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

CÂMPUS DE JABOTICABAL

BIODISPONIBILIDADE DE FONTES DE METIONINA E NÍVEIS DE METIONINA + CISTINA EM DIETAS PARA FRANGOS DE CORTE

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JABOTICABAL – SÃO PAULO – BRASIL

2018

A627b

Antayhua, Fernando Andrés Prado
Biodisponibilidade de Fontes de Metionina e Níveis de Metionina + Cistina em Dietas para Frangos de Corte / Fernando Andrés Prado Antayhua. – – Jaboticabal, 2018
viii, 56 p. : il. ; 29 cm

Dissertação (mestrado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2017
Orientadora: Nilva Kazue Sakomura
Banca examinadora: Matheus de Paula Reis, Nei André Arruda Barbosa
Bibliografia

1 Fontes de metionina. 2 Frangos de corte. 3 Aminoácidos sulfurados. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 636.087:636.5

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

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

JABOTICABAL – SÃO PAULO – BRASIL

Fevereiro de 2018

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: BIODISPONIBILIDADE DE FONTES DE METIONINA E NÍVEIS DE METIONINA + CISTINA EM DIETAS PARA FRANGOS DE CORTE

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Jaboticabal, 26 de janeiro de 2018

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DEDICATORIA

Aos meus pais Pilar e Cesar pela força e compreensão nas decisões e momentos mais difíceis da vida e pelo amor e carinho em mim.

A minha avó e meu avô pelos ensinos quando eu era criança e que fizeram que eu ande pelo caminho certo e que desde o céu me cuidam.

A Helen pela paciência, amor e por sempre estar nos momentos felizes e de tristeza

AGRADECIMENTOS

À Deus por ser a luz da vida.

À minha família pelo apoio incondicional e por estarem sempre ao meu lado mesmo estando longe.

À professora e minha orientadora Dra. Nilva Kazue Sakomura pela confiança em mim no desenvolvimento desta dissertação e em outras pesquisas em que estive envolvido.

Aos membros da banca de defesa da dissertação Dr. Nei André Arruda Barbosa, Profa. Dra. Nilva Kazue Sakomura e Dr. Matheus de Paula Reis pela contribuição na dissertação.

Ao professor Dr. Edney Pereira da Silva pela contribuição como membro da banca da qualifição do presente estudo e pelos ensinos da vida.

Aos membros de graduação, pós-graduacao e pós-doutorandos do LAVINESP pela amizade e grande ajuda no presente trabalho e que fazem as coisas acontecer e cujo esforço se reflete em cada estudo desenvolvido.

Aos caros funcionários do setor de avicultura, Izildo, Robson e Vicente, pela grande ajuda durante o desenvolvimento da presente pesquisa e pela dedicação e esforço ao longo dos anos para fazer que as pesquisas aconteçam e o LAVINESP seja reconhecido no Brasil e no mundo inteiro.

Aos companheiros de outros grupos de pesquisa da UNESP-Jaboticabal pela amizade e companherismo.

A Erick, Hilda, Karla, Rony por fazerem me sentir em casa.

Aos moradores da República Amoribunda por fazer que os dias durante o mestrado sejam de convivência, respeito e confraternização.

Aos meus amigos e amigas brasileiros, colombianos, mexicanos, argentinos, venezuelanos, bolivianos e de outros países pela amizade e momentos de confraternização.

A agencia CNPq pela concessão da minha bolsa de estudos durante esses dois anos de mestrado.

A todos aqueles que contribuíram para a realização deste trabalho.

Muito obrigado por tudo.

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RESUMO – A avaliação de fontes de metionina e níveis de metionina + cistina (Met+Cis) em dietas para frangos de corte torna-se relevante para fazer ajustes nutricionais nas fórmulas. Foram conduzidos dois experimentos com frangos de corte macho. No experimento I, foi avaliada a biodisponibilidade relativa(BDR) da metionina hidroxi análoga cálcica(Ca-MHA) em relação a DL-metionina(DL-Met) em duas fases de criação de 1 até 21 dias e 21 até 42 dias. Em ambas as fases foram utilizadas 1890 aves macho da linhagem Cobb 500 que foram alimentadas com uma dieta basal com concentrações adequadas em todos os nutrientes com exceção da Met+Cis. Em cada fase, a dieta basal foi suplementada com níveis equimolares de cada fonte de metionina. Em ambas as fases, foram mensurados o ganho de peso, consumo de ração e conversão alimentar. Na segunda fase de criação, aos 42 dias foi determinado o rendimento de carcaça e peito. Através do uso da regressão multi-exponencial foi estimada a BDR média para ambas as fases de 60 e 72% da Ca-MHA em relação à DL-Met na base produto-produto e na base equimolar, respetivamente. No experimento II, foi determinado a concentração ótima biológica e econômica de Met+Cis em dietas de frangos de corte macho em duas fases de 1 até 21 dias, e 21 até 42 dias. Desta forma foram utilizados 1050 aves da linhagem Cobb 500 para cada fase. Na primeira fase, foram avaliadas os níveis de 5.60, 6.08, 7.11, 8.65, e 10.71 g/kg de Met+Cis, em quanto que na segunda fase foram avaliados os níveis 5.40, 5.91, 6.94, 7.96, e 10.02 g/kg de Met+Cis. Para atender os níveis de Met+Cis foi utilizada a DL-metionina como fonte de metionina. Foi avaliado o ganho de peso e conversão alimentar em ambas as fases, já no caso da segunda fase aos 42 dias foi determinado o rendimento de peito e gordura abdominal dos frangos de corte. Em ambas as fases a resposta biológica aos níveis de Met+Cis foi exponencial e que não permitiu estimar uma máxima resposta, porém sim uma estimativa em relação à assíntota. Finalmente, a concentração ótima econômica não coincidiu com a concentração ótima biológica para as variáveis de desempenho e carcaça da segunda fase de criação. Desta forma, é possível ajustar as concentrações de Met+Cis de acordo com o preço da DL-metionina e objetivos de produção de cada criação de frangos de corte.

Palabras-chave: fontes de metionina, frangos de corte, aminoácidos sulfurados, modelo multi-exponencial

BIOAVAILABILITY OF METHIONINE SOURCES AND DIETARY LEVELS OF MET+CYS IN BROILER DIETS

ABSTRACT – Methionine is the first limiting amino acid in poultry diets based on corn and soybean meal. Thus, the assessment of methionine sources and dietary levels of methionine plus cystine (Met+Cys) in broiler diets is important to adjust nutritional composition of broiler diets. It was conducted two experiments with male broilers. In experiment I, it was evaluated the relative bioavailability (RBA) of calcium salt of methionine hidroxi analogue(Ca-MHA) in relation to DL-methionine(DL-Met) in two broiler phases, 1 to 21 days and 21 to 42 days. In both of phases, it was used 1890 birds of Cobb 500 strain that were fed with a basal diet adequate in all nutrients with exception of Met+Cys. In each phase, the basal diet was supplemented with equimolar levels of each methionine source. In both of phases, it was measured body weight gain, feed intake and feed conversion ratio. In the second phase, at 42 days of age it was determined the carcass and breast yield of broilers. By the use of multi-exponential regression analysis it was estimated an average RBA of 60 and 72% of Ca-MHA in relation to DL-Met in a product-to-product basis and on an equimolar basis, respectively. Through the conduction of experiment II, it was determined a biological and economical concentration of Met+Cys of male broiler diets of both of phases 1 to 21 and 21 to 42 days. Thus, it was used 1050 birds of Cobb 500 strain in each phase. In the first phase, it was assessed dietary levels of 5.60, 6.08, 7.11, 8.65, and 10.71 g/kg of Met+Cys, whereas in the second phase it was assessed dietary levels of 5.40, 5.91, 6.94, 7.96, and 10.02 g/kg of Met+Cys. To meet dietary levels of Met+Cys, it was used DL-methionine as a methionine source. It was measured body weight gain, feed intake and feed conversion ratio for both of phases. In the case of the second phase, at 42 days of age it was determined breast and abdominal fat yield of broilers. In both of phases, response of all traits to dietary levels of Met+Cys was exponential and this did not lead to estimate a maximum response, however it was possible to estimate a concentration related to the asymptote. Finally, optimum dietary economic levels of Met+Cys did not coincide with optimum dietary biological levels for both performance and carcass traits of the second rearing phase. In this sense, it is possible to adjust dietary levels of Met+Cys according to DL-methionine price and broiler production goals.

Keywords: Methionine sources, broilers, sulfur amino acids, multi-exponential model

CAPÍTULO I – CONSIDERAÇÕES GERAIS

1.1 Introdução

A nutrição e alimentação de frangos de corte recebe valor dentro da indústria por ser o componente com maior participação nos custos de produção de frangos de corte. Na região ocidental, as dietas são majoritariamente compostas por milho e farelo de soja uma vez que desde um ponto de vista logístico apresentam a vantagem de fácil acesso à compra, porém com a desvantagem nutricional por ser limitante em metionina o que conduz à suplementação de fontes deste aminoácido para atender as exigências nutricionais dos frangos de corte.

A nutrição de aminoácidos para frangos de corte visa expressar o máximo potencial genético através de uma adequada nutrição, no qual inclui o balanço de aminoácidos na dieta. Neste contexto, a metionina é um aminoácido essencial devido a limitada capacidade de síntese das aves, também é um aminoácido limitante pois é encontrado em baixa quantidade nos ingredientes que compõe a dieta dos frangos de corte. Desta forma, os nutricionistas se encontram frente a um cenário onde a suplementação de substancias com atividade de metionina em dietas de frangos de corte torne-se uma opção acertada para garantir que o potencial genético das aves seja expresso.

A suplementação dessas substancias com atividade de metionina em dietas para frangos de corte é uma pratica contínua na indústria. Cada substância, possui estrutura e forma isomérica própria, é absorvida no intestino e metabolizada em diferentes tecidos. O produto final do metabolismo dessas substâncias será a forma levogira do aminoácido pelo fato dessa configuração se encontrar de forma natural na composição química do corpo. Desta forma, o conhecimento das reações químicas envolvidas no metabolismo de fontes de metionina permite dar valor econômico as mesmas, pois a transformação inclui processos mais ou menos eficientes de acordo com cada substancia.

A atividade de metionina de cada substancia pode ser expressa em termos da biodisponibilidade e possui valor econômico para a indústria. De acordo com cada cenário nutricional e ambiência, a biodisponibilidade de uma substancia com atividade de metionina pode mudar, já que o metabolismo deste aminoácido envolve outros nutrientes que, em excesso ou deficiência, modificam o metabolismo da metionina. Diante do exposto, o valor da biodisponibilidade de diferentes fontes de metionina torna-se decisivo no ajuste de matrizes nutricionais e, consequentemente, na formulação de rações para frangos de corte.

1.2 Revisão de literatura

1.2.1 Aminoácidos

Desde um ponto de vista químico e biológico, os aminoácidos são substancias orgânicas compostas por um grupo carbóxilo e amino que conferem características aniônicas e catiônicas e que se unem através de ligações peptídicas para formar peptídeos, oligopeptídeos ou proteínas do corpo (Zubay et al., 1995). Aliás, pelo fato dos aminoácidos participarem de diversas funções como regulação da ingestão de alimento, síntese de enzimas, expressão gênica, fosforilação de proteína e síntese de hormônios (Wu, 2013), estes podem ser denominados substancias multifuncionais.

Os aminoácidos podem ser classificados de acordo com a essencialidade ou não no animal, assim a essencialidade baseia-se no fato que certos aminoácidos não podem ser sintetizados ou não são sintetizados em quantidades suficientes nas células do corpo (Tabela 1).

Tabela 1. Aminoácidos essenciais e não essenciais

Aminoácidos não sintetizados pelas aves		Aminoácidos sintetizados a partir de outros aminoácidos*	Aminoácidos não essenciais	
Arginina	Valina		Hidroxiprolina	Alanina
Lisina	Metionina	Cistina	Acido Aspártico	Glicina**
Histidina	Treonina	Tirosina	Asparagina	Serina**
Leucina	Triptofano	Hidroxilisina	Acido glutâmico	Prolina***
Isoleucina	Fenilalanina		Glutamina	

* Tirosina é sintetizada da fenilalanina, cistina da metionina e hidroxilisina da lisina.

**Nas primeras semanas de vida a glicina e serina nas aves não é suficiente para acompanhar o rápido crescimento das aves.

