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DOSE RESPOSTA A NÍVEIS CRESCENTES DE DL-2-HIDROXI-4-METIL-TIO-BUTANÓICO SOBRE O DESEMPENHO ZOOTÉCNICO E RENDIMENTO DE CORTES COMERCIAIS DE FRANGOS DE CORTE NO PERÍODO DE 28 A 42 DIAS DE IDADE

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DOSE RESPOSTA A NÍVEIS CRESCENTES DE DL-2-HIDROXI-4-METIL-TIO-BUTANÓICO SOBRE O DESEMPENHO ZOOTÉCNICO E RENDIMENTO DE CORTES COMERCIAIS DE FRANGOS DE CORTE NO PERÍODO DE 28 A 42 DIAS DE IDADE

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Orientador: Sergio Luiz Vieira

RESUMO – Esta dissertação teve como objetivo avaliar o desempenho zootécnico e rendimento de cortes comercias de frangos de corte no período de 28 a 42 dias de idade utilizando níveis crescentes de DL-2-hidroxi-4metil-tio-butanóico 88% (HMTBA) como fonte de suplementação de metionina (MET) na dieta. Foram alojados 2.106 frangos de corte machos Cobb x Cobb 500 com 1 dia de idade, distribuídos aleatoriamente em 81 boxes (1,65 x 1,65 m) com 26 aves cada, recebendo uma dieta padrão para todos. Aos 28 dias, os boxes foram rearranjados de modo que não houvesse diferença no peso vivo entre os tratamentos. Um delineamento completamente casualizado foi utilizado para gerar curvas de regressão de respostas zootécnicas em função da suplementação com HMTBA. Uma dieta basal, sem adição de HMTBA, foi formulada a base de milho, farelo de soja e farinha de carnes. Esta dieta apresentava 3.200 kcal/kg e 19,35% de proteína bruta, sendo 0,52% de metionina e cisteína digestível (Met + Cis dig.), 0,95% de lisina digestível (dig)., 0,64% de treonina dig., 0,78% de valina dig., 0,69% de isoleucina dig., 1,50% de leucina dig., 1,16% de arginina dig. e 0,18% de triptofano dig. Oito níveis de suplementação de HMTBA foram adicionados à dieta basal (0,07; 0,14; 0,21; 0,28; 0,35; 0,42; 0,49 e 0,56% de inclusão na dieta). Avaliou-se o desempenho zootécnico dos 35 aos 42 dias de idade. Aos 42 dias 6 aves por unidade experimental foram abatidas para avaliação de rendimento de carcaça e cortes comerciais. As respostas de ganho de peso (GP), conversão alimentar, (CA), rendimento de peito e gordura abdominal tiveram ajustes quadráticos com a inclusão de HMTBA na dieta. Estimativa de respostas máximas para suplementação de HMTBA foram de 0,35% para GP, de 0,37% para CA, 0,33% para gordura abdominal e 0,35% para rendimento de carne de peito. O presente estudo fornece dados de regressão para aqueles que utilizam HMTBA como um suplemento dietético de MET.

Palavras-chave: Avicultura, Nutrição, Metionina análoga.

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DOSE RESPONSE TO INCREASING DIETARY LEVELS OF 2-HYDROXY-4-(METHYLTHIO)-BUTANOIC ACID ON LIVE PERFORMANCE AND CARCASS YIELDS OF BROILERS FROM 28 TO 42 DAYS OF AGE

Author: Cesar Augusto Pontin
Advisor: Sergio Luiz Vieira

ABSTRACT – This dissertation aimed to evaluate the performance and commercial cuts yield of broilers from 28 to 42 days of age by increasing levels of methionine hydroxy analogue 88% (HMTBA) as a source of methionine (MET) supplementation in the diet. A total of 2,106 male broilers Cobb x Cobb 500 were housed with one day of age, receiving a standard diet for everyone. At 28 days, birds were rearranged to have no difference in body weight among treatments. A completely randomized design was used to calculate equation regression of growth performance in response to HMTBA supplementation. A basal diet without adding HMTBA, was made from corn, soybean meal and meat meal. This diet had 3,200 kcal / kg and 19.35% crude protein, with 0.52% of digestible methionine and cysteine (dig. Met + Cis), 0.95% dig. lysine , 0.64% of dig. threonine, 0.78% to dig valine., 0.69% to dig isoleucine., 1.50% of dig. leucine, 1.16% of dig arginine and 0.18% of dig. tryptophan. Eight HMTBA supplementation levels were added to the basal diet (0.07, 0.14, 0.21, 0.28, 0.35 0.42 0.49 and 0.56% of inclusion in diet). Growth performance was evaluated from 35 to 42 days. In adittion, six birds per pen were slaughtered at 42 days to evaluate carcass and commercial cut yields. The body weight gain (BWG), feed conversion ratio (FCR), breast meat and abdominal fat yields were quadratic fits with the inclusion of dietary HMTBA. Maximum responses estimated using HMTBA supplementation were estimated at 0.35% for BWG, 0.37% for FCR, 0.33% for abdominal fat and 0.35% for breast meat yield. The present study provides regression data for those using HMTBA as a MET dietary supplement.

