W80, 2020). The lighting program followed the recommended guidelines and water, and feed were provided *ad libitum* throughout the entire experiment period.

The measurements (biological assays) were periodically conducted in groups of six experimental units per assay, since the respirometry system have capacity for six chambers. All the experiment consists of 30 experimental diets with four replicated, totalizing 120 experimental units of birds randomly distributed. At each assay, six diets were randomly selected to compose a group (1 diet per chamber). Before the measurement, 36 laying hens were selected from the initial population based on average body weight (BW) and egg production and allocated in six birds per cages (0.60 m × 0.20 m × 0.10 m). Birds were fed experimental diets for five days to allow diet adaptation. After this period, the birds were transferred to the Respirometry Laboratory and habituated to the chambers for two days. Afterwards, the data collection began under feeding conditions with the respective experiment diet for four days. On the last day, the feed was withdrawn, and the gas exchange measurements were conducted under fasting conditions for 24 hours.

Experimental diets

A total of 30 experimental diets were formulated to have a wide variation in the metabolizable energy (ME) value (Table 1). Additionally, a criterion of feed formulation was adopted considering the same ratio between the digestible lysine and energy (Lys SID: AME = 2.95%). The experimental diets were performed using traditional and non-traditional ingredients to provide flexibility in the nutritional composition and allow a wide range of energy intake to be evaluated between experimental diets. The variation in the nutritional composition of the diets, on a dry matter basis, ranged from 2419 to 3096 kcal/kg of metabolizable energy (ME), 12.88 to 22.75% crude protein (CP), 1.34 to 10.25% ether extract (EE), 1.36 to 5.35% crude fiber (CF) and 35.83 to 45.24% starch. Additionally, crystalline amino acids were supplemented to the diets to correct the balance between AME and amino acids, thus ensuring that the variation in feed intake did not limit amino acid intake. The concentration of Ca (4.51%) and available P (0.36%) were attended according to the management guide (Hy-line W80, 2020).

Respirometric chambers and gas analyzer system

Six open-circuit respirometry chambers (0.90 m × 0.85 m × 0.80 m) with temperature and humidity automatic controllers were used in this trial. The average of temperature and humidity were 21˚C and 59% respectively, as recommended by the management guide (Hy-line W80, 2020). The respirometry system consisted of a mass flow pump (FK-100; Sable System, USA) that pulled the atmospheric air into the chamber under negative pressure at a flow rate of 12 L/min to maintain the CO_2 concentration below 1% (Lighton, 2018). 160 mL/min air sample was constantly extracted by a pull-mode sub-sample pump (SS4; Sable System, USA) and flowed through the analyzer system. The air analyzers consisted of the water vapor pressure analyzer (RH -300; Sable System, USA), and scrubbing columns with 99.5% calcium sulfate ($CaSO_4$) to capture the water vapor molecules (Drierite, W. A. Hammond Drierite Co., LTD). Subsequently, the dry air was analyzed by an infrared analyzer of CO_2 (CA-10A; Sable System, USA) and a paramagnetic analyzer of O_2 (PA- 10; Sable System, USA). The flowmeter and analyzer signals were coded by a universal interface (UI-2; Sable System, USA) and saved one record per second using ExpeData software (version 1.9.22, Sable Systems).

Gas sampling was programmed from each chamber and baseline using a multiplexer (MUX RM-8; Sable System, USA) in an intermittent sequence: initial baseline (180 seconds), chambers (540 seconds per chamber), and final baseline (180 seconds). This procedure was repeated in a loop for 24 hours per day. At the beginning and the end of each data collection, the gas analyzer was calibrated with concentrated gas (>99.99% N_2) and a certified gas mixture (21% O_2 and 1% CO_2).

Performance and heat production calculations

Body weight (BW), feed intake (FI), egg mass (EM) and laying were recorded daily. Additionally, total excreta collection was performed during the feeding phase (3 days) to determine apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen (AMEn).

The air ingoing flow (F_{in}), volumetric O_2 consumption (VO_2, L/d), and CO_2 production (VCO_2, L/d) were calculated following the methodology described by Lighton (2018) for an open circuit indirect calorimetry.

$$F_{in}(L/d) = F_{out} \times \frac{(100\text{-}[O_2]_{out}\text{-}[CO_2]_{out})}{(100\text{-}[O_2]_{in}\text{-}[CO_2]_{in})} \times 1.440 \text{ minutes} \text{ (Eq. 1)}$$

$$VO_2(L/d) = F_{in} \times [O_2]_{in} - F_{out} \times [O_2]_{out} \text{ (Eq. 2)}$$

$$VCO_2(L/d) = F_{out} \times [CO_2]_{out} - F_{in} \times [CO_2]_{in} \text{ (Eq. 3)}$$

Where F_{out} is the air outgoing flow, $[O_2]_{in}$ and $[CO_2]_{in}$ are the atmospheric gas concentrations or baseline.

The total heat production (THP) and fasting heat production (FHP) were calculated using the Brouwer equation (1965):

$$HP\ (kJ/d) = 16.17 \times VO_2 + 5.02 \times VCO_2 \text{ (Eq. 4)}$$

The FHP was determined as the plateau of HP decline under fasting condition (around after 14 hours of fasting) during the dark period (Bennett and Harvey, 1987).

Chemical analysis and calculations

Samples of eggs, feed and excreta were collected and homogenized for laboratory analysis. All samples were subjected to dry matter content determination through forced air oven drying at 105˚C for 16 hours. In eggs, the dry matter was determined through freeze-drying for 72 hours. The nitrogen content of the samples was measured using the Kjeldahl method (AOAC, 2000), and the crude protein content was calculated using the coefficient of 6.25 g of crude protein per g of nitrogen. The ether extract of the feeds was determined through extraction with petroleum ether (ANKOM Technology® apparatus) and, the gross energy was obtained by the total combustion of samples using the adiabatic calorimeter (IKA C 2000 basic, USA).

The AME was determined using the total excreta collection method (Bourdillon et al., 1990). The AME values were converted to AMEn using the nitrogen balance and the coefficient 8.22 kcal/g of N as a correction factor (Hill and Anderson, 1958) for zero N retention in the body and eggs. The intake of apparent metabolizable energy (ME_i) was determined by multiplying the difference between the gross energy in the feed and excreta by the respective intake and excreta production values. The total energy retention (RE) was determined by the difference between ME_i and total heat production (THP), then RE was partitioned as RE in the body (RE_{body}) and RE in the egg (RE_{egg}).

The RE_{egg} was obtained by multiplying the gross energy value of the egg (GE_{egg}), determined using adiabatic bomb calorimetry, by the egg mass (EM): $ER_{egg} = EM \times GE_{egg}$. The RE_{egg} was partitioned into RE in the egg as protein ($RE_{egg\text{-}pt}$ = EM × N content in the egg × 6.25 g N/g protein × 5.69 kcal/g protein) and the RE in the egg as fat ($RE_{egg\text{-}fat}$), which was obtained by subtracting the $RE_{egg\text{-}pt}$ from RE_{egg}. Finally, the RE_{body} was determined by subtracting the RE_{egg} from RE.

Retained energy in the body (RE_{body}) was determined by subtracting the RE_{egg} from RE. The partitioning of RE_{body} into protein and fat was calculated from nitrogen retention (NR = N intake – N excreted) to determine the total RE as protein (RE_{pt} = NR

× 6.25 gN/g Protein × 5.69 kcal/g Protein). The energy retained as fat (RE_{fat}) was calculated from the difference between RE and RE_{fat}. Finally, the RE_{body} was partitioned into $RE_{body\text{-}pt}$ (RE_{pt} - $RE_{egg\text{-}pt}$) and $RE_{body\text{-}fat}$ (RE_{fat} - $RE_{egg\text{-}fat}$).

Design of models

Linear and nonlinear mixed models were used to describe energy utilization by laying hens during the production phase. BW, BWG, EM and energy retention as total, body and eggs were used to determine the requirements for maintenance based on ME and NE (kcal/kg$^{0.75}$). For all models the allometric coefficient of 0.75 was used to determine metabolic BW to allow a direct comparison with other reports.

Model 1 was used to determine the ME_m using a simple linear regression model of RE as a function of AME_i as proposed by Farrell (1974).

$$\mathrm{RE} = a + b\mathrm{AMEi} + \varepsilon \text{ (Eq. 5)}$$

Where the metabolizable energy for maintenance (ME_m) can be estimated by the ratio between *a* and *b* (RE=0).

Model 2 used multiple linear regression to determine the energy retention efficiencies in the body and egg based on the energy difference between ME and ME_m.

$$\mathrm{ME} - \mathrm{ME}_m = \frac{1}{k_{body}} \times \mathrm{RE}_{\mathrm{body}} + \frac{1}{k_{egg}} \times \mathrm{RE}_{\mathrm{egg}} + \varepsilon \text{ (Eq. 6)}$$

In this model, k_{body} and k_{egg} represent the energy cost for retaining 1 kcal of energy as body and egg, respectively.

In model 3, heat production was calculated using a simple linear regression by its logarithmic relationship with AME_i as proposed by Lofgreen & Garret, (1968).

$$\log_{10} \mathrm{THP} = a + b \times \mathrm{AME}_i + \varepsilon \text{ (Eq. 7)}$$

The net energy for maintenance (NE_m) was assumed to be the intercept of *a*.

In model 4 a nonlinear mixed model was used to describe NEi utilization by using the parameters *a, c,* and *d* to express the energy required for maintenance, weight gain, and production, respectively.

$$\mathrm{NE}_i = a \times \mathrm{BW}^{0.75} + c \times \mathrm{BWG} + d \times \mathrm{EM} + \varepsilon;\ \mathrm{NE}_i \sim \mathrm{N}\ (\mu, \sigma^2) \text{ (Eq. 8)}$$

For the MEi (model 5), additional parameters were included to estimate the efficiencies of utilization for maintenance (k_m), weight gain (k_{body}), and production (k_{eeg}).

$$\mathrm{ME}_i = a\frac{1}{k_m}\mathrm{BW}^{0.75} + c\frac{1}{k_{body}}\mathrm{BWG} + d\frac{1}{k_{egg}}\mathrm{EM} + \varepsilon;\ \mathrm{ME}_i \sim \mathrm{N}\ (\mu, \sigma^2)(\mathrm{Eq.}\,9)$$

The predictability of the models was subjected through residual size comparisons. The models were compared by assessing their accuracy in predicting the kcal/bird/day energy requirements using ME or NE. Each model was evaluated using the root mean square errors (RMSE), which was calculated through the comparison of observed and estimated values using the following equation.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(Y_{obs} - Y_{pred})^2}{n}}$$

Statistical analysis

Before the model construction, the dependence between variables was tested using Pearson correlation between the performance and energy metabolism parameters. This analysis was performed using the PROC CORR procedures. Additionally, it was fitted using the PROC REG to perform the linear regression analysis to estimate the parameters of Eq. 5, 6 and 7. On the other hand the nonlinear mixed-effects models were fitted using the PROC NLMIXED procedure from SAS Institute (SAS Systems Inc., Cary, NC).

The parameters adjusted in the models were considered statistically significant when the P<0.05.

Results

A total of 10 observations were removed from the 120 total measurements due to low FI or technical issues in the respirometry chambers. The results of performance and energy metabolism variables with their respective average, minimum, maximum, and standard deviation values are shown in (Table 2). The variables of performance, BW, FI, EM and BWG varied from 1.48 to 1.69 kg, 0.088 to 0.124 g/b×d, 50.33 to 65.02 g/b×d and -13.10 to 11.90 g/b×d respectively. Also, the variables of energy metabolism response of AMEi, THP, FHP and RE varied from 214.56 to 301.01 kcal/b×d, 120.30 to 139.75 kcal/kg$^{0.75}$×d, 63.37 to 84.34 kcal/kg$^{0.75}$×d and 56.93 to 106.28 kcal/kg$^{0.75}$×d respectively.

The Pearson correlation was obtained to determine the degree of linear relationship between the main variables included in the models (Table 3). A high degree of variability in nutrient partitioning response was achieved exclusively through the variation of the chemical composition of experimental diets, without the restricted feed intake. A positive correlation between daily feed intake and AME_i (r=0.48; P<0.001) showed considerable variation. FI ranged from 0.088 to 0.124 (kg/bird×d), and AME_i from 214.56 to 301.01 kcal/b×d. These results were favorable for observing energy retention in birds as this is the main parameter for constructing ME and NE models.

The correlation between AME_i and RE, as well as AMEi and RE_{body}, were r=0.95 (P<0.001) and r=0.77 (P<0.001) respectively. These results indicate a strong degree of linear association between these variables. The values of RE ranged from 46.93 to 106.28 kcal/kg$^{0.75}$×d, while RE_{body} values varied from -37.03 to 29.09 kcal/$^{kg0.75}$×d, indicating significant catabolism and anabolism in the laying hens. On the other hand, the different levels of AMEi showed no influence on RE_{egg}, as no correlation was observed between these variables (r=-0.06; P>0.05). The relationship between RE, RE_{body} and RE_{eggs} with AME_i is illustrated in Figure 1-A.

Exploring the relationship between AMEi and RE_{body} partitioning, a strong correlation between AMEi and $RE_{body\text{-}fat}$ is evident (r=0.78; P<0.001). However, no such correlation is observed between AMEi and $RE_{body\text{-}pt}$ (r=0.12; P>0.05). Similarly, the components of RE_{egg}: $RE_{egg\text{-}fat}$ and $RE_{egg\text{-}pt}$, were unaffected by the varying levels of AME_i. Additionally, a significant correlation was observed between FHP and AME_i (r=-0.22; P<0.01), indicating that the different levels of metabolizable energy intake had a substantial effect on the maintenance of laying hens.