***Quando as dietas são purificadas, a suplementação de prolina pode ser necessária para atingir o máximo desempenho.

Adaptado de Leeson and Summers (2001).

1.2.2 Metionina

As fontes de metionina, sob a forma de pó ou liquido, são produzidas industrialmente para complementar nutricionalmente dietas limitantes em esse aminoácido. A metionina pode ser produzida sob a mistura racêmica de levogiras e dextrogiras, a onde a ave transformará a forma dextrogira para levogira. Alem disso, a metionina é um dos aminoácidos essenciais que se caracteriza pela presença de enxofre na estrutura química (Figura 1) e cuja formula química é $C_5H_{11}NO_2S$.

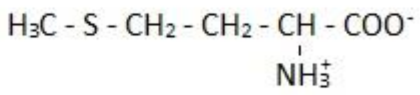

Figura 1. Estrutura química da metionina (Wu, 2013)

A importância deste aminoácido deve-se à iniciação na síntese de proteínas no processo de tradução de ADN. Essa função tem valor biológico e econômico pois em situação de insuficiente concentração de metionina nas células, a síntese de proteínas nos diferentes tecidos será limitada e será refletida no ganho de peso da ave.

O enxofre na estrutura da metionina confere propriedades devido às ligações de enxofre que estabilizam a estrutura terciaria de proteínas estruturais como colágeno e queratina presentes na pele e penas das aves, respectivamente. Além do enxofre conferir propriedade estrutural, este pode participar na eliminação de substancias reativas ao oxigênio devido à baixa eletronegatividade do enxofre através da formação de metionina sulfóxido que posteriormente será reduzida pela metionina sulfóxido redutase para retornar a metionina (Luo and Levine, 2009).

Parte do metabolismo da metionina, inclui a produção de S-adenosilmetionina e S-adenosilhomocisteina. Nessas reações, o grupo metilo é liberado e pode ser utilizado na metilação de ADN e síntese de carnitina e creatina (Simon, 1999).

1.2.3 Precursores de metionina

O estudo de substancias com atividade de metionina para frangos de corte, galinhas poedeiras, matrizes, etc. é extenso (Carvalho et al., 2009; Elwert et al., 2008; Hoehler et al., 2005; Kluge et al., 2015; Xiao et al., 2017). Cada fonte de metionina possui características próprias como concentração de substancias ativas, estrutura química ou processo de fabricação. Os principais precursores de metionina apresentam diferentes substancias ativas como ácido DL-2-amino-4-metiltiobutanoico ou ácido DL-2-hidroxi-4-metiltio-butanoico cujas estruturas químicas se encontram na Figura 2.

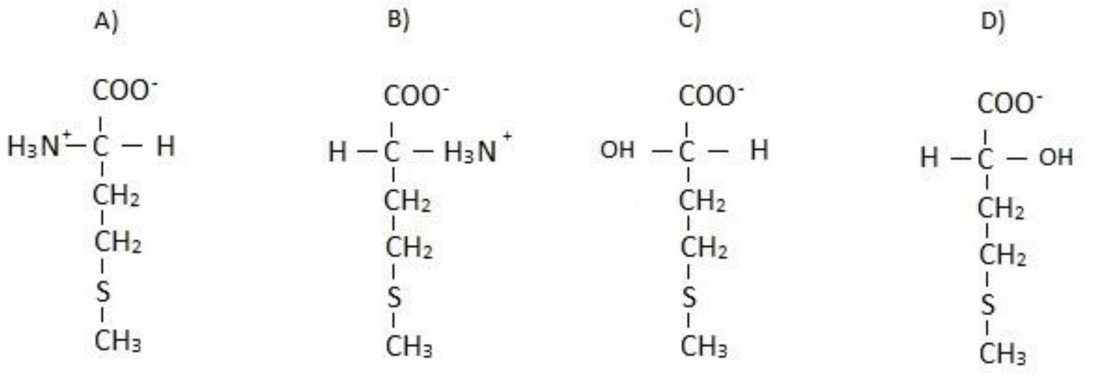

Figura 2. Estrutura química das substancias ativas das fontes de metionina. A) Ácido L-2-amino-4-metiltiobutanoico; B) Ácido D-2-amino-4-metiltiobutanoico; C) Acido L-2-hidroxi-4-metiltio-butanoico; D) Ácido D-2-hidroxi-4-metiltio-butanoico.

Fonte: Nelson et al. (2008)

1.2.4 Utilização de fontes de metionina

A digestão de proteínas compreende a quebra de estruturas complexas em estruturas mais simples de um ingrediente, que no caso das aves começa no proventrículo sob a presença de ácido clorídrico e pepsina. Após a ação de enzimas exopeptidases e endopeptidases do pâncreas, a ação das enzimas ligadas à membrana celular e citosol dos enterocitos do intestino delgado produzirão moléculas menores como oligopeptídeos, tripeptídeos ou dipeptídeos que serão capazes de ser utilizadas para o metabolismo dos aminoácidos (Krehbiel and Matthews, in Mello 2003).

A absorção das substancias ativas dos precursores de metionina tem sido pesquisada sob métodos *in vitro* (Knight and Dibner, 1984a; Richards et al., 2005), para simular a absorção que acontece *in vivo* e para entender o mecanismo de absorção dessas substancias. Pesquisas feitas *in vivo* (Drew et al., 2003; Lingens and Molnar, 1996; Mitchell and Lemme, 2008) tem evidenciado que a absorção das substancias ativas pode ser afetada pelas bactérias intestinais, condições ambientais ou nível de suplementação da fonte de metionina.

O avanço em pesquisas relacionadas à absorção de substancias ativas das fontes de metionina continuam acrescentando para esclarecer melhor a utilização de precursores de metionina e assim explicar os mecanismos biológicos que influenciam a biodisponibilidade deles na nutrição de aves. Nesse sentido, Zhang et al. (2017) encontraram efeito da suplementação de fontes de metionina na expressão genica de transportadores de substancias ATB^{+} e MTC1 do duodeno e jejuno, respectivamente. Estudos ligados à expressão genica permitirão buscar enteder os fatores biológicos envolvidos na absorção dos precursores de metionina sob diferentes condições nutricionais e ambiência.

1.2.5 Metabolismo de precursores de metionina

Uma vez que os precursores de metionina são absorvidos, a metabolização destes acontecerá de acordo com o estado fisiológico da ave. O ácido L-2-amino-4-metiltiobutanoico é a forma final que será disponível para a ave, pois a composição de metionina dos tecidos se encontra sob essa configuração química. Portanto, as formas ácido D-2-amino-4-metiltiobutanoico e ácido DL-2-hidroxi-4-metiltiobutanoico precisam ser metabolizadas até a forma ácido L-2-amino-4-metiltiobutanoico.

No caso do metabolismo da DL-2-hidroxi-4-metiltiobutanoico, o processo de conversão até ácido L-2-amino-4-metiltiobutanoico envolve processos metabólicos para cada forma isomérica. No metabolismo do L-isômero, está envolvida a enzima L-2-hidroxi-acido oxidase que se encontra nos peroxisomas do fígado e rins. Já no caso do D-isomero, o processo envolve a enzima D-2-hidroxi acido desidrogenase que tem atividade na mitocôndria da mucosa intestinal, musculo esquelético, fígado, rins, cérebro e baço (KNIGHT and Dibner, 1984b). Desta forma, o produto final de ambas reações será o alfa-keto-gamma-metiltiobutirato que finalmente sofrerá uma reação de transaminação para produzir o ácido L-2 amino 4-metilbutanoico.

Já no caso do ácido D-2-amino-4-metiltiobutanoico, a conversão numa molécula com atividade de metionina acontece nos tecidos do fígado, rins, cérebro e coração e consiste na oxidação para produzir o alfa-keto-gamma-metiltiobutirato que posteriormente será convertido em ácido L-2-amino-4-metiltiobutanoico através da reação enzimática de transaminação (Wu, 2013).

1.2.6 Biodisponibilidade de nutrientes

Uma vez que a proteína de um ingrediente foi digerida até as unidades fundamentais chamadas aminoácidos, estas passam a ser absorvidas e metabolizadas. Assim, o aminoácido torna-se biodisponível para ser utilizado em uma função como síntese proteica, doação de grupos metílicos, entre outras. Em contraste com o conceito de digestibilidade, a biodisponibilidade inclui o processo de metabolismo ou utilização do nutriente (Parsons, 2002). De acordo com essa distinção de termos, uma substancia com atividade de metionina é absorvida e metabolizada por mecanismos específicos e diferentes a outra fonte de metionina, devido à estrutura química delas.

Por outro lado, para que os aminoácidos provenientes de fontes proteicas sejam metabolizados, é indispensável o processo de digestão química das proteínas dos ingredientes. Existem fatores que podem influenciar a biodisponibilidade dos aminoácidos dos ingredientes tais como condições de processamento, presença de anti-nutrientes, composição química e física da proteína e conteúdo de fibra do ingrediente (Sauer and Ozimek, 1985; Sibbald, 1987); isto permite que as matrizes nutricionais das rações sejam ajustadas e suplementadas com aminoácidos cristalinos com digestibilidade assumida de 99%, porém com biodisponibilidade relativa diferente entre as fontes de aminoácidos cristalinos.

1.2.6.1 Determinação da biodisponibilidade de nutrientes

Sob o contexto que uma substancia é biodisponível quando ela é utilizada para o metabolismo após ter sido absorvido (Ammerman et al., 1995), procura se dar importância biológica e econômica aos precursores de nutrientes que possuem valor nutricional para o animal e ajudam a manter a homeostase.

Através de ensaios dose-resposta é possível estimar a biodisponibilidade relativa de precursores de nutrientes, a posterior aplicação da regressão linear ou não linear nos dados permite estimar o valor, geralmente expressado em porcentagem, da biodisponibilidade de uma substancia em relação à outra. A utilização da regressão linear ou não linear dependerá da resposta e do ajuste dos dados em cada modelo, neste caso da resposta da variável dependente sob estudo à diferentes níveis de ingestão ou suplementação de um precursor de nutriente, como foi evidenciado por Vieira et al. (2004).

Devido à abordagem estatística envolvida no estudo da biodisponibilidade de precursores de nutrientes, precisa-se adotar certas exigências de índole estatística quando aplica-se a regressão dos dados. Já no caso da regressão linear, a resposta deve ser linear para fonte de nutriente, o intercepto deve ser o mesmo e a resposta ao zero nível de suplementação deve ser igual ao intercepto, no entanto, devido à variação própria dos dados, essas condições podem não ser cumpridas (Littell et al., 1997). No caso da regressão não linear, é assumida que a resposta dos precursores de nutrientes tem uma máxima resposta (a+b) para ambos precursores de um nutriente como expressado na Figura 3.

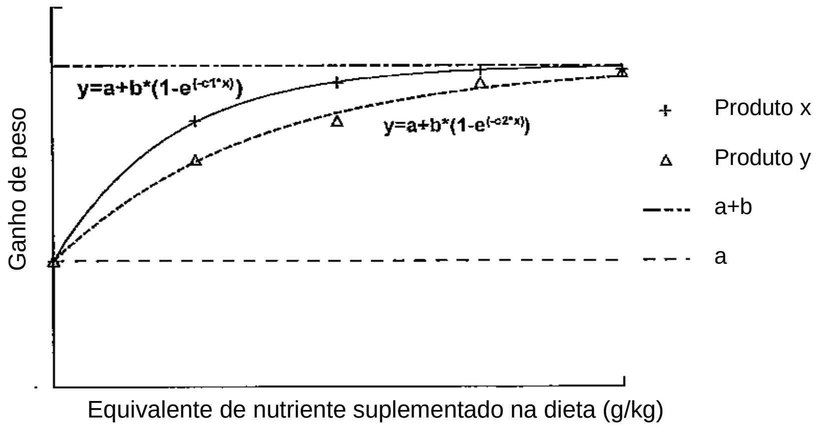

Figura 3. Representação matemática da resposta não linear de duas fontes, produto x e y, de um nutriente.

Adaptado do Jansman et al. (2003)

1.2.6.2 Fatores envolvidos na utilização de fontes de metionina

A regulação das vias metabólicas visa manter a homeostase do corpo diante um desafio nutricional ou ambiência. Consequentemente, qualquer desordem nutricional ou alteração na ambiência modifica o metabolismo dos nutrientes e é relevante ter em consideração as condições nutricionais em que as pesquisas são feitas como relação metionina: cistina, deficiências ou excessos desses aminoácidos ou outros nutrientes relacionados ao metabolismo da metionina.

