UNIVERSIDADE ESTADUAL PAULISTA-UNESP

CÂMPUS DE JABOTICABAL

EXIGÊNCIA DE ISOLEUCINA PARA FRANGOS DE CORTE

Mirella Cunha Melaré

Zootecnista

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UNIVERSIDADE ESTADUAL PAULISTA - UNESP

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DADOS CURRICULARES DO AUTOR

MIRELLA CUNHA MELARÉ, filha de Sérgio Matheus Melaré e Zélia da Cunha, nascida no dia 9 de março de 1992 em Osasco, São Paulo. Em 2010, iniciou o cursinho pré-vestibular fornecido pela Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista – campus de Jaboticabal, São Paulo. Ingressou no curso de Zootecnia em 2011 na mesma universidade, finalizando a graduação em 2015. Neste período, realizou duas iniciações cientificas, sendo bolsista de iniciação científica pela PROPe, no período de março de 2012 a março de 2013, sob orientação da Profª. Drª. Lizandra Amoroso, e pelo CNPq, no período de abril de 2013 a abril de 2014, sob orientação do Prof. Dr. Luciano Hauschild. Iniciou o curso de Mestrado em Zootecnia em março de 2016 na mesma instituição com bolsa da FAPESP e sob orientação da Profª. Drª. Nilva Kazue Sakomura.

À minha mãe Zélia da Cunha

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SUMÁRIO

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CERTIFICADO

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

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EXIGÊNCIA DE ISOLEUCINA PARA FRANGOS DE CORTE

RESUMO - A utilização de modelos matemáticos na área de nutrição avícola está se tornando frequente entre as empresas do ramo, que manipulam essa ferramenta com o intuito de melhorar a produtividade e o lucro da atividade. Neste contexto, existe a necessidade de mais pesquisas, visando o estudo das respostas dos animais, estabelecendo valores corretos de eficiência de utilização e assim produzir modelos acurados para predição da ingestão ideal de nutrientes, como a ingestão de Isoleucina (Ile). Dessa forma, o objetivo do presente estudo foi avaliar as respostas das aves submetidas a dietas com níveis crescentes de isoleucina de modo a estimar a exigência de consumo desse aminoácido, além de determinar a eficiência de utilização e desenvolver modelos de predição da exigência de isoleucina para frangos de corte. Foram realizados seis ensaios, três para machos e três para fêmeas, utilizando 640 frangos de corte Ross 308 em cada ensaio. Avaliou-se as fases inicial (1 a 14 dias), crescimento (15 a 28 dias) e final (29 a 42 dias). No total, 8 tratamentos foram distribuídos aleatoriamente em 4 repetições de 20 aves cada. Os tratamentos consistiram em 7 níveis crescentes de Ile e um contra-prova, para confirmar que a Ile foi o primeiro aminoácido limitante na dieta. A técnica de diluição foi aplicada para produzir os níveis de Ile e manter a relação de aminoácidos com a lisina. As variáveis de resposta foram: consumo de ração (CR), ganho de peso corporal (GP) e conversão alimentar (CA). No primeiro artigo, foram ajustados três modelos matemáticos, linear *broken-line* (LBL), quadrático *broken-line* (QBL) e polinomial quadrático (QUAD), com o intuito de determinar o nível ideal de Ile. O critério de informação de Akaike (AIC), uma análise de sensibilidade e uma simulação econômica foram aplicados para avaliar cada modelo. A ingestão óptima de Ile estimada variou de 114,81 a 316,83, 535,49 a 1052,43 e 694,54 a 1610,04 mg de Ile por ave/dia, para as fases inicial, crescimento e final, respectivamente. O modelo QUAD obteve o menor valor de AIC. Após uma redução de 10% na Ile ideal estimada, o LBL foi o modelo com maior impacto na variável resposta (12% de redução no GP). De acordo com a simulação econômica, o nível de Ile estimado pelo modelo QBL tem o menor custo entre todos os modelos. Com base nos resultados deste estudo, o modelo QBL apresentou os melhores resultados entre os modelos utilizados. No segundo artigo, foi determinada a eficiência de utilização (EU) e produzidos modelos fatoriais para a ingestão ideal de Ile. A EU foi determinada por meio de duas técnicas distintas, considerando o corpo inteiro da ave, ou considerando a eficiência para a deposição do aminoácido no corpo livre de penas (Bff) e nas penas. Foram desenvolvidos três modelos fatoriais distintos, M1, M2 e M3. O modelo M2 foi avaliado para uma ou duas EU (Bff + Pena), sendo denominado M2 e M3. Os modelos foram testados utilizando o ajuste do erro o predito. A EU para o corpo inteiro foi estimada em 64%. Quando a eficiência foi particionada, foram determinados os valores de 53 * e 69% para a pena e Bff. A estimativa ideal de ingestão de Ile para cada modelo foi de 275, 882, 1399 mg de Ile/ave/ dia (M1); 258, 806, 1307 mg de Ile/ave/ dia (M2); e finalmente 289, 819, 1278 mg d de Ile/ave/ dia (M3) para as fases inicial, crescimento e final, respectivamente. De acordo a análise imparcialidade do modelo, os resultados sugerem que o M3 é o modelo menos tendencioso, que poderia estar relacionado à EU, que foi particionado para Bff e pena. A EU particionada para o corpo livre de pena e pena reduziu a tendência do modelo. O modelo M1 estima valores mais elevados para a ingestão de Ile do que os modelos M2 e M3, o que pode estar relacionado a diferença em contabilizar o ganho de peso corporal (M1) ou apenas o ganho de proteína (M2 e M3) para estimar a quantidade de aminoácido necessária para o frango de corte em cada fase.

Palavras-chave: aminoácido, dose-resposta, método fatorial, eficiência de utilização

ISOLEUCINE REQUIREMENT FOR BROILERS

ABSTRACT – The use of models in poultry nutrition is becoming decisive among companies, which uses such tool to improve their productivity and profit. In this context, there is a necessity of more research, to study the responses of birds, to elucidate the efficiency of utilization and to produce models to predict an optimal nutrient intake, such as Isoleucine (Ile) intake. The objective of this work was to evaluate the responses of birds submitted to diets with increasing levels of Ile in order to estimate the requirements of this amino acid, as well as to determine the efficiency of isoleucine utilization and to develop models of prediction of Ile requirements for broiler chickens. Six dose response trials were carried out, three for males and three for females, with 640 Ross 308 in each studied phase. The initial (1 to 14 days), grower (14 to 28 days), and finisher (28 to 42 days) phases were evaluated to cover the growing phase of the broiler chicken. In total, 8 treatments were randomly distributed to 4 replicates of 20 birds each. The treatments consisted in 7 crescent levels of Ile and one counter proof, to ensure that Ile was the first limiting amino acid in the diet. Dilution technique was applied to produce the levels of Ile and keep the amino acid ratio with lysine. The response variable where feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR). In the firs paper, three different mathematical models (linear broken line (LBL), quadratic broken line (QBL), and polynomial quadratic (QUAD)) were adjusted to observed data, producing the optimal Ile level. The Akaike Information Criteria, a sensibility analysis and an economic simulation were applied to evaluate each model. The optimal Ile intake estimated ranged from 114.81 to 316.83, 535.49 to 1052.43, and 694.54 to 1610.04 mg of Ile per bird daily, for starter, growing and finisher phases, respectively. The QUAD model have the lower AIC value. After a reduction of 10% in the optimal Ile estimated, the LBL was the model with the higher impact in the response variable. According to the economic simulation, the feed formulated with the Ile level estimated by QBL model have the lower cost among all models. Based on the results of this study, QBL model have the best results for economic analysis applied. In the second paper, was to determine the EU and produce factorial models for optimal Ile intake. A different procedure to determine the EU was developed and tested in the models produced. The EU were determined to account for whole body or partitioned for feather free body (Bff) and feather. Two distinct factorial models were developed, M1, M2, and M3. The M2 model were evaluated for one or two EU, being denominated as M2 and M3. Models were tested using the residual against predicted technique. The EU determined considering the whole body was 64%. When the efficiency was partitioned, the values of 53 and 69% for feather and Bff were determined. The optimal Ile intake estimate for each model were of 275, 882, 1399 mg of Ile/bird/day (M1); 258, 806, 1307 mg of Ile/bird/day (M2); and finally 289, 819, 1278 mg of Ile/bird/day (M3) for initial, grower, and finisher phases, respectively. According to the error against predicted method, the results suggests that the M3 is the less biased model, which could be related to the EU, which was partitioned for Bff and feather and applied to M2'. In conclusion, the EU partitioned for feather free body and feather reduced the biased of the M3. The model M1 estimate higher values for Ile intake than models M2 and M3,

which may be the difference in account for body weight gain (M1) or only protein gain (M2 and M3) to estimate the amount of amino acid required for broiler chicken in each phase.

Keywords: amino acid, dose-response, factorial method, efficiency of utilization

CAPÍTULO 1 - Considerações gerais

Introdução

Há anos a produção de frangos de corte é destaque no setor de carnes por ser uma fonte de proteína barata e de rápida obtenção, sendo mais acessível ao consumidor de diversos perfis socio-econômicos. De forma a obter produtos de melhor qualidade em maiores quantidades, exigiu-se a tecnificação dessa indústria que vai desde a melhoria das instalações e avanços nas elaborações de medidas sanitárias preventivas até a busca pelo fornecimento de ração que atendam às necessidades para a mantença e produção do animal. Contudo, o avanço genético das aves necessita de atualizações constantes das exigências nutricionais e programas de alimentação (SAKOMURA et al., 2015).

O fornecimento de uma dieta balanceada em aminoácidos essenciais é fundamental para o atendimento das exigências nutricionais, resultando em melhor aproveitamento de nutrientes, síntese e deposição proteica, desempenho animal, além de redução de custos e poluição ambiental. Dentre esses aminoácidos, a isoleucina (Ile) é de suma importância. É considerada o quinto aminoácido limitante em dietas a base de milho e farelo de soja para frangos de corte (FERNANDEZ et al., 1994; KIDD; BURNHAM; KERR, 2004; BERRES et al., 2010) e em dietas com baixa proteína (PARK; AUSTIC, 2000), podendo interferir no aproveitamento de outros nutrientes pelo animal caso sua exigência não seja atendida.

A maior parte dos estudos sobre exigências de aminoácidos baseiam-se em duas abordagens: a empírica e a fatorial. Na empírica, as exigências de aminoácidos são estimadas em função da resposta do desempenho das aves que receberam dietas contendo níveis crescentes de um aminoácido em determinada idade (GOUS, 1998). Apesar desta técnica ser mais utilizada pelos nutricionistas, é feita uma predição estática dos aminoácidos e valida apenas para as condições em que os experimentos foram realizados (OVIEDO-RONDÓN; WALDROUP, 2002). O método fatorial se baseia na partição das exigências de aminoácidos para a mantença do corpo, crescimento de todos os componentes do corpo e na eficiência de utilização do aminoácido. A utilização da abordagem fatorial aliada a predição do crescimento corporal possibilita a estimação dinâmica das exigências dos aminoácidos, que por sua vez permite determinar a máxima margem bruta da atividade por meio da elaboração de programas nutricionais flexíveis (GOUS, 1998)

Para predizer a exigência de aminoácidos utilizando o método fatorial, é fundamental que se conheça a eficiência de utilização do aminoácido em questão. Apesar disso, a literatura sobre Ile e sua eficiência de utilização é escassa, o que é algo a ser explorado visto que parte das diferenças entre estimativas existentes ocorre devido às eficiências (DORIGAM, 2016).

Diante da escassez de trabalhos envolvendo Ile, o desenvolvimento de estudos que ajudem na elaboração de modelos de predição é de extrema importância para determinação das exigências de aminoácidos essenciais para frangos de corte.

1- Revisão de Literatura

2.1 Importância do uso de aminoácidos na nutrição avícola

No passado, as aves eram alimentadas com rações que levavam em consideração a exigência em proteína bruta (PB) e não em aminoácidos (FIRMAN; BOLING, 1998). Com os avanços na nutrição, os nutricionistas começaram a aplicar o conceito de proteína ideal, que é definido como o balanceamento ideal de aminoácidos capaz de atender, sem excessos ou falta, às exigências dos aminoácidos essenciais, sendo expressos como porcentagem de lisina, considerada o aminoácido referência (KIDD et al., 1997; FURUYA et al., 2005).

A proteína desempenha funções importantes, sendo constituinte de diversos tecidos como pena, pele, ligamentos, matriz óssea, órgãos e músculos (NRC, 1994). Entretanto, o conhecimento do perfil aminoacídico da proteína é importante, pois as aves apresentam exigências nutricionais de aminoácidos, e não da proteína em si (CORZO; LOAR II; KIDD, 2009). Assim, as exigências dos aminoácidos essenciais devem ser atendidas seguindo-se a ordem de limitação dos mesmos. Quando não atendidas em consideração à ordem de limitação, não há síntese proteica e o desempenho das aves pode ser prejudicado (COSTA et al., 2014).