The estimated parameters obtained from the models for ME and NE energy requirements, based on the BW$^{0.75}$, BWG, EM and RE, are presented in Table 4. All the models and parameters showed significance (P<0.05). Using linear regression approach, model 1 shows that the values of ME_m (for RE=0) and FHP (for AMEi=0) are 106.41 and 82.47 kcal/kg$^{0.75}$×d, respectively, with an efficiency of AMEi utilization for energy retention of 77.5%. For model 2, a multiple linear regression was calculated using the difference between ME and ME_m as the dependent variable (y), and RE_{body} and RE_{egg} as independent variables. From the reciprocals of the coefficients, it was observed that birds deposit energy in the body with an efficiency of 84% and in the egg with an efficiency of 78% from the metabolizable energy for production (ME_p). In other words, this results in a cost of 1.186 and 1.283 kcal/b×d of metabolizable energy for each gram of energy deposited in the body and eggs, respectively.

In model 3, a simple linear regression between the logarithmic values of HP and AMEi was used to determine the FHP. According to Lofgreen and Garret (1968), extrapolating a linear regression model between HP and AME_i to an energy intake of zero results in a more accurate estimate of fasting heat production. The logarithm of the fasting heat production by birds is thereby found to be 1.956 ± 0.0306, in this case the antilogarithm ranges from 84.21 to 96.96 kcal/kg$^{0.75}$×d, with an average value of 90.36 kcal/ kg$^{0.75}$×d.

To describe the utilization of NE_i and ME_i, models 4 and 5 were used, respectively, using metabolic body weight, body weight gain and egg mass as variables to predict model parameters. The purpose of these models was to overcome the limitations of linear models, which have traditionally been employed as the classical method for energy partitioning in birds. By incorporating nonlinearity and accounting for random and fixed variability in the relationships between the independent and dependent variables, models 4 and 5 offer a more comprehensive approach. Determining the requirements of NE_i and ME_i using these models would be more appropriate, given the influence of multiple variables on the response, which is crucial in the context of energy partitioning.

All estimated parameters in models 4 and 5 showed significant differences ($P<0.05$). While in model 4, the value for parameter a which represents NE for maintenance, was estimated as 81.17 kcal/b×d, in model 5, a value of 94.15 kcal/b×d for ME_m was observed, resulting in an energy utilization efficiency for maintenance (km) of 0.862. Moreover, the assigned values for the body weight gain variable expressed as parameter c were 0.4169 and 0.901 in models 4 and 5, respectively, leading to a body energy deposition efficiency (k_{body}) of 0.463. Finally, the assigned values for egg mass, represented by parameter d were 1.512 and 2.37 in models 4 and 5, respectively, corresponding to an efficiency of egg energy deposition (k_{egg}) of 0.638. Furthermore, the predictive quality of the models was assessed using RMSE, with the NE prediction system showing higher accuracy (RMSE = 11.25) compared to the ME system (RMSE = 14.00).

Discussion

Based on the results obtained in this study, it becomes evident that the variation in metabolizable energy of the diets and the levels of energy intake affected THP, RE, and FHP, as a result of a strong correlation between these variables. The linearity between THP, RE, and AME_i has been well established and studied by several authors (Lofgreen and Garrett, 1968; Leeson et al., 1973; Reid et al., 1977; Luiting, 1990), suggesting that these variables are sensible to changes on MEi. However, even with the extensive studies about energy metabolism, some questions still persist, especially regarding the accurate measurements of FHP. Luiting (1990) suggested that FHP is an intrinsic characteristic of the animal and should be measured under specific conditions, such as fasting birds for at least 24 hours, in a stress-free and thermoneutral environment. In other studies, like the one reported by Burlacu and Baltec (1971), FHP was measured in birds subjected to different feed levels based on *ad libitum* intake. However, despite its widespread use, the approach of using feeding levels has received criticism due to the possible adaptation of the animal to a catabolic condition, which may not accurately represent the true energy expenditure of fasting, underestimating the real value of FHP (Koong 1977, 1982; De Lange et al., 2006; Labussière et al., 2011).

In the current study, FHP was measured using indirect calorimetry in laying hens fed *ad libitum* and previously adapted to the diets but subjected to a prior fasting period before measurement. This method is considered the most accurate for measuring FHP, according to Noblet et al., (2022). It can be supported by the RQ, a parameter with biological meaning related to the nutrient sources used (Gerrits et al., 2017), FHP was also predicted using classic energy partition models (models 1 and 3) and nonlinear mixed effect models where the NE_m was assigned to the coefficient *a* in model 4 (Table 4). The respirometry chamber trials results showed an average value of 75.05 kcal/kg$^{0.75}$, with a variation ranging from 63.37 to 84.34 kcal/kg$^{0.75}$ due to the diets (Table 2). For the regression models, the value obtained was 82.47

kcal/kg$^{0.75}$ for the regression between RE and AME$_i$ (model 1) and 90.27 kcal/kg$^{0.75}$ for the regression between logHP and AME$_i$ (model 3). The estimation from the regression depends on the dataset, and their variations, also, it should be closer that observed, as was shown in our study. These values agree with the variation reported in the literature, ranging from 69.28 kcal/kg$^{0.75}$ reported by Reid et al. (1977) to 90 kcal/kg$^{0.75}$ reported by Wu et al. (2016), using linear models. Model 3 showed a higher FHP value than the other models and the values obtained from the respirometry trials. This result may be related to what Noblet et al., (2022) defined as the relative energy utilization efficiency. In other words, the coefficient of energy utilization efficiency (*km*), represented by the slope of model 3, is not an absolute value, as it considers the efficiency of using dietary energy for maintenance and the efficiency of using energy from body reserves. In this sense, *km* represents the average of energy efficiency utilization by body and from the diets. Although tissue mobilization was observed in animals subjected to different experimental diets in this study, there was no feed intake restriction to determine the FHP. This model may explain the higher value obtained for this variable. Additionally, the independent regression of each component (HP and RE) from the ME$_i$ has a statistical implication since the extrapolation of each parameter presents their shelf error, and when this is integrated to predict the requirement by the factorial approach, the error is additive, inducing a higher bias (Romero et al., 2022).

Linear regression analysis between RE and AME$_i$ estimated the ME$_m$ by extrapolating RE=0 in model 1. The 106.41 kcal/kg$^{0.75}$ value for ME$_m$ obtained in this study is close to the results obtained by Waring and Brown (1965) who reported a value of 105.80 kcal/kg$^{0.75}$. The *km*, obtained through the slope of the equation, was 0.775. This value is close to that obtained by Barzegar et al. (2019), which was 0.75. However, the ME$_m$ obtained by Barzegar was higher (120 kcal/kg$^{0.75}$) than the one presented in the present study. The difference between ME$_m$ values can be explained by the approach adopted by Barzegar et al. (2019) who formulated diets with the same AME levels to minimize the effects of energy on feed intake.

Although the linear model is widely used due to its simplicity and a strong data fit (Birkett & De Lange, 2001), this model has been criticized for its limitation in expressing the accurate values of maintenance components. The energy expenditure associated with production can be portioned among several components, such as, RE$_{body}$ and RE$_{egg}$, as well as RE$_{protein}$ and RE$_{fat}$. Each of these components have a coefficient of efficiency associated, however, model 1 compromise the ME_m by standardizing the utilization efficiencies for all components (Emmans, 1995, Sakomura 2004). Furthermore, there is also the effect of multicollinearity due to the high correlation among the variables used as input in the model (Slinker and Glantz, 1985). In this regard, alternative approaches and methodologies should be considered to develop robust models that predict coefficients with higher accuracy, considering the main intrinsic factors that influence energy utilization.

The mixed-effects models have been used as an alternative to overcome the limitations of the linear model once it does not assume independence between the components of partitioning or collinearity. Additionally, this model considers important parameters that are correlated with the maintenance energy requirement of laying hens, such as body weight, daily body weight gain, and egg mass. The NE_m value obtained through the mixed model fitted in this study was found to be 81.72 kcal/kg$^{0.75}$×d (Table 4). This value is in accordance with the range of FHP obtained in the calorimetry trials. Moreover, it corresponds to the maintenance requirement of 82.46 kcal/kg$^{0.75}$×d reported by Sakomura (2004). The ME_m obtained by $NE_m \times 1/k_m$ was calculated as 94.15 kcal/kg$^{0.75}$×d, with k_m=0.84. This value is lower than those reported by Reid (1977) and Sakomura (2004), who reported values of 111 and 112 kcal/kg$^{0.75}$×d, respectively. The lower ME_m requirement observed in model 5 can be explained by the higher energy utilization efficiency (k_m=0.86) than the values reported in the literature, ranging from 62.35% (Reid, 1977) to 80.1% (Burlacu & Baltec, 1971). According to Azevedo et al. (2005) the estimates of ME_m were highly dependent on the approach and assumptions used to determine this variable. These authors reported higher and less accurate values for ME_m and k with the factorial approach than with multivariate analysis.

In addition, a linear relationship exists between AMEi levels and the retained energy in body. However, the same behaviour is not observed in energy retention in eggs (Table 3, Figure 1-A). This suggests that the energy used for egg formation is constant, regardless of the energy intake level (Chwalibog, 1991; Teofilo et al., 2023). It seems that laying hens adopt a hierarchical system for energy utilization, prioritizing the egg production, as suggested by Leeson and Summers (2009). Thus, to sustain metabolic work and egg laying the efficiency of energy utilization changes according to the nutritional composition of the diets, and energy intake. Excess or lack of energy triggers variations in body energy retention (Romero et al., 2009; Leeson and Summers, 2009; Zuidhof, 2019; Teofilo et al., 2023). Analysing the Pearson correlation in Table 3 and the graphical response between AMEi and body energy partitioning (Figure 1-B), evidencing that the energy intake have higher clear effect on the adipose than the protein tissue deposition. In other words, higher energy intake results in greater fat tissue retention, while mobilization of reserves is observed at lower energy intake levels. These variations are part of a fluctuating energy dynamism aimed at supporting egg production.

The energy utilization efficiencies for body and egg obtained by multiple linear regression (model 2) were 0.84 and 0.78 respectively. These values are slightly higher than those Barzegar et al. (2019) reported, who found values of 0.75 and 0.60 for k_{body} and k_{egg}, respectively (recalculated values). Analysing the same coefficients obtained from the mixed-effects model (model 5), a drastic reduction in the efficiency of these parameters can be observed (k_{body}=0.46 and k_{egg}=0.63). The obtained values are similar to those reported by Romero et al. (2009), who obtained k_{body}=0.49 and k_{egg}=0.66 using the same mixed-effects model approach for broiler breeder hens. These discrepancies in values found for the same parameters but analysed with different approaches was reported by van Milgen and Noblet (1999) in trials with growing pigs. According to these authors, using mixed-effects models allows simultaneous analysis of multiple dependent variables (RE_{body}, RE_{egg}) to determine AME_i (independent variable), which positively affects k. The analysis procedure using mixed-effects models allows considering that the energy utilization efficiencies in the body and egg are the results of the variation in energy intake, rather than the reverse, as assumed in the factorial approach. Unfortunately, information about using more complex models to explore the relationships between variables in the energy metabolism of laying hens is limited in the literature, making it challenging to compare NE_m, ME_m, and k coefficients. Also, the accuracy in determining the requirements using an integrated model that takes into consideration all energy parameters, can be helpful in estimating the energy requirement in different systems.

In conclusion, only through the variation in the nutritional composition of diets was it possible to determine the partitioning of energy between the body and egg, as well as the maintenance values of ME and NE, without subjecting the birds to feed intake restriction. Furthermore, the wide variation in AME_i has no effect on the REegg, which remains constant regardless of the diets. This suggests that laying hens prioritize the use of energy to maintain constant demand for egg formation. Additionally, both evaluated models provided estimates of ME and NE requirements, with the NE system offering more accurate information about energy utilization for laying hens. However, nonlinear mixed-effect models demonstrated a more precise fit than the linear models. These models considered the variation in body energy retention and egg production, improving the fit and reducing bias in the estimates. Thus, the nonlinear mixed-effect models proposed in this study proved to be more accurate than the linear models in terms of quantifying energy partitioning, estimating ME and NE requirements, and evaluating diets based on the estimated coefficients.

Ethics approval

This study was conducted at the Poultry Science Laboratory of the School of Agricultural and Veterinary Sciences, São Paulo State University (Unesp). All procedures were approved by the Animal Care and Use Committee of Unesp (CEUA, Protocol n° 5402/20).

Data and model availability statement

The data/models were not deposited in an official repository. Information can be made available from the authors upon request**.**

Declaration of generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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All authors read and approved the final manuscript.

Declaration of interest

We declare that we have no financial and personal relationships with other people or organizations that could inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Table 1

Composition and nutritional content of experimental diets (n=30).