Key words: Aviculture, Nutrition, Methionine analogue.

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RELAÇÃO DE ABREVIATURAS

AA	Aminoácidos
CA	Conversão Alimentar
Dig	Digestível
GP	Ganho de Peso
HCl	Ácido clorídrico
MET	Metionina
MET+CIS	Metionina + cisteína
AAST	Aminoácidos sulfurados totais
SAM	S-adenosilmetionina
MAT	Metionina adenosiltransferase
CIS	Cisteína
S	Enxofre
MTPA	Metil-tio-aldeído-propionico
HCN	Cianeto de hidrogênio
OH	Hidróxido
NH_2	Amina
L-AOX	L-2-hidróxi ácido oxidase
D_HAD	D-2 hidróxi ácido desidrogenase
$DAAO_x$	D aminoácido oxidase

CAPÍTULO I

INTRODUÇÃO

Aminoácidos (AA) sintéticos são utilizados em rações de frangos de corte há muitos anos para reduzir a quantidade de AA em excesso na dieta, visando minimizar o custo de produção e permitir à ave expressar o seu real potencial genético (Vieira e Angel, 2012). Essa suplementação pretende maximizar os índices zootécnicos, bem como melhorar os rendimentos de cortes comerciais, especialmente da carne de peito. A concentração de AA dietéticos que maximiza o ganho de peso vivo (GP), a conversão alimentar (CA) ou o rendimento de carne de peito são diferentes. Por exemplo, o nível de aminoácidos sulfurados totais (AAST) ótimo para o rendimento da carne de peito tem se mostrado mais elevado que o recomendado para rendimento de carcaça inteira, CA ou GP.

A metionina (MET) tem um papel importante na síntese proteica e também uma ampla interação com a exigência de mantença, além de ser a principal fonte de enxofre para a síntese de outros componentes (Maenz e Engele-Schaan, 1996). Esse AA é o primeiro limitante em frangos de corte alimentados com dietas a base de milho e farelo de soja. Portanto, essas dietas são comumente suplementadas com DL-metionina (DL-MET) na forma pó com 99% de MET ou DL-2-hidroxi-4-metil-tio-butanóico (HMTBA) com 88% de substância ativa. A HMTBA é similar à DL-Met, exceto por conter em sua composição um grupo hidroxilia ao invez de um grupo amino, considerada então como um ácido orgânico (Boebel e Baker, 1982). Por isso, a HMTBA não é considerada um AA, necessitando ser convertida em D-aminoácido (dextrógiro) e/ou L-aminoácido (levógiro). Apenas a forma L-aminoácido é utilizada para a síntese proteica pelas aves, assim as formas D-aminoácidos devem ser convertidas para L-aminoácidos por duas reações, oxidação do alfa-carbono para céto-análogo e transaminação do céto-análogo para L-aminoácido (Dibner, 2003).

Em comparação com todos os outros AA produzidos por fermentação de microrganismos geneticamente modificados, a MET é obtida através de síntese química. Esta síntese resulta numa mistura racêmica de 50% D e 50% de L-metionina. No entanto, a MET é o único AA em que os dois estereoisómeros D e L são utilizados quase da mesma intensidade por todos os animais vivos. Além disso, a sua forma hidroxi-análogo pode ser totalmente convertido em L-metionina para participar da síntese proteica (Vazquez-Anon *et al.*, 2006).

Usualmente, a exigência de AAST é estabelecida baseada nas respostas obtidas com DL-Met. Erros são observados, pois formuladores usam estes dados para dietas formuladas com HMTBA como fonte de MET. Diante da importância da MET nas dietas de frangos de corte, tornam-se indispensáveis estudos quanto à eficiência das fontes disponíveis no mercado. Dessa forma, objetivou-se avaliar a suplementação de HMTBA para a fase final de frangos de corte, determinando nível para obter melhor desempenho e rendimento de cortes comerciais.