Dilger and Baker (2008) estudaram o efeito da mudança da relação entre aminoácidos sulfurados sendo a utilização da DL-2-hidroxi-4-metiltiobutanoico mais sensível do que a DL-2-amino-4-metiltiobutanoico às concentrações de cisteina na ração de aves não comerciais. Por outro lado, a interação entre a relação arginina: lisina com a DL-2-hidroxi-4-metiltiobutanoico e DL-2-amino-4-metiltiobutanoico se reflete no consumo de ração e ganho de peso quando as aves são submetidas à condições de estresse térmico, desta forma se demonstram os efeitos dessa condição de ambiência na absorção de arginina e controle de saciedade quando as dietas de frangos de corte são suplementadas com o precursor da metionina DL-2-amino-4-metiltiobutanoico (Chen et al., 2003).

No caso dos minerais, tem sido demonstrado que o potássio tem interação com a substancia DL-2-hidroxi-4-metiltiobutanoico quando suplementada em dose maior que 1%, em quanto que com o precursor DL-2-amino-4-metiltiobutanoico não foi reportada interação para consumo de ração de frangos de corte submetidos à estresse térmico, isto leva a pensar na interação desses precursores com outros nutrientes e processos metabólicos (Ribeiro et al., 2008).

Os fatores nutricionais e de ambiência mencionados acima são diretamente manejáveis pelo nutricionista e influenciam diretamente a utilização dos precursores de metionina. No entanto, Shen et al. (2015) encontrou que a utilização dos isômeros de DL-2-amino-4-metiltiobutanoico mudou com o decorrer da idade dos frangos de corte o que é possivelmente explicado pela limitada expressão da D-amino acido oxidasa em animais jovens (D'Aniello et al., 1993). Como mencionado acima, o avanço de outros métodos de estudo permitirão entender melhor os fatores inerentes à ave e externos, como nutrição e de ambiência, envolvidos na utilização de precursores de metionina.

1.2.7 Ótimo biológico e econômico de aminoácidos sulfurados

Estudos relacionados a avaliar diferentes concentrações de aminoácidos no desempenho de frangos de corte visam obter a máxima resposta da ave em ganho de peso ou menor conversão alimentar (Schutte and Pack, 1995; Vieira et al., 2004; Eits et al., 2005; Pesti, 2009; Ojediran et al., 2017). A avaliação da resposta dos frangos de corte à concentrações de metionina + cistina é ampla(Albino et al., 1999; Oliveira Neto et al., 2007; Rakangtong e Bunchasak, 2011) e continuamente feita pois a melhora genética em essas aves é constante e cada vez mais exigente aos nutrientes.

Desde um ponto de vista biológico, a concentração de um nutriente como exigência nutricional pode ser fixada na formulação de uma ração para frangos de corte e pode ser feita sem levar em conta os preços das matérias primas e do produto final, bem seja frango processado ou vivo. Já desde um ponto de vista econômico, precisa-se levar em conta os preços dos ingredientes a serem incluídos na dieta e os preços do produto final para definir uma concentração ótima econômica que vise o máximo lucro por unidade de produto final produzido como descrito por Siqueira et al., (2011).

O uso de concentrações econômicas de um nutriente ajuda na quebra do paradigma de formulação ao mínimo custo pois sob esta abordagem visa-se uma concentração de um nutriente em função de preços e que garantam o maior retorno econômico por kilograma de produto final.

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CAPÍTULO 2 - Methionine sources for broilers: Relative bioavailability of DL-methionine Hydroxy Analogue Calcium Salt vs. DL-Methionine

Este capítulo é apresentado de acordo com as normas da Animal Feed Science and Technology.

Tittle: Methionine sources for broilers: Relative bioavailability of DL-methionine Hydroxy Analogue Calcium Salt vs. DL-Methionine

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ABSTRACT

Two experiments were conducted to determine the relative bioavailability (RBA) of the calcium salt of DL-2-hydroxy-4-(methylthio) butanoic acid (Ca-MHA) compared to DL-methionine (DL-Met) as methionine sources for broilers. In experiment 1, 1890 d-old Cobb 500 male broilers were fed a basal diet (BD) adequate in all nutrients, but deficient in methionine + cystine (Met+Cys) from d 1 to 21. The BD was supplemented with 4 graded levels of DL-Met (0.49, 1.53, 3.08, and 5.16 g/kg) or equimolar levels of Ca-MHA (0.57, 1.79, 3.61, and 6.03 g/kg). In experiment 2, 1890 three-wk-old Cobb 500 male broilers were fed a BD adequate in all nutrients but deficient in Met + Cys from d 21 to 42. The BD was supplemented with 4 graded levels of DL-Met (0.52, 1.56, 2.59, and 4.67 g/kg) or equimolar levels of Ca-MHA (0.61, 1.82, 3.04, and 5.46 g/kg). In both trials, body weight gain (BWG), feed intake, and feed conversion ratio (FCR) were measured. At 42 days, carcass yield (CY) and breast yield (BY) were determined. Contrast analysis was used to compare both sources, whereas multi-exponential regression analysis was used to determine the RBA of Ca-MHA compared to DL-Met based on growth performance and carcass traits. In trial 1, chicks fed diets supplemented with DL-Met had greater BWG and FCR than chicks fed Ca-MHA ($P < 0.001$). The RBA of Ca-MHA to DL-Met were 69 and 57% for FCR and BWG, respectively on product-to-product basis. In trial 2, broilers fed diets supplemented with DL-Met had improved FCR, CY and BY than broilers fed Ca-MHA ($P < 0.05$). The RBA of Ca-MHA to DL-Met were 60 and 65% for BWG and FCR and 54 and 56% for CY and BY, respectively. Based on these experiments, the average RBA of Ca-MHA to DL-Met is 60 and 72% on product-to-product basis and on an equimolar basis, respectively, and broilers fed diets with DL-Met had greater FCR, CY and BY than those fed Ca-MHA.

Key words: Amino acid, bioavailability, multiple regression, methionine, broiler chicken

INTRODUCTION

Methionine is the first limiting amino acid (AA) in broiler diets. To solve this nutritional deficiency and to precisely meet the sulfur AA requirements of broilers, diets are routinely supplemented with commercially available methionine sources such as DL-methionine (DL-Met; 99% pure), L-methionine (99% pure) or DL-2-hydroxy-4-(methylthio) butanoic acid (HMTBA) products. The latter is available either in liquid form with 12% water and 88% HMTBA or in dry form as calcium salt with 1% water, 12% calcium and 84% HMTBA (Ca-MHA).

The relative bioavailability (RBA) of HMTBA products compared to DL-Met in broilers has been extensively investigated. While the majority of studies available in the literature have compared the liquid form of HMTBA to DL-Met, fewer publications are available comparing Ca-MHA and DL-Met. In addition, many of the previous published Ca-MHA studies used experimental diets that were not similar to commercial diets today (Tipton et al., 1966; Smith, 1966; Katz and Baker, 1975; Boebel and Baker, 1982) while most recent studies comparing Ca-MHA with DL-Met vary in diet composition, broiler´s strains and nutrient levels (Elwert et al., 2008; Li et al., 2010; *Drażbo* et al., 2015).

Recent studies with practical diets (Elwert et al., 2008 and Li et al., 2010) suggest an average RBA of Ca-MHA relative to DL-Met of 63 and 62% for growth performance and carcass traits, respectively. Those values of bioavailability are of great importance for poultry nutritionist to have updated information to formulate cost-effective balanced diets for different stages of growth and production. Therefore, the objective of the present study was to determine the RBA of Ca-MHA in comparison to DL-Met using corn and soybean meal diets for starter and finisher broilers.

MATERIALS AND METHODS

Animal Ethics and Welfare Committee approval

Animal Ethics and Welfare Committee of UNESP (CEUA) approved all experimental procedures of this study under the protocol number 1970/16. The present study was divided in two independent and successive experiments that were conducted at the Poultry Science Laboratory of São Paulo State University, UNESP Jaboticabal, São Paulo, Brazil.

Experiment 1

Animals, Design and Housing

A total of 1890 d-old male Cobb 500 chicks, with initial BW of 38.23 ± 0.55g, were weighed and distributed in a completely randomized design that consisted of 9 dietary treatments with 7 replicate pens of 30 birds each. Birds were housed in floor pens of 1.05 m width by 2.96 m in length. The temperature in the poultry house was kept constant with negative pressure ventilation system, according to breeder´s guidelines. Light was provided for 24 h per day. Birds and feeders were weighed at the beginning and end of the trial to measure mean body weight (BW) and feed intake (FI), enabling to calculate feed conversion ratio (FCR) and body weight gain (BWG). Dead birds were removed and weighed to adjust FCR in this period.

Experimental diets

A corn-soybean meal based diet (BD) was formulated to meet or exceed the nutritional recommendations of Rostagno et al. (2011), except for digestible methionine + cystine (Met + Cys) which was 35% deficient (Table 1). To reduce mixing errors, a master basal batch was manufactured which was then divided into 9 equal aliquots. Each aliquot from the BD was supplemented with either 4 graded levels of DL-Met (0.49, 1.53, 3.08, and 5.16 g/kg) or equimolar levels of Ca-MHA (0.57, 1.79, 3.61, and 6.03 g/kg) that were added at the expense of sand which was used as an inert filler. The digestible Met+Cys levels of the basal and the corresponding 4 inclusion levels of DL-Met or Ca-MHA diets were: 5.60, 6.08, 7.11, 8.65, and 10.71 g/kg. Feed and water were supplied *ad libitum.*

Table 1. Composition of the basal diet of starter and finisher broilers

Ingredients, (g/kg as-fed)	Experiment 1 Starter, 1-21 d	Experiment 2 Finisher, 21-42 d
Corn	585.4	607.7
Soybean meal, (460 g/kg CP)	336.1	313.6
Soybean oil	32.9	43.0
Dicalcium phosphate	17.0	12.4
Limestone	9.0	8.2
Salt	2.0	3.3
Sodium bicarbonate	3.8	1.8
Inert[a]	6.0	5.4
L-Lysine-HCl (78%)	4.3	2.5
L-Threonine (98.5%)	1.3	0.5
L-Valine (96.5%)	0.3	-
L-Tryptophan (98%)	0.4	-
Starter supplement[b]	1.0	
Grower supplement[c]	-	1.0
Coccidiostat[d]	0.5	0.5
Analyzed nutritional composition[e]		
Metabolizable Energy, MJ/kg	12.76	13.18
Crude Protein (N x 6.25)	207.5 (201.5)	197.3 (203.8)
Digestible methionine	2.8 (2.8)	2.7 (2.7)
Digestible Met + Cys	5.6 (6.2)	5.4 (6.0)
Digestible lysine	12.5 (12.9)	11.0 (11.9)
Digestible threonine	8.1 (9.0)	7.1 (8.0)
Digestible arginine	12.9 (13.5)	12.4 (13.2)
Calcium	8.7	7.2
Available phosphorus	4.2	3.3

[a] Inert material was sand.

[b] Starter vitamin and mineral supplement (supplied per kilogram of diet): 110 mg of Zn; 96 mg of Fe; 156 mg of Mn; 20 mg of Cu; 1.4 mg of I; 0.36 mg of Se; 30000 IU of vitamin A; 6 mg of vitamin B1; 0.06 mg of vitamin B12; 12 mg of vitamin B2; 12 mg of vitamin B6; 6000 IU of vitamin D_3; 60 IU of vitamin E; 8 mg of vitamin K_3; 3 mg of folic acid; 80 mg of niacin; 30 mg of pantothenic acid; 0.24 mg of Biotin; 0.125 mg of Butylated hydroxytoluene (*BHT*).

[c] Finisher vitamin and mineral supplement (supplied per kilogram of diet): 110 mg of Zn; 96 mg of Fe; 156 mg of Mn; 20 mg of Cu; 1.4 mg of I; 0.36 mg of Se; 10000 IU of vitamin A; 2 mg of vitamin B1; 0.02 mg of vitamin B12; 4 mg of vitamin B2; 4 mg of vitamin B6; 2000 IU of vitamin D_3; 20 IU of vitamin E; 2 mg of vitamin K_3; 1 mg of folic acid; 30 mg of niacin; 10 mg of pantothenic acid; 0.06 mg of Biotin; 0.125 mg of Butylated hydroxytoluene (*BHT*).