A composição de aminoácidos das proteínas é variável. Os aminoácidos praticamente não ocorrem em quantidades iguais em uma proteína. Alguns podem estar presentes em grandes quantidades ou até mesmo ausentes em determinados tipos de proteína (NELSON; MICHAEL, 2014), podendo afetar a ordem de limitação dos aminoácidos (CORRENT; BARTELT, 2011). Isso demonstra a importância do conhecimento da composição em aminoácidos dos ingredientes e da suplementação dos mesmos visando o atendimento da exigência.

A suplementação de aminoácidos essenciais na dieta permite equilibrar sua composição e reduzir a proteína na dieta sem que o crescimento e o desempenho sejam prejudicados. Além disso, pode diminuir a poluição ambiental, aumentar a eficiência alimentar e o retorno econômico, e contribuir para o aumento global de recursos proteicos (WU, 2013).

Tendo em vista as vantagens da suplementação dietética de aminoácidos e a necessidade de fornecer uma dieta que atenda as exigências nutricionais, a suplementação de aminoácidos sintéticos na dieta se torna uma alternativa para suprir as exigências de aminoácidos limitantes (MICHELS; CELLA; BERTOLDO, 2011). Entretanto, para suprir a deficiência dos aminoácidos é necessário que se tenha o conhecimento das exigências.

Há valores fixos compilados em tabelas de exigências que são utilizados para formulação de dietas que visam o custo mínimo. No entanto, fatores como a diversidade genética, potencial de crescimento e composição da carcaça não são considerados (GOUS, 1998). Também é difícil avaliar até que ponto a seleção genética pode alterar os níveis nutricionais exigidos (EMMANS, 1995).

Para garantir o atendimento à exigência nutricional, é necessário que a ave tenha ingerido a quantidade ideal de cada nutriente, o que é possível com a aplicação de modelos matemáticos que possibilitam estimar a ingestão ideal de um nutriente para garantir o desempenho de uma população (SILVA; SAKOMURA; HAUSCHILD, 2014).

2.2 Isoleucina e suas características

A isoleucina (Ile) é membro da família alifática de aminoácidos hidrofóbicos, presente principalmente no interior de proteínas e enzimas (DUARTE, 2009). É classificada como aminoácido apolar, pois possui grupo R com caráter de hidrocarboneto que não interage com a água e, por isso, frequentemente localiza-se no interior da molécula proteica (MARZZOCO; TORRES, 2015). É considerada cetogênica, por liberar corpos cetônicos por meio da conversão do acetoacetil-CoA em acetona e beta-hidroxidobutirato, e glicogênica por ser convertida em succinil-CoA e transformada em glicose e glicogênio (NELSON; MICHAEL, 2014).

Assim como leucina (Leu) e valina (Val), a Ile sensibiliza tecidos aos hormônios insulina e fator de crescimento semelhante à insulina tipo 1 (IGF-1), sinaliza para os tecidos periféricos que há nutrientes disponíveis para síntese proteica (D'MELLO, 2003) e faz parte do processo de síntese de glutamina e também do equilíbrio entre aminoácidos isoleucina, leucina e valina, conhecidos como aminoácidos de cadeia ramificada (ACR) (WU, 2009).

Dentre os aminoácidos essenciais, Ile é considerada o quinto aminoácido na ordem de limitação em rações à base de milho e farelo de soja para frangos de corte (FERNANDEZ et al., 1994; KIDD; BURNHAM; KERR, 2004; BERRES et al., 2010) e em dietas com baixa proteína (PARK; AUSTIC, 2000). Por vezes, Ile e Val são limitantes ao lado da treonina , que é o terceiro aminoácido limitante (CORZO; LOAR; KIDD, 2009).

Para que os processos metabólicos funcionem corretamente, é preciso que haja o fornecimento ininterrupto dos aminoácidos, principalmente em locais de síntese proteica, já que qualquer déficit pode prejudicar a utilização dos aminoácidos restantes, reduzir a síntese de proteínas e afetar outros processos fisiológicos (D'MELLO, 2003).

Em estudo com frangos de corte em crescimento, observou-se que dietas deficientes em isoleucina resultam em menor rendimento de peito (KIDD et al., 2000). Corzo et al. (2008) relataram redução no ganho de peso e piora da conversão alimentar com dietas contendo níveis reduzidos de isoleucina. Ademais, foi observado que com a suplementação de Ile na dieta houve resposta linear no ganho de peso e melhora linear na conversão alimentar à medida que Ile foi adicionada. Burnham et al. (1992) também relataram o efeito depressor de concentrações dietéticas mais baixas de isoleucina sobre o consumo de ração e o crescimento de frangos de corte.

O desbalanceamento entre ACR pode gerar problemas ao organismo animal como formação anormal de penas em formato côncavo que se direcionam em sentido contrário ao das outras penas, redução de peso corporal e piora na conversão alimentar (RIBEIRO; KRATZ, 2002). De acordo com Park (2006), dietas desbalanceadas retardam o crescimento e reduzem o consumo alimentar de frangos em crescimento.

2.3 Antagonismo entre aminoácidos de cadeia ramificada

O antagonismo é definido como a interação que ocorre quando a ingestão excessiva de um aminoácido aumenta a exigência de outro estruturalmente semelhante.

Esse desequilíbrio ocorre devido a conformação estrutural semelhante dos ACR, os quais compartilham os mesmos sistemas de transporte através de membranas celulares, visto que são degradados pelas mesmas enzimas (aminotransferases de cadeia ramificada, α-ceto-ácido desidrogenase de cadeia ramificada; HARPER, 1984).

Devido a baixa atividade de transaminase nas mitocôndrias das células hepáticas para os ACR (WU, 2013), o catabolismo de Ile, assim como de Leu e Val, não ocorre no fígado como os demais aminoácidos, e sim, nos tecidos muscular esquelético (WILTAFSKY et al., 2010), adiposo, renal e cerebral (NELSON; MICHAEL, 2014).

O processo de transaminação reversível é intermediado pela aminotransferase de cadeia ramificada, resultando na produção de α-cetoácidos ramificados (WILTAFSKY et al., 2010; NELSON; MICHAEL, 2014). Posteriormente, o complexo da desidrogenase dos α-cetoácidos ramificados catalisa a descaboxilação da isoleucina, liberando o grupo carboxíla como CO_2 e produzindo acil-CoA (NELSON; MICHAEL, 2014). Em contrapartida, a atividade da desidrogenase dos α-cetoácidos ramificados é maior no fígado em comparação a outros tecidos como músculo esquelético, intestino delgado, tecido adiposo, placenta, coração, rins e cérebro (HARPER et ai, 1984). Desse modo, a maior parte dos α-cetoácidos ramificados produzidos pelos tecidos extra-hepáticos é liberado para circulação e posterior absorção e catabolismo pelo fígado (WU, 2013). De forma simplista, o músculo esquelético, absorve os ACR do sangue arterial, em seguida sintetiza alanina e glutamina dos ACR e α-cetoglutarato e libera esses dois aminoácidos na circulação (WU, 2009).

Os efeitos resultantes do antagonismo entre os ACR são: baixa taxa de crescimento, baixa eficiência alimentar, conversão alimentar elevada e anormalidade da pena associada à toxicidade à leucina (FARRAN; THOMAS, 1992). Também podem ser observadas alterações dos ACR nos níveis plasmáticos e nos tecidos (HARPER, 1984). De acordo com Park (2006), o transporte competitivo entre os aminoácidos tem grande influência na redução da concentração do aminoácido limitante no cérebro.

A concentração de valina no plasma é sensível aos níveis de leucina e isoleucina, sendo que a leucina também pode interferir no destino da isoleucina e valina (D'MELLO; LEWIS, 1970). De acordo com Cemin (2013), os α-cetoácidos de Ile, Leu e Val são, respectivamente, α-cetometilvalerato (KMV), α-cetoisocaproato (KIC) e α-cetoisovalerato (KIV). Após o consumo de níveis excessivos de leucina, os níveis de isoleucina e KMV e de valina e KIV no plasma sanguíneo e nos tecidos encontram-se reduzidos, demonstrando o efeito do excesso de leucina sobre as exigências de isoleucina e valina (HARPER, 1984). Calvart et al. (1982) observaram que o excesso de leucina reduziu o consumo de ração, ganho de peso e eficiência de utilização em pintos machos, porém a suplementação de 0,80% de Ile e 1,07% de Val foi capaz de impedir a piora do desempenho animal.

O excesso de isoleucina também afeta a valina e a leucina, pois há um aumento de isoleucina na concentração plasmática, inibindo o transporte dos outros dois aminoácidos até o cérebro, que resulta em redução do consumo de ração (PEGANOVA; EDER, 2002; 2003). Como providencia, algum aminoácido estruturalmente semelhante, como leucina ou valina, deve ser fornecido para aliviar o efeito antagonista (PARK, 2006).

Portanto, o conhecimento das exigências nutricionais, do metabolismo e interações entre os aminoácidos são fundamentais para garantir bons resultados na produção animal. Além disso, possibilita a formulação de ração para frangos de corte baseada no atendimento das exigências aminoacídicas, melhorando o desempenho, reduzindo a excreção de nitrogênio ao ambiente (CORZO, 2004) e evitando a ocorrência de problemas relacionados ao antagonismo de aminoácidos.

2.4 Determinação das exigências de aminoácidos das aves

A técnica de formulação mais utilizada para determinar as exigências nutricionais é a de suplementação. Contudo, críticas são feitas ao método por, diversas vezes, as dietas serem formuladas com excesso de nutrientes e pelo aumento sucessivo do aminoácido limitante, que ocasionam no desbalanceamento dos demais aminoácidos, afetando a resposta dos animais (SAKOMURA; ROSTAGNO, 2007).

Como alternativa, a Técnica de Diluição da Dieta desenvolvida por Fisher e Morris (1970) é baseada no método dose-resposta que pode reduzir a presença de antagonismo entre os aminoácidos. Essa técnica consiste em diluir sequencialmente uma dieta com alto teor de proteína com uma dieta isoenergética e isonutritiva, isenta de proteína. Dessa forma, obtém-se níveis intermediários de aminoácidos, mantendo a relação ideal entre eles, e as respostas dos animais ao aminoácido limitante sem interferência de outros nutrientes da dieta (GOUS; MORRIS, 1985). A técnica é um método aprimorado que proporciona resultados mais confiáveis do que a técnica de suplementação (GOUS, 1980).

Dentre os resultados existentes, Klain et al. (1960) indicaram que o nível de Ile na dieta foi de 0,73% para frangos até 21 dias de vida. Thomas et al. (1992) recomendaram que para frangos de corte fêmeas de 22 a 42 dias de idade, o nível de Ile foi de 0,69% da dieta. Hale et al. (2004) demonstraram o impacto de diferentes níveis de Ile no desempenho e rendimento de carcaça de frangos de corte de 30 a 42 dias de idade. Para ganho de peso, consumo de ração e conversão alimentar, a exigência de Ile foi, respectivamente, de 0,67, 0,66 e 0,68% de Ile na dieta. Para rendimento de peito e asas, a exigência foi estimada em 0,63% de Ile na dieta em Ile.

Os métodos dose-resposta e fatorial são comumente utilizados para determinar as exigências dos aminoácidos e elaborar modelos de predição. Diversos trabalhos utilizam o método dose-resposta para definir os níveis nutricionais das dietas correlacionando com o desempenho animal (SAKOMURA; ROSTAGNO, 2007).

O método dose-resposta determina as exigências do animal de acordo com a resposta na composição corporal ao aumento da ingestão de determinado nutriente. Nesse método, quando um nutriente limitante é adicionado na ração, mantendo-se os níveis adequados dos demais nutrientes, ocorre crescimento do animal até que sua exigência seja atendida (EUCLYDES; ROSTAGNO, 2001). No método fatorial, as exigências são estimadas de acordo com a demanda dos nutrientes para manutenção, crescimento, engorda e produção (SAKOMURA; ROSTAGNO, 2007). O método dose-resposta é útil para fornecer informações como a eficiência de utilização para elaboração de modelos fatoriais, a qual é fundamental para predição das exigências de aminoácidos.

A escassez de trabalhos sobre Ile e a importância da determinação da exigência de aminoácidos para nutrição avícola realçam a necessidade de novos estudos para atualização do nível ótimo e da eficiência de utilização deste aminoácido.