REVISÃO BIBLIOGRÁFICA

Digestão da proteína e absorção dos aminoácidos

A digestão da proteína da dieta envolve diversas interações entre estômago glândular (proventrículo) e muscular (moela), terminando com o transporte de AA e peptídeos pela membrana basolateral do intestino delgado (D'mello, 2003). A digestão gástrica inicia com a secreção de ácido clorídrico (HCl) pelas células oxintopepticas do proventrículo (D'Mello, 2003). Quando secretada na forma de zimogênio e ativada pelo HCl, a pepsina promove a quebra as estruturas terceárias e quartenárias das proteínas, facilitando a ação das endopeptidases pancréticas presentes no intestino delgado (Guan e Green, 1996). A principal função da digestão no proventrículo é quebrar a proteína em polipeptídeos.

Após a digesta passar pelos estômagos ela segue para os intestinos onde glândulas anexas auxiliam na digestão. O pâncreas é responsável pela secreção de diversas enzimas proteolíticas, como: tripsinogênio, Elastase, quimiotripsinogênio e procarboxipeptidase A e B (Erickson *et al.*, 2015). A enteroquinase (liberada pela presença de proteínas desnaturadas no intestino) ativa a tripsina, que por sua vez, ativa outros zimogênios (quimiotripsinogênio e procarboxipepridase A e B) (D'Mello, 2003). Ao final da digestão pancreática são encontrados oligopeptídeos com até seis aminoácidos (aproximadamente 60%) e aminoácidos livres (aproximadamente 40%) (Evenepoel, 2001).

A última fase de digestão é realizada pelas peptidases citosólicas na membrana em forma de escova no intestino delgado. As endopeptidases são capazes de quebrar os oligopeptídeos da digestão pancreática em menor número de aminoácidos. Estas enzimas estão presentes no enterócito, na região apical e no citosol (Freeman e Kim, 1978). Muitos tri e dipeptídeos são absorvidos intactos pelos enterócitos e clivados por estas enzimas (D'mello, 2003).

Peptídeos e aminoácidos presentes no citosol dos enterócitos podem ser incorporados as proteínas das células, metabolizado dentro do epitélio do intestino delgado ou transportado através da membrana basolateral, chegando a circulação hepática através da veia porta (D'Mello, 2003). No fígado, parte dos AA são fixados pelas células hepáticas e o restante é liberado na corrente sanguínea formando um *pool* extracelular de AA livres. Nos tecidos, após absorvidos pelas células, são convertidos em outros metabólitos ou ligam-se a um RNA tranpostador específico para ser utilizado na síntese protéica no ribossomo (Rathmacher, 2000).

Metabolismo da Metionina

A MET desempenha inumeras funções no organismo animal. Possui participação importante na síntese proteica, proporcionando melhores rendimentos de carcaça e de peito, além de reduzir o teor de gordura abdominal (Rodrigueiro *et al.*, 2000). Também tem um papel importante na manutenção do organismo.

A MET é precursora de diversos compostos. Seu metabolismo começa com o processo de metilação, com a sua ativação a S-adenosilmetionina, (SAM) pela enzima metionina-adenosiltransferase (MAT) (figura 1), (Bydlowski *et al.*, 1998). A SAM é um cofator enzimático envolvido na transferência de grupo metilo, o que é importante, por exemplo, na síntese de colina (forma a lecitina) e creatina (aminoácido não proteico). A introdução de um grupo metila altera a estrutara da molécula metilada e consequentemente sua função. O DNA por exemplo sofre metilação, e é através dessa metilação que o gene se manifesta ou não. O produto da desmetilação da SAM é a S-adenosil-homocisteína, único precursor de homocisteína (aminoácido sulfurado não formador de proteínas). A homocisteína formada pode seguir duas vias: transulfuração ou remetilação (Nerbass *et al.*, 2005). A remetilação é o retorno da homocisteína em metionina, processo realizado através da ação de duas enzimas: metionina-sintetase e a betaína-homocisteína-metiltransferase. (Stipanuk e Ueki, 2011). Outra via da homocisteína é a transulfuração, processo pelo qual há formação da cisteína (CIS). A homocisteína fornece um um grupo S (enxofre) para a L-serina, (através da ação da enzima cistationina β-sintetase e vitamina B_6) formando uma molécula de cisteína (Brosnan e Brosnan, 2006).