[d] Coxistac 12% with salinomycin sodium.

[e] Analyzed compositions are presented inside parenthesis. Ca-MHA content of the starter basal diet was <0.2 g/kg, and it contained <0.1, 2.27, 1.32 and 0.32 g/kg of free Met, Lys, Thr and Val, respectively. Ca-MHA content of the finisher basal diet was <0.2 g/kg, and it contained <0.1, 1.6, 0.54 and <0.20 g/kg of free Met, Lys, Thr and Val, respectively.

Experiment 2

Animals, Design and Housing

A total of 1890 three-wk-old male Cobb 500 chicks were weighed and distributed in a completely randomized design that consisted of 9 dietary treatments, with 7 replicate pens of 30 birds each. Birds were reared in floor pens of 1.05 m width by 2.96. m in length and fed a common pre- and starter diet from 1 to 21 d of age. Broilers were fed the experimental diets from 21 to 42 d of age. The average initial BW was 832.11 ± 7.35g. Using a negative pressure system, the environmental temperature was regulated according to broiler´s age. However, maximum and minimum records of temperature for the 4th, 5th and 6th wk were 30.57 and 24.67, 30.65 and 22.79, 31.41, and 21.77°C, respectively. The lighting program was continuous during the 21 days. Birds and feeders were weighed at the beginning and end of the trial to measure mean BW and FI, and to calculate FCR and BWG. Dead birds were removed and weighed to adjust FCR in this period. Also, 4 birds per replicate whose BW were ±10% of the average pen weight were selected, stunned by electronarcosis and subsequently killed to measure carcass and breast weights. Carcass weight was measured without neck and feet, whereas breast meat weight was measured without skin and with bones. Breast and carcass yield were calculated as a percentage of BW of birds.

Experimental diets

The BD was formulated to meet or exceed the nutritional recommendations of Rostagno et al. (2011), except for digestible Met+Cys, which was limiting by 29%. Similar to experiment 1, a master basal batch was manufactured and divided into 9 equal aliquots to make the corresponding experimental diets (Table 2). Each aliquot from the BD was supplemented with either 4 graded levels of DL-Met (0.52, 1.56, 2.59, and 4.67 g/kg) or equimolar levels of Ca-MHA (0.61, 1.82, 3.04, and 5.46 g/kg) that were added at the expense of sand. The digestible Met+Cys levels of the BD and the corresponding 4 inclusion levels of DL-Met or Ca-MHA diets were 5.40, 5.91, 6.94, 7.96, and 10.02 g/kg. Feed and water were provided *ad libitum*.

Statistical Analysis

Experimental data were analyzed using SAS 9.2 statistical software (SAS Institute, 2010). Each pen was considered the experimental unit. Orthogonal contrasts statements were used to compare the main effect responses obtained by the two sources of methionine compared to the BD. Statistical differences between means were considered significant with $P < 0.05$. Probabilities obtained in the range of P>0.05 and $P < 0.10$ were considered a tendency. In order to determine the RBA of Ca-MHA compared to DL-Met, BWG, FCR, carcass and breast yield data were subjected to simultaneous multiple regression analysis using the NLIN procedure (PROC NLIN) as described by Littell et al. (1997) as follows:

$$Y = a + b*(1 - e^{-(c_1*x_1 + c_2*x_2)}) \qquad \text{Eq. (1),}$$

where y is the performance or carcass measure; a is the performance or carcass attained with the basal diet; b is the asymptotic response; a + b is the common asymptote (maximum performance level); c_1 is the slope coefficient of the DL-Met curve; c_2 is the slope coefficient of the Ca-MHA curve; x_1 is the dietary level of DL-Met; x_2 is the dietary level of Ca-MHA. As suggested by Littell et al. (1997), the RBA of Ca-MHA was obtained by dividing the slope coefficient of Ca-MHA curve with the slope coefficient for DL-Met curve.

RESULTS

Experiment 1

Chicks fed diets supplemented with increasing levels of DL-Met or Ca-MHA had greater BW gain and FCR (*P* < 0.001) than chicks fed the BD (Table 3). Chicks fed diets supplemented with DL-Met had increased (*P* < 0.001) BWG and FCR than chicks fed diets supplemented with Ca-MHA (Table 2).

Table 2. Growth performance of broilers fed diets with DL-Met or Ca-MHA in Experiment 1 from 1 to 21 days.

Variable[a]	BD	DL-Met, g/kg				Ca-MHA, g/kg				SEM	*P-value*[b]		
		0.49	1.53	3.08	5.16	0.57	1.79	3.61	6.03		BD vs. DL-Met	BD vs. Ca-MHA	DL-Met vs. Ca-MHA
BWG, kg	0.592	0.801	0.864	0.881	0.880	0.762	0.850	0.872	0.857	0.012	<0.0001	<0.0001	<0.0001
FCR, kg/kg	1.432	1.330	1.248	1.237	1.220	1.347	1.261	1.238	1.240	0.009	<0.0001	<0.0001	<0.0001

BWG= Body weight gain, FCR= Feed conversion ratio, BD= Basal diet, SEM = Standard error of the mean, DL-Met= DL-Methionine,

Ca-MHA= Methionine hydroxy analogue calcium salt.

[a] Mean values representing 7 floor pens of 30 birds each.

[b] Contrast analysis with 5% of significance.

According to the multi-exponential regression analysis, broilers fed diets supplemented with DL-Met had a greater performance than those fed Ca-MHA (Figure 1). On product-to-product basis the RBA of Ca-MHA in relation to DL-Met were 57 and 69% for BWG and FCR, respectively, whereas on an equimolar basis they were 68 and 82%, respectively.

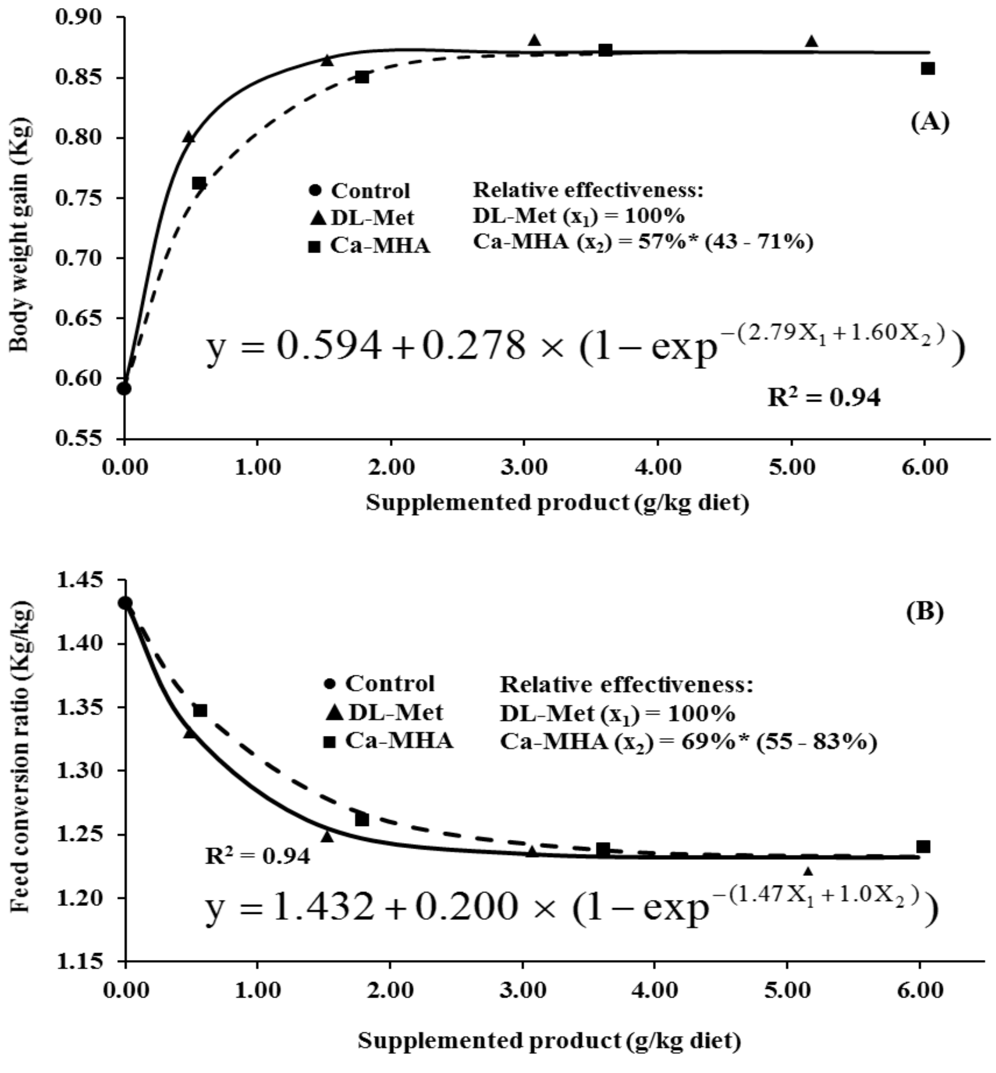

Figure 1. Body weight gain (A) and feed conversion ratio (B) of broilers fed diets with increasing supplementation of DL-Met (99%, DL-Met) and methionine hydroxy analogue calcium salt (84%, Ca-MHA) from 1 to 21 days. Values in parenthesis indicate 95% confidence interval.

Experiment 2

Birds fed diets supplemented with DL-Met or Ca-MHA had improved BWG and FCR (*P* < 0.001) compared to chicks fed the BD (Table 3). A lower FCR was found in broilers fed diets supplemented with DL-Met than those fed diets supplemented with Ca-MHA (*P* < 0.001). Responses in BWG tended to improve (P = 0.061) when broilers were fed diets supplemented with DL-Met compared to birds fed Ca-MHA (Table 3). On product-to-product basis, the RBA of Ca-MHA compared to DL-Met were 60 and 65% for BWG and FCR, respectively. In addition, on an equimolar basis, the RBA of Ca-MHA compared to DL-met were 71 and 77% for BWG and FCR, respectively.

Table 3. Growth performance of broilers fed diets with DL-Met or Ca-MHA in Experiment 2 from 21 to 42 days.

Variable[a]	BD	Inclusion, g/kg DL-Met				Inclusion, g/kg Ca-MHA				SEM	*P-value*[b]		
		0.52	1.56	2.59	4.67	0.61	1.82	3.04	5.46		BD vs. DL-Met	BD vs. Ca-MHA	DL-Met vs. Ca-MHA
BWG, kg	1.830	1.958	2.055	2.107	2.110	1.927	2.042	2.054	2.110	0.017	<0.0001	<0.0001	0.0601
FCR, kg/kg	1.838	1.744	1.647	1.622	1.598	1.777	1.668	1.624	1.608	0.016	<0.0001	<0.0001	0.0003
CY, g/kg BW	745.72	761.64	762.69	775.14	775.25	759.15	757.33	771.07	772.78	0.011	<0.0001	<0.0001	0.0125
BY, g/kg BW	232.34	252.50	263.46	277.23	286.48	246.81	256.04	271.16	278.48	0.165	<0.0001	<0.0001	0.0046

BWG= Body weight gain, FCR= Feed conversion ratio, CY= Carcass yield, BY=Breast yield, BW = body weight, BD= Basal diet, SEM = standard error of the mean, DL-Met= DL-Methionine, Ca-MHA= Methionine hydroxy analogue calcium salt.

[a] Mean values representing 7 floor pens of 30 birds each.

[b] Contrast analysis with 5% of significance.

Increasing supplemental levels of both methionine sources to the BD, improved carcass and breast yields ($P < 0.001$). Broilers fed diets supplemented with DL-Met had greater carcass and breast yields ($P < 0.05$) than broilers fed diets supplemented with Ca-MHA (Table 3). On product-to-product basis, the RBA of Ca-MHA in relation to DL-Met were 54 and 56% for carcass yield and breast yield, respectively. In addition, on an equimolar basis the RBA of MHA-Ca in relation to DL-Met were 64 and 67% for carcass yield and breast yield, respectively. Graphs of all traits are shown in Figures 2 and 3.