2.5 Mantença de Isoleucina

A mantença é definida como situação de equilíbrio de nitrogênio (N), no qual a ingestão de N é igual a quantidade de N excretada, de forma que o conteúdo de N corporal permaneça constante (SAKOMURA; COON, 2003). Para sua determinação, o uso de aves adultas representa uma forma mais adequada, visto que embora essas aves não estejam acumulando proteínas, requerem aminoácidos dietéticos para funções de manutenção, e não apresentam exigências para crescimento (OWENS; PETTIGREW, 1989). Além disso, o uso de aves adultas tem sido feito em diversas pesquisas (LEVEILLE; FISHER, 1960; BURNHAM; EMMANS; GOUS, 1992; NONIS; GOUS, 2008; BONATO et al. 2011; SIQUEIRA et al., 2011; De Lima et al., 2016).

As exigências de aminoácidos para mantença podem ser expressas com base no peso metabólico ou em função do peso proteico (SAKOMURA; ROSTAGNO, 2016). Emmans e Fisher (1986) afirmaram que as exigências de mantença relacionam-se mais intimamente com o conteúdo de proteína corporal, sendo uma forma mais apropriada para expressar as exigências, uma vez que o conteúdo de lipídios pode variar mesmo entre aves com pesos corporais semelhantes. Assim, propuseram uma equação (MP = 0,008.Pm 0,73.u); baseando-se na exigência de proteína, onde MP é a exigência de proteína para a mantença (g/dia), 0,008 é uma constante (kg de proteína ideal/ unidade de mantença; Pm 0,73.u), Pm é o peso de proteína à maturidade (kg) e u é o grau de maturidade da proteína no tempo t (u = Pt / Pm = 1 para aves adultas, nas quais Pt = Pm). Burnham, Emmans e Gous (1992) e Nonis e Gous, 2008 utilizaram este princípio para estimar as exigências de isoleucina para a mantença de galos Leghorn (60 mg/kg/dia ou 300 mg/ $Pm^{0,73}$.u/dia) e de lisina (182 mg/$Pm^{0,73}$.u/dia), respectivamente.

De Lima et al. (2016), através da técnica de balanço de nitrogênio, usando diferentes sistemas de unidades, determinou as exigências de isoleucina, valina e triptofano para mantença. Com base no peso proteico, as exigências foram 24, 134 e 37 mg / kg BP m 0,73 por d para valina, isoleucina e triptofano, respectivamente. Estes autores inferem que os cálculos baseados no peso da proteína e no grau de maturidade expressam melhor as exigências de aminoácidos para manutenção de aves.

A determinação das exigências de aminoácidos para a mantença das aves e da eficiência de utilização dos aminoácidos possibilita a elaboração de modelos de predição das exigências com base no método fatorial (SIQUEIRA, 2009).

2.6 Eficiência de utilização de isoleucina

A eficiência de utilização (EU) de aminoácidos se refere a relação entre a quantidade de aminoácido depositada e ingestão do aminoácido para deposição, indicando como os recursos são convertidos em resultados. Para tanto, a quantidade de aminoácido ingerida direcionada para mantença deve ser descontada do total ingerido (SAKOMURA; ROSTAGNO, 2016).

A regressão linear é comumente utilizada para determinar a eficiência de utilização de animais em crescimento, através da relação da deposição de aminoácido no corpo em função da ingestão de aminoácido para ganho. Neste caso, a deposição é a variável dependente, a ingestão é a variável independente e o coeficiente de inclinação da reta representa a eficiência de utilização do aminoácido (EMMANS, 1981).

A determinação da eficiência de utilização de aminoácidos é necessária para melhorar a precisão de modelos de predição. No entanto, a eficiência de utilização de Ile é escassa na literatura e necessita ser estudada. Além disso, a eficiência de utilização muda entre os nutrientes (SIQUEIRA et al., 2011; FERREIRA et al., 2016; DONATO et al., 2016; DORIGAM et al., 2016), o que dá a necessidade de avaliá-la para cada aminoácido. Emmans (1989), utilizou em seu trabalho o valor de eficiência de utilização de 0,80 para todos os aminoácidos. Martin, Bradford e Gous (1994) em experimento para determinar as exigências dietéticas de aminoácidos das frangas poedeiras, utilizaram essa eficiência de utilização de 0,80, em razão de não terem muitos resultados sobre eficiência e considerando que este valor era independente de variações provocadas por genótipo, grau de maturidade, tipo e concentração de aminoácidos. Burnham, Emmans e Gous (1992) após realizar alguns experimentos com frangos de corte, estimaram valores de eficiência de utilização de Ile entre 0,60 e 0,73.

A eficiência de utilização é considerada a mesma para o corpo inteiro, porém, o estabelecimento das exigências em aminoácidos poderia ser mais preciso se fossem utilizadas eficiências diferentes para corpo livre de penas e penas em vista da variação na composição de aminoácidos do corpo e das penas (FISHER et al., 1981; STILBORN et al., 1997). Com base nisso, Kielanowski (1965) foi um dos primeiros a usar uma técnica de regressão linear múltipla para determinar da EU de energia depositada como gordura e proteína, considerando a deposição de cada componente químico e a ingestão de energia acima da manutenção. Este autor considerou o consumo de energia como a variável dependente e a deposição de proteínas e lipídios como variáveis independentes, utilizando o coeficiente de regressão para explicar a proporção em que a energia consumida é convertida em proteína corporal e lipídios corporais. Nos últimos tempos, a mesma técnica foi utilizada por outros autores (KOONG, 1977; AZEVEDO et al., 2005).

Outro aspecto importante de abordar é a variação da eficiência de utilização devido a fatores como: tipo de aminoácido e de seu nível na dieta (HEGER; FRYDRYCH, 1985), idade e sexo (D'MELLO, 2003) e genótipo das aves (FATUFE et al., 2004). Como exemplo, a eficiência de utilização de aminoácidos diminui com o aumento da idade dos animais (SILVA et al., 2014).

Diante da necessidade de atualização dos valores existentes de eficiência de utilização para cada aminoácido e devido sua importância para elaboração de modelos de predição para determinar as exigências em aminoácidos, nos seguintes capítulos serão abordados os métodos dose-resposta, fatorial e a determinação da eficiência de utilização de Ile para frangos de corte.

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CAPÍTULO 2 - RESPONSE OF BROILERS TO INCREASING LEVELS OF ISOLEUCINE

Abstract

The Isoleucine could be the fourth or fifth limiting amino acid in a diet of broilers, depending on the feed ingredients, denoting it importance to broiler nutrition. Furthermore, an evaluation of the requirement of a nutrient should be based in both biological and economic results. In this study, the optimum Ile intake was determined using mathematical modeling, and three procedures were applied aiming to produce information that will be helpful to a deep understand of each model. A total of six trials were carry out, three for male and three for females, using 1280 broiler chickens randomly distributed in 7 treatments, with 4 replicates of 20 birds each. The experiments were set to represent three phases of the growing broiler, initial (1 to 14 days old), growing (15 to 28 days old), and finishing (29 to 42 days old). Treatments consisted in different proportion of a summit diet diluted with a nitrogen free diet, producing seven levels of Ile for each phase, and keeping the ratio of all other essential amino acids constant. The response variable where feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR). Three different mathematical models (linear broken line (LBL), quadratic broken line (QBL), and polynomial quadratic (QUAD)) were adjusted to observed data, producing the optimal Ile level. The Akaike Information Criteria, a sensibility analysis and an economic simulation were applied to evaluate each model. The optimal Ile intake estimated ranged from 114.81 to 316.83, 535.49 to 1052.43, and 694.54 to 1610.04 mg of Ile per bird daily, for starter, growing and finisher phases, respectively. The QUAD model have the lower AIC value. After a reduction of 10% in the optimal Ile estimated, the LBL was the model with the higher impact in the response variable. According to the economic simulation, the feed formulated with the Ile level estimated by QBL model have

the lower cost among all models. Based on the results of this study, all models have a good adjustment to the data observed and QBL model have the best results for economic analysis applied.

INTRODUCTION

Studies evaluating the responses of broiler chickens to protein and amino acid intake contribute to advances in poultry economic, nutritional, and environmental sectors (Vieira, 2004). In this context, the application of ideal protein concept and the supply of adequate essential amino acid (EAA) levels, promoted a reduction of crude protein in feed. As a consequence, an improvement on broilers performance, a reduction on feed cost (Siqueira et al., 2011) and nitrogen excretion (Donato et al., 2016) are expected.

The nutrient requirements constantly change due to the productive advance of modern broilers (Zuidhof et al., 2014; Dorigam, 2016). Moreover, different nutritional requirements among broiler chicken genders is very well known, in which males have faster growth rate for body protein compared to relative growth rates of females (Havenstein et al., 2003; Gonçalves, 2017) and demanding more amino acids intake to supply the maintenance and the growth.

Despite the amino acids commonly studied (lysine, sulfur amino acids, threonine, etc), only few studies have tried to elucidate the responses of broiler chickens receiving increased levels of isoleucine (Ile). The values of Ile requirement found in literature ranged from 0.60% to 0.89% (NRC, 1994; Park and Austic, 2000; Rostagno et al., 2017). Differences in the genetic, environment, and age could have contributed to this variation (Baker et al., 2002), as well as the antagonism between amino acids. The excess of leucine in feed is known to reduce the plasmatic levels of Ile (Harper et al., 1984). This observation has lead certain investigations such as the necessity to keep the amino acid ratios in a dose response study. The dilution technique has been used to formulate diets in dose-response studies, enabling to produce feeds with crescent levels of amino acid and keeping the ratio among EAA (Fisher and Morris, 1970) to reduce or avoid the antagonist effect between amino acids.

The mathematic model used to determine a nutrient requirement in a dose-response trial could also be a source of variation (Vedenov and Pesti, 2008; Pesti et al., 2009). Moreover, despite the variation in predicted requirement, mathematic models have different paths up to the optimal intake, indicating that the description of the model characteristics need to be given into consideration.

Considering the exposed differences and the procedures applied to determine the amino acids requirements, it is necessary to know and update the precise requirement of each essential amino acid. Thus, the objective of this study was to determine the optimum Ile intake using mathematical modeling to describe the performance of male and female broilers, in different growing phases.

MATERIALS E METHODS

Ethics approval

The experiments were performed in accordance to the Ethical Committee on the Use of Animals of the São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences, Jaboticabal (Process 009999/14; approved on 06 June 2014).

Birds, housing and experimental design

The experiments were conducted at the Poultry Science Laboratory of São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences. One thousand two hundred eighty day-old Ross 308® broilers (640 male and 640 female) were obtained from a commercial hatchery where they were vaccinated against Marek's disease. Six assays were performed, three for males and three for females, in the periods from 1 to 14, 15 to 28, and 29 to 42 days. In each trial, birds were randomly distributed in 8 treatments with 4 replicates.

At the beginning of each assay the birds were individually weighed and distributed in experimental unit which had homogeneous weight. Water and feed was supplied *ad libitum* throughout the experimental period. Control of temperature, humidity and air renewal was carried out automatically through pad cooling and exhaust fans, being programmed according to the age of the birds. The lighting program used in the tests was 24 hours of light. The birds were vaccinated on the first day of life against Marek and Gumboro, at 12 days of age they were vaccinated against Newcastle (via drinking water) and at 16 days against Gumboro (via drinking water).

Experimental diets

Two diets (Ile summit and protein-free) were formulated (Tables 1 and 2) using the dilution technique according to Fisher and Morris (1970). The summit diet contained 1.19 times the digestible Ile levels suggested by Rostagno et al. (2011) and 1.49 times for the other amino acids to obtain relative isoleucine deficiency of 30% and maintain an ideal ratio of the other amino acids. Sequential dilutions of the high-protein diet with the iso-energetic protein-free (N free) diet were performed, resulting in diets with intermediate levels of Ile (1-14 d: 0.23 to 1.14%; 15-28 d: 0.21 to 1.05%, and 29-42 d: 0.19 to 0.97%).

Before feed processing, the ingredients soybean, peanuts meal, soybean meal, corn gluten, and corn were analyzed for amino acid and crude protein content, using near-infrared spectroscopy (NIR). Through the total amino acids amount acquired with the analyses, feed was formulated based on digestible amino acids according digestibility coefficient in the AMINODAT (Fickler et al., 2016).