Ingredients (%)	**Mean**	**Minimum**	**Maximum**	**SD**
Corn, Grain (7.86% CP)	42.73	1.13	68.80	18.11
Rice Bran	7.87	1.74	16.10	4.57
Sorghum Grain, Low Tannin	26.37	16.70	36.84	7.08
Millet, Grain	28.95	14.50	49.00	13.57
Soybean Meal (45% CP)	9.74	1.84	26.84	6.46
Peanut Meal	3.93	1.99	8.86	2.17
Corn, Gluten Meal (60% CP)	6.11	0.62	11.76	4.03
Soybean Protein Conc.	6.67	3.27	10.15	2.93
Meat and Bone Meal (48% CP)	6.75	2.37	9.52	2.13
Feather & Poultry by-product meal	2.80	2.00	5.00	1.30
Fish Meal (54% CP)	2.47	0.39	6.78	2.25
Oil, Soybean	2.50	0.08	7.00	2.07
Fat, Poultry	2.95	0.12	5.31	1.59
Canola Meal	3.23	2.10	4.00	0.70
Barley, Grain	9.50	3.00	15.00	4.93
Cottonseed Meal (39% CP)	5.22	0.75	8.23	2.46
Wheat Bran, Midds	12.84	1.45	18.80	6.21
Wheat, Grain	19.37	7.62	35.00	8.51
Inert	2.38	0.24	3.80	1.08
Limestone	10.55	9.03	11.00	0.58
Dicalcium Phosphate	1.19	0.00	1.76	0.53
Salt	0.10	0.02	0.26	0.06
Sodium Bicarbonate	0.59	0.36	0.96	0.14
Potassium Carbonate	0.28	0.00	1.01	0.24
L-Lysine HCl	0.47	0.00	0.73	0.14
DL-Methionine	0.41	0.20	0.57	0.08
L-Threonine	0.23	0.04	0.36	0.08
L-Tryptophan	0.06	0.00	0.12	0.03
Arginine	0.17	0.00	0.40	0.10
L-Valine	0.33	0.00	0.59	0.15
Isoleucine	0.21	0.00	0.37	0.09
Choline chloride (70%)	0.10	0.10	0.10	0.00
Mineral Premix[1]	0.10	0.10	0.10	0.00
Vitamins Premix[2]	0.10	0.10	0.10	0.00
Nutritional content				
ME[3], kcal/kg	2773	2419	3096	169.9
Crude Protein[3], %	17.64	12.88	22.75	2.02
Total Ca, %	4.55	4.51	4.74	0.07
AvP, %	0.41	0.36	0.55	0.05
Lysina SID, %	0.81	0.67	0.92	0.06
Methionine + Cystine SID, %	0.78	0.67	0.90	0.05

Threonine SID, %	0.61	0.49	0.71	0.05
Ether Extract[3] %	5.09	1.34	10.25	2.27
Crude Fiber [3] %	2.90	1.36	5.35	0.97
Starch [3] %	39.52	35.83	45.24	2.09
NE[3],kcal/kg	2029	1695	2432	147

SD: Standard deviation, **CP**: Crude protein, **Avp**: Available phosphorus, Content/kg of premix: [1]Iron: 22 g, Copper: 4500 mg, Manganese: 25 g, Zinc: 25 g, Iodine: 500 mg, Selenium: 125 mg; [2]Vit. A: 4,850,00 UI, Vit. D3: 1,350,000 UI, Vit.E: 8500 UI, Vit. K3: 1395 mg, Vit. B1: 1000 mg, Vit. B2: 2570 mg, Pantothenic acid: 5295 mg, Vit. B6: 1525 mg, Vit. B12: 7500 mcg, Niacin: 19.45 g, Folic acid: 500 mg, Biotin: 41.50 mg; [3]Nutrients assayed.

Table 2

Mean, minimum, and maximum values of performance, calorimetry parameters, and retained energy of laying hens.

Variable[1]	Mean	Minimum	Maximum	SD
Performance				
BW (kg)	1.60	1.48	1.69	0.05
Feed intake (g/b*d)	0.105	0.088	0.124	0.01
Egg mass (g/b*d)	58.19	50.33	65.02	3.48
Body weight gain (g/b*d)	-0.36	-13.10	11.90	5.01
ME intake (kcal/b*d)	249.89	214.56	301.01	17.25
ME intake (kcal/$kg^{0.75}$*d)	208.25	177.83	238.96	14.32
Calorimetry parameters (kcal/$kg^{0.75}$*d)				
THP	129.29	120.30	139.75	4.64
FHP	75.06	63.37	84.34	4.68
HI	54.22	42.50	68.28	5.52
RQ	1.006	0.923	1.070	0.025
Retained energy (kcal/$kg^{0.75}$*d)				
Total	78.96	56.93	106.28	11.65
As protein	40.49	28.73	57.91	5.54
As fat	38.47	15.87	69.46	12.36
Egg	79.84	58.78	101.92	8.11
Body	-0.88	-37.03	29.89	14.95
Body as fat	-9.57	-39.15	29.40	14.42
Body as protein	8.96	-3.55	26.60	6.03

[1]The value of each variable represents the average of six birds. **BW**: Body weight, Egg mass: Weight of eggs laid calculated as the total weight of eggs/number of days; **THP**: Total heat production, **FHP**: Fasting Heat production, **HI**: Heat increment, **SD**: Standard deviation.

Table 3

Paired Pearson Correlation between variables (kcal/kg$^{0.75}$*d)

		FI	AME	THP	FHP	RE	RE_{Body}	RE_{egg}	EM	$RE_{Body\text{-}Ptn}$
AME	R	0.471***								
THP	R	0.396***	0.683***							
FHP	R	-0.077 *ns*	-0.228*	0.301**						
RE	R	0.420***	0.955***	0.437***	-0.403***					
RE_{Body}	R	0.390***	0.778***	0.290**	-0.450***	0.841***				
RE_{egg}	R	-0.096 *ns*	-0.019 *ns*	0.112 *ns*	0.233*	-0.069 *ns*	-0.597***			
EM	R	0.273**	0.474***	0.394***	-0.085 *ns*	0.425***	0.072 *ns*	0.496***		
$RE_{Body\text{-}Ptn}$	R	0.375***	0.107 *ns*	-0.087 *ns*	-0.287**	0.168 *ns*	0.395***	-0.480***	-0.120 *ns*	
$RE_{Body\text{-}Fat}$	R	0.220**	0.785***	0.343***	-0.340**	0.828***	0.904***	-0.439***	0.143 *ns*	0.015 *ns*

FI: Feed intake; **AME**: Apparent metabolizable energy; **THP**: Total heat production; **FHP**: Fasting heat production; **RE**: Total retained energy; **RE_{body}**: Retained energy in body; **RE_{egg}**: Retained energy in eggs; **EM**: Egg mass; **$RE_{body\text{-}Pt}$**: Retained energy in body as protein; **$RE_{body\text{-}Fat}$**: Retained energy in body as fat. Pearson correlation significance level expressed as *(P<0.05), **(P<0.01), ***(P<0.001), and *ns* is non-significative.

Table 4

Parameters of metabolizable and net energy utilization models

Model	Parameters	Estimated	SE	t-value	P-value	RMSE
1	$\mathrm{RE} = a + b\mathrm{AMEi}$					35.84
	a	-82.47	4.824	-17.1	<0.001	
	b	0.775	0.023	33.62	<0.001	
2	$\mathrm{ME} - \mathrm{MEm} = \frac{1}{k_{body}}\mathrm{RE}_{\mathrm{body}} + \frac{1}{k_{egg}}\mathrm{RE}_{\mathrm{egg}}$					35.91
	k_{body}	1.186	0.026	42.57	<0.001	
	k_{egg}	1.283	0.005	257.1	<0.001	
3	$\mathrm{logTHP} = a + b\mathrm{AMEi}$					43.38
	a	1.956	0.0161	121.39	<0.0001	
	b	0.00075	0.0001	9.70	<0.0001	
4	$\mathrm{NEi} = a\mathrm{BW}^{0.75} + c\mathrm{BWG} + d\mathrm{EM}$					11.25
	a	81.172	13.116	6.19	<0.001	
	c	0.4169	0.209	2.00	0.048	
	d	1.5127	0.271	5.59	<0.001	
5	$\mathrm{MEi} = 81.17\frac{1}{k_m}\mathrm{BW}^{0.75} + 0.416\frac{1}{k_{body}}\mathrm{BWG} + 1.512\frac{1}{k_{egg}}\mathrm{EM}$					14.00
	km	0.862	0.151	5.710	<0.001	
	k_{body}	0.463	0.136	3.390	0.001	
	k_{egg}	0.638	0.092	6.960	<0.001	

a is the estimated parameter per unit for maintenance (kcal/kg0.75), ***b*** is the energy efficiency for AMEi utilization, ***c*** and ***d*** are the estimated parameters for weight gain (kcal/g*d) and egg production (kcal/g egg mass) respectively, ***km*** is the efficiency of energy utilization for maintenance, ***kbody*** is the efficiency of tissue deposition for body weight gain for the ME system, ***kegg*** is the efficiency for egg production, **RE**: Retained energy, **AMEi**: Metabolizable energy intake, **ME**: Metabolizable energy; **MEm**: Metabolizable energy for maintenance, **$\mathbf{RE_{body}}$**: Retained energy in body, **$\mathbf{RE_{egg}}$**: Retained energy in eggs, T**HP**: Total heat production; **BW**: Body weight, **BWG**: Daily body weight gain, **EM**: Egg mass; **SE**: Standard error; **RMSE**: root mean square errors.

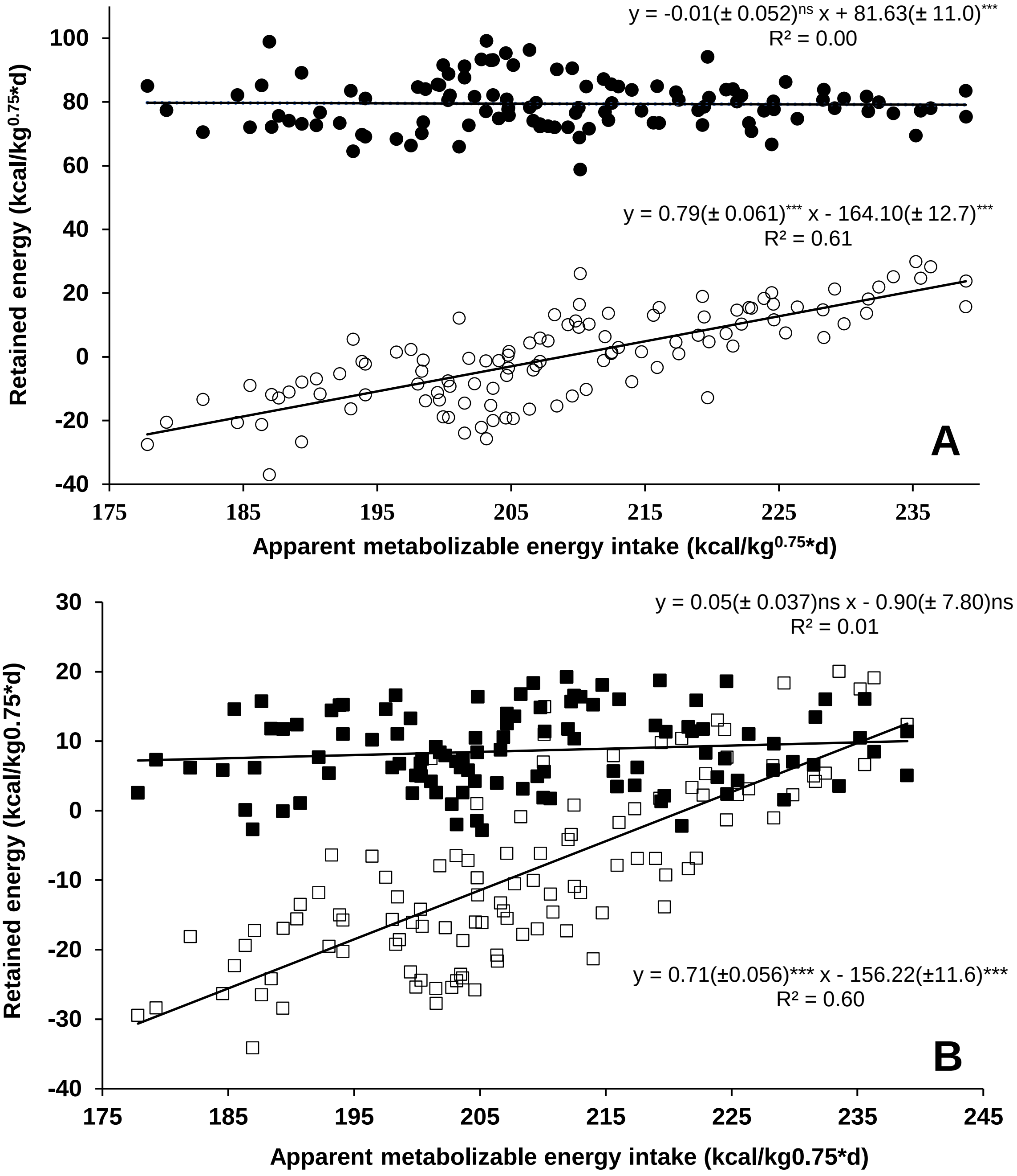

Figure 1. Dispersion plots are used to compare the relation between the retained energy and apparent metabolizable energy. **(A)** The relation between the intake of metabolizable energy and the simultaneous retention in the body and eggs (n=110), ○ Retained energy in body, ● Retained energy in eggs. **(B)** The relation between the intake of metabolizable energy and the simultaneous retention in the body as protein and fat energy (n=110), □ Retained energy in body as fat, ■ Retained energy in body as protein. Linear regression significance level expressed as *(P<0.05), **(P<0.01), ***(P<0.001), and *ns* is non-significative.