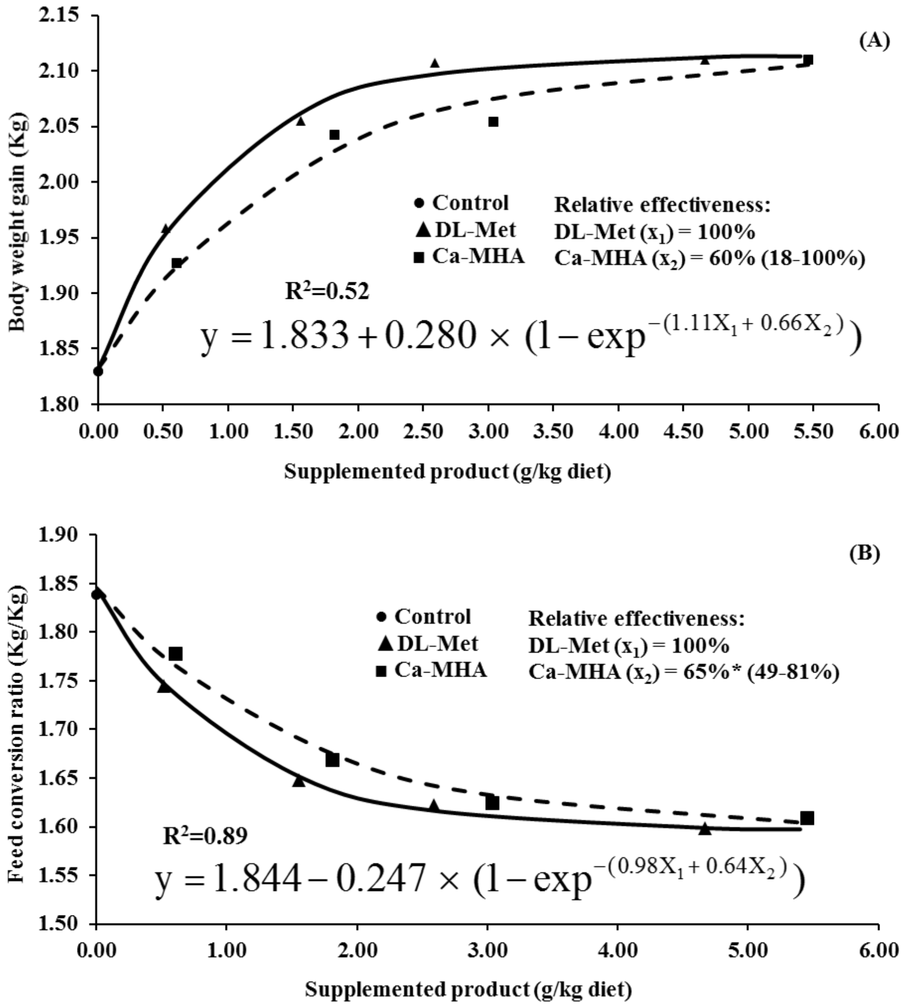

Figure 2. Body weight gain (**A**) and feed conversion ratio (**B**) of broilers fed diets with increasing supplementation of DL-Met (99%, DL-Met) and methionine hydroxy analogue calcium salt (84%, Ca-MHA) from 21 to 42 days. Values in parenthesis indicate 95% confidence interval

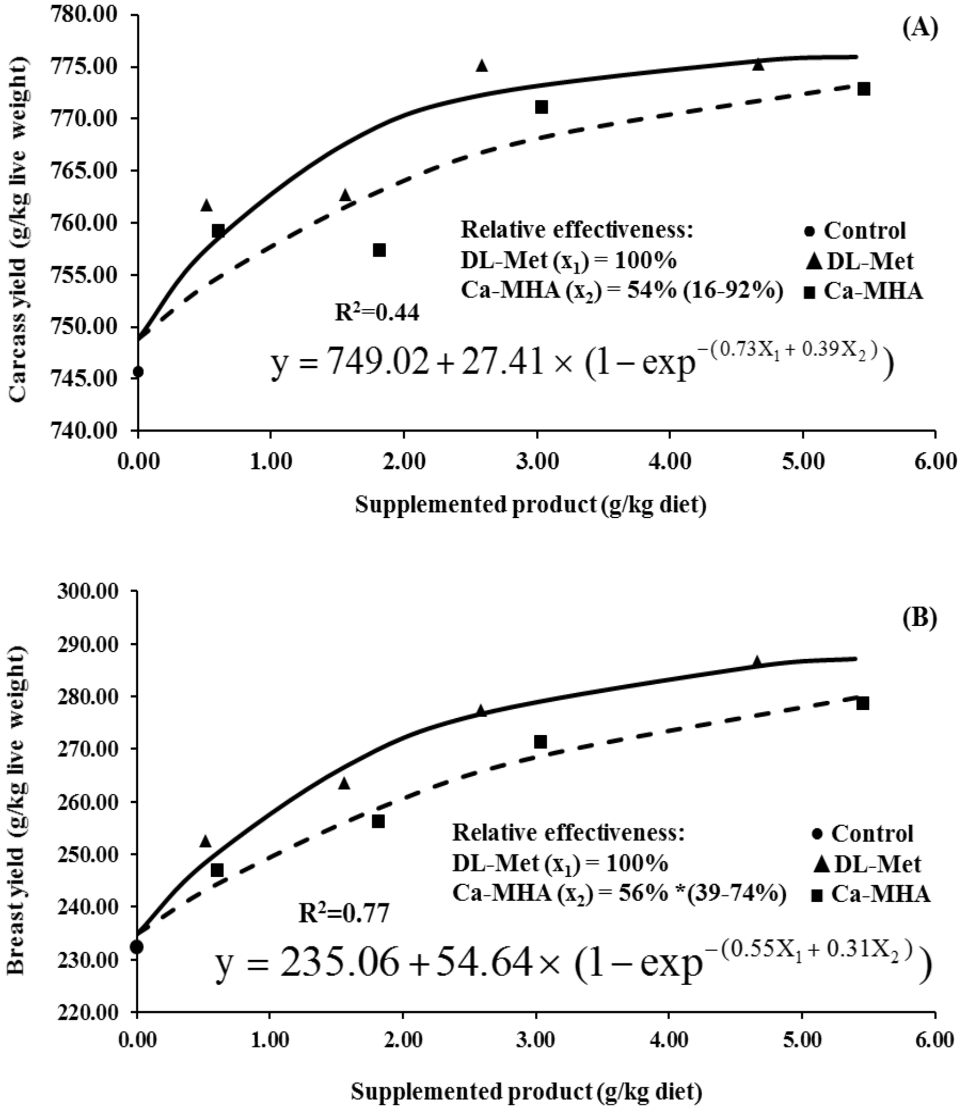

Figure 3. Carcass (A) and breast yield (B) of broilers fed diets with increasing supplementation of DL-Met (99%, DL-Met) and methionine hydroxy analogue calcium salt (84%, Ca-MHA) from 21 to 42 days. Values in parenthesis indicate 95% confidence interval

DISCUSSION

The RBA of Ca-MHA in relation to DL-Met was determined by comparing the responses of broilers to increasing supplementation levels of both sources in starter and finisher phases.

Growth and carcass performance achieved in the current experiments were in agreement with Cobb 500 performance objectives guidelines (Cobb-Vantress, 2015). Different studies demonstrated lower efficacy of Ca-MHA relative to DL-Met under different conditions such as diet composition, feeding phases, and nutritional recommendations (Dilger and Baker, 2008; Elwert et al., 2008; Li et al., 2010). In the current experiments, male broilers were fed corn-soybean meal diets and the range of digestible Met+Cys of the dietary treatments were established to be above and below the nutritional recommendations of Rostagno et al. (2011).

In both experiments, the BD resulted in significantly reduced growth performance and carcass traits, which confirmed the dietary deficiency of digestible Met + Cys. As recommended by Jansman et al. (2003), the deficiency of the nutrients in the BD is a criterion to detect differences in the effectiveness of methionine sources in poultry. In the current experiments, supplementing the BD diet with increasing levels of either methionine source resulted in a significant curvilinear increase ($P<0.0001$) in growth and carcass performance. This trend is in agreement with Vieira et al. (2004) who found a non-linear response in broiler performance as the dietary sulfur amino acid content increased. Furthermore, as the level of supplementation increased for both methionine sources, broiler response followed the law of diminishing returns as evidenced by Lemme et al. (2007b).

In Experiment 1, contrast analysis showed that in average, broiler fed diets supplemented with DL-Met had better FCR and BWG than broilers fed diets with Ca-MHA. Based on BWG and FCR data, the RBA of Ca-MHA in comparison to DL-Met was on average 63% on product-to-product basis or 75% on an equimolar basis for male broilers from 1 to 21 days.

These results differ with those reported by Conde-Aguilera et al. (2016) who reported similar broiler performance for DL-Met and DL-HMTBA. A possible explanation for the different results can be due to the procedures followed by these authors such as a restricted allowance to feed of approximately 12% to the *ad libitum* intake capacity to guarantee similar feed intake between treatments. Thus, the ability of broilers to adjust their feed intake according to nutritional composition of a diet was not taken into consideration. Consequently, the nutritional value of each methionine source can be underestimated in broilers whose feeding is usually *ad libitum* under commercial conditions. Our results are in agreement with those reported by Dilger and Baker (2008) who reported an improved performance for starting broilers fed diets with DL-Met than those fed diets with Ca-MHA in their dose-response study. Additionally, their finding of RBA on an equimolar basis was 78% and it is similar to the RBA obtained in the current study.

In Experiment 2, the BWG of broilers fed diets supplemented with DL-Met tended to be greater than broilers fed diets supplemented with Ca-MHA (P = 0.060). This finding is not in agreement with Balnave et al. (1999) who reported similar BWG in growing male broilers that received diets supplemented with both sources under elevated environmental temperatures. With regards to FCR, our results are not in agreement with the results obtained by *Drażbo* et al. (2015) who found no statistical differences in FCR for broilers fed diets with DL-Met compared to those fed diets with Ca-MHA, however they found differences in BWG and feed intake. Therefore, it seems that under *ad libitum* feeding, broilers are able to adjust their feed intake to diets supplemented with either DL-Met and Ca-MHA as found in the starter phase of the current study (data not shown) and as previously reported by Lemme et al. (2008a) which could be related to the ability of broilers to detect differences in diet composition.

Carcass traits such as breast yield and carcass yield were improved by both methionine sources compared to the BD. These results do not agree with those found by Elwert et al. (2008) who did not find significant differences in breast and carcass yield between broilers fed the basal diet and those fed diets supplemented with both sources. In contrast, *Drażbo* et al. (2015) found an improvement in carcass and breast traits due to the supplementation of both sources. That contrast could be explained by the degree of deficiency of Met+Cys used in those studies. In our study, the digestible Met + Cys concentration of the basal diet was 5.4 g/kg and it was similar to that used by *Drażbo* et al. (2015).

Our experiments showed that birds fed diets supplemented with DL-Met had better carcass yield and deposited more breast than those fed diets supplemented with Ca-MHA. These results are in agreement with those found by Esteve-Garcia and Llaurado (1997) who found that breast deposition was higher for DL-Met than for an equimolar amount of methionine from liquid MHA. Those results could be explained due to a higher methionine availability in muscle tissues of broilers fed diets supplemented with DL-Met than those fed Ca-MHA which is based in a higher protein synthesis stimulated by metabolic reactions such as mainly methylation of DNA (Waterland, 2006). Therefore, a high availability of Met in broilers is expressed on muscle growth and this could have occurred in our study because a much higher bioavailability of DL-Met in relation to Ca-MHA was found for carcass and breast yield.

In Experiment 2, the average RBA of Ca-MHA in relation to DL-Met for both growth performance and carcass traits were 62 and 55%, respectively on product-to-product basis, whereas 74 and 65% on an equimolar basis, respectively. Values of RBA of Ca-MHA to DL-Met achieved in this experiment are in agreement with recent studies developed by Li et al. (2010) who reported on an equimolar basis an average of 65% for performance and carcass traits, respectively. Additionally, Elwert et al. (2008) reported similar values such as 68 and 54% for body weight gain and breast yield, respectively on product-to-product basis.

Although experiment 2 was intended to be conducted under controlled conditions of temperature and humidity, the negative pressure ventilation system of the experimental was not sufficient to effectively control the high environmental temperatures. Therefore, the maximum temperature inside the poultry house recorded was 31.41°C whereas the minimum was 21.77°C during the complete experimental period. Although these were unfavorable conditions of heat stress, broilers fed diets supplemented with DL-Met showed a tendency for higher BWG than those fed Ca-MHA.