A CIS está presente na constituição de muitas proteínas como a queratina. No caso das aves é o principal constituinte das penas, um dos fatores da MET ser principal limitante na dieta de frangos. Imunoglobulinas, compostas por duas cadeias de enxofre, tem um papel importante no sistema imune, através da neutralização de toxinas e opsonização de antígenos. Insulina, formada através de ligações de dissulfeto, esta proteína é de extrema importância para síntese de proteínas e armazenamento de lipídios. Além das funções já citadas, a CIS ainda participa da formação de cistina pela junção de duas moléculas de CIS através de seus grupos S. A CIS é utilizada na síntese de vários compostos contendo enxofre incluindo a taurina, um aminoácido que participa na formação do ácido biliar (importante na digestão de lipídeos). Também atua na formação de glutationa (um tripeptídeo) juntamente com a glicina e o glutamato. A glutationa é um antioxidante mais frequente nas células das aves.

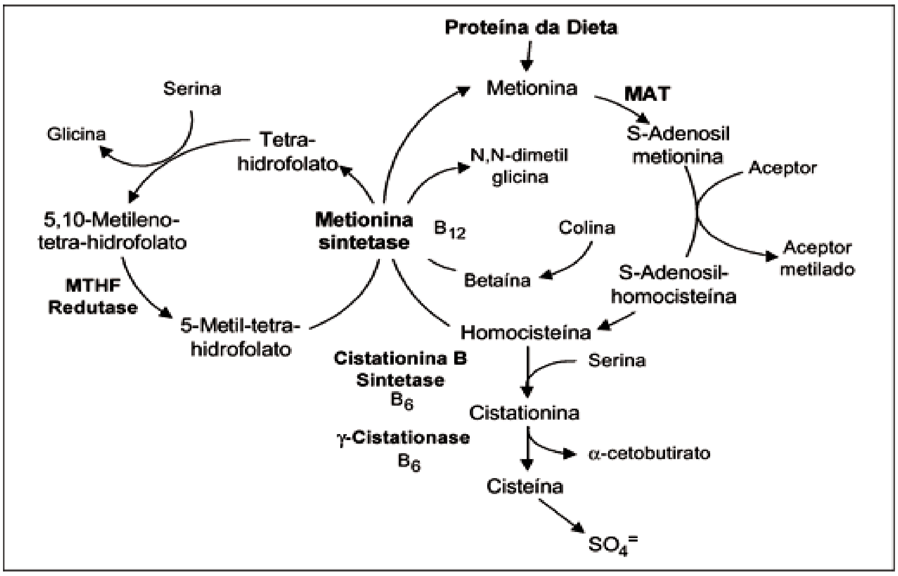

Figura 1. Via de metabolismo da metionina.

Fontes de Metionia

A inclusão do aminoácido MET nas dietas de frangos de corte, iniciou-se a partir da década de 70. As fontes deste aminoácido encontradas em escala industrial hoje no mercado são: A DL-MET e a DL-2-hidroxi-4-meti-butanóico (HMTBA). DL-MET é um produto em pó com a cor branca contendo 99% de substância ativa, que é a fonte usualmente empregada como padrão na comparação de fontes de metionina. Seu peso molecular é 149,2 g/mol e sua forma molecular é C5H11NO2S. A HMTBA é um líquido de cor escura contendo 88% de metionina na sua forma ativa e 12% de água. Seu peso molecular é 150,2 g/mol e sua formula molecular é C5H10O3S. É composta por 66% de monômeros, 19% de dímeros, 1 a 3% de oligômeros (Baker e Boebel, 1980). As duas substâncias, HMTBA e DL-Met, possuem os dois isômeros L e D.

A HMTBA não é um aminoácido, mas um precursor de metionina. A estrutura química das moléculas mostra a diferença no carbono alfa da molécula (Figura 2), nesse ponto a DL-Met contém um grupo amina (NH_2), enquanto que a HMTBA contém um grupo hidroxila (OH).