Table 1 - Centesimal composition of the basal diets used in the experiment

Ingredients (%)	Diets	
	Ile Summit	N-Free
Corn (7.88%)	32.19	-
Soybean meal (45%)	26.30	-
Soy Protein Concentrate (62.7%)	12.00	-
Peanut Bran (48.2%)	11.93	-
Soybean oil	8.06	6.69
Corn gluten meal (60%)	2.83	-
Dicalcium phosphate	1.84	2.54
L-Lysine HCl (78.5%)	0.99	-
Limestone	0.90	0.43
DL-Methionine (99%)	0.79	-
L-Threonine (96.1%)	0.51	-
Salt	0.36	0.51
L-Valine (98%)	0.42	-
L-Arginine (98.5%)	0.15	-
L-Histidine (98%)	0.09	-
L-Tryptophan (98%)	0.05	-
Sodium bicarbonate	0.20	-
Premix Mineral and Vitamin (1)	0.516	0.516
Maize starch	-	65.00
Rice husk	-	15.00
Sugar	-	7.15
Potassium chloride	-	2.51
Composition (%)		
Metabolizable Energy (Kcal / kg)	3200	3200
Crude protein	30.40	0.45
Met. + Cys. Dig.	1.53 (1.54)	-
Methionine dig.	1.15 (1.10)	-
Lysine dig.	2.00 (2.20)	-
Isoleucine dig.	1.14 (1.23)	-
Threonine dig.	1.38 (1.47)	-
Arginine dig.	2.30 (2.30)	-
Valine dig.	1.64 (1.73)	-
Tryptophan dig.	0.36	-
Leucine dig. (%)	2.28 (2.39)	-
Phenylalanine dig.	1.42 (1.48)	-
Phe. + Tyr. dig.	2.45	-
Calcium	0.92	0.92
Phosphorus	0.47	0.47

(1)Content per kg - vit. A = 10575 IU; Vit. D3 = 2554 IU; Vit. K = 1.8 mg; Vit. E = 14.87 mg; Vit. B1 = 2.00 mg; Vit. B2 = 4.5 mg; Vit. B6 = 2.50 mg; Vit. B12 = 2.00 mg; Niacin = 30.00 mg; Folic acid = 0.75 mg; Calcium pantothenate = 11.74 mg; Biotin = 0.01; Iron = 43.44 mg; Zinc = 43.35 mg; Copper = 8.56 mg; Manganese = 56.00 mg; Iodo = 0.56 mg; Selenium = 0.34 mg; Antioxidant = 4.20 mg. Salinomycin (12%) = 550 mg. Adsorbent = 2 g. (2)Values between parentheses refer to total analyzed amino acid.

Table 2. Proportions of summit and N-free diets and the calculated concentration of limiting amino acid

Diets	1-14 days			15-28 days			29-42 days		
	Summit	N-free	Ile	Summit	N-free	Ile	Summit	N-free	Ile
	%	%	g/kg	%	%	g/kg	%	%	g/kg
D1	20	80	2.3	19	81	2.1	17	83	1.9
D2	36	64	4.1	33	67	3.7	30	70	3.4
D3	51	49	5.9	47	53	5.3	43	57	4.9
D4	67	33	7.7	61	39	6.9	55	45	6.3
D5	83	17	9.5	74	26	8.5	68	32	7.8
D6	92	8	10.5	83	17	9.5	77	23	8.8
D7	100	0	11.4	92	8	10.5	85	15	9.8
D8	20	80	4.1[1]	19	81	3.7[2]	17	83	3.4[3]

[1]D8 = D1 + 1.81 g l-isoleucine/kg.
[2]D8 = D1 + 1.61 g l-isoleucine/kg.
[3]D8 = D1 + 1.48 g l-isoleucine/kg.

Data collection

At the beginning and the end of each trial, birds and feed were weighed for the measurements of body weight gain (BWG) and feed intake (FI). Mortality was verified daily to adjust FI and calculate feed conversion ratio (FCR). The isoleucine requirement for performance data (feed intake, body weight, body weight gain, and feed conversion) was determined as a function of isoleucine intake.

Models description

The mean value of Ile intake (g/kg) was considered as independent variable and BWG or FCR was the dependent variable. The linear broken line (LBL), quadratic broken line (QBL), and polynomial quadratic (QUAD) were used to adjust the responses of each variable, as described by Robbins et al. (2006). In the segmented models (LBL and QBL), the intersection of the ascending curve with the plateau determines the optimal level of Ile. In the LBL a simple linear equation is used to describe the response of the dependent variable in function of independent variable, which is smooth connected to the plateau segment. Therefore, two functions were used to describe both segments:

$$LBL\colon Y_i = \begin{cases} L + U * (R - X) + e_{ij} & if\ X < R \\ L + U * (0) + e_{ij} & if\ X \geq R \end{cases}$$

Where, Y is the dependent variable (BWG or FCR), L is the breaking point in Y, U is the slope of the first segment, X is the response variable (amino acid intake), and ε is the error.

Same approach was adopted for QBL as follows:

$$QBL\colon Y_i = \begin{cases} L + U * (R - X)^2 + e_{ij} & if\ X < R \\ L + U * (0)^2 + e_{ij} & if\ X \geq R \end{cases}$$

For QUAD, the equation used is a second order regression equation, linear in the parameters, defined by:

$$QUAD\colon Y_i = a + b * X + c * X^2 + e_{ij}$$

Where a is the intercept in X axis, b is the regression parameter for linear component, and c is the regression parameter for the quadratic component.

Analyzing the Sensibility of the models

Beside the statistic criteria used to compare the models, a validation analysis was also performed according to Wessels et al. (2016). Since the models (LBL, QBL, and

QUAD) estimate different intakes for Ile, it is interesting to evaluate how the independent variable affect the dependent variable, which may be different for each model. In this sense, it was applied a reduction of 10% in the optimum amino acid intake estimated by each model aiming to evaluate the variation predicted for the response variable (e.g. reducing 10% of the predicted Y when X is in the breaking point for LBL and QBL, or when X reach the inflection point for the QUAD model, evaluating the variation in X).

Isoleucine as a limiting amino acid

Isoleucine is considered the 5th limiting amino acid in a corn and soybean meal based feed (Rostagno et al., 2017); however, to our knowledge there is no report in literature determining in which level of crude protein a supplementation of Isoleucine becomes necessary, and if it's economic viable. Therefore, a simulation was performed to identify in which crude protein level the Ile becomes limiting in the feed. Initially, 200 feeds were formulated using the package Solver from Microsoft Excel, which calculate a feed formula based on minimum cost through linear program, when a given constrain is set. The nutrient requirement was set in accordance to Brazilian Table (Rostagno et al., 2017), except for Ile, which was not constrained in the feed. The ingredients price (U$/kg) were obtained in Brazilian market in December of 2017 and the ingredients that changed among diets were: corn – U$ 0.15; soybean meal – U$ 0.31; DL-methionine – U$ 3.08; L-lysine – U$ 2.40; L-threonine – U$ 5.54; L-tryptophan – U$ 20.34; and L-valine – U$ 13.56. All feeds were equal in nutrients, changing only in crude protein (CP) and Ile contents. The variation from a diet to the next one was 0.034% of CP, starting at 19.85% for the first feed until 16.86% of CP for the last feed.

Statistical analyses

Descriptive statistics was used to analyze the normality assumption and to identify outliers by a Proc Univariate and Proc Means procedures. The Dunnett's one-tailed t test was used to identify if diet 1 was significantly less or greater then diet 8 for BWG or FCR, by Proc GLM option DUNNETTL or DUNNETTU, respectively.

Linear and nonlinear regressions were employed to determine ideal levels of isoleucine, through Proc Reg and Proc NLMixed procedures. To evaluate the fit among models, information criteria (AIC) and the coefficient of determination (R^2) were applied. All statistical analyses procedures were performed in SAS (Statistical Analysis System, version 9.3).

RESULTS

In order to determine the requirement of an amino acid, this compound must be the first limiting nutrient present in the diet. Indeed, it is guaranteed that the results obtained herein are solely based on birds' responses to different levels of isoleucine (Ile). The Ile limitation was confirmed by the slightly increase in BWG and a reduction of FCR data obtained from diet 8 (D8) compared with D1, which was confirmed statistically by Dunnett's one-tailed t test ($P<0.05$). The averages of performance data from females and males of broilers submitted to diets with increasing levels of Ile are summarized in Table 3.

Birds that received diets with lower levels of Ile had reduced BWG and increased FCR. On the other hand, increasing Ile levels led to the improvement of BWG, FI and FCR. Moreover, the maximum feed intake was found close to the fourth level of Ile either to males or females. Nevertheless, FI slightly decreased in so far as Ile levels in diets were increasing.

In all phases, there was effect ($P<0.0001$) of Ile levels on FI, Ile intake, BWG, and FCR. Based in these results, the models were adjusted to determine optimal Ile intake to BWG and FCR. In all phases, the plateau for BWG occurred between the third and the fourth levels of Ile, resulting in three levels on the slope.

Table 3. Effects of different dietary levels of isoleucine for female and male during initial, grower and finisher phases

		Female				Male			
Diet	Ile level	FI[1]	Ilei[2]	BWG[3]	FCR[4]	FI	Ilei	BWG	FCR
1-14d									
D1	2.3	12.7	29.1	3.59*	3.55*	12.9	29.7	4.19	3.16*
D2	4.1	22.2	90.9	12.2	1.82	20.8	85.3	12.0	1.78
D3	5.9	26.1	154	18.7	1.40	29.1	172	21.7	1.34
D4	7.7	28.2	217	23.4	1.20	31.6	243	25.4	1.24
D5	9.5	26.6	253	23.9	1.11	30.0	285	28.2	1.06
D6	10.5	27.3	286	24.6	1.11	26.0	273	25.9	1.00
D7	11.4	26.8	305	23.9	1.12	27.5	313	26.2	1.05
D8	4.1	12.5	51.2	6.03	2.08	14.1	57.8	5.65	2.51
SEM[5]		1.01	18.5	1.44	0.16	1.23	19.6	1.63	0.14
P-value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
15-28d									
D1	2.1	87.7	188	25.4*	3.45*	94.9	203	25.8*	3.69*
D2	3.7	110	410	51.2	2.15	119	444	54.7	2.18
D3	5.3	115	614	70.3	1.64	130	693	78.7	1.66
D4	6.9	115	792	81.1	1.41	127	880	92.9	1.37
D5	8.5	107	913	79.2	1.36	118	1000	90.4	1.30
D6	9.5	103	976	76.7	1.34	115	1095	92.2	1.25
D7	10.5	101	1065	74.1	1.37	111	1171	87.0	1.28
D8	3.7	93.7	349	29.6	3.16	97.3	363	29.2	3.33
SEM		1.76	57.2	3.62	0.14	2.19	63.8	4.55	0.16
P-value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
29-42d									
D1	1.9	167	326	27.5*	6.33*	191	372.9	22.5*	8.58*
D2	3.4	180	613	60.6	2.98	215	729.5	70.0	3.10
D3	4.9	185	895	80.3	2.29	219	1061	97.3	2.25
D4	6.3	183	1155	90.2	2.03	217	1371	108	2.02
D5	7.8	173	1341	91.1	1.90	211	1636	117	1.81
D6	8.8	173	1516	77.6	2.23	211	1846	101	2.08
D7	9.8	165	1608	88.7	1.86	198	1928	115	1.73
D8[7]	3.4	168	570	35.9	4.67	186	633	36.8	5.08
SEM		0.035	84.9	4.13	0.30	2.20	96.8	6.08	0.42
P-value		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

*Mean is statistically smaller from treatment 8 (P < 0.05) by Dunnett test.

The adjusted model allowed to determinate the optimal Ile levels for broilers. The equations were adjusted appropriately for the data (P<0.001). The R^2 for all models indicated good adjustments. For both sexes, Ile intakes were estimated by LBL model which were lower than those obtained with QBL and QUAD (Tables 4 and 5). The same was observed for FCR. The results showed that the optimal Ile intake varies according to the model used. In some cases, the QUAD model showed a response slope to treatments with Ile levels above the requirement.

The Akaike (AIC) criteria were used for all models. The QUAD model showed the lowest values among all models, suggesting that the data is better predicted by QUAD model rather than by broken line models. When the optimal intake estimated was reduced by 10% (Table 6) the LBL model showed a great decline in BWG when compared to QUAD, evidencing a considerable impact on performance once the recommended dosage was not used. On the other hand, the quadratic broken-line model presented a slight decrease in weight gain similarly to what was observed for the quadratic model. The performance reduction difference is showed in table 6.