Possible physiological explanation to understand the differences in RBA relies on the different chemical structure of both sources. DL-Met is 99% pure and is a neutral amino acid; whereas the Ca-MHA is an organic acid precursor to methionine, with a purity of 84%. The two methionine sources are absorbed by different mechanisms and regions of the gastrointestinal tract (Richards et al., 2005).

Different researchers have focused on providing evidence about mechanisms and transporters involved in the uptake of active substances of methionine sources by the gastrointestinal tract (Dibner and Knight, 1984; Drew et al., 2003; Martin-Venegas et al., 2008; Zhang et al., 2016). In particular, Ca-MHA and DL-Met absorption were studied by Lingens and Molnar (1996), who observed that of the ingested 14C, 17% of the 14C Ca-MHA and 4.4% of the 14C DLM were recovered in the excreta, support some of the differences in the bioavailability of both methionine sources obtained in the current study.

In conclusion, the average RBA of Ca-MHA compared to DL-Met was 60 and 72% on product-to-product basis and on an equimolar basis, respectively, and they were significantly lower than the active content of 84% of Ca-MHA. Male broilers fed diets with DL-Met had improved FCR, carcass and breast yield than those fed diets with Ca-MHA.

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CAPÍTULO 3 - Biological and economical assessment of dietary levels of sulfur amino acids for broilers

Este capítulo é apresentado de acordo com as normas da Revista Brasileira de Zootecnia

Running title: Sulfur amino acids in broiler nutrition

Manuscript category: Animal Science and Pastures

Biological and economical assessment of dietary levels of sulfur amino acids for broilers

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ABSTRACT

Methionine is the first limiting amino acid in broiler diets based in corn-soybean meal. In the current study, biological and economical dietary levels of digestible methionine plus cystine (Met+Cys) were assessed in broilers. From 1 to 21 days, 1050 day-old Cobb 500 male chicks received five diets with 5.60, 6.08, 7.11, 8.65, and 10.71 g/kg of Met+Cys. From 21 to 42 days, 1050 three-week-old Cobb 500 male broilers were fed diets with 5.40, 5.91, 6.94, 7.96, and 10.02 g/kg of Met+Cys. Traits such as body weight gain (BWG), feed conversion ratio (FCR), breast yield (BY) and abdominal fat (AF) were measured. Nonlinear regression analysis was performed to adjust an exponential model. Optimal dietary level of Met+Cys was estimated as 95 or 105% of the asymptotic response of each trait. Estimation of the optimal economical dietary level of Met+Cys was performed by a fixed market sale such as live body weight or breast meat and different DL-met prices. For both phases, performance parameters responded nonlinearly to the supplementation of DL-met in diets. In the first phase (1 to 21 days), optimal dietary level of Met+Cys for BWG and FCR were 6.41 and 6.45 g/kg, respectively. For the second phase (21 to 42 days), optimal dietary level of Met+Cys were 6.29, 6.52, 7.80 and 6.58 g/kg for BWG, BY, FCR and AF, respectively. The optimal economical dietary level of Met+Cys did not coincide with the optimal biological dietary level obtained for both performance and carcass traits of broilers for the second phase. Due to differences in economic sceneries of broiler industry, it is possible to adjust concentrations of Met+Cys in broiler diets according to each broiler production goals.

Key Words: broilers, carcass quality, nonlinear model, sulfur amino acid

Introduction

Methionine is the first limiting amino acid in poultry diets based on corn and soybean meal and it is one of the most important amino acids for body protein deposition. Therefore, this amino acid is commonly supplied in the diet to meet requirements for maximum performance of birds under different growth phases, therefore any deficiency of this nutrient will be reflected in poor performance.

Genetic advance in broilers increased growth rate with a higher protein deposition rate of these birds (Henn et al., 2014; Zuidhof et al., 2014). Environmental conditions and nutritional status are factors which need to be balanced to lead the expression of genetic potential of broilers. In this context, the assessment of dietary levels of amino acid in broiler diets aims to meet the needs for the genetic potential of the birds.

Nutritional recommendations are based on determination of an optimal dietary level of a nutrient to produce a maximum biological response such as body weight gain. Thus, regression models such as linear plateau, exponential or quadratic have been evaluated and utilized to estimate an optimal level to improve the biological response (Pesti et al., 2009). On the other hand, carcass traits such as breast yield or abdominal fat, which have economic significance in poultry industry, need different requirements to attain market target of breast yield and minimum abdominal fat.

Likewise, it is aimed a dietary level of a nutrient produces the highest gross margin correspondent to feeding component (Pesti and Miller, 1999). The knowledge of biological response of broilers to any alteration in the concentration of an amino acid leads to apply a precise economic analysis of the addition of a source of that nutrient to meet a concentration which maximize the profit.

Due to the importance of sulfur amino acids in broiler nutrition, the current study aimed to assess the biological and economical response of broilers fed diets with different levels of methionine plus cystine.

Material and methods

The current broiler study was approved by the institutional committee on animal use (1970/16). Two independent and consecutive experiments were conducted in Jaboticabal, in the state of Sao Paulo, Brazil (21º15'16" S; 48º19'19" W, altitude 607 m), one for the phase from 1 to 21 days and another from 21 to 42 days.

In the first experiment, a total of 1050 one day-old male of the Cobb 500 strain were weighed and distributed in a completely randomized design which consisted in five dietary treatments with seven replicates of 30 birds each. Bird were allocated in floor pens of 1.05 x 2.96 m where environmental temperature was maintained constant through negative pressure and pad-cooling system according to the age of the bird. Lighting program consisted on continuous 24 hours/day during the whole phase. It was measured the live weight and feed consumption to calculate the weight gain and feed conversion ratio. Mortality was registered daily to adjust the feed conversion ratio.

Experimental diets for this phase were based in corn and soybean meal and followed nutritional recommendations of Rostagno et al. (2011). Concentrations of digestible methionine plus cystine (Met+Cys) were 5.60, 6.08, 7.11, 8.65, and 10.71 g/kg of Met+Cys. The diet with 5.60 g/kg was used as a basal diet to be supplemented with DL-methionine. Nutritional composition of this diet is presented in Table 1. During this phase, feed and water were offered *ad libitum* through tubular feeder and nipple drinker, respectively.

Table 1. Composition of the basal diet of starter and finisher broilers

Ingredients, (g/kg as-fed)	Experiment 1 Starter, 1-21 d	Experiment 2 Finisher, 21-42 d
Corn	585.4	607.7
Soybean meal, (460 g/kg CP)	336.1	313.6
Soybean oil	32.9	43.0
Dicalcium phosphate	17.0	12.4
Limestone	9.1	8.2
Salt	2.0	3.3
Sodium bicarbonate	3.8	1.8
Inert[a]	6.0	5.4
L-Lysine-HCl (78%)	4.3	2.5
L-Threonine (98.5%)	1.3	0.5
L-Valine (96.5%)	0.3	-
L-Tryptophan (98%)	0.4	-
Starter supplement[b]	1.0	
Grower supplement[c]	-	1.0
Coccidiostat[d]	0.5	0.5
Nutritional composition[e]		
Metabolizable Energy, Kcal/kg	3050	3150
Crude Protein (N x 6.25)	207.5 (201.5)	197.3 (203.8)
Digestible methionine	2.8 (2.8)	2.7 (2.7)
Digestible Met + Cys	5.6 (6.2)	5.4 (6.0)
Digestible lysine	12.5 (12.9)	11.0 (11.9)
Digestible threonine	8.1 (9.0)	7.1 (8.0)
Digestible arginine	12.9 (13.5)	12.4 (13.2)
Calcium	8.7	7.2
Available phosphorus	4.2	3.3

[a] Inert material was sand.
[b] Starter vitamin and mineral supplement (supplied per kilogram of diet): 110 mg of Zn; 96 mg of Fe; 156 mg of Mn; 20 mg of Cu; 1.4 mg of I; 0.36 mg of Se; 30000 IU of vitamin A; 6 mg of vitamin B1; 0.06 mg of vitamin B12; 12 mg of vitamin B2; 12 mg of vitamin B6; 6000 IU of vitamin D_3; 60 IU of vitamin E; 8 mg of vitamin K_3; 3 mg of folic acid; 80 mg of niacin; 30 mg of pantothenic acid; 0.24 mg of Biotin; 0.125 mg of Butylated hydroxytoluene (*BHT*).
[c] Finisher vitamin and mineral supplement (supplied per kilogram of diet): 110 mg of Zn; 96 mg of Fe; 156 mg of Mn; 20 mg of Cu; 1.4 mg of I; 0.36 mg of Se; 10000 IU of vitamin A; 2 mg of vitamin B1; 0.02 mg of vitamin B12; 4 mg of vitamin B2; 4 mg of vitamin B6; 2000 IU of vitamin D_3; 20 IU of vitamin E; 2 mg of vitamin K_3; 1 mg of folic acid; 30 mg of niacin; 10 mg of pantothenic acid; 0.06 mg of Biotin; 0.125 mg of Butylated hydroxytoluene (*BHT*).
[d] Coxistac 12% with salinomycin sodium.
[e] Analyzed compositions are presented inside parenthesis.

In the second phase, 21 day-old broilers of Cobb 500 strain (n=1050) were weighed and distributed in a completely randomized design which consisted on five dietary treatments with seven replicates of 30 birds each. Birds were reared in floor pens of 1.05 x 2.96 m. Temperature and humidity were adjusted according to bird's age through negative pressure and pad-cooling system in the experimental broiler house. It was adopted a lighting program of 24 continuous hours of light during the whole experiment. Performance traits such as body weight gain and feed conversion ratio were evaluated. Mortality of birds was registered daily to correct feed intake and adjust feed conversion ratio. At 42 days, four birds whose weight were ±10% of the average body weight of the pen were selected, identified, stunned by electro-narcosis, sacrificed and de-feathered to measure carcass weight, abdominal fat and breast. Abdominal fat was collected from abdomen part of each bird. Carcass weight was measured without neck and feet, whereas breast weight did not include skin neither bone. Breast yield and abdominal fat were calculated as a percentage of live weight.

In this second phase, it was formulated a basal diet (Table 1) based on corn and soybean meal according to the nutritional recommendations of Rostagno et al. (2011). The basal diet contained 5.40 g/kg of digestible Met+Cys and was supplemented with DL-methionine to achieve concentrations of 5.91, 6.94, 7.96, and 10.02 g/kg of digestible Met+Cys. Birds had free access to feed and water during the whole experiment.

Economic analysis to estimate the concentration of Met+Cys which produced the highest gross margin for this phase consisted on estimate gross margin per quilogram of weight gain of birds or per quilogram of breast per quilogram of live weight for each level of Met+Cys. The estimation of gross margin was done as described by Siqueira et al. (2011):

$$GM = PB - FC \qquad \text{Eq. 1}$$

Where GM= gross margin per kilogram of broiler or breast meat (U$); PB= price per kilogram of whole broiler or breast meat (U$.kg^{-1}); and FC= feeding cost per kilogram of weight gain (U$.kgWG^{-1}) or kilogram of breast meat (U$.kg).

Therefore, optimal concentration of Met+Cys was established as that with lower feeding cost and higher gross margin per kilogram of whole broiler or breast yield. Due to the fluctuations in amino acid prices throughout the year and between broiler industry, it were considered three prices (U$.kg^{-1} 3.12, U$.kg^{-1} 4.37 and U$.kg^{-1} 5.31) of DL-methionine. Referential prices of feedstuffs such as corn and soybean meal were U$.kg^{-1} 0.13 and 0.32, respectively. Furthermore, price of the basal diet for both first and second phase were U$.kg^{-1} 0.272 and 0.243, respectively.

Performance and carcass traits were analyzed by ANOVA procedure of software SAS (Statistical Analysis System, version 9.1) using Met+Cys levels as a source of variation. Broiler response of both phases to digestible sulfur amino acids were adjusted to the nonlinear model described by Rodehutscord and Pack (1999):

$$Y = a + b * [1 - EXP(-c * (x - d))] \quad \text{Eq. 2}$$

Where Y= carcass or performance trait; a= performance achieved with the basal diet; b= difference between minimum and maximum response achieved by the addition of DL-methionine; c=slope of the curve; x= level (g/kg) of Met+Cys of the experimental diets; d= level (g/kg) of Met+Cys of the basal diet. Optimal dietary level of Met+Cys was estimated for 95% of the maximum response or 105% of the minimum response for both traits abdominal fat and feed conversion ratio. The PROC NLIN procedure of software SAS (Statistical Analysis System, version 9.1) was used to adjust nonlinear models to dataset.