CAPÍTULO 3: NET ENERGY PREDICTION EQUATIONS OF FEEDSTUFFS FOR LAYING HENS

This chapter was written in the form of international scientific paper according to the standards Animal Journal

METABOLISM AND NUTRITION

Net energy prediction equations of feedstuffs for laying hens

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Abstract

The net energy (NE) system provides a more precise way to express the energy content of feed and the energy requirements of birds. However, most of the available data in this area has been developed for swine and broilers, making it challenging for this system to be successfully applied to laying hens. Therefore, the aim with this study was to develop prediction equations of NE for laying hens during the production phase. Multiple linear regression models were fitted based on the nutritional composition and metabolizable energy (ME) of thirty-two diets. Gas exchanging measurements (VO_2 and VCO_2) and heat production were measured in Hy-line W80 laying hens using an open-circuit respirometric chambers. Employing a completely randomized design, each diet was assigned to a chamber and replicate four times, with six birds per replication. Birds were previously adapted to the experimental diets and respirometric chambers for five and two days, respectively, before data collection. Daily measurements of feed intake (FI), excreta production, individual body weight (BW), egg mass (EM), and total heat production (THP), were collected over a four-day period. Fasting heat production (FHP) was measured for 24 hours after the last day of data collection. NE values ranged from 1725 to 2424 kcal/kg, and the efficiency of energy utilization of diets (NE/AME) ranged from 69 to 79%. The results demonstrated that AME and NE equations can be predicted based on the nutritional composition of the diets. Prediction equations for AME and AMEn were positively related to ether extract (EE) (41.20; 40.17 kcal/kg) and starch (11.53; 10.55 kcal/kg), while crude protein (CP) showed no significant effect in the model ($P>0.05$). The fitted model for NE revealed a significant ($P<0.05$) and positive effect for AME (0.765), AMEn (0.779), and EE (18.24; 18.65 kcal/kg) for this system. However, CP was negatively related to NE (-8.95; -6.35 kcal/kg), while starch showed no significance in the model ($P>0.05$). The NE equations developed in this study exhibited a low residual standard deviation (RSD), indicating a high level of reliability of the adjusted coefficients.

Keywords: Energy metabolism, indirect calorimetry, heat production, fasting heat production.

Introduction

Providing the appropriate nutrients and energy for laying hens is a crucial strategy to ensure a proper development and improve the efficiency of production and better resources utilization. Specifically, the dietary energy requires a special attention, as it is used for maintenance, growth, and egg production. It is widely known that the feed energy content is the main driver of feed intake in laying hens, impacting directly on the performance. (Noblet., 1994; Kong & Adeola., 2014). Additionally, the energy content accounts for a significant portion of the cost of poultry production, representing approximately 70% of the total feeding cost. (van der Klis et al., 2010). This high cost is mainly related to the variability in prices of high-energy ingredients caused by a rising demand for energy yielding commodities (Yu et al., 2006; Harri et al., 2009, Wu et al. 2019). Therefore, accurate estimations of the available energy values in the ingredients are crucial for enhancing animal performance and improving the profitability over feeding cost (De Groote, 1974; Carré et al., 2014).

The poultry industry has predominantly relied on metabolizable energy (ME), and its fraction corrected for zero nitrogen retention (MEn) as the main energy system. For many years, ME and MEn have been the basis for feed formulation, mostly due to the relative constancy of the values and also because this system express the energy of the bird's requirements and nutrient content on the same basis (Reid et al., 1977; Lopez & Leeson., 2008). Despite the ME system providing estimates of energy with acceptably low variability and repeatability, this system does not explain the bird's energy utilization, as it does not explain the partitioning of energy into all components. Additionally, modern laying hen strains are highly productive due to advances in genetics, and consequently these birds present a high dynamic of energy utilization to support a higher levels of egg production requiring a more precise formulation techniques, particularly in terms of energy levels (Pirgozliev e Rose., 1999; Gous, 2007; Sakomura et al., 2019). Formulating diets for poultry based on net energy (NE) may be a potential alternative to represent in a more relevant way the energy utilization from the feed according to the productive levels (Wu et al., 2019).

A precise description and understanding of the metabolic pathway of nutrients and how it is expressed in terms of energy utilization, considering its partitioning as maintenance (i.e., fasting heat production), the dynamic of tissue retention and mobilization (protein and fat), and production (eggs), can be achieved through the NE system. The accuracy associated with this system is related to its consideration of all components of energy loss, including the heat increment (HI), often seen as a measure of system inefficiency, since this energy fraction is not utilized for production (Birkett et al., 2001). In previous studies by De Groote et al. (1994), improved feed efficiency in broilers was demonstrated using the NE system, suggesting an economic advantage when considering precisely the energy utilization that comes from the nutrients. In addition, Pirgozliev & Rose (1999) and Carré et al. (2014), through their analysis of different ingredients with a wide range of energy levels, demonstrated that the ME system tends to overestimate ingredients with high protein content, while ingredients rich in fat are underestimated in comparison to carbohydrates. The application of these concept has yielded the development of the practical models and equations for prediction the NE of feed and feedstuffs. These models aim to estimate the energy derived from the nutritional composition, offering a more accurate evaluation of the available energy content on the diet than can be efficiently used by the animals, as opposed to the traditional ME system. This advancement enhances our ability to accurately optimized the feed energy utilization (Schiemann et al., 1972; Just, 1982; Noblet et al., 1994).

In this context, NE represents the most readily available portion of energy in the diet, offering a potential solution to overcome the limitations of the current ME system. Moreover, this approach finds an extensive application in the formulation of diets for cattle and pigs. (Ferrell and Oltjen, 2008; Noblet et al., 2010). Thus, several authors have developed models to predict NE for poultry, aiming to incorporate this system into poultry nutrition (Schiemann et al., 1972; Hoffmann & Klein., 1980; Swick et al., 2013; Carré et al., 2014; Barzegar et al. 2019; Wu et al., 2019; Cerrate et al., 2019). Nevertheless, the majority of these studies have focused on broilers and broiler breeders. Therefore, there is still a considerable variability in the NE values derived from these studies, making it challenging their potential application in the nutrition of laying hens. A previous study developed by Barzegar et al. (2019) indicated that laying hens exhibit greater sensitivity to variations in fat and protein levels in the diet, suggesting that formulating feed based on NE would bring greater benefits for laying hens compared to broilers. Despite this, there is still limited data for the implementation of the NE system for laying hens (Chudy et al., 2003; Sakomura, 2004; Sakomura et al., 2005).

This present study aims to propose equations for predicting net energy for laying hens during the production phase, fed with 32 diets with a wide variation of chemical composition.

Material and Methods

Birds and Experimental Design

A total of 288 Hy-line W80 laying hens were obtained at 19 weeks of age from a local commercial production (Granja Mayra, Minas Gerais, Brazil) and allocated in a climate-controlled poultry house with temperature and humidity control. Birds were randomly allocated in individual cages (0.20 m × 20 m × 10 m) equipped with feeders and nipple drinkers.

The trial was divided into the pre-experimental and experimental period. During the pre-experimental period, birds were fed with a standard commercial diet based on corn and soybean meal, formulated to meet the nutritional recommendation of the genetic guidelines (Hy-line W80, 2019). Water and feed were provided *ad libitum* throughout the entire experiment period, the lighting program adopted was to provide 16L:8D following the guidelines recommendation.

When the birds reached sexual maturity (more than 50% of egg production) and peak of egg production (around 24-old-wks), they were distributed under a completely randomized design and divided in different batches (assay) of allocation to determine the net energy content of 32 diets. The different batches of bird’s allocation to the experiment are needed since the respirometry laboratory is equipped with six chambers. For this reason, the measurements were performed by grouping six experimental diets per assay. Six diets were randomly selected for each assay to compose a group (1 diet per chamber) replicating each diet four times. Before the measurement, 36 laying hens were selected from the initial population (288) based on average body weight (BW) and egg production and divided into six groups. Each group was allocated in cages (0.60 m × 0.20 m × 0.10 m), and the experimental diets were randomly assigned to each group (6 birds/diet). Birds were fed with the experimental diets by five days to allow diet adaptation. After this period, the birds were transferred to the respirometry laboratory and acclimatized in the chambers for two days. Afterwards, the data collection began under feeding conditions with the respective experiment diet by four days. On the last day, the feed was withdrawn, and the gas exchange measurements were conducted under fasting conditions for 24 hours.

Experimental Diets

A total of 32 experimental diets were formulated to have a wide variation in the nutrient composition and metabolizable energy (ME) value (Table 1). Additionally, a criterion of feed formulation was adopted considering the same ratio between the digestible lysine and energy (Lys SID: AME = 2.95%). The experimental diets were formulated using traditional and non-traditional ingredients to provide flexibility in the nutritional composition and allow a wide range of energy intake to be evaluated between experimental diets. The variation in the nutritional composition of the diets, on a dry matter basis, ranged from 2441 to 3096 kcal/kg of apparent metabolizable energy (AME), 11.50 to 19.75% crude protein (CP), 2.99 to 9.97% ether extract (EE), 1.77 to 4.02% crude fiber (CF) and 32.00 to 47.00% starch. The high variation between dietary nutrients was established with the objective of facilitating the application of statistical procedure to achieve either no or minimal correlation among predictors (nutrients). This was done to reduce the effects of multicollinearity and enhance the likelihood of developing robust net energy prediction equations.

The diet formulation was made based on the energy and nutritional composition of the ingredients as described on the Brazilian Tables of Poultry and Swine (Rostagno et al., 2017). Additionally, crystalline amino acids were supplemented to the diets to correct the balance between AME and amino acids, thus ensuring that the variation in feed intake did not limit amino acid intake. The concentration of Ca (4.55%) and available P (0.41%) were attended according to the management guide (Hy-line W80, 2019).

Respiratory Chambers and Gas Analyzer System

Six open-circuit respirometric chambers (0.90m x 0.85m x 0.80m) with temperature and humidity automatic controllers were used in this trial. The respirometry system consisted of a mass flow pump (FK-100; Sable System, USA) that introduce atmospheric air into the chamber under negative pressure at a flow rate of 12 L/min to maintain the CO_2 concentration below 1% (Lighton, 2018). 160 mL/min air sample was constantly extracted by a pull-mode sub-sample pump (SS4; Sable System, USA) and flowed through the analyzer system. The air analyzers consisted of the water vapor pressure analyzer (RH -300; Sable System, USA), and scrubbing columns with 99.5% of calcium sulfate ($CaSO_4$) to capture the water vapor molecules (Drierite, W. A. Hammond Drierite Co., LTD). Subsequently, the dry air was analyzed by an infrared analyzer of CO_2 (CA-10A; Sable System, TX. USA) and a paramagnetic analyzer of O_2 (PA- 10; Sable System, USA). The flowmeter and analyzer signals were coded by a universal interface (UI-2; Sable System, USA) and saved one record per second using ExpeData software (version 1.9.22, Sable Systems, TX. USA).

Gas sampling was programmed from each chamber and baseline using a multiplexer (MUX RM-8; Sable System, TX. USA) in an intermittent sequence: initial baseline (180 seconds), chambers (540 seconds per chamber), and final baseline (180 seconds). This procedure was repeated in a loop for 24 hours per day. At the beginning and at the end of each data collection the gas analyzer was calibrated with concentrated gas (>99.99% N_2) and a certified gas mixture (21% O_2 and 1% CO_2).

Performance and Heat Production Calculations

Body weight (BW), feed intake (FI), egg mass (EM) and laying were recorded daily. Additionally, total excreta collection was performed during the feeding phase (4 days) to determine apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen (AMEn). The egg mass (EM) was calculated from the average egg weight multiplied by the laying rate (%) for 10 days (adaptation period and measurement period).

The calculations employed in this study were based on the methods described by Lighton (2018) and Lizana et al. (2023). The air ingoing flow (F_{in}), volumetric O_2 consumption (VO_2, L/b*d), and CO_2 production (VCO_2, L/b*d) were calculated was:

$$F_{in}(L/d)=(F_{out}\times\frac{(100-[O_2]_{out}-[CO_2]_{out})}{(100-[O_2]_{in}-[CO_2]_{in})}\times 1.440 \quad \text{(Eq. 1)}$$

$$VO_2(L/d)= F_{in}\times[O_2]_{in}-F_{out}\times[O_2]_{out} \quad \text{(Eq. 2)}$$

$$VCO_2(L/d)= F_{out}\times[CO_2]_{out}-F_{in}\times[CO_2]_{in} \quad \text{(Eq. 3)}$$

Where F_{out} is the air outgoing flow, $[O_2]_{in}$ and $[CO_2]_{in}$ are the atmospheric gas concentrations or baseline.

The total heat production (THP) and fasting heat production (FHP) were calculated using the Brouwer equation (1965):

$$HP\ (kJ/d)=16.17\times VO_2+5.02\times VCO_2 \qquad \text{(Eq. 4)}$$

The FHP was measured on the last day of data collection, over a 24-hour of fasting period, following eleven days in which the birds consumed the experimental diets. Only values within the plateau observed in the asymptote of FHP were used, as this region is identified as the minimum HP with zero physical activity, corresponding to the most accurate value of FHP as suggested by Milgen et al. (1998).

Chemical Analysis and Calculations

Samples of eggs, feed and excreta were collected and homogenized for laboratory analysis. All samples were subjected to dry matter content determination through forced air oven drying at 105˚C for 16 hours. In eggs, the dry matter was determined after 72 hours of lyophilization. The nitrogen content of the samples was measured using the Kjeldahl method (AOAC, 2005), and the crude protein content was calculated using the coefficient of 6.25 g of crude protein per g of nitrogen. The ether extract of the feeds and excreta was determined through extraction with petroleum ether (ANKOM Technology® apparatus) and, the gross energy was obtained by the total combustion of samples using the adiabatic calorimeter (IKA C 2000 basic, USA).