A MET é essencial para mantença e crescimento de frangos de corte, além de ser o primeiro aminoácido limitante em dietas a base de milho e farelo de soja. Portanto, DL-MET ou HMTBA são frequentemente utilizados nas formulações para ajustar metionina na dieta, suprir as exigências dos animais, maximizar o desempenho da produção e reduzir a excreção de nitrogênio. A metionina e seus análogos são os aminoácidos mais tóxicos. Excesso desses aminoácidos na dieta é tão prejudicial quanto sua deficiência, pois pode levar a redução no ganho de peso e aumento na conversão alimentar (Edmonds e Baker, 1987). A adição de 2% de DL-MET resultou em redução no ganho de peso em um estudo de dose-resposta com frangos de corte (Harter e Baker, 1978). No entanto, alguns trabalhos demonstram que a HMTBA apresenta menor toxicidade do que a DL-MET (Lemme *et al.*, 2002; Vazquez-Anon *et al.*, 2006).

A maioria dos aminoácidos são produzidos por fermentação de microorganismos geneticamente modificados. A metionina é produzida por síntese química, a partir de metil-tio-aldeído propiónico (MTPA) e cianeto de hidrogénio (HCN), na presença de um catalisador (Klose *et al.*, 1985). MTPA e HCN são obtidos por síntese química, utilizando matérias-primas de origem petroquímica. Essa síntese resulta em uma mistura racêmica de 50% D e 50% L-metionina, pois sua molécula tem carbono alfa assimétrico. (Leite *et al.*, 2009). Os aminoácidos podem existir em duas formas, referidas como D e L isômeros. Para a síntese de proteínas e a formação de S-adenosilmetionina, apenas o L-isómero estéreo de Met pode ser usado. A eficácia dos isômero D aminoácidos para frangos de corte é alta, cerca de 90% da D-Met é convertida para L-Met (Ly *et al.*, 2012). Para essa essa conversão são necessárias duas reações químicas, a desaminação oxidativa do D isômero e a transaminação do cetoácido para L-aminoácido (Dibner, 2003).

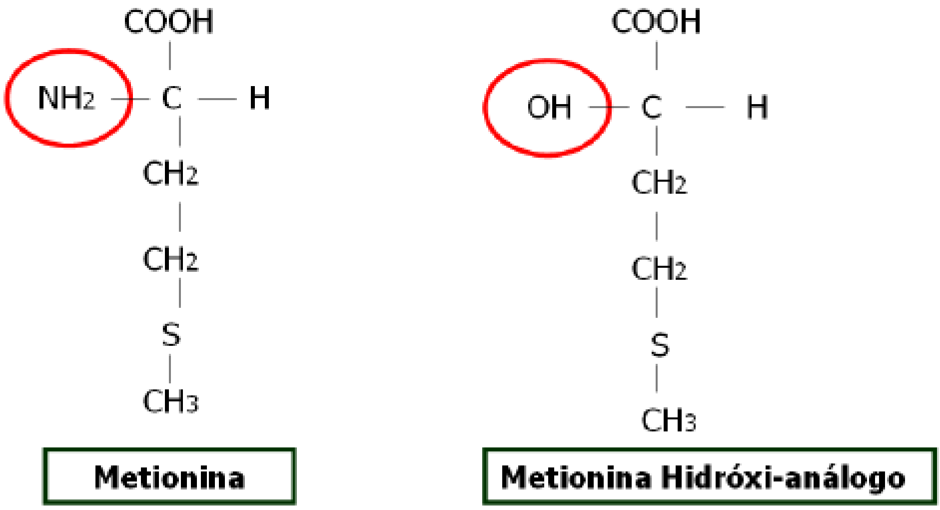

Figura 2. Estrutura química da metionina e do hidróxi análogo de metionina.