Table 4. Models adjusted to body weight gain and feed conversion ratio in function of digestible isoleucine intake for female broilers from 1-14 (initial phase), 14–28 (growth phase) and 28–42 days (finisher phase) old

Model[1]	Equation	Ilei[2]	BWG[3]	AIC	R^2
	Body weight gain				
	01-14 d				
LBL	Y = 23.99-0.12*(194.47-Ile)	194.47	23.99	102.60	0.97
QBL	$Y = 24.23-0.0004*(275.80-Ile)^2$	275.80	24.23	92.10	0.97
QUAD.	$Y = -1.65+0.18*Ile-0.0003*Ile^2$	296.79	24.69	41.21	0.97
	15-28d				
LBL	Y = 77.76-0.11*(676.93-Ile)	676.93	77.76	136.30	0.98
QBL	$Y = 77.75-0.0001*(878.07-Ile)^2$	878.07	77.75	141.80	0.98
QUAD.	$Y= -9.05-0.20*Ile-0.0001*Ile^2$	880.38	78.53	78.55	0.99
	29-42d				
LBL	Y = 86.91-0.09*(942.94-Ile)	942.94	86.91	182.40	0.93
QBL	$Y = 87.56-0.00007*(1251.96-Ile)^2$	1251.96	87.56	372.10	0.94
QUAD.	$Y = -21.38+0.17*Ile-0.0001*Ile^2$	1281.13	90.21	127.05	0.92
Model	Equation	Ilei	FCR[4]	AIC[5]	R^2
	Feed conversion rate				
	01-14 d				
LBL	Y = 1.19+0.03*(114.81-Ile)	114.81	1.19	-23.10	0.98
QBL	$Y = 1.17+0.0001*(166.60)^2$	166.60	1.17	-26.40	0.98
QUAD.	$Y = 4.03-0.03*Ile-0.00005*Ile^2$	241.01	0.87	-52.11	0.93
	15-28d				
LBL	Y = 1.42+ 0.01*(535.49-Ile)	535.49	1.42	-36.70	0.97
QBL	$Y = 1.37-0.00001*(808.41-Ile)^2$	808.41	1.37	-61.80	0.99
QUAD.	$Y = 4.63-0.01*Ile+0.000004*Ile^2$	893.99	1.30	-101.90	0.96
	29-42d				
LBL	Y = 2.07+0.01*(694.54-Ile)	694.54	2.07	46.40	0.98
QBL	$Y = 2.06+0.00002*(866.30-Ile)^2$	866.30	2.06	59.50	0.98
QUAD.	$Y = 9.45-0.01*Ile+0.000005*Ile^2$	1255.46	1.66	6.88	0.84

[1]LBL, linear broken line; QBL, quadratic broken line; QUAD, polynomial quadratic;
[2]Ilei, Isoleucine intake;
[3]BWG, Body weight gain;
[4]FCR, Feed conversion ratio;
[5]AIC, Akaike information criterion;
R^2, Coefficient of determination.

Table 5. Models adjusted to weight gain and feed conversion rate in function of digestible isoleucine intake for male broilers from 1-14 (initial phase), 14–28 (growth phase) and 28–42 days (finisher phase) old

Model[1]	Equation	Ilei[2]	BWG[3]	AIC	R^2
	Body weight gain				
	01-14 d				
LBL	Y = 26.44-0.12*(206.85-Ile)	206.85	26.44	114.00	0.95
QBL	Y = 27.06-0.0003*(308.79-Ile)²	308.79	27.06	108.70	0.95
QUAD.	Y = -0.91+0.18*Ile-0.0003*Ile²	316.83	27.20	57.30	0.95
	15-28d				
LBL	Y = 90.61-0.11*(793.60-Ile)	793.60	90.61	153.70	0.98
QBL	Y = 91.14-0.00009*(1058.40-Ile)²	1058.40	91.14	159.90	0.97
QUAD.	Y = -11.35-0.20*Ile-0.0001*Ile²	1052.43	88.56	105.75	0.98
	29-42d				
LBL	Y = 110.25-0.11*(1150.82-Ile)	1150.82	110.25	195.50	0.93
QBL	Y = 111.83-0.00006*(1598.62-Ile)²	1598.62	111.83	316.00	0.95
QUAD.	Y = -39.04+0.19*Ile-0.00006*Ile²	1610.04	113.94	140.52	0.93
Model	**Equation**	**Ilei**	**FCR[4]**	**AIC[5]**	**R^2**
	Feed conversion rate				
	01-14 d				
LBL	Y= 1.14+0.02*(115.25-Ile)	115.25	1.14	16.50	0.87
QBL	Y = 1.14+0.0001*(164.20)²	164.20	1.14	16.90	0.88
QUAD.	Y = 3.49-0.02*Ile-0.00004*Ile²	255.73	1.04	-29.55	0.85
	15-28d				
LBL	Y = 1.37+0.01*(575.94-Ile)	575.94	1.37	-21.60	0.96
QBL	Y = 1.31+0.000005*(904.62-Ile)²	904.62	1.31	-39.00	0.99
QUAD.	Y = 4.93-0.01*Ile+0.000004*Ile²	1005.90	1.24	-82.55	0.96
	29-42d				
LBL	Y = 1.98+0.02*(805.13-Ile)	805.13	1.98	26.10	0.99
QBL	Y = 1.98+0.00002*(972.71-Ile)²	972.71	1.98	21.20	0.99
QUAD.	Y = 13.10-0.02*Ile+0.00001*Ile²	1489.12	1.70	16.96	0.83

[1]LBL, linear broken line; QBL, quadratic broken line; QUAD, polynomial quadratic;
[2]Ilei, Isoleucine intake;
[3]BWG, Body weight gain;
[4]FCR, Feed conversion ratio;
[5]AIC, Akaike information criterion;
R^2, Coefficient of determination.

Table 6. Effect of reduction on Ile recommendation on performance for female and male broilers from 1-14 (initial phase), 14–28 (growth phase) and 28–42 days (finisher phase) old.

	Female			Male		
	LBL[4]	QBL[5]	QUAD[6]	LBL	QBL	QUAD
1-14d						
L[1]	23.99	24.23	24.68	26.44	27.06	27.20
R[2]	194.47	275.80	300.00	206.85	308.79	316.83
Decrease (%)[3]	9.94	1.13	0.86	9.70	1.06	1.03
15-28 d						
L	77.76	77.75	78.00	90.61	91.14	91.32
R	676.93	878.07	880.38	793.60	1058.40	1052.43
Decrease (%)	9.18	1.90	1.12	9.49	1.11	1.12
29-42 d						
L	86.9	87.56	90.15	110.25	111.83	113.34
R	848.65	1251.96	1280.80	1150.82	1598.62	1610.04
Decrease (%)	10.07	1.25	1.24	11.42	1.37	1.34

[1]L, BWG (g/bird/d) by the models;
[2]R, Ile estimated by the models (mg/bird/d)
[3]Decrease in BWG with -10% in the Ile intake estimated by models;
[4]LBL, Linear broken line;
[5]QBL, Quadratic broken line;
[6]QUAD, Polynomial quadratic;

Isoleucine is considered the 5th limiting amino acid in a corn and soybean meal based feed (Rostagno et al., 2017). Therefore, a simulation was performed to identify in which crude protein level the Ile becomes limiting in the feed and also to evaluate the variation of the feed cost against Ile. According to this simulation and following the requirement of broiler from 34 to 42 days old (Rostagno et al., 2017), Ile starts to be limiting in the feed when CP reaches 18.03% in the feed. The feed cost decrease until an Ile reduction of 0.711% (18.40% of CP), then feed cost starts to increase (Figure 1).

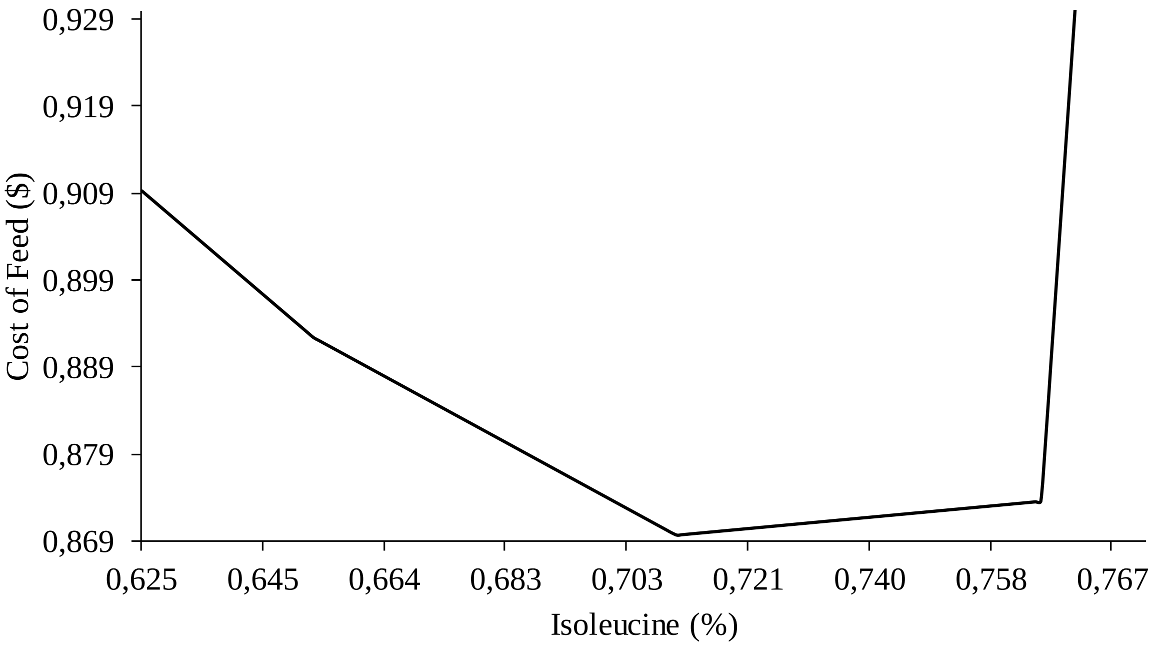

Figure 1. Variation of feed cost according to crescent levels of isoleucine.

DISCUSSION

In this study, Ile intake to maximize performance was estimated through the response of broilers to diets with increasing levels of Ile. The responses obtained with diet 8 showed that Ile was the first limiting amino acid in the diet, since as BWG and FCR responses were between diets 1 and 2, improving the performance of the birds through the supplementation of Ile in diet 8 ($P<0.05$). The results attested that the variation observed in broiler response is due to Ile deficiency, the dilution technique allows a constant balance between amino acids, producing a relative deficiency of the studied amino acid. This response validates this study confirming that the obtained response is due to the amino acid tested, as proposed in the dilution technique (Fisher and Morris, 1970; Gous and Morris, 1985).

The responses obtained in this study showed that lower Ile levels in the diet affected performance. This is in agreement with Burnham et al. (1992). Other studies that did not use this formulation method, such as Kidd et al. (2004) and Corzo et al. (2010) also observed lower weight gain and worst feed conversion for broilers fed with Ile-deficient diets. In another study with broiler chickens, it was observed that broilers fed 0.42% Ile had low BWG and FI and higher FCR than broilers fed 0.72% Ile (Hale et al., 2004).

It has been demonstrated that excessive levels of protein lead to a feed intake reduction. The main hypothesis is that the metabolism of excess protein will increase the concentration of ammonia in blood reducing feed intake (Namroud et al., 2008). Herein, a similar result was observed, where broilers fed diets containing higher levels of Ile demonstrate a lower feed intake.

The application of statistical models allows to understand the questions about adequate feeding levels (Pesti and Vedenovi, 2011). Thus, models were used to predict the requirements of animals. In the present study, the results obtained with LBL model suggested the lower Ile level, whereas QUAD model estimate the highest Ile level.

In general, the levels determined in this study were higher than other literature results, mainly for initial phase (Rostagno et al., 2017; Farran and Thomas, 1990; NRC, 1994). Many factors may be influencing this difference (environment, diet, and genetic), however, the mathematical model used to describe the broiler response to crescent levels of Ile is also a source of variation. Thus, an important factor to be considered while choosing for an amino acid requirement is how this level was set. In the present study, AIC was used to evaluate the models and its values demonstrated that the QUAD model has the best adjustment to observed data. Although, it is risky to state which is the best intake of an amino acid for certain genetic, age and sex only based on the prediction of a mathematical model which obtained a better selection criterion. Statistically, the model's selection criteria facilitate the adoption of a model, however this choice may not compensate economically when choosing a model that recommends higher levels of an amino acid.

In fact, each model has advantages and disadvantages. The LBL and QBL models are considered as nonlinear models (Robbins et al., 2006), in which the parameters have a biological meaning, facilitating the interpretation of the equation adjusted. In both broken line models applied in this study, the parameter L indicate the amount of Ile necessary to maximize the response variable, in this sense, the parameter R indicate the maximum value predicted for the response value. The difference among LBL and QBL rely on the ascension portion of the curve, where any increase in the studied nutrient will reflect a variation of the response variable, however, the QBL assumes a quadratic ascension, which might best mimic the biologic phenomena (Lamberson and Firman, 2001). On the other hand, the QUAD model is considered a linear model, in relation to it's parameters, could be simple to fit the equation to observed data, however, without any calculus of the original equation, the parameters itself do not have any biological meaning, being necessary a first derivative of the equation in order to find the maximum response and consequently the optimal dose of the nutrient studied. According to Robbins et al. (2006), the BL model considers that the changing rate with the nutrient dose diminished as the dose approaches to its requirement; however, the QBL as well as LBL model cannot identify the toxic concentration of a nutrient (Pesti et al., 2009).