The AME was determined using total collection of excreta. The AME values were converted to AMEn using the nitrogen balance and the coefficient 8.22 kcal/g of N as a correction factor (Hill and Anderson, 1958) for zero N retention in body and eggs. The intake of apparent metabolizable energy (AMEi) was determined by multiplying the difference between the gross energy in the feed and excreta by the respective intake and excreta production values. The total energy retention (RE) was determined by the difference between AMEi and total heat production (THP), while the NE values of the experimental diets were obtained through the sum of FHP and RE.

Statistical analysis

The data of performance and energy balance were expressed on a bird per-day basis. Feed intake and dietary energy values of the diets were expressed on a dry matter basis, and energy utilization values were expressed as percentages. All data were analyzed using software SAS (SAS Systems Inc., Cary, NC).

Pearson correlation analysis was performed using the PROC CORR procedure between nutrients of diets. The formulation process of experimental diets was conducted independently to ensure the absence or minimal correlation between predictor nutrients in the equations, thus, reducing the effect of multicollinearity among variables in the model.

Multiple linear regressions were applied to create prediction equations of metabolizable and net energy through the PROC REG procedure. These models were developed considering the chemical components of the variables and employing a stepwise approach, with or without intercept. The significance level for variables inclusion or exclusion in the model was established at P<0.05. The regression model was as follows:

$$\mathrm{y} = \beta 0 + \beta 1\mathrm{x}1 + \beta 2\mathrm{x}2 + \ldots + \beta \mathrm{nxn} + \varepsilon$$

Where *x1, x2 ... xn* are the predictor nutrients of the equations, *β1, β2, …βn* are the coefficients of the respective nutrients, and *ε* is the corresponding error of the model.

The predictability of the models was subjected through residual size comparisons. The models were compared by assessing their accuracy in predicting energy values in kcal/kg of AME and NE based on the nutritional composition of the diets. Each model was evaluated using the root mean square errors (RMSE), which was calculated through the comparison of observed and estimated values using the following equation.

$$RSD = \sqrt{\frac{(Y_{obs} - Y_{pred})^2}{n}}$$

Internal and external model evaluation

The NE models were evaluated internally and externally. The internal validation was performed to determine the robustness of the models using the bootstrapping resampling method from the initial database (32 diets with four replicates), generating one thousand sub-sample using a Macro procedure developed in SAS Institute (SAS Systems Inc., Cary, NC). The macro was constructed to classify the dataset into training and validation. Subsequently, the parameters of the independent variables were estimated for each sub-samples, and the R-square, standard deviation (SD) and confidence interval (CI) of the whole sub samples were used as a criterion to evaluate the models.

On the other hand, external evaluation was conducted using the analysed composition of diets and the net energy values available from the study developed by Barzegar et al. (2019). Sixteen diets with a wide range of nutritional composition were used. Models were fitted, and the predicted NE values were compared to the observed NE values by Barzegar et al. (2019). The model adjustments, and the error decomposition, are presented in Figure 1.

Results

The results of performance and energy metabolism variables with their respective average, minimum, maximum, and standard error of means values are presented in table 2. The wide variation in the nutritional composition of the diets affected significantly ($P<0.005$) all the traits studied, except laying production. The average of ME in experimental diets was 2746 kcal/kg DM ranging between 2441 to 3096 kcal/kg DM. This range led to variations of 1.29 to 1.69 kg in body weight (BW), 69.16 to 111.17 g/DM in feed intake (FI) and 50.33 and 69.64 g in egg mass (EM). The metabolizability of energy (AME/GE) was 79% ranging from 70 and 86%. Diets with higher starch values are correlated with grater energy efficiency of utilization. Levels above 45% of starch in the diets resulted in values greater than 80% for AME/GE. The obtained values of AMEn/GE were slightly lower than those observed for AME/GE, with an average of 76% and a range between 67% and 82%.

The different levels of ME (kcal/kg DM) between experimental diets resulted in a range of 166.73 to 268.67 kcal/(kg$^{0.75}$×d) of AME intake, which had a significantly effect on total heat production (THP), heat increment (HI) and retained energy (RE). The variation in THP ranged from 120.30 to 144.25 kcal/(kg$^{0.75}$×d), while the variation of HI and RE ranged from 42.50 to 72.13 kcal/(kg$^{0.75}$×d) and 43.54 to 128.92 kcal/(kg$^{0.75}$×d), respectively. Birds with higher AME intake were associated with higher levels of total retained energy, while those with the lowest AME intake (166.73 kcal/(kg$^{0.75}$×d)) showed the highest degree of body fat energy mobilization (-56.76 kcal/(kg$^{0.75}$×d)), evidenced by the negative value. On the other hand, the variation in AME intake did not affect the energy retained in the eggs. Similarly, the composition of the diets had an effect in the metabolic heat production. Higher levels of protein were associated with higher levels of THP, while diets with higher levels of EE are correlated with lower HI. In this study, the high-fat (10.25% of EE) and high-protein diets (22.75% of CP) were correlated for the lowest and highest HI values, respectively. Additionally, the respiratory quotient (RQ) ranged from 0.923 to 1.071 among the experimental diets. It was observed that diets with higher levels of EE (>5%) exhibited lower RQ values, whereas high-starch diets (>45%) were associated with higher RQ values.

Pearson correlation analysis was proceeded to evaluate the relationship between the analyzed nutritional components and the determined AME of the experimental diets. The results obtained are presented in Table 3. There was no shown significant correlation between ME, CP and Starch. On the other hand, ME was negatively correlated with CF ($R=-0.662$, $P<0.01$), NDF ($R=-0.666$, $P<0.01$), and positively correlated with EE ($R=0.563$, $P<0.01$). Furthermore, a high positive correlation was observed between CF and NDF ($R=0.840$, $P<0.01$). These correlations were expected since the interactions between these nutrients are inevitable and logical. The correlation between predictor variables in a model is related to the effects of multicollinearity, which can make it challenging to determine the individual effect of each variable in the dependent variable. However, in this study, the Pearson correlation analysis demonstrated an absence of correlation between the main nutrients used for predicting NE, indicating the feasibility to perform the multiple analyses regression for NE prediction equations.

The net energy values ranged from 1725 to 2424 kcal/kg DM, with an average of 2033 kcal/kg DM. The average of energy utilization efficiencies was 74 and 77 for NE/AME and NE/AMEn, respectively. The efficiency of converting AMEn to NE was found to be higher than that of AME to NE, mainly because of the correction for the nitrogen balance, assuming zero nitrogen retention coming from the diet. Lower NE/AME efficiencies were observed in diets with lower levels of EE and high CP levels, while higher NE/AME efficiencies were shown in diets with EE levels exceeding 8% in the formulation and high starch content. Additionally, the NE/AME ratio was associated with higher values of AME and energy retention in the body. A higher NE/AME ratio (79%) also resulted in greater egg mass (69.64 g) and higher body energy retention (36.12 kcal/kg$^{0.75}$*d).

From the individual energy values of the main nutrients used in diet formulation, it was possible to determine their contributions to GE, AME, AMEn and NE. Multiple linear regressions without an intercept was employed to determine the caloric contribution of CP, EE, Starch, and RES to each energy system. The residue (RES) was determined through the portioning of organic matter (OM) and subtracting the fractions of CP, EE, and starch. Consequently, the RES component in this study can be predominantly described as crude fiber and simple sugars. The coefficient estimated of these analyses are presented in table 4. The ratios between AME and GE coefficients for each nutrient in the equations provide an estimate of the metabolizability (including the rate of digestibility of the nutrients in energy terms), while the ratios between NE and AME coefficients provide an estimate of AME efficiency for NE of each nutrient. The coefficients associated with CP showed a reduction from 57.55 for GE to 32.69 for AME, and then to 16.32 for NE, representing a reduction in energy utilization efficiency between AME/GE (56.80%) and NE/AME (49.92%). Similarly, the coefficients associated with the metabolizability and energy efficiency of starch, exhibited a similar behavior, being 95.87% and 74.05% for AME/GE and NE/AME, respectively. On the other hand, the values obtained for the coefficient of EE for NE were slightly higher than those obtained for AME, being 76.16% and 74.09%, respectively. Thus, the efficiency utilization of EE for AME/GE was 80.51% and 102.79% for NE/AME. Similarly, RES had higher coefficient for energy efficiency (NE/AME) than for metabolizability (AME/GE), being 83.23% and 71.87%, respectively. Furthermore, the coefficients associated with AMEn were slightly lower when compared to AME, except for RES, where the values were rather similar.

Prediction equations for metabolizability (AME/GE) and energy efficiency (NE/AME) were determined through multiple linear regressions using the stepwise criteria to determine the best independent variables. Coefficients and intercepts that showed significance ($P<0.05$) were assigned to the models and can be observed in Table 5. The results obtained suggest that the AME/GE ratio is negatively associated with CP and positively associated with starch. Additionally, the coefficients of CP and starch for AMEn/GE prediction equation have a similar behavior to those in the AME/GE equation but with slightly higher values for CP due to the correction for zero nitrogen. Furthermore, there is a difference in the intercepts between the two equations, with AME/GE having a higher intercept (67.34) compared to AMEn/GE (62.90). On the other hands, starch did not exhibit a statistical significance ($P>0.05$) as a predictor in the efficiency equations, while EE was assigned to the equations with a positive relationship with both, NE/AME and NE/AMEn. The CP, as seen in the metabolizability prediction equations, showed a negative effect on NE/AME, while the intercept values were 77.11 for NE/AME and 79.03 for NE/AMEn.

Crude and digestible nutrients were used as predictors in the equations to determine the values of AME, AMEn and NE of the diets. The statistical procedure used was the same as previously described, and the results obtained can be observed in Table 6. The coefficients associated for EE using crude nutrients were positively associated to AME (41.20), AMEn (40.17), and NE (18.24; 18.65). Similarly, the values of EE associated with digestible nutrients were 54.78, 55.59 e 79.24 for the AME, AMEn and NE, respectively. Likewise, the coefficient associated with starch were significant ($P<0.05$) and positively related to the AME (11.53) and AMEn (10.55) equations for crude nutrients, while digestible carbohydrates (dCHO) exhibited the same behavior with values of 22.8 for AME, 22.75 for AMEn, and 29.9 for NE. In contrast to the other nutrients, CP showed significance ($P<0.05$) but negative relationship with NE for nutrients. For digestible nutrients, CP was significant ($P<0.05$) and positively associated with AME and NE. In all equations, the intercepts were analyzed for their significance. The AME and AMEn prediction equations displayed significant intercepts when the Stepwise procedure was applied. However, none of the intercepts showed significance ($P>0,05$) when the NE equations were being developed. Instead, AME and AMEn demonstrated significance ($P<0.05$) and a positive relationship with NE, and they were incorporated in the models, with coefficients of 0.765 and 0.779 for AME and AMEn, respectively.

Discussion

In the present study, a wide range in the chemical composition of diets was intentionally formulated to get a NE equation based on the nutritional composition, considering non-correlation between the independent variables. The different levels of ME, CP, EE and starch led to substantial variation in feed intake and thereby, influencing the parameters of energy metabolism. Thus, to ensure adequate amino acids supply for the birds, a procedure was adopted to balance diets in terms of amino acid supplementation and metabolizable energy, as recommended by Noblet et al. (2002) As a result, lysine intake exhibited a range from 562 to 802 mg/b×d between treatments. Despite this, there was minimal mobilization of RE as body protein (-3.549 kcal/kg$^{0.75}$×d) (Table 2), even in diets with low crude protein intake. On the other hand, as expected, some diets did not provide sufficient energy to meet the production demands of the birds. Consequently, there was a significant mobilization of RE as body fat in some diets, reaching up to 56.61 kcal/kg$^{0.75}$×d of body mobilization. These results were anticipated, given that some diets were formulated with ME values below those recommended by the manual guidelines (Hy-line, 2019). Therefore, the net energy equations predicted in this study correspond to a broad range of metabolic situations, similar to previous observation provided by Carré et al., (2014).

Fasting heat production was measured for each experimental diet. Unlike some authors who assigned a mean value of FHP to all experiment diets, we chose to attribute the corresponding FHP value to each diet with the aim of obtaining more accurate values of NE and minimizing bias in estimation. Consequently, the average FHP was 76.15 kcal/kg$^{0.75}$×d with a range from 63.37 to 86.31 kcal/kg$^{0.75}$×d. These values slightly differ from those reported by Barzegar et al. (2019), for Hy-Line Brown laying hens, and Farrell (1975) for White Leghorn hens, who observed values of 88 and 95 kcal/kg$^{0.75}$×d, respectively. Additionally, the values obtained in this study are slightly higher than those obtained by Reid et al. (1978) (69 kcal/kg$^{0.75}$×d) and closer to the values reported by Sakomura (2004), who observed a FHP of 80 kcal/kg$^{0.75}$×d in laying hens raised in cages and determined through the regression method extrapolating the THP for AME intake equal to zero. The wide variability of FHP values assigned to laying hens in the literature can be attributed mainly to variations in birds genetics and the various methods that can be used to determine this variable. Nevertheless, our results suggest that the FHP values obtained in this study align with those found in the literature, providing accurate and representative data for calculating the net energy value of diets.

In the present study, as expected, EE showed a higher energy contribution across all systems when compared with other nutrients (Table 4). Interestingly, CP exhibited a higher energy value for the GE (57.55) when compared to starch (32.40) and RES (37.18). However, the CP lost efficiency and showed a lower contribution to ME and NE, while starch and RES demonstrated higher energy contribution for these systems. In other words, the hierarchy among nutrients may vary between GE, ME and NE systems. Assuming that NE is the most accurate estimation of the "true" energy values, these differences in coefficients assigned to nutrients suggest that in the ME systems, high-protein feeds will be overestimated, while high-fat feed will be underestimated (Noblet et al., 1994). Furthermore, when analyzing the differences between the coefficients obtained in the GE and ME equations, it is observed that most of the energy lost between these systems comes primarily from CP. The average energy loss related to CP was 24.86%, representing the energy loss in the form of nitrogen in the urine and feces. Similar values were reported by Barzegar et al (2019), in studies with Hy-line brown hens, where these authors obtained 20.90% of energy loss related to CP between GE and ME.