Os efeitos das diversas fontes de metionina sobre os índices zootécnicos de frangos de corte tem sido estudada há muitos anos. A primeira referência conhecida sobre a utilização de HMTBA foi de um trabalho publicado na década de 1930, onde o produto proporcionou um aumento no crescimento de ratos alimentados com dietas deficientes em cistina (Jackson e Block, 1932). Desde então, começou-se a pesquisar sobre fontes análogas de metionina como doadoras de metionia. Ou seja há mais de 85 anos de pesquisa na produção e efeito de suplementação desse análogo de metionina. Com isso, muitas controvérsias surgiram sobre a bioeficácia desse produto, atribuído em parte aos diferentes modelos estatísticos utilizados para sua avaliação e a conversão de HMTBA em L-MET (Ferjancic-Biagini *et al.*, 1995). Por exemplo, em um trabalho demonstrando diferentes modelos estatísticos para avaliar dose resposta de fontes de metionina, os autores indicam o modelo estatístico de regressão com platôs separados ao invés do mesmo platô como muitas vezes é utilizado, pois as fontes possuem respostas diferentes (Kratzer and Littell, 2006). Outro experimento avaliou dose resposta de DL-Met e HMTBA utilizando na mesma base equimolar (Gonzales-Esquerra et al., 2007). Os autores observaram um aumento linear no desempenho das aves utilizando HMTBA e quadrático com a inclusão de DL-Met na dieta, demonstrando diferentes respostas entre as fontes utilizadas. Quatro experimentos foram realizados com frangos de corte utilizando DL- Met e HMTBA como suplementação de metionina na mesma base equimolar (Vazquez-Anon *et al.*, 2006). Neste experimento foram utilizados os modelos estatísticos linear, quadrático e exponencial para as respostas de desempenho avaliadas. Foi verificado que cada resposta apresentava uma curva com formato diferente para cada fonte avaliada. Então, a biodisponibilidade pode variar em função da dose (Kratzer and Littell, 2006) e em função da resposta em estudo. Em um experimento utilizando quatro níveis de suplementação na dieta basal de frangos de corte (0,6; 0,12; 0,18 e 0,24%) de DL-MET e HMTBA, observou-se diferentes respostas de desempenho produtivo. A biodisponibilidades do análogo foi de 68, 67 e 62% para ganho de peso, conversão alimentar e rendimento de carcaça, respectivamente, em relação a fonte tradicional (Lemme *et al*., 2002).

Absorção da metionina

Os aminoácidos são absorvidos ao longo de todo trato intestinal, principalmente no jejuno, devido a necessidade de hidrólise de proteínas pelas enzimas secretadas no pâncreas. Em aves e suínos, a absorção de HMTBA é concluída no final do duodeno enquanto que a absorção de DL-MET é completa no final do jejuno (Jendza *et al.*, 2011). Por outro lado, um estudo mostrou que a HMTBA é absorvida em todo o trato digestivo inclusive no ceco (Dibner *et al.*, 1988). A DL-Met é transportada por um único mecanismo, processo ativo sódio dependente, o sistema de transporte ativo tem uma maior afinidade por L-met do que por D-Met (Dibner *et al.*, 1988), o que permite que sejam transportados contra um gradiente de concentração, com gasto energético. Por outro lado, os isômeros de HMTBA são absorvidos de forma passiva, que requer a passagem de um meio mais concentrado para menos concentrado. Devido ao pH mais alto na parte superior do trato gastro intestinal, a absorção por difusão da HMTBA é favorecida (Knight e Dibner, 1984). Esses pesquisadores avaliaram as duas fontes de metionina e comprovaram que a absorção de HMTBA foi tão rápida quanto a DL-Met. Sendo um acido monocarboxilico, sua absorção se assemelha a de outros ácidos orgânicos, de cadeia curta (Jongbloed *et al.*, 2000), em particular o acido láctico. Estudos demonstram o efeito do H^+ sobre a absorção dessa fonte, onde seu transportador possui características semelhantes ao transportador do ácido láctico (monocarboxylate transporter1) (Martin-Venegas *et al.*, 2009). Em um trabalho com frangos de corte machos, encontraram uma absorção para DL-Met de 100% e aproximadamente 96 a 99% para HMTBA, concluído que as diferenças de biodisponibiliades não estão relacionadas a absorção das duas fontes (Huyghebaert, 1993). Utilizando as duas fontes de metionina na mesma base equimolar, (Han *et al.*, 1990) demonstraram que não houve diferença na absorção entre elas (98,8% de absorção para HMTBA e 99,7% para DL-MET).

No fígado das aves e em outros tecidos, tem se encontrado enzimas capazes de oxidar os isômeros D e L de HMTBA, (Knight e Dibner, 1984). A enzima específica para L-HMTBA é a L-2 hidroxi ácido oxidase (L-AOX), encontrada nos peroxissomos do fígado e rins das aves. A D-HMTBA requer a enzima mitocondrial D-2-hidroxi ácido desidrogenase (D-HAD) encontrada em vários tecidos, incluindo, fígado, rins, músculo esquelético, intestino, pâncreas, baço e cérebro. (Knight e Dibner, 1984). Já a enzima necessária para conversão de D-MET é a D-aminoácido oxidase (DAAOx). Essas enzimas são amplamente presentes nos tecidos do corpo (rins, fígado) mas também ao longo de todo tracto digestivo. (Geraert e Mercier).

HIPÓTESES E OBJETIVOS

Hipóteses

A HMTBA é uma fonte eficiente para suplementação de metionina na dietas.