It's necessary to look for consistent methods to evaluate a model, in which some characteristics attributed to a particularly model, enable the user to understand the consequences of a changing in the optimum dose estimated. Based on this, a procedure adopted from Wessels et al. (2016) was applied in this study, aiming to observe prediction differences among models. Using this approach, its possible to observe how is the impact in the response variable if a reduction of 10% is applied to the optimum amino acid intake determined in the model. Applying this procedure to LBL model, resulted in a diminished of more than 10 % of BWG compared to 1 to 1.5 % with the QUAD model. This result denotes the importance to understand the model, not only the final prediction determined by it. Its notable the way that the variable response goes through until reaches the optimal point is different. This BWG reduction by LBL model is much more accentuate than the QBL and QUAD models.

In a nutrient requirement study is important to be attentive to the diet crude protein (CP) level and to the feed cost which varies with a higher or lower requirement. Based on this, 200 corn and soybean meal-based feeds were formulated in order to obtain a protein variation ranging from 19.85 to 16.86 % with a 0.015 % of crude protein range between each diet. With this exercise is possible to determine in which CP level the Ile becomes necessary and what is the feed cost in this specific level. The simulation started with the higher value of CP, where the Ile level was calculated as 0.767% in the diet, which demonstrates that the optimum value estimated for QUAD model was not completely satisfied. In the opposite, the feed formulated to contain the lower value of CP contained 0.625% of Ile, which is higher than the value estimated by LBL models. In both cases, it seems expensive to produce a feed to meet the optimum Ile intake determined by LBL and QUAD models. Nevertheless, the values estimated by QBL models (males-0.66; females-0.63) was the closest to the feed with the lower cost among all 200 diets formulated. Considering the impact in the response variable when a changing in the isoleucine estimated is performed and a corn and soybean meal based feed, formulated in minimum cost, the optimum isoleucine level estimated using quadratic broken line model was more appropriate in an economic point of view. As demonstrated in this study not only the nutrient requirement must be known, also the mathematical model used to determine the requirement and its characteristics.

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CAPÍTULO 3 – Factorial models to estimate isoleucine requirements for broilers

Abstract

The use of models in poultry nutrition is becoming decisive among companies, where such tool is applied to improve productivity and profit. In this context, there is a necessity of more research, to elucidate the Efficiency of Utilization and to produce models to predict an optimal nutrient intake, such as Isoleucine (Ile) intake. The objective of this work was to determine the EU and produce factorial models for optimal Ile intake. Six dose response trials were carried out, three for males and three for females, with 640 Ross 308 in each studied phase. The initial (1 to 14 days), grower (15 to 28 days), and finisher (29 to 42 days) phases were evaluated to cover the growing phase of the broiler chicken. In total, 8 treatments were randomly distributed to 4 replicates of 20 birds each. The treatments consisted in 7 crescent levels of Ile and one counter proof, to ensure that Ile was the first limiting amino acid in the diet. Dilution technique was applied to produce the levels of Ile and keep the amino acid ratio with lysine. At the beginning and the end of each trial, representative individual were slaughter to determine protein and amino acid deposition. The EU were determined to account for whole body or partitioned for feather free body (Bff) and feather. Two distinct factorial models were adjusted, M1 and M2. The M2 model were evaluated for one or two EU, being denominated as M2 and M3. Models were tested using the residual against predicted technique. When the efficiency was partitioned, the values of 53% and 69% for feather and Bff were determined. The optimal Ile intake estimate for each model were of 275, 908, 1412 mg of Ile/bird/day (M1); 258, 829, 1321 mg of Ile/bird/day (M2); and finally 284, 835, 1288 mg of Ile/bird/day (M3) for initial, grower, and finisher phases, respectively. The results suggests that the M3 is the less biased model. The EU partitioned for feather free body and feather reduced the biased of the model M3. Overall, higher values of Ile intake is estimated when model M1 is used, which may be the difference in account for body weight gain (M1) or only protein gain (M2 and M3) to estimate the amount of amino acid required for broiler.

Key-words: isoleucine, mathematical modeling, efficiency of utilization

INTRODUCTION

The mathematical modeling in poultry nutrition has been applied for several years. The use of the models contributes to understand biological phenomena and to evaluate optimal levels of nutrients. Thus, its applicability affords costs reduction due to the supply of a diet formulated in accordance to the recommended levels predicted by the models, to the improvement of animal performance and finally, to the reduction of both nitrogen and environmental contamination (Donato et al., 2016).

In this context, the correct determination of the efficiency of utilization (EU) of an amino acid could be the difference between a good or a poor prediction of both requirement and animal performance. In addition, the EU changes among nutrients (Siqueira et al., 2011; Tavares et al., 2016; Donato et al., 2016; Dorigam et al., 2016), which gives a necessity to evaluate the EU for each amino acid. Furthermore, there are distinct procedures to determine the EU of a nutrient, and some techniques are addressed in this study.

The EU could be determined by a simple regression equation, in which the deposition of the amino acid in the body is the dependent variable and the intake of the amino acid for gain is the independent variable (Emmans, 1981). In this approach, the efficiency how an amino acid is deposited in different tissues is considered as the same, however, may be different for body and feathers deposition (Emmans, 1989). Kielanovski (1965) determined the EU of energy deposited as fat and protein, considering the deposition of each chemical component and the intake of energy above maintenance. The protein composition of feather-free body and feathers are different (Fisher et al., 1981; Stillborn et al., 1997), being reasonable to determine EU separately, as done by Kielanovski (1965). In some literature factorial models to determine amino acid requirements utilizes the same efficiency for feather-free body (Bff) and feathers (Rostagno et al., 2017). However, the prediction of the amino acid requirement should be more precise if different efficiencies for Bff and feathers were used in view of the different amino acid composition of body and feathers (Fisher et al., 1981; Stilborn et al., 1997). To our knowledge there is no literature work in such issue.

Furthermore, determining requirements based on physiological parameters as maintenance and production enables to do more accurate recommendations (Fisher, 2013). The methodologies used to settle poultry responses to amino acids are the empirical and factorial methods. The first one does not reflect the requirement other than that in which the research was developed (Oviedo-Rondon; Waldroup, 2002). On the other hand, the factorial method is based on the partition of nutrients requirements, e.g. maintenance, growth of body components, and productive potentials (Sakomura et al., 2015). Factorial models are also dynamic, which gives flexibility to nutritionists in produce feeds to meet the requirement of broiler for every age. Thus, models are important to determine precisely the requirements of essential amino acids for broilers and to update nutritional recommendations. Among the essential amino acids, isoleucine (Ile) is considered the fifth limiting amino acid in a soybean meal and corn based diet (Berres et al., 2010). Previous researches showed that this amino acid has an important role on protein synthesis processes once it acts as substrate to glutamine (Wu, 2009), improves intestinal immune response (Zhao et al., 2014) and also sensitizes tissues to the hormones Insulin an Insulin Growth Factor-1 (IGF-1), reinforcing the importance in study Ile requirement.

Models to determine Ile requirements to broilers taking into account deposition and efficiency utilization of this amino acid on body and feathers could contribute to update requirements and improvement of nutritional programs. Thereby, we aimed to determine the efficiency of utilization of isoleucine on the feather-free body and feathers and elaborate factorial models to estimate broilers' Ile requirements.

MATERIALS E MHETODS

Ethics approval

Experiments were performed in accordance to the Ethical Committee on the Use of Animals of the São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences, Jaboticabal (Process 009999/14; approved on 06 June 2014).

Birds, housing and experimental design

The experiments were conducted at the Poultry Science Laboratory of Sao Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences. One thousand two hundred eighty day-old Ross 308® broilers (640 male and 640 female) were obtained from a commercial hatchery where they were vaccinated against Marek's disease. Six assays were performed, three for males and three for females, in the periods from 1 to 14, 15 to 28, and 29 to 42 days. In each trial, birds were randomly distributed in 8 treatments with 4 replicates.

At the beginning of each assay birds were individually weighed and distributed in experimental unit which had homogeneous weight. Water and feed were supplied *ad* *libitum* throughout the experimental period. Control of temperature, humidity and air renewal was carried out automatically by the means of pad cooling and exhaust fans, being programmed according to the age of the birds. The lighting program used in the tests was 24 hours of light. The birds were vaccinated on the first day of life against Marek and Gumboro, at 12 d-old they were vaccinated against Newcastle (via drinking water) and at 16 d-old against Gumboro (via drinking water).

Experimental diets

Two diets (Ile summit and protein-free) were formulated (Tables 1 and 2) using the dilution technique according to Fisher and Morris (1970). The summit diet contained 1.19 times the digestible Ile levels suggested by Rostagno et al. (2011) and 1.49 times for the other amino acids to obtain relative isoleucine deficiency of 30% and maintain an ideal ratio among amino acids. Sequential dilutions of the high-protein diet with the iso-energetic protein-free (N-free) diet were performed, resulting in diets with intermediate levels of Ile (1-14 d: 0.23 to 1.14%; 15-28 d: 0.21 to 1.05%, and 29-42 d: 0.19 to 0.97%).

Table 1 - Centesimal composition of the basal diets used in the experiment

Ingredients (%)	Diets	
	Ile Summit	N-Free
Corn (7.88%)	32.19	-
Soybean meal (45%)	26.30	-
Soy Protein Concentrate (62.7%)	12.00	-
Peanut Bran (48.2%)	11.93	-
Soybean oil	8.06	6.69
Corn gluten meal (60%)	2.83	-
Dicalcium phosphate	1.84	2.54
L-Lysine HCl (78.5%)	0.99	-
Limestone	0.90	0.43
DL-Methionine (99%)	0.79	-
L-Threonine (96.1%)	0.51	-
Salt	0.36	0.51
L-Valine (98%)	0.42	-
L-Arginine (98.5%)	0.15	-
L-Histidine (98%)	0.09	-
L-Tryptophan (98%)	0.05	-
Sodium bicarbonate	0.20	-
Premix Mineral and Vitamin(1)	0.516	0.516
Maize starch	-	65.00
Rice husk	-	15.00
Sugar	-	7.15
Potassium chloride	-	2.51
Composition (%)(2)		
Metabolizable Energy (Kcal / kg)	3200	3200
Crude protein	(30.40)	(0.45)
Met. + Cys. Dig.	1.53 (1.54)	-
Methionine dig.	1.15 (1.10)	-
Lysine dig.	2.00 (2.20)	-
Isoleucine dig.	1.14 (1.23)	-
Threonine dig.	1.38 (1.47)	-
Arginine dig.	2.30 (2.30)	-
Valine dig.	1.64 (1.73)	-
Tryptophan dig.	0.36	-
Leucine dig. (%)	2.28 (2.39)	-
Phenylalanine dig.	1.42 (1.48)	-
Phe. + Tyr. dig.	2.45	-
Calcium	0.92	0.92
Phosphorus	0.47	0.47

(1)Content per kg - vit. A = 10575 IU; Vit. D3 = 2554 IU; Vit. K = 1.8 mg; Vit. E = 14.87 mg; Vit. B1 = 2.00 mg; Vit. B2 = 4.5 mg; Vit. B6 = 2.50 mg; Vit. B12 = 2.00 mg; Niacin = 30.00 mg; Folic acid = 0.75 mg; Calcium pantothenate = 11.74 mg; Biotin = 0.01; Iron = 43.44 mg; Zinc = 43.35 mg; Copper = 8.56 mg; Manganese = 56.00 mg; Iodo = 0.56 mg; Selenium = 0.34 mg; Antioxidant = 4.20 mg. Salinomycin (12%) = 550 mg. Adsorbent = 2 g. (2)Values between parentheses refer to total analyzed amino acid.

Table 2. Proportions of summit and N-free diets and the calculated concentration of Isoleucine (Ile)

Diets	1-14 days			15-28 days			29-42 days		
	Summit	N-free	Ile	Summit	N-free	Ile	Summit	N-free	Ile
	%	%	g/kg	%	%	g/kg	%	%	g/kg
D1	20	80	2.3	19	81	2.1	17	83	1.9
D2	36	64	4.1	33	67	3.7	30	70	3.4
D3	51	49	5.9	47	53	5.3	43	57	4.9
D4	67	33	7.7	61	39	6.9	55	45	6.3
D5	83	17	9.5	74	26	8.5	68	32	7.8
D6	92	8	10.5	83	17	9.5	77	23	8.8
D7	100	0	11.4	92	8	10.5	85	15	9.8
D8	20	80	4.1[1]	19	81	3.7[2]	17	83	3.4[3]

[1]D8 = D1 + 1.81 g L-isoleucine/kg of feed.
[2]D8 = D1 + 1.61 g L-isoleucine/kg of feed.
[3]D8 = D1 + 1.48 g L-isoleucine/kg of feed.