Results

Performance and carcass traits response to Met+Cys concentrations in broiler diets were studied in both phases 1 to 21 and 21 to 42 days-old of broiler. According to variance (Table 2 and 3) analysis of each trait, at least there is a concentration of Met+Cys that produce a response statistically different in performance, breast yield or abdominal fat (*P<0.0001*).

Table 2. Growth performance of broilers fed dietary levels of Met+Cys from 1 to 21 days.

Variable[a]	Met+Cys, g/kg					*P-value*	*CV[b]*
	5.60	6.08	7.11	8.65	10.71		
BWG, kg	0.592	0.801	0.864	0.881	0.880	P<0.0001	6.07
FCR, kg/kg	1.432	1.330	1.248	1.237	1.220	P<0.0001	13.27

BWG= Body weight gain, FCR= Feed conversion ratio.

[a] Mean values representing 7 floor pens of 30 birds each.

[b] Coefficient of variation

Table 3. Growth and carcass performance of broilers fed dietary levels of Met+Cys from 21 to 42 days.

Variable[a]	Met+Cys, g/kg					*P-value*	*CV[b]*
	5.40	5.91	6.94	7.96	10.02		
BWG, kg	1.830	1.958	2.055	2.107	2.110	P<0.0001	6.89
FCR, kg/kg	1.838	1.744	1.647	1.622	1.598	P<0.0001	5.67
BY, g/kgLW	745.72	761.64	762.69	775.14	775.25	P<0.0001	8.07
AF, g/kg LW	232.34	252.50	263.46	277.23	286.48	P<0.0001	16.31

BWG= Body weight gain, FCR= Feed conversion ratio, BY=Breast yield, LW = Live weight.

[a] Mean values representing 7 floor pens of 30 birds each.

[b] Coefficient of variation

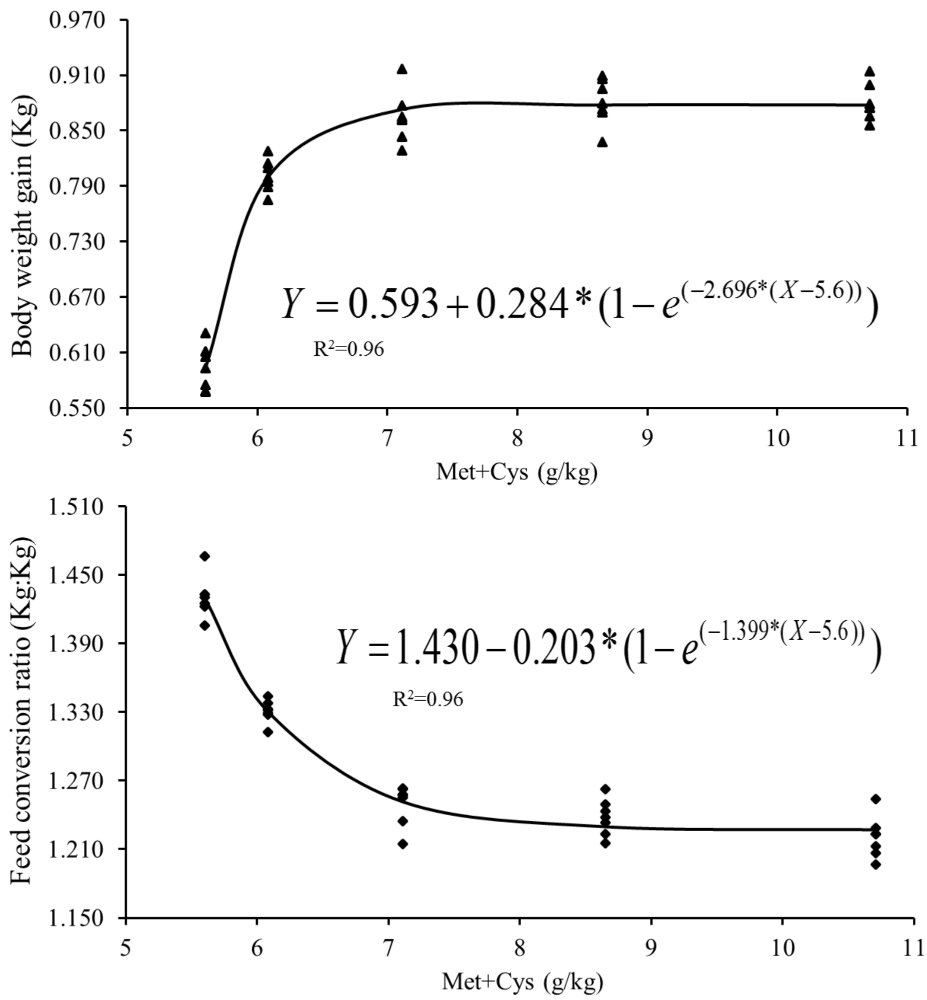

Figure 1. Performance of broilers from 1 to 21 days-old fed with increasing dietary levels of Met+Cys

In the first phase, body weight gain and feed conversion ratio responded non-linearly (Figure 1) to dietary levels of Met+Cys (*P<0.0001*). In addition, the 95% of asymptote of body weight gain curve corresponded to 0.877 kg which was achieved by 6.41 g/kg of Met+Cys in the broiler diet. In the case of feed conversion ratio curve, due to its diminution trend as Met+Cys concentration increased, it was utilized the value of 105% of the asymptote which corresponded to 6.45 g/kg of Met+Cys.

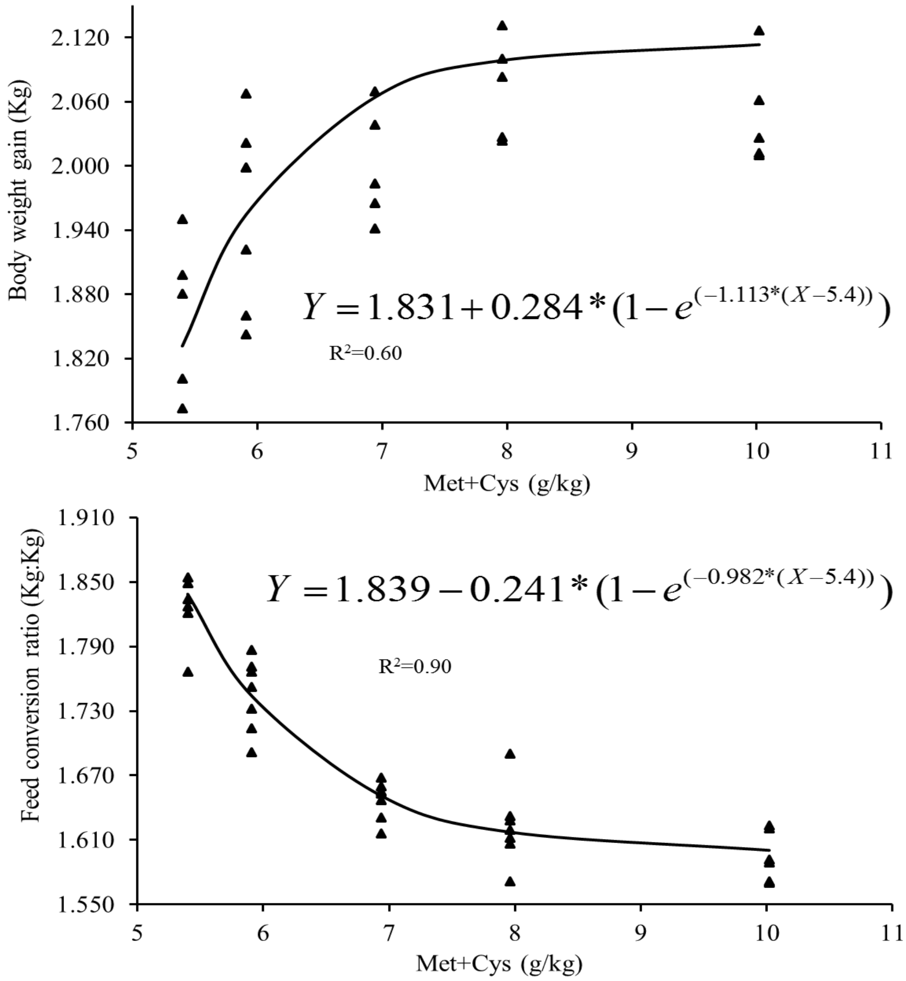

Figure 2. Performance of broilers from 21 to 42 days-old fed with increasing dietary levels of Met+Cys

With regards to the second phase, body weight gain, feed conversion ratio, breast yield and abdominal fat yield responded non-linearly (Figure 2 and 3) as concentrations of Met+Cys increased (P<0.0001). Furthermore, it was utilized 95 and 105% of the asymptote of body weight gain and breast yield, and feed conversion ratio and abdominal fat yield, respectively. Therefore, optimal dietary levels of Met+Cys in broiler diets for body weight gain, breast yield, feed conversion ratio and abdominal fat yield were 6.29, 6.52, 7.80 and 6.58 g/kg, respectively.

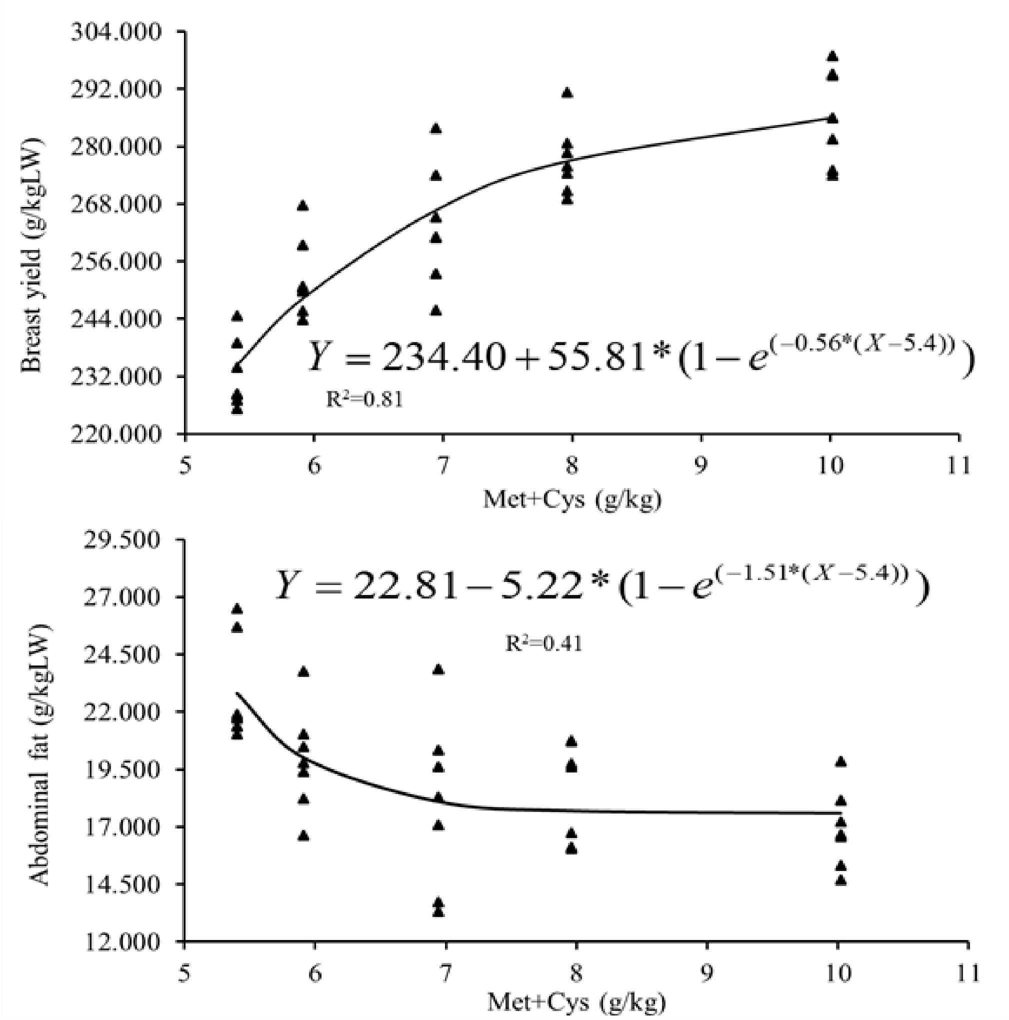

Figure 3. Carcass traits of broilers fed with increasing dietary levels of Met+Cys from 21 to 42 days old

In relation to economic analysis, it is shown that as Dl-met price increased then the optimal dietary Met+Cys level was reduced to produce the maximum gross margin, this economic trend was evidenced when broilers are sold as live weight or as cut-part (Figure 4). When broilers are sold as live weight, then for each DL-methionine price U\$ kg^{-1} 3.12, U\$ kg^{-1} 4.37 and U\$ kg^{-1} 5.31 corresponded the following optimal Met+Cys dietary levels 8.38, 8.23 and 8.01 g/kg respectively. In addition, when economic analysis is performed as breast meat yield, then for each DL-methionine price U\$ kg^{-1} 3.12, U\$ kg^{-1} 4.37 and U\$ kg^{-1} 5.31 corresponded the following optimal Met+Cys dietary levels 8.98, 8.89 and 8.79 g/kg, respectively.