A inclusão de HMTBA tem um nível ótimo para frangos nas fases crescimento e final.

O desempenho zootécnico de frangos de corte suplementados com níveis crescentes de HMTBA apresenta comportamento quadrático.

Objetivos

Avaliar a utilização de níveis crescentes de sobre o desempenho e o rendimento de carcaça e cortes comerciais de frangos de corte na fase final.

Determinar o ponto de máximo desempenho para todas as variáveis analisadas.

Desenvolver informação relevante sobre dose resposta de Met+Cys em aves quando usarmos HMTBA como fonte de Met suplementar.

CAPÍTULO II[1]

[1] Artigo elaborado conforme as normas da revista Animal Feed Science and Technology.

Response to increasing dietary levels of 2-hydroxy-4-(methylthio)-butanoic acid: Live performance and carcass yields of broilers fed from 28 to 42 d

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ABSTRACT

As the first limiting amino acid in corn-soy broiler diets, methionine (Met) supplementation from commercial synthetic sources is demanded to obtain economic formulations. The Met analogue 2-hydroxy-4-(methylthio)-butanoic acid (HMTBA) is largely used with that intention. The objective of the present study was to obtain responses of broilers fed with increased levels of HMTBA from 28 to 42 d. A total of 2,106 Cobb × Cobb 500 1-d-old male broilers were randomly placed into 81 floor pens (2.7 m^2 each). Birds were fed conventional starter (1 to 14 d: 230 g/kg CP, 12.95 MJ/kg) and grower diets (15 to 27 d: 220 g/kg CP and 13.05 MJ/kg). Starting at 28 d of age, replicate pens of 26 birds were randomly allocated into 9 treatments with 9 replications having increased levels of supplemented HMTBA (0.00, 0.07, 0.14, 0.21, 0.28, 0.35, 0.42, 0.49, and 0.56%). These were prepared by mixing different proportions of corn-soy dilution and summit diets, which had the same concentration of formulated nutrients and energy (197 g/kg CP, 9.0 g/kg Ca, 4.5 g/kg Av. P, 9.5 g/kg digestible Lys, and 13.20 MJ/kg), with the exception of HMTBA (0.56% in the summit but not supplemented in the corn-soy dilution diet). Growth performance was evaluated until 42 d when carcass yield and commercial cuts were evaluated using 6 birds randomly taken from each pen. Body weight gain, FCR, yields of breast fillets and abdominal fat were adjusted using quadratic polynomial regressions ($P < 0.05$). Estimation of maximum responses for supplemented HMTBA using quadratic regressions were 0.35% for BWG, 0.37% for FCR, 0.35% for breast fillets yield and 0.33% for abdominal fat. The present study provided regression data for those using HMTBA as a Met dietary supplement.

Keywords: broiler, methionine analogue, performance, sulfur amino acids

Abbreviations: AA, amino acids; BWG, body weight gain; CP, crude protein; Cys, cysteine; d, days; dig, digestible; FCR, feed conversion ratio; FI, feed intake; HMTBA, 2-hydroxy-4-(methylthio)-butanoic acid; Lys, lysine; Met, methionine; SBM, soybean meal; Thr, threonine; TSAA, total sulfur amino acids.

1. Introduction

Adding synthetic and crystalline forms of amino acid (AA) in broiler diets has allowed extensive investigation in AA requirements for broilers in the last decades. Responses of broilers to variations in dietary AA remain important subjects for investigation because of the improvements in live performance and carcass yield constantly delivered by genetics. Nutrient recommendations for broilers intend to optimize growth performance as well as the proportions of individual carcass components, especially breast meat, to a point that delivers best economic returns. The dietary concentration of amino acids that maximize BW gain (BWG), FCR, and breast meat yield can be different for each one of these parameters. For instance, optimum total sulfur amino acids (TSAA) levels for breast meat yield have been shown to be higher when compared with those for whole carcass yield, FCR, or BWG and are dependent of broiler genetics (Vieira et al., 2004).

For broilers fed corn-soy diets, methionine (Met) is first limiting AA. This AA plays important roles for growing broilers because its limited concentration in feeds affect muscle accretion and feather synthesis, as well as the biochemical processes dependent of the presence of methyl groups donated by Met (NRC, 1994). Methionine is the initiating amino acid in the synthesis of virtually all eukaryotic proteins (Lucas-Lenard and Lipmann, 1971) and its metabolism begins with its activation to S-adenosylmethionine, which is used in methylation reactions and is also converted to cysteine (Cys) by the transsulfuration pathway (Brosnan and Brosnan, 2006).