Before feed processing, the ingredients soybean, peanut meal, soybean meal, corn gluten, and corn meal were analyzed for amino acid and crude protein content, using near-infrared spectroscopy (NIR). Feed was formulated based on digestible amino acids according digestibility coefficient from AMINODAT® (Fickler et al., 2016).

Data collection

At the beginning and the end of each trial, birds and feed were weighed for the measurements of body weight gain (BWG) and feed intake (FI). Mortality was verified daily to adjust FI and calculate feed conversion ratio (FCR). The isoleucine requirement for performance data (body weight gain, and feed conversion) was determined as a function of isoleucine intake.

Body nutrient deposition

To evaluate body nutrient deposition (g/bird/day) on feather-free body and feathers, the comparative slaughter technique was performed. Thus, 24 chickens (12 females and 12 males) at the beginning (referential slaughter) and 128 (two from each experimental unit) at the end of each assay were slaughtered.

After 24 hour-fasting, birds were weighed and euthanized by CO_2 inhalation. A sample of feathers were used to analysis and the rest of them was removed. Feather-free body was weighed, identified and frozen at -20 ° C for further processing. The frozen Bff was sawed using a band saw, milled on industrial grinder, homogenized, weighed and frozen at -80 ° C for subsequent pre-drying. Samples were freeze-dried over 72 hours at -80 °C and -10 ATM (Edwards SuperModulo, Thermo Fisher Scientific, Waltham, MA), after dried were weighted and milled on a microgrinder. Samples were analyzed for ether extract, dry matter, ash and crude protein. Feather samples were dried in a forced-ventilated oven at 55 ° C for 72 h, weighed, grounded in a milled for ash analysis, dry matter and nitrogen according to AOAC (2005). To determine the total dry matter content, samples were dried for 16 h in a conventional oven at 105 ° C (method 920.39).

Nitrogen content of samples was measured by Kjeldahl procedure (Foss Kjeltec 8400, method 2001.11) multiplying nitrogen content by 6.25. Carcass samples were previously defatted to quantify the total nitrogen content. Ash analysis was performed incinerating the sample in a muffle oven at 550°C per 4 hours (method 942.05). Total fat extraction was done with ether petroleum as a solvent in the fat extractor AnkomXT15 (ANKOM Technology, Macedon, NY) (method 920.39).

The percentages of Ile in the protein of *Bff* and feather used in the present study are derived from aminogram analyses of carcass and feather from animals with 1, 14, 28, and 42 days old. To amino acid quantification, High-Performance Liquid

Chromatography (HPLC) were used. The isoleucine in *Bff* and feather were 3.69 and 4.36, respectively, expressed in mg per 100 g of protein. Ile deposited in *Bff* and feather was obtained by difference of Ile found in the end and beginning of the trial.

Efficiency of utilization

The efficiency of utilization, was determined considering the whole body or particion in EU for *Bff* and feathers. Data collected from treatments 1 to 4 were used to determine the EU. It is assumed that maintenance has first priority, and the remaining amino acid will be directed to body deposition. Hence, the amino acid consumed for gain was determined subtracting the amount required for maintenance (de Lima et al., 2016) from the total amino acid intake.

Statistical analysis

Simple linear regression

EU is the slope of regression equation adjusted with Ile consumed for gain (independent variable) and Ile deposited (dependent variable), as in the equation 1:

$$Daa = a + EU * Iaag \qquad \text{[Eq. 1]}$$

Where, Daa is Ile deposition in the whole body, a is the intercept of the equation, and $Iaag$ is the intake of the amino acid for gain.

The efficiency of utilization varies with age, level of the amino acid consumed, body weight, among other factors (Silva et al., 2014). Thus, to consider BW in EU, an exponential equation was fitted, as described:

$$Y = A + B * (1 - e^{c*(BW)}) \qquad \text{[Eq. 2]}$$

Where *Y* is the efficiency of utilization for Ile, *A* is the intercept in X axes, *B* is the rate of decrease, *C* is the slope of the exponential curve; *BW* is the body weight and *e* is the base number of natural logarithm (2.718282).

Multiple linear regression

A multiple linear regression was adjusted, the Ile consumed for gain (*Iaag*) (mg/bird/day) as a function of Ile deposition in *Bff* and feathers (Kielanowski, 1965; Azevedo et al., 2005), as the equation:

$Iaag = b1 * Bff + b2 * Feather$ [Eq. 3]

The slopes values b1 and b2 are the amino acid conversion ratio and represent the amount of *Iaag* deposited in *Bff* and feathers, respectively. The *EU* for *Bff* (EU_{Bff}) and feathers (EU_F) is obtained by $\frac{1}{b1}$ and $\frac{1}{b2}$.

Models to predict Ile requirements

In order to predict broilers' digestible Ile requirement, three models were developed. The Model 1 (empiric), does not differentiate the composition of *Bff* and feathers (Siqueira, 2009). The Model 2 (mechanistic) consider the maintenance and growth of *Bff* and feathers (Emmans, 1981; Siqueira, 2009). The Model 3 (mechanistic) consider two efficiencies of utilization, for *Bff* and feather.

Empirical Model

In this model, the Ile requirement is estimated without considering the differences in *Bff* and feathers composition. The parameter which expresses Ile requirement for maintenance is based on metabolic body weight (32 mg/kg $BW^{0.75}$ d), according to de Lima et al. (2016). The digestible Ile requirement is estimated by the model:

M1: $Ile_r = (32 * BW^{0.75}) + (\frac{a+b*(BWG)}{EU})$ [Eq. 4]

Where Ile_r is the digestible isoleucine requirement (mg/ day); *BW* stands for body weight (kg); *EU* is the efficiency of isoleucine utilization for body deposition; *a* and *b* are linear regression coefficients between deposition of Ile (*DIle)* and *BWG.*

Mechanistic Model

The Ile requirement for maintenance used in this model was based on protein unit, which consider the body protein weight at maturity (*PBm*) and the degree of maturity of body protein (u). The parameter that expresses the Ile requirement for maintenance of *Bff* protein (134 mg/kg $BPm^{0.73}$) was according to de Lima et al. (2016). The protein requirement for maintenance of feather is considered equivalent to loss of feathers (Emmans, 1989). The proposed Model 2 is:

M2*:* $Ile_r = [(AA_m c * BP_m^{0.73} * u) + (FL * FP * AA_f)] + [\frac{(AA_b*BPD)}{EU} + \frac{(AA_f*FPD)}{EU}]$ [Eq. 5]

Where Ile_r is the requirement of isoleucine (mg/ day); AA_mc represents the amino acid requirement for maintenance (mg/$PB_m^{0.73}* u$.dia); $BP_m^{0.73}$ refer to body protein weight at maturity (kg); u is the degree of maturity of body protein (u = BP_t / BP_m); *FL* is the feather protein loss (g) assuming that it is equivalent to 0.001 g/ g of feathers per day (Emmans, 1989); FP is the feather protein weight at a certain time t (g/ day); AA_f represents the amino acid content in feather protein (mg/ g); AA_b refers to amino acid content in body protein (mg/ g); *BPD* is the protein deposition in *Bff* (g/ day); *FPD* is the protein deposition in feathers (g/ day); *EU* implies the amino acid utilization efficiency for deposition in *Bff* and feathers.

Model 3

Prediction of amino acid intake were performed using two *EU,* for *Bff* (0.69) and feather (0.53), determined by multiple linear regression in Eq 3.

M3: $Ile_r = [(AA_m c * BP_m^{0.73} * u) + (FL * FP * AA_f)] + [\frac{(AA_b * BPD)}{0.69} + \frac{(AA_f * FPD)}{0.53}]$ [Eq. 6]

Application and evaluation of the models

Gompertz function were used to generate an animal that represents the average characteristics of the population. The parameters necessary to describe such animal were obtained from Gonçalves (2017) (Table 3). Factorial models (M1, M2, and M3) were applied to the broiler generated and compared with the requirements described in the manual guideline (Ross 2014) and in Brazilian Tables of Poultry and Swine (Rostagno et al., 2017).

Table 3. Gompertz function parameters of body and protein growth of broiler chickens.

Parameters*	Body weight	Body protein	Feather protein
Wm (kg)	8.3755	1.266	0.217
B (g/day)	0.04	0.039	0.039
Wi (kg)	0.0437	0.006	0.002

[1] Wm = Mature weight, B = deposition rate, and Wi = initial weight of the component
[2]Data from Gonçalves (2017).

To evaluate the bias of each model, the residual (observed – predicted) was plotted against the predicted values, using the following equation according to St-Pierre (2003).

$e_i = \beta_0 + \beta_1 * cPIle + \varepsilon_i,$ [Eq. 7]

where e_i is the value obtained subtracting the predicted values from the observed values, βo is the intercept and indicates the overall prediction bias, β1 is the slope and indicates the linear prediction bias, cPIle is the centred predicted value (predicted value of Ile minus the mean of all predicted values of Ile) and εi is the error of the regression of the residuals on the predicted values.

RESULTS

The linear equation adjusted using deposition of amino acid in function of amino acid intake was $EU = 0.64(\pm 0.01)\ x + 6.69(\pm 0.52)$. The efficiency of how Ile is utilized for body deposition was estimated as 64%, although this value is static.

Exponential equation was applied to adjust the *EU* according to BW. The prediction of *EU* ranged from 76 to 64%, and the equation determined was:

$$EU = 1.177 - 0.5895\ x\ (1 - EXP(-5.3312\ x\ BW))$$

The *EU* was also determined for Bff and feathers (F), partitioning the intake of Ile above maintenance for Bff and F deposition, according to multiple linear regression.

$$Iaa_g = 1.455\ (\pm 0.13) \times Bff + 1.892\ (\pm 0.55) \times F$$

The reciprocal of regression coefficients gives the *EU* for Bff ($K_{Bff} = 0.69$) and for feather ($K_{feather} = 0.53$). The *EU* for Bff was 23% superior than the efficiency estimated for feathers, suggesting that there are differences of amino acids utilization among them.

The efficiencies were used in the models described hereafter. In Model 1, the amino acid requirement is determined considering the maintenance and Ile deposition for the whole body, hence, only the efficiencies obtained with the exponential model was used. In order to estimate Ile for growing, it is necessary to determine Ile deposition. It were estimated by linear regression of Ile deposition against BWG, according to equation:

$$DIlec = 7.505x - 17.621$$

Based on these results, it were developed the models:

Model 1:

$$Ile = (32 \times BW^{0.75}) + [(-17.621 + 7.5073 \times BWG) / EU]$$

In the model 2 was based on the requirements of Bff and feathers, considering the requirement for both maintenance and protein deposition, separately. In M2 it was used the EU obtained with the exponential model.

Model 2:

$$Ile = [(134 \times BP^{0.73}m \times u)+(0.01 \times FP \times 43.6]+[(36.9 \times BPD)/EU+(43.6\times FPD)/ EU]$$

In model 3 were used EU for Bff (0.69) and other for feathers (0.53), obtained by multiple linear regression.

Model 3:

$$Ile= [(134 \times BP^{0.73}m \times u)+(0.01 \times FP \times 43.6)]+[(36.9 \times BPD)/0.69+(43.6\times FPD)/0.53]$$

An average individual was generated based on growth parameters of Ross 308 (Table 3), to obtain values of BW and BWG applied in Model 1 and protein deposition in Bff and feathers applied in Model 2 and 3. The Ross 308 broilers' Ile requirements estimated by models are demonstrated in table 4. From 1 to 7 days, M3 provided higher Ile intakes than the predictions of M1 and 2. This situation reverses from 14 days of age, when the Model 1 estimate higher requirement than other models, and this persist until 42 days of age.

Table 4. Digestible Ile requirements for Ross 308 broilers estimated by empirical (M1) and mechanistic (M2 and M3) model.