Applying the same interpretation between ME and NE, it is possible to determine the energy loss as HI. In this context, EE showed lower energy loss between these two systems, while CP demonstrated a higher energy loss (16.37%) among the predictor nutrient of the equation. These results are consistent with previous studies conducted in growing pigs (Noblet et al., 1994), and broilers (Wu et al., 2016), confirming the data collection and proximate analysis quality of the present study.

The energy metabolism derived from different nutrients exhibits considerable variation. From the coefficients obtained in the linear regressions presented in Table 4 it is possible to infer the digestibility of the main nutrients used as energy source. The results demonstrate a digestibility rate of 56.80% for CP, 80.51% for EE, and 95.87% for starch. However, the AMEn/GE values for the same nutrients were slightly lower due to correction for zero nitrogen retention. Starch and ether extract represent the major digestible fraction of energy, contributing predominantly to ME, while CP contributes only 51.40% to the conversion from GE to MEn. These results are in accordance with previous studies on laying hens (Barzegar et al., 2019) and broilers chicken (Wu et al., 2019), reporting digestibility values of 60.34% and 57.32% for CP, 78.35% and 113.00% for EE and 94.64% and 92.61% for starch, respectively. According to Murugesan et al. (2017), broilers have superior efficiency in utilizing energy from lipid sources compared to laying hens. These results support the effect of these nutrients in the AME/GE prediction equations presented in Table 5. In model 12 and 13, just the coefficients associated with CP and starch showed significance (P>0.05) in the model. The high digestibility of starch and low digestibility of CP, positively (0.427) and negatively (-0.437) influenced the AME/GE ratio, respectively. In contrast, Wu et al. (2019) demonstrated that in addition to CP and starch, EE also has a significant (P<0.05) and positive effect on predicting AME/GE for broilers. While starch is almost completely digested by the birds, the digestibility of protein is often challenging to interpret. The digestibility of CP in this model does not distinguish between the indigestible portion of the diet and the endogenous fraction, attributed to the protein turnover in birds. Additionally, protein catabolism generates nitrogenous residues that require energy to be completely excreted (Musharaf and Latshaw, 1999).

Through the analysis of energy utilization obtained in this study, the highest efficiency was observed for EE (102.79%), followed by starch (74.05%) and CP (49.92%) (Table 4). These values are close with those reported by Barzegar et al. (2019) in previous studies. However, the coefficients assigned to CP and EE in this study differ from those obtained by Carré et al. (2014) for broilers and Noblet et al. (1994) for growing pigs. Comparing the coefficients of AME/GE and NE/AME, it becomes evident that starch and EE exhibit a positive relationship with both, ME and NE, while CP is less efficient than the other nutrients in both systems. Furthermore, a change in the hierarchy of energy utilization efficiency is noticeable between EE and starch between different energy systems. While starch demonstrates higher efficiency in the ME system, EE is the most efficient nutrient in the NE system. This discrepancy is due to the ME system, which considers only the digestibility of each nutrient and not necessarily its utilization by the animal. In this context, starch has a digestibility close to 96%, being practically fully absorbed by the birds. However, net energy is determined from the heat increment of each nutrient and not only by their digestibility. In other words, the most efficient nutrients for the NE system are those with higher oxidative capacity. In previous studies, Jansman et al. (2004) developed NE equations based on the ATP yield of carbohydrates, amino acid, glycerol, fatty acids, and volatile fatty acids. From these results, the authors determined the energy production in the form of ATP for each nutrient: 9.70 KJ/g of protein, 25.31 KJ/g for EE, and 11.73 KJ/g for starch. Therefore, despite starch exhibiting high digestibility, its oxidative efficiency is lower than that of EE. The effect of these nutrients on NE/AME can also be observed in models 14 and 15 of Table 5. In these models, starch did not show significance ($P>0.05$) in predicting NE/AME, while EE was positively (0.671, 0.688) related to energy utilization efficiency. Additionally, similar to previous models predicting AME/GE, CP also showed a negative relationship (-0.364, -0.300) with the fitted models.

Based on the previous discussions, it is evident that the ME and NE of ingredients can be predicted from the chemical composition of diets. In this context, energy predictions based on nutrients are attractive due to their practical feasibility, simplicity, and cost-effectiveness (Cerrate et al., 2019). Therefore, in the present study, crude and digestible nutrients were used to predict AME, AMEn, and NE through multiple linear regressions using the stepwise procedure. Intercept values were included to the models when significant ($P<0.05$) to reduce the RSD (Residual Standard Deviation) and ensure better precision of the estimates. The coefficients estimated to the nutrients can be considered representative due to the wide range in the nutritional composition of the diets. Additionally, the correlation between the predictor nutrients is either nonexistent or low, reducing model bias and ensuring high reliability due to the minimal effect of multicollinearity among them.

According to Carré et al. (2013), the estimation of AME value is influenced by nutrient levels in the diet and digestibility. In this regard, this study indicates that AME can be predicted from EE and starch (Table 6). This confirms the previous results, demonstrating that EE and starch influenced more than the other components on the ME feed bases. Additionally, CP was not significant when included in the models for AME and AMEn prediction, also, this last system indirectly takes into account a fraction of protein intake that is retained when considered the nitrogen balance correction. These results are consistent with several authors who observed a greater influence of

EE and starch on ME, and less effect of CP (Barzegar et al. 2019, Cerrate et al. 2019, Wu et al. 2018, Carré et al. 2013). Furthermore, when comparing the coefficients in AME and AMEn equations, a reduction of 2.50% for EE and 8.49% for starch was observed. This reduction, imposed by nitrogen correction was also observed by Lopez & Leeson (2008) in a comparison between the classic AME and AMEn systems.

Similarly, equations based on digestible nutrients show a positive effect for digestible ether extract (dEE) and digestible carbohydrate (dCHO) in predicting AME and AMEn, while apparent digestible crude protein (dCP) was positively and significantly (P<0.05) related only to AME (Table 6). Apparently, the influence of dCP is reduced and loses significance when AME is corrected for zero nitrogen retention. Results from Cerrate et al. (2019) demonstrate a 22% decrease in the estimated coefficient for dCP, illustrating a reduced effect of dCP on AMEn in their study. Likewise, Carré et al. (2014) demonstrated that digestible protein undergoes a substantial reduction when converting AME to AMEn, while digestible fat and starch shows a minimal reduction (2%) between these systems. The higher loss of sensitivity of dCP between AME and AMEn models is directly related to the influence that nitrogen has on ME. Among the other nutrients commonly used for energy prediction, CP has the lowest metabolizability. Consequently, correction by zero nitrogen retention further reduces the variability of ME estimates, especially for high-protein ingredients. This is reflected in model 19, where the impact previously attributed to dCP in the AME is replaced by the intercept in the AMEn system.

Subsequently, equations for predicting NE were determined using the same statistical procedures described earlier (Table 6). Models 20 and 21 were adjusted using nutrients and AME values from 32 diets. The AME and AMEn positively affected NE as expected, and the coefficients (0.765, 0.779) estimated are in accordance with the NE/AME values obtained in the experimental diets. Moreover, the results obtained are in agreement with several authors (Noblet et al. 1994, Pirgozliev and Rose, 1999, Barzegar et al. 2019, Wu et al. 2019), who observed a variation between 0.711 and 0.808 in the AME coefficients for NE prediction in different species. Additionally, it was demonstrated that EE increase the value of NE, while CP reduces it. Similar results where CP is negatively associated with NE were described by Hartel (1977), Emmans (1994), Swick et al. (2013), Wu et al. (2019) and Barzegar et al. (2019). These authors attribute the negative effect of CP to the high HI of this nutrient, which penalizes the NE system.

In other hand, the energy value attributed to dCP was positively related (22.85 kcal/kg) to NE in this study. Similarly, the positive effect of dCP on net energy in different species has been reported by Cerrate et al. (2019) and Hoffmann and Schiemann (1980) who estimated a value of 20 kcal/kg and 25.84 kcal/kg to dCP, respectively, in previous studies. In the same way, De Groote (1974), Pirgozliev and Rose (1999) and Carré et al. (2014) observed positive values, although higher for dCP than those obtained in the present study.

Unlike what was observed in the AME prediction models, starch did not show significance ($P>0.05$) to be included in the NE models. According to Borggreve et al. (1975), NE alone is not capable of explaining all the variation in the transformation from ME to NE in high-starch diets. This suggests that the starch fraction in NE models is attributed to the coefficient related to AME, as starch digestibility is nearly 96% in laying hens, as demonstrated previously. Similarly, Wu et al. (2019), and Barzegar et al. (2019) did not found any significant effect for starch in NE prediction equations for broilers and laying hens, respectively. According to Wu et al. (2019), this is likely because the levels of starch assigned to the diets did not have enough variation to demonstrate significant effects in NE models. In the present study, the variation in crude starch was approximately 10%, while Wu et al. (2019) and Barzegar et al. (2019) utilized diets with a variation of approximately 17% and 19%, respectively. In the study by Noblet et al. (1994), the accuracy of models was reduced when starch was not considered in the prediction. However, despite starch not being included in the NE prediction models, the equation predicted in this study showed high accuracy, as indicated by the relatively low RSD values.

The internal validation process of the NE models was conducted through bootstrap resampling method (Table 7). The diets were individually randomly removed from the database at least once to generate new models and identify the individual influence that each diet exerted on the equations. This approach allowed for a comprehensive analysis of model performance, considering data variability, and assessing its generalization ability across different datasets. Thus, a database of approximately 50 thousand new samples was generated from all possible combinations between diets and replications. The new models obtained from the bootstrap samples showed coefficients very similar to those obtained in the linear regression analyses with the experimental data. This similarity demonstrates consistency of the coefficients in the NE equations, indicating the robustness of the proposed model. In this regard, no individual effect of diets on the model performance were observed, suggesting that the proposed NE equations provide good inferences of parameter estimates.

The percentage of significance of the regressors (PSR) was considered high, as AME, CP, and EE showed significance in 100% of the models generated from the bootstrap samples. Scalon, Freire, and Cunha (1998) recommend a minimum value of 50% for the PSR index in bootstrap samples to validate the predictive capacity of regression models. The high significance of these predictors in the validation process highlights the ability of AME, CP, and EE to explain variations in NE in birds. Additionally, the estimates parameters were within the CI obtained during the validation process. This indicates that the dispersion of the original parameters of models 20 e 21 closely matched within 95% of CI relative to the dispersion of the parameter generated by bootstrap. Except for the EE coefficient of model 20, which is slightly below the lower 95% CI.

The external validation aimed to challenge the NE models of the present study to predict the net energy values of diets from other sources and formulated under different approaches. The dataset from Barzegar et al. (2019), developed using Hy-line Brown laying hens and diets containing exogenous enzymes, was utilized. The NE values predicted from models 20 and 21 were close to the observed values (Figure 1). The fitness of the equations was evidenced by the R-squared of 0.934 and 0.937 for models 20 and 21 respectively. Additionally, the proposed models exhibited a high degree of fit, as evidenced by the slope, suggesting a low trend error in the models. Furthermore, error decomposition showed that the main sources of error were random, accounting for 85% for model 20 and 77% for model 21. These results evidenced a favourable robustness of the parameters and overall adequacy of the models to predict NE values of diets for laying hens.

In conclusion, the nutritional variations in the experimental diets were sufficient to develop robust and accurate equations for net energy. Analyzing the fitted models, a clear hierarchy among nutrients and energy systems was observed. Crude protein, ether extract, and starch were identified as key predictors in ME and NE equations. Both starch and EE showed a positive and significant relationship in the models. Due to the high digestibility, starch plays a significant role in predicting AME, while EE represents the major contribution for NE system. In other hands, CP demonstrated a negative relation with NE and a lower caloric contribution in the ME system compared to other nutrients. Furthermore, it has been demonstrated that when metabolizable energy is corrected for zero nitrogen retention (MEn), the effects that CP has on energy models are mitigated. In other words, the accuracy of predicting energy from CP using AMEn and NE is relatively close. Thus, the main advantage of NE system in poultry nutrition is its greater accuracy in predicting the energy values of EE in diets, an aspect underestimated by ME and MEn systems. Therefore, transitioning to the net energy system in poultry nutrition would offer a more accurate representation of nutrient utilization by birds, especially regarding EE. This transition would result in improved performance prediction and reduced feed costs.

Ethics approval

This study was conducted at the Poultry Science Laboratory of the School of Agricultural and Veterinary Sciences, São Paulo State University (Unesp). All procedures were approved by the Animal Care and Use Committee of Unesp (CEUA, Protocol n° 5402/20).

Data and model availability statement

The data/models were not deposited in an official repository. Information can be made available from the authors upon request**.**

Declaration of generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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All authors read and approved the final manuscript.

Declaration of interest

We declare that we have no financial and personal relationships with other people or organizations that could inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Table 1.