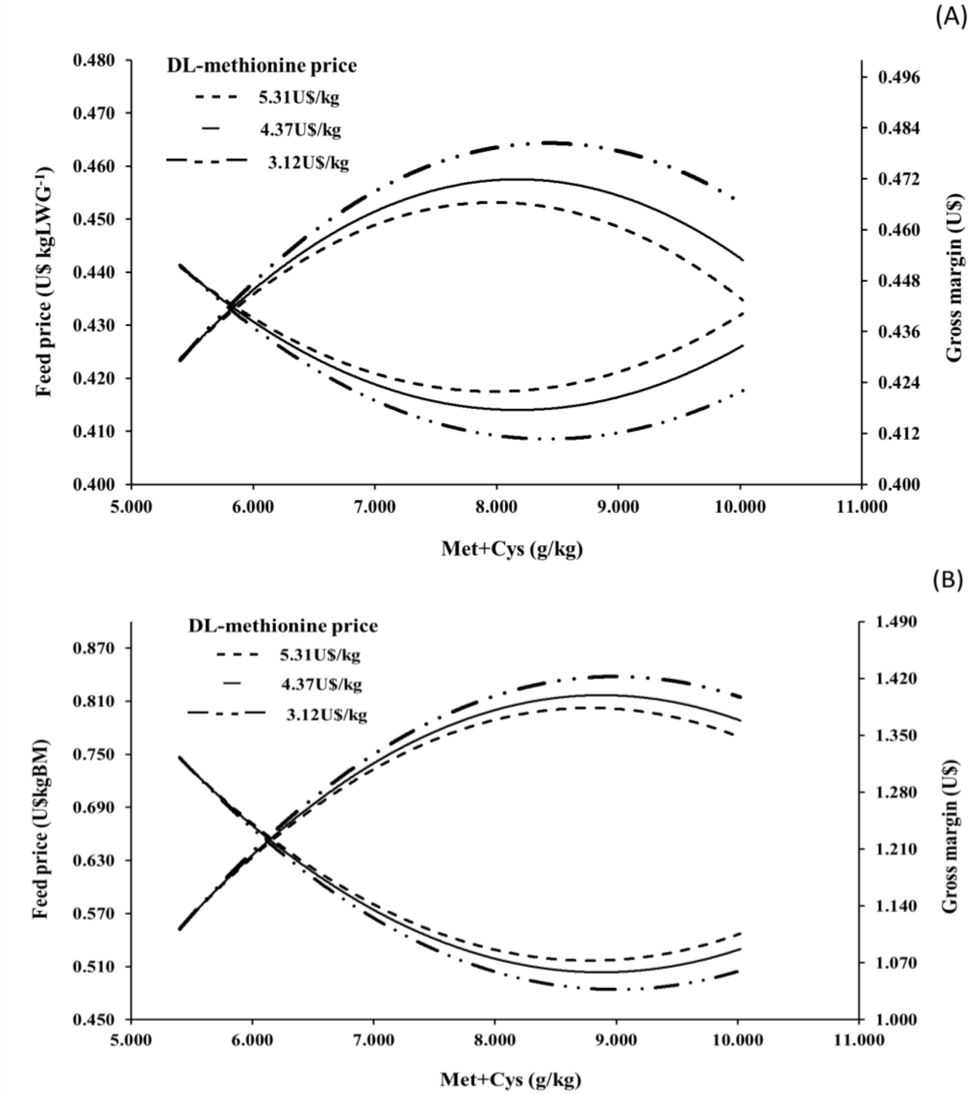

Figure 4. Feed cost and gross margin per kilogram of live weight gain (A) or breast meat (B).

Discussion

For both of phases, the non-linear response lead fitting to the model described by Rodehutscord and Pack (1999) which was built with performance response data of broilers fed dietary Met+Cys levels. According to broiler response of the current study, it is possible to verify that the basal diet was limiting in Met+Cys and it was reflected in a poor performance and carcass traits in relation to those diets that were supplemented with DL-methionine. In this sense, as the basal diet was supplemented with DL-methionine broiler response increased non-linearly and Met+Cys was less limiting until the response was near an asymptote as evidenced in Figure 1. These non-linear behavior of broiler response was in accordance with results found by Vieira et al. (2004), these findings show the non-linear performance response of broilers to Met+Cys levels.

Due to the non-linear characteristic of exponential model, it is not possible to determine a maximum break point which can be utilized as a nutritional recommendation, therefore the use of a percentage of the asymptote response, such as 95 or 105%, is a common choice and arbitrarily described and used in the literature (Mack et al. 1999).

Literature regarding the assessment of dietary levels of Met+Cys on carcass traits of broilers are scarce in comparison to performance traits such as body weight gain or feed conversion ratio. Studies have reported higher optimal concentration of Met+Cys in broiler diets when a carcass trait such as breast yield was under assessment (Schutte and Pack, 1995; Vieira et al. 2004; Rakangtong and Bunchasack, 2011) as a consequence of potential genetic of modern broiler for increased muscle mass growth. Over the last years, genetic advances in broilers have highlighted a higher protein deposition in breast meat, this potential genetic improvement is expressed with ideal environmental conditions and adequate supply of nutrients with the methionine as an essential amino acid and methyl group donor in biological processes such as DNA methylation for protein synthesis. Therefore, the correct supply of methionine in broiler diets will lead the genetic potential expression of breast yield in broilers as evidenced in data of the second phase.

With regards to abdominal fat, it is observed a nonlinear decrease as dietary level of Met+Cys increased in broiler diets, there results are in accordance with Rodrigueiro et al. (2000) and Junior et al. (2005) whose results showed a decrease of abdominal fat in carcass of broilers as dietary levels of Met+Cys increased. However, results obtained by Oliveira Neto et al. (2007) showed no influence of dietary level of Met+Cys in abdominal fat which could be explained by the narrow range of concentrations of Met+Cys. A possible explanation of the reduction of abdominal fat by increasing levels of Met+Cys obtained in the current study can be related by the reduction of lipogenesis made by methionine evidenced by (Takahashi et al. 1994; Takahashi and Akiba, 1995).

With regards to exponential model parameters obtained from data of the current study, the deficiency of the basal diet was reflected as poor response in performance and carcass traits yield, this was also expressed as the parameter corresponded as the difference between maximum response of DL-met supplementation and that of broiler received the basal diet, therefore the deficiency of the basal diet was expressed in all performance and carcass traits of broilers and it was in agreement with parameters obtained by Vieira et al. (2004).

In addition, curvature steepness obtained from data of both experiments, was lower for feed conversion ratio compared to body weight gain, this finding means that maximum body weight gain was achieved by a lower dietary level of Met+Cys in comparison to feed conversion ratio, these observations are consistent with previous studies (Faridi et al. (2013); Vieira et al. (2004); Schutte and Pack, 1995). The contrast of the curvature steepness made above is consistent with the dietary levels of Met+Cys obtained by applying 95 and 105% of the asymptote for body weight gain and feed conversion ratio, respectively, which resulted in higher dietary levels of Met+Cys for feed conversion ratio than for body weight gain.

Ideal ratio between indispensable amino acids without excess and deficiencies is part of the ideal protein concept (Emmert and Baker, (1997)). In this context, it was aimed to maintain amino acid ratios with lysine as the amino acid reference by the use of sand as an inert in both of phases of the current study, however ratio of Met+Cys with respect to Lysine increased as supplementation of DL-methionine increased. According to Rostagno et al. (2011), it is recommended a ratio of Met+Cys: Lys of 0.72 and 0.73 for 1 to 21 and 22 to 42 days, respectively. These ratios are near the optimal levels of Met+Cys obtained for performance traits, however for the optimal dietary level achieved for breast yield it was found a ratio of Met+Cys: Lys of 0.84, which could be explained by the protein deposition in this tissue of modern broilers. This finding, support the importance of establishing dietary levels of Met+Cys according to the production aims for each broiler industry.

Since a biological point of view, nutritional recommendations of literature are concerned in a fixed dietary levels of an amino acid to achieve the maximum performance of broilers without taking into account variations on ingredients or market products prices. Feeding broilers based in corn and soybean meal has advantages since a logistic point of view due to the availability in the market of these ingredients or reduction of nutritional variability of diets. However, these feedstuffs have nutritional disadvantages such as a poor amino acid balance that leads the supplementation of any source of amino acids limiting in broiler diets.

The supplementation of crystalline amino acids in broilers diets is a frequent strategy to meet nutritional requirements of broilers. It is habitually to observe oscillations in methionine sources prices throughout the year or between broiler industries, therefore the understanding about optimal economical dietary Met+Cys is necessary to produce cost-effective diets. Under this approach, for the second phase it was fixed a sell price of breast meat (1.875 U$ kg^{-1}) and live weight of broilers (0.875 U$ kg^{-1}). As shown in Figure 4, for both way of sale, as dietary level of Met+Cys increased in broiler diets gross margin increased until a maximum point and decreased until a minimum feeding cost. The dietary level of a nutrient looking forward maximum performance does not coincide with that which gives the maximum gross margin.

This economic analysis shows that optimal biological dietary level of Met+Cys does not necessarily coincide with the concentration that provide maximum gross margin. When sale criteria changed to broiler breast meat, the dietary level of Met+Cys increased in comparison as live weight sale. In the same way, as DL-methionine price increases the economical dietary level of Met+Cys tends to be lower as observed in Figure 4.

In this sense, dietary level of Met+Cys in broiler diets can be adjusted to price variations of DL-methionine and how broilers are marketed, this approach reinforces the relevance of formulation of cost-effective broiler diets by including feedstuffs or additives prices in the optimization of the diets aiming maximization of gross margin.

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CAPÍTULO 4 - IMPLICAÇÕES

Um balanço adequado de aminoácidos na nutrição de frangos de corte garante que a ave expresse o potencial genético sob condições ideais de criação. As dietas de frangos de corte podem ser compostas majoritariamente por ingredientes de origem vegetal como milho e soja por serem de fácil acesso e por garantirem menor risco de transmissão de doenças por ter um inadequado processamento das farinhas de origem animal. O primeiro aminoácido limitante em dietas compostas por milho e soja é a metionina, isto leva à suplementação de uma fonte desse aminoácido para garantir que a ave expresse o potencial genético.

As fontes de metionina disponíveis no mercado podem estar sob a forma cristalina ou liquida. Conhecer o valor biológico dessas fontes tem importância para os nutricionistas no ajuste nutricional das formulas e para fazer a valoração econômica de cada fonte de metionina. A través do estudo da biodisponibilidade de um nutriente é possível fazer estimativas do valor biológico de cada fonte de um nutriente. No caso da metionina, cada fonte possui uma estrutura química que deverá ser absorvida e metabolizada até a forma levogira da metionina que se encontra em forma natural nas proteínas do corpo.

A avaliação de níveis de metionina + cistina na resposta em desempenho e rendimento de carcaça em frangos de corte permite fazer estimativas de uma concentração ótima que garanta a máxima resposta biológica. No entanto, desde um ponto de vista econômico, a utilização da concentração de um nutriente que garanta o maior retorno econômico por unidade produzida é aquela que produz o melhor estado financeiro da criação de frangos.

Conhecer a resposta biológica da metionina + cistina em diferentes concentrações permite estimar com maior precisão uma concentração ótima econômica que garanta o maior lucro. Isto envolve o ajuste na concentração da metionina + cistina de acordo com fatores externos como o preço da fonte de metionina a ser utilizada na fórmula.