Age (days)	KIle[1]	Growth estimates[2]						Isoleucine intake[3]		
		BW	BWG	BPW	BPD	FPW	FPD	M1	M2	M3[4]
		g	g/d	kg	g/d	g	g/d	(mg/d)	(mg/d)	(mg/d)
1	0.77	53.70	10.85	6.77	1.38	1.91	0.35	87	95	109
7	0.74	157.70	25.06	20.17	3.26	5.12	0.75	238	225	253
14	0.70	416.10	49.97	54.21	6.66	12.54	1.39	526	480	510
21	0.67	866.20	78.61	115.10	10.76	24.78	2.10	889	810	818
28	0.65	1508.00	103.41	204.06	14.52	41.62	2.68	1218	1120	1101
35	0.64	2292.00	118.80	315.60	17.09	61.75	3.03	1426	1331	1297
42	0.64	3145.00	123.20	439.80	18.13	83.39	3.11	1497	1420	1383

[1]KIle-efficiency of utilization of isoleucine;
[2]BPD-body protein deposition; FPW-feather protein weight; FPD-feather protein deposition; BW: body weight; BWG: body weight gain; BPW-body protein weight;
[3]IleM1-Ile intake estimated by model 1; IleM2-Ile intake estimated by model 2; IleM3-Ile intake estimated by model 3;
[4]M3, Model 2 with efficiency for *Bff* and feathers determined by multiple linear regression.

Ile requirements of both models were compared to those described in Ross 308 lineage guideline (Ross, 2015) and in Brazilian Tables for Poultry and Swine (BT) (Rostagno et al., 2017) (Figure 1). In general, with the exception of the first phase, the requirement predicted by M1 were closely to those stablished by BT. On the first phase, the prediction of obtained by M3 provided requirements close to the BT and the guideline. On the second phase, Model 1 estimated Ile requirement higher than others models, and this changes in the following phases. On the third phase, model 1 is close to BT and the guideline presented the lowest intakes closely models 2 and 3. On the last phase, the Ile requirements estimated by models 2 and 3 are lower than model 1 and of the BT and guideline.

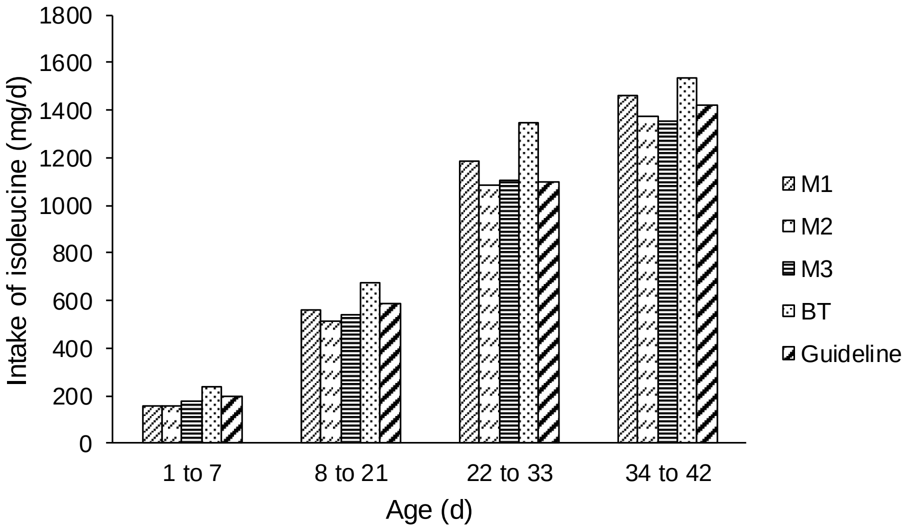

Figure 1. Ile requirements to broilers at different raising phases, estabilished by predictive models 1 (M1), 2 (M2) and 3 (M3), Brazilian Tables for Poultry and Swine (BT) and Ross 308 lineage guideline (Guideline)

The relation between error and the value predicted gives the bias of the model St-Pierre (2003). The results of adjusting the error against the predicted value can be observed in figure 2. Observing the slope of the linear equation generated, values close to zero could be considered unbiased. In this sense, graph A was generated from M1 and have the higher slope (0.117), followed by M2 (0.091). According to the results, M3 produced the smallest slope (0.023) among all factorial models tested.

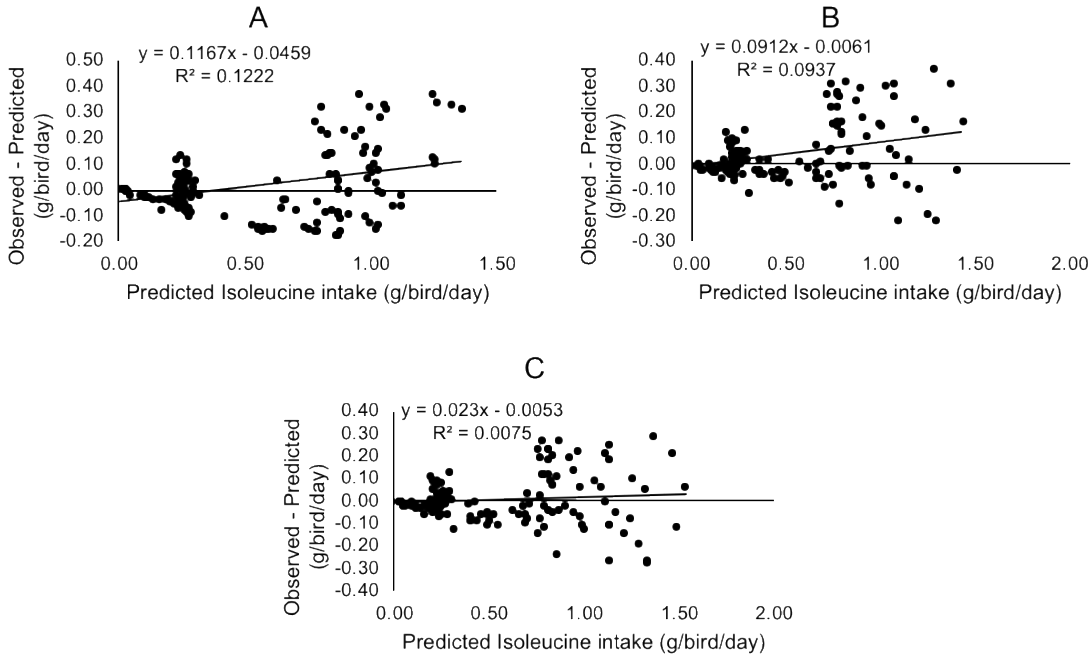

Figure 2. Ratio between error (g/bird/day) and predicted value for Isoleucine intake (g/bird/day) according to model 1 (A), model 2 (B), and model 3 (C)

DISCUSSION

A great variation between the amino acids requirements estimated by models occurs due to the efficiency of utilization. Thus, the knowledge of this relation is fundamental (Sakomura; Rostagno, 2016). Nevertheless, to predict the efficiency, it is relevant that the maintenance requirement is determined in advance and subtracted from the total amino acid intake. Therefore, the first step described in this study take into account the amount of Ile required for maintenance, as determined by de Lima et al. (2016), in order to obtain an accurate amount of Ile consumed that is directed for body deposition.

The EU could be a source of variation when dealing with a nutrient modelling. Thus, the methodology applied to determine the EU needs to be consistent. The EU change as broilers are getting old (Silva 2014), broilers at the first five days old still have a yolk sac, which could complement the source of nutrient from the feed and influence the EU, generating results that could be higher than 100%. Hence, it seems reasonable to adjust the EU in function of BW. In this study, the efficiency of how Ile is utilized for deposition in the whole body was an average 0.64, during broilers growth. Burnham et al. (1992) estimate a range of Ile efficiency of utilization between 0.60 and 0.73, after a series of three experiments with broiler chickens. Applying the exponential equation of EU as function of BW, the EU relied between 0.64 and 0.77, which is close related with what was found by the authors cited. Emmans (1989), for simplistic reasons, suggested that the efficiency of digestible amino acid utilization should be considered as 0.80, however, a correct estimation of the proportion of the amino acid consumed for gain that is deposited in the body improves the accuracy of a model that will use this information.

Considering the variation in amino acid composition in feather-free body and feathers of broiler chickens (Fisher et al., 1981; Stilborn et al., 1997, 2010), determine the EU separately for these parts could increase the accuracy of the models developed to predict amino acid requirement. In this study, the Iaa_g was split into deposition in Bff and feather to determine EU, by multiple linear regression. Using a similar approach of Kielanowisk (1965) but applied for amino acid (Eq 2), the Iaa_g (dependent variable) was regressed against the amino acid deposited in Bff and feathers (independent variable) to determine the reciprocal of the regression coefficient, in other words, the efficiency of utilization for Bff and feathers. The values estimated herein was 0.69 and 0.53 for Bff and feather, respectively, which still closely related with what was found for the whole body.

Comparing the efficiencies of amino acid deposition in body and feather, Bff efficiency was higher than the feather. There is a lack of studies that investigate in which efficiency of utilization an amino acid is deposited in different tissues, which difficult the comparison of the results obtained herein. To our knowledge, there is only one study that evaluate the efficiency of utilization of Ile for broiler chickens (Burnham et al., 1992) and was determined for whole body and not partitioned in different tissues.

Usually, the requirement of amino acids for broiler described in literature are based on dose response studies (observed performance to increasing levels of an amino acid). The results obtained in these studies are expressed as a concentration of diet which define nutritional tables. This empiric approach does not consider factors as genetics (growth potential) or carcass composition (Gous, 1998). In this case, it is expected that the amino acids recommendations are higher than those provided by mechanistic models. Among the factors that could explain this scenario, the use of quadratic models to adjust the observed data to amino acid level is known to be a factor that could generate higher estimates (Vedenov et al., 2008; Pesti et al., 2009). Furthermore, when a requirement of an amino acid is determined using body weight gain, the values could be overestimated. Body weight gain is a consequence of all nutrients deposition rather than amino acid deposition only.

Comparing the estimates obtained with each model, its possible to observe that M1 estimates higher requirements in relation to M2. We attribute this result based on the reason that M1 consider BWG, which involves mainly proteins and lipids gains. A more mechanistic model that takes into account the protein depositions on defeathered body and on feathers to estimate the requirement seems to be more reasonable, which was accomplished applying models M2 and M3.

In order to compare the values estimated by the models adjusted herein with published nutritional recommendation, the requirement was estimated for a feed program with four different phases. As expected, the requirements of Ile for male broilers available in The Brazilian Tables for Poultry and Swine (Rostagno et al., 2017) are higher than what was estimated using models M2 and M3 (Figure 1). Among models adjusted in this study, the Ile requirement estimated by M1 gives closer values to what is described in Brazilian Tables; however, models M2 and M3 were closer related to Ross lineage guide.

The values estimated when model M1 is used are 156, 562, 1183 and 1463 mg/bird/day per bird at 1 to7 (pre-initial), 8 to 21 (initial), 22 to 35 (growth), and 36 to 42 days-old (finisher), respectively, whereas according to M2 the estimated values for the same phases were 155, 514, 1088 and 1375 mg/bird/day per bird. On the other hand, the values found by M3 were 176, 539, 1103 and 1355 mg/bird/day per bird. Taking into account both male and female requirements, Rostagno et al. (2017) described the values to pre-initial, initial, growth and finisher phases as 235, 672, 1345 and 1534 mg/bird/day, respectively, whereas the Ross 308 lineage guide recommends the ingestion of Ile as 199, 588, 1097 and 1422 mg/bird/day per bird.

In a previous study, Burnham et al. (1992) using broilers from 8 to 21 day-old, found a requirement of 0.73 to 0.85 % in the diet. Converting to mg, the value were closer to 608 mg/bird/day, which is higher than the amount estimated herein. Hale et al. (2004) related digestible levels of Ile of 0.59 and 0.64% in the diet, which is equivalent to 1141 mg/bird/day for broiler from 30 to 42 days-old, being lower to the results estimated in the present study.

Beside the values predicted by the models compared to other literature results, such as tables of requirements and manual guideline, the models adjusted in this study allow nutritionists to determine the requirement of Ile for any specific age, which gives an advantage of its use. However, the models could have a bias at some point of the prediction, which may affect the values estimated. In this sense, an unbiased model is preferred, since it will give confidence to nutritionists that the values predicted will be close to the required by the birds for any age. Analyzing the models for bias (St-Pierre, 2003), model M3 have the lowest value of regression coefficient, which could be a result of split the efficiency of utilization for Bff and feathers. This result suggest that using two efficiencies instead of one increase the unbiased of the model.

CONCLUSION

The use of exponential equation was efficient to determine the efficiency of utilization according to body weight, however the value estimated is related to whole body. Using multiple linear regression gives reasonable values of efficiency of utilization partitioned for feather-free body and feather. Models adjusted in this study predicted different values of Isoleucine requirement, where model M1 predicted the higher values. Models M2 and M3 are close related, being the difference observed on the estimations attributed to the efficiency of utilization used. The efficiency of utilization partitioned for feather free body and feather reduce the bias of the model, as observed in model M3.

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