Composition and nutritional content of experiment diets (n=32)

Ingredients (%)	**Mean**	**Minimum**	**Maximum**	**SD**
Corn, Grain (7.86% CP)	42.71	1.13	68.69	19.43
Rice Bran	7.11	1.74	16.10	4.81
Sorghum Grain, Low Tannin	22.86	7.61	36.84	13.37
Millet, Grain	31.89	14.50	49.00	18.56
Soybean Meal (45% CP)	10.87	1.84	26.84	7.79
Peanut Meal	3.87	1.70	8.86	2.49
Corn, Gluten Meal (60% CP)	5.32	0.75	11.76	4.16
Soybean Protein Conc.	5.69	0.32	10.15	3.77
Meat and Bone Meal (48% CP)	6.82	2.37	9.52	3.75
Feather & Poultry by product meal	2.80	2.00	5.00	1.53
Fish Meal (54% CP)	2.32	0.39	6.78	1.96
Oil, Soybean	2.64	0.08	7.00	2.08
Fat, Poultry	3.12	0.12	5.31	1.96
Canola Meal	3.23	2.10	4.00	1.72
Barley, Grain	11.80	3.00	20.00	6.84
Cottonseed Meal (39% CP)	4.33	0.75	8.23	3.04
Wheat Bran - Midds	12.49	3.20	18.80	7.56
Wheat, Grain	18.89	10.30	27.05	10.54
Inert	2.05	0.24	3.80	1.41
Limestone	10.45	9.03	11.00	0.64
Dicalcium Phosph.	1.17	0.00	1.76	0.54
Salt	0.10	0.02	0.26	0.06
Sodium Bicarbonate	0.57	0.36	0.96	0.13
Potassium Carbonate	0.30	0.00	1.01	0.22
L-Lysine HCl	0.48	0.00	0.73	0.15
DL-Methionine	0.41	0.20	0.57	0.08
L-Threonine	0.24	0.04	0.36	0.09
L-Tryptophan	0.06	0.00	0.12	0.03
Arginine	0.17	0.00	0.40	0.12
L-Valine	0.32	0.00	0.59	0.15
Isoleucine	0.20	0.00	0.37	0.09
Choline chloride (70%)	0.10	0.10	0.10	0.00
Mineral Premix[1]	0.10	0.10	0.10	0.00
Vitamins Premix[2]	0.10	0.10	0.10	0.00
Nutritional content				
ME[3], kcal/kg	2746	2441	3096	154
Crude Protein[3], %	15.21	11.50	19.75	1.85
Total Ca, %	4.55	4.51	4.74	0.07

AvP, %	0.42	0.36	0.63	0.07
Lysina SID, %	0.81	0.67	0.92	0.05
Methionine + Cystine SID, %	0.79	0.67	0.90	0.05
Threonine SID, %	0.61	0.49	0.71	0.05
Ether Extract[3] %	5.95	2.99	9.97	1.94
Crude Fiber[3] %	2.69	1.77	4.02	0.63
Starch[3] %	39.80	32.00	47.00	4.11

SD: Standard deviation, **ME:** Metabolizable energy, **CP**: Crude protein, **Avp**: Available phosphorus, Content/kg of premix: [1]Iron: 22 g, Copper: 4500 mg, Manganese: 25 g, Zinc: 25 g, Iodine: 500 mg, Selenium: 125 mg; [2]Vit. A: 4,850,00 Ul, Vit. D3: 1,350,000 Ul, Vit.E: 8500 Ul, Vit. K3: 1395 mg, Vit. B1: 1000 mg, Vit. B2: 2570 mg, Pantothenic acid: 5295 mg, Vit. B6: 1525 mg, Vit. B12: 7500 mcg, Niacin: 19.45 g, Folic acid: 500 mg, Biotin: 41.50 mg; [3]Nutrients assayed.

Table 2.
Mean, minimum, and maximum values of performance, calorimetry parameters, and retained energy of laying hens (n=128).

Variable[1]	Mean	Minimum	Maximum	SEM	P-value
Performance					
BW, kg	1.59	1.29	1.69	0.005	0.040
Daily Feed Intake, g DM	90.50	69.16	111.17	0.073	<0.001
Laying, %	95.81	83.00	100.00	0.363	ns
Egg mass, g	58.33	50.33	69.64	0.305	0.001
FCR	1.55	1.23	1.94	0.012	<0.001
Energy balance (kcal/kg$^{0.75}$×d)					
AME intake	207.80	166.73	268,67	1.63	<0.001
THP	130.10	120.30	144.24	0.45	<0.001
HI	53.94	42.50	72.13	0.53	<0.001
FHP	76.23	63.36	86.31	0.47	-
RE					
Total	77.65	43.54	128.92	1.44	<0.001
As protein	41.16	28.85	57.91	0.52	<0.001
As fat	36.58	6.03	85.99	1.36	<0.001
Energy values, kcal/kg DM					
AME	2746	2441	3096	12.60	<0.001
AMEn	2635	2354	2981	12.30	<0.001
NE	2033	1725	2424	11.50	<0.001
Energy utilization, %					
AME/GE	79	70	86	0.32	<0.001
AMEn/GE	76	67	82	0.31	<0.001
NE/AME	74	69	79	0.17	<0.001
NE/AMEn	77	72	82	0.17	<0.001

[1] The value of each variable represents the average of six birds. **BW**: Body weight, Egg mass: Weight of eggs laid calculated as the total weight of eggs/number of days; **FCR**: Feed conversion ratio, **THP**: Total heat production, **HI**: Heat increment, **AME**: Apparent metabolizable energy, **AMEn:** Apparent metabolizable energy corrected for zero nitrogen retention, **NE:** Net energy**, GE:** Gross energy, **SEM**: Standard error of means.

Table 3.

Paired Pearson Correlation between nutrients (%) and energy values (kcal/kg) of experimental diets (n=32).

		ME	CP	EE	CF	NDF	Starch	NFE
CP	R	0.035*ns*						
EE	R	0.563***	-0.112*ns*					
CF	R	-0.661***	-0.083*ns*	-0.067*ns*				
NDF	R	-0.665***	-0.015*ns*	-0.163*ns*	0.840**			
Starch	R	0.035*ns*	-0.022*ns*	-0.219*ns*	-0.071*ns*	-0.004*ns*		
NFE	R	0.108*ns*	-0.302*ns*	-0.465*	-0.301*ns*	-0.188*ns*	0.519**	
NE	R	0.938***	-0.156*ns*	0.751***	-0.519**	-0.563***	-0.081*ns*	-0.015*ns*

ME: Metabolizable energy; **CP:** Crude protein, **EE:** Ether extract, **CF:** Crude fiber, **NDF:** Nitrogen detergent fiber, **NFE:** Nitrogen-free extract. **NE:** Net energy. Pearson correlation significance level expressed as *(P<0.05), **(P<0.01), ***(P<0.001), and *ns* is non-significative.

Table 4.

Analysis of contribution, metabolizability, and efficiency of utilization of the main energy-yielding nutrients in the diets (% DM basis) GE, AME, AMEn and NE (kcal/kg DM basis) in laying hens (n=128).

Model	Energy	Equation[1]				RSD
		CP	EE	Starch	RES	
8	GE	57.55	92.03	33.40	37.18	94
9	AME	32.69	74.09	32.02	26.72	106
10	AMEn	29.58	72.86	30.84	26.9	106
11	NE	16.32	76.16	23.71	22.24	90
AME/GE		56.8	80.51	95.87	71.87	-
AMEn/GE		51.4	79.17	92.34	72.35	-
NE/AME		49.92	102.79	74.05	83.23	-
NE/AMEn		55.17	104.53	76.88	82.68	-

[1] Linear regression without intercept. Stepwise procedure was not used. **GE:** Gross energy, **AME:** Apparent metabolizable energy, **AMEn:** Apparent metabolizable energy corrected for zero N retention; **NE:** Net energy, **CP:** Crude protein, **EE:** Ether extract, **RES:** Organic matter minus CP, EE and starch, **RSD:** Residual standard deviation.

Table 5.

Prediction of energy metabolizability and efficiency (%) from diet composition (% of DM) in laying hens (n=128).

Model	Energy	Equations[1]				RSD
		Intercept	CP	EE	Starch	
12	AME/GE	67.34	-0.437	-	0.427	3.51
13	AMEn/GE	62.90	-0.482	-	0.447	3.63
14	NE/AME	77.11	-0.364	0.671	-	1.08
15	NE/AMEn	79.03	-0.300	0.688	-	1.24

[1] Predicted equations from multiple linear regression using Stepwise procedure. **GE:** Gross energy, **AME:** Apparent metabolizable energy; **AMEn:** Apparent metabolizable energy corrected for zero N retention, **NE:** Net energy, **CP:** Crude protein; **EE:** Ether extract, **RSD:** Residual standard deviation.

Table 6.
Prediction of AME, AMEn and NE (kcal/kg DM) from nutritional composition of diets (% of DM) and ME content (kcal/kg DM) in laying hens (n=128)

Model	**Energy**	**Equations**[1]									**RSD**
		Intercept	**AME**	**AMEn**	**CP**	**EE**	**Starch**	**dCP**	**dEE**	**dCHO**	
16	AME	2024				41.20	11.53				108
	SE	*258.85*				*5.56*	*5.58*				
17	AMEn	1962				40.17	10.55				108
	SE	*235.56*				*5.06*	*5.08*				
18	AME	1242						15.45	54.78	22.88	91
	SE	*127.37*						*6.3*	*4.82*	*2.03*	
19	AMEn	1284							55.59	22.75	91
	SE	*112.84*							*4.71*	*2.02*	
20	NE		0.765		-8.95	18.24					31
	SE		*0.011*		*1.46*	*1.58*					
21	NE			0.779	-6.35	18.65					33
	SE			0.015	2.02	2.21					
22	NE							22.85	79.24	29.9	91
	SE							*5.59*	*4.02*	*0.95*	

[1] Predicted equations from multiple linear regression using Stepwise procedure. **AME:** Apparent metabolizable energy; **AMEn:** Apparent metabolizable energy corrected for zero N retention, **NE:** Net energy, **CP:** Crude protein; **EE:** Ether extract, **dCP:** Apparent digestible crude protein, **dEE:** Digestible ether extract, **dCHO:** Digestible carbohydrate, **SE:** Standard error, **RSD:** Residual standard deviation.

Table 7.
Analysis of parameter estimates of the net energy models using 1000 bootstrap replications.

Model	Regressor	Average estimates	PSR (%)	Standard deviation	Lower 95%	Upper 95%	R^2
20	AME	0.765	100	0.008	0.765	0.765	0.989
	CP	-8.964	100	1.21	-8.97	-8.95	
	EE	18.247	100	1.34	18.24	18.26	
21	AMEn	0.779	100	0.010	0.779	0.779	0.988
	CP	-6.360	100	1.38	-6.372	-6.348	
	EE	18.686	100	1.37	18.674	18.698	

AME: Apparent metabolizable energy; **AMEn:** Apparent metabolizable energy corrected for zero N retention; **CP:** Crude protein; **EE:** Ether extract; **PSR:** Percentage of significance for regressor.

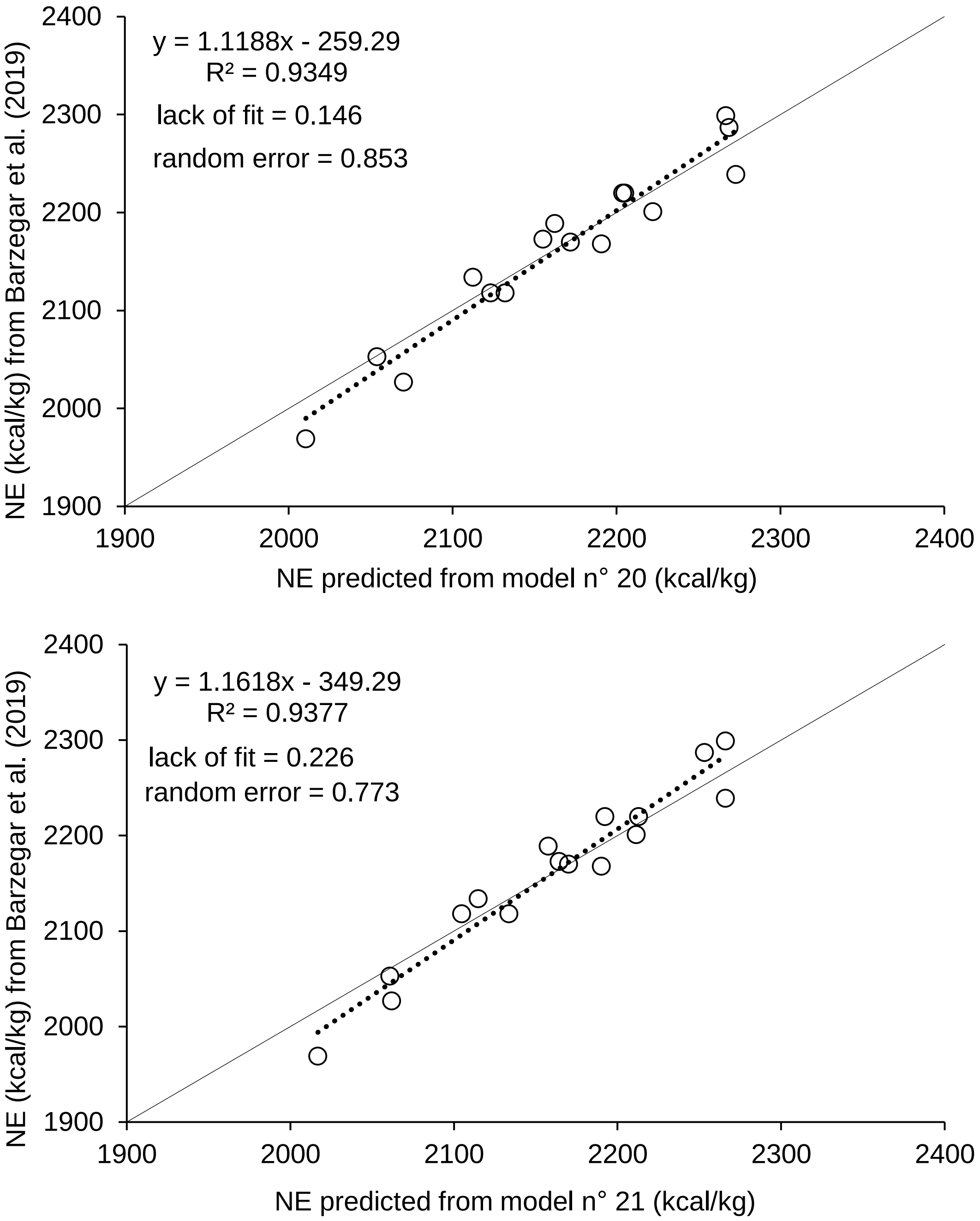

Figure 1. Validation of net energy prediction models through comparisons between predicted and observed NE values of 16 diets from Barzegar et al. (2019).

CAPÍTULO 4: IMPLICATIONS

IMPLICATIONS

The present study offers a detailed analysis of the energy metabolism in birds with the aim of assisting in the implementation of the net energy system in laying hen nutrition. Adopting this system in practical nutrition would bring several benefits, including a better estimation of performance parameters based on energy intake, reduced environmental impact through decreased nitrogen excretion, and improved economic outcomes due to reduced costs associated with formulation and use of non-traditional ingredients. Despite being widely used in swine and cattle nutrition, there is still limited information about the application of NE in poultry nutrition, especially for laying hens. Thus, this study expands the discussion on the use of NE system for hens by proposing different models for determining nutritional requirements and the energy value of ingredients, both on an NE basis.

Determining the NE nutritional requirements of birds is a crucial step for implementing this system in practical formulations. Currently, the metabolizable energy system is widely used by nutritionists, but it does not accurately describe the bird's "true" requirement as it does not consider the animal heat production and tends to overestimate protein ingredients and underestimate energy ingredients. Thus, based on the models proposed in Chapter 2, it is possible to determine the NE requirement based on live body weight, body weight gain, and egg mass of laying hens. This provides nutritionists a reference value to use during the diet formulation process.

Figure 1 illustrates the results of an exercise where performance parameters of two strains of light and heavy hens were used to estimate the requirements of NE and ME using the models proposed in Chapter 2 (models 4 and 5). It can be observed that the efficiency between NE and ME is approximately 75% on average for the production phases. Furthermore, considering the requirement for NE (figure 1. A and B) in birds implies a reduction in the requirement for ME (figure 1 C and D). In our exercise, the NE and ME requirements were determined for the three main production phases (Peak, Layer 2 and 3), showing a difference in ME requirement between the models and the manual guidelines of 350 kcal/kg and 71 kcal/kg on average for Hy-Line W80 and Lohmann hens, respectively. These results illustrate that the currently used ME values could be much lower if we adopted the NE system as the basis for determining the birds' energy requirement.

Once the laying hen’s energy requirement is determined, it is essential to know the NE values of the ingredients that will be used in the diets. Since measuring the NE of ingredients is complex and requires specific equipment, it is more feasible to use prediction equations. In this study, NE prediction equations were developed based on the chemical composition of the ingredients (models 20 and 21 in chapter 3). In addition to being essential for the implementation of the NE system, these models assist in the use of non-traditional ingredients in poultry farming.

One of the factors contributing to the relevance of the ME system for birds is the widespread use of corn and soybean meal as the main energy and protein ingredients in the diet, respectively. However, these grains are commodities and face a wide price fluctuation throughout the year, in addition to being ingredients that are part of human nutrition, which raises debates about their use in animal feed. In this sense, NE prediction models helps to reduce the dependence on conventional ingredients with high demand and offer more economical alternatives, considering market conditions. The NE values of the main traditional and non-traditional ingredients used for bird feeding are presented in Table 1. Currently, most studies on NE for birds focus on broiler chickens, with limited information available for laying hens. However, it is interesting to note that there are similarities between the values obtained with the models in this study and the equations proposed by Barzegar et al., (2019). This reinforces the feasibility of predicting NE values based on nutritional composition. Also, this approach facilitates the implementation of this system since the crude protein and ether extract contents of ingredients can be easily consulted in nutritional tables and specific literature.

Finally, the use of this system would bring economic and environmental benefits, as formulating diets based on NE promotes a reduction in crude protein content, which is primarily responsible for the largest fraction of dietary heat increment. This reduction in crude protein content encourages the use of crystalline amino acids, thereby reducing nitrogen excretion. Figure 2 illustrates that diets with higher NE/AME ratios show lower nitrogen excretion by birds, aligning with current goals in animal production to reduce environmental pollution levels. This reduction in nitrogen excretion not only minimizes the risks of environmental pollution but also ensures a formulation with more accurate levels and consequently with lower costs.

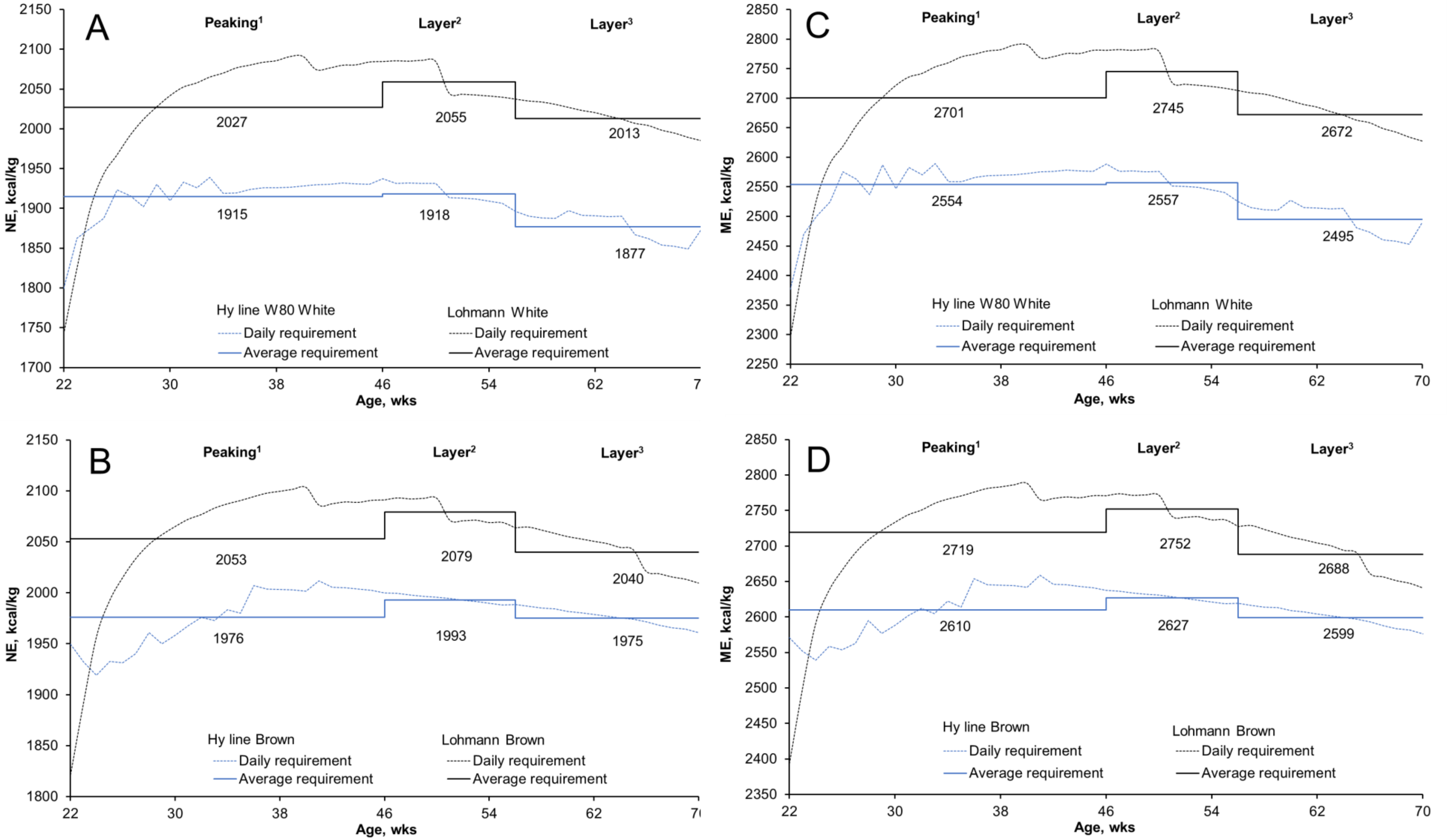

Figure 1.
Determination of net energy (NE) and metabolizable energy (ME) requirements in different strains of light and heavy hens, using models 4 and 5 presented in Chapter 2.

Table 1.

Nutritional composition of feedstuffs and the net energy estimated based on the equations proposed on the Chapter 3.

Ingredients	CP[1], %	EE[1], %	GE[1], kcal/kg	AME[2], kcal/kg	NE[3], kcal/kg	NE[4], kcal/kg	NE[3]/AME
Corn, Grain	7.25	3.50	3971	3364	2573	2603	76
Rice Bran	17.49	14.29	4344	2583	2116	2104	82
Sorghum Grain, Low Tannin	7.49	2.40	3988	3204	2434	2461	76
Millet, Grain	13.64	7.40	4221	3189	2402	2420	75
Soybean Meal 45% CP	46.36	1.66	4283	2258	1357	1296	60
Peanut Meal	42.97	2.85	4219	2228	1290	1225	58
Corn, Gluten Meal 60% CP	65.08	2.76	5352	3705	2320	2250	63
Soybean Protein Conc.	57.11	1.40	4550	2635	1463	1376	56
Meat and Bone Meal, 48%	47.36	13.14	3643	2373	1615	1534	68
Feather & Poultry Bypr. Meal	66.27	12.83	5211	3264	2162	2051	66
Fish Meal 54% CP	54.61	10.12	4038	2851	1839	1758	65
Canola Meal	36.92	5.30	4342	1743	1056	1005	61
Barley, Grain	8.83	2.30	3886	2701	2001	2019	74
Cottonseed Meal 39% CP	41.68	2.65	4230	1951	1203	1151	62
Wheat Bran - Midds	18.09	3.39	4064	1810	1312	1303	72
Wheat, Grain	14.30	2.11	3992	3039	2251	2273	74

CP: Crude protein, **EE:** Ether extract, **GE:** Gross energy, **AME:** Apparent metabolizable energy, **NE:** Net energy,

[1] Values obtained through Near-Infrared Spectroscopy (NIRS) analysis by Evonik Industries AG (Hanau, Germany);

[2] Brazilian table of poultry and swine;

[3] Net energy values estimated based on the equation n° 20. $NE=0.765\times AME-8.95\times CP+18.24\times EE$;

[4] Net energy value based on the equation proposed by Barzegar et al. (2019). $NE=0.781\times AME-11.00\times CP+16.24\times EE$.

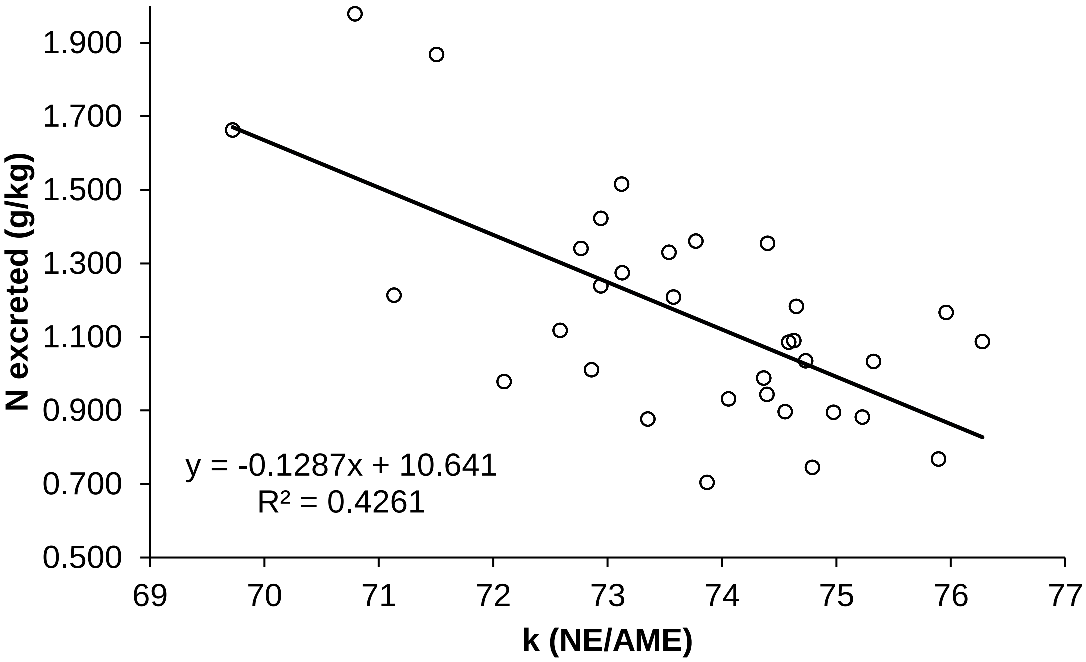

Figure 2.
Relationship between efficiency of energy utilization (NE/AME) and nitrogen excretion (g/kg) of laying hens fed with different levels of net energy and metabolizable energy (n=32; each point represents the average of four experimental units).