Two commercially available synthetic products are the main precursors of Met routinely supplemented in broiler feeds: DL-Met and 2-hydroxy-4-(methylthio)-butanoic acid (HMTBA). The use of these Met precursors in poultry nutrition has been usual for many years allowing the reduction in the dietary inclusion of protein feedstuffs without affecting bird performance (Warnick and Anderson, 1968; Wallis, 1999). Both of these Met synthetic sources have demonstrated to be readily converted into L-Met (Dibner and Knight, 1984; Martín-Venegas et al., 2006), which is the final form of Met used in the animal metabolism. However, these are different molecules that follow unique processes from absorption to final transformation into L-Met. For instance, the absorption of DL-Met follows an active sodium dependent pathway and it is finally used as L-Met before undergoing the process of oxidative deamination and transamination of its keto acid (Gilbert et al., 2008). On the other hand, HMTBA has a hydroxyl group on the alpha carbon rather than the amino group present in DL-Met (Dibner and Knight, 1984). Therefore, as an organic acid, HMTBA is passively absorbed and later converted into L-Met by the body cells (Dibner and Knight, 1984; Martín-Venegas et al., 2011).

Whereas bioequivalence of DL-Met and HMTBA remains controversial (Wallis, 1999; Lemme et al., 2002), HMTBA is largely used in broiler diets being approximating 70% of the total MET supplementation used in the US poultry industry (USDA, 2015). Therefore, there is a need to routinely review the response of commercial broiler traits to increased addition of HMTBA, such that users can plot their own objectives to the increasing doses of this supplement. The objective of this study was to evaluate growth performance and carcass and commercial cuts yields of male broilers fed with increasing levels of HMTBA as the sole TSAA supplement in corn-soy diets, such that estimators of maximum responses are provided for each of these responses.

2. Material and methods

All procedures adopted throughout this study avoided unnecessary animal discomfort and were approved by the directives of Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

2.1. General bird husbandry

A total of 2,106 male Cobb × Cobb 500 slow feathering broiler chickens were randomly placed into 81 floor pens (1.65 × 1.65 m), 26 birds in each with new rice hulls as bedding. Each pen was equipped with a tube feeder and a bell drinker. The environmental temperature was controlled to maintain bird comfort throughout the study (Cobb-Vantress, 2013), which was reduced by 1ºC every 2 d through the use of thermostatically controlled heaters, fans and foggers. Lighting was continuous until 7 d of age, with a 14L:10D cycle used afterwards. Birds had *ad libitum* access to water and mash feed.

2.2. Dietary treatments

Birds were fed common corn-soy mash starter (1 to 14 d: 230 g/kg CP, 12.95 MJ/kg) and grower diets (15 to 27 d: 220 g/kg CP and 13.05 MJ/kg). following Brazilian industry standards (Rostagno et al., 2011). At 28 d, replication pens having bird with individual BW = 1.469 ± 53.0 g were allocated into a completely randomized design with 9 dietary treatments with 9 replications. Treatments were produced by mixing a non-supplemented HMTBA diet (dilution diet) with a summit diet having an inclusion of 0.56% HMTBA. Manufacturer recommendation of 88% equivalence of HMTBA to TSAA was used to formulate experimental diets (Alimet, Novus International, Indaiatuba, SP, Brazil).

Analyses of AA in ingredients and diets were conducted according to the method 914.12 using an HPLC auto analyzer and employed performic acid oxidation of the feed sample prior to acid hydrolysis (AOAC International, 1998). Analysis of HMTBA in the supplemental diets was also done using HPLC (Ontiveros et al., 1987).

2.3. Measurements

Live performance was evaluated from 28 to 42 d when feed intake (FI), BWG, and FCR corrected for mortality were estimated. Mortality was recorded daily. At 42 d, 6 birds were randomly selected from each pen and processed for carcass and commercial cuts evaluation. Broilers were fasted for 8 h and individually weighed prior to slaughter. Birds were humanely rendered insensible using electrical stunning (45 V for 3 s), then bled through a jugular vein cut for 3 min, scalded at 60ºC for 45 s, and lastly defeathered. Evisceration was manually performed and carcasses were statically chilled in ice for approximately 3 h. Eviscerated carcasses (without feet and neck but with lungs) were hung for 3 min to remove excess water prior to weighing. Commercial cuts were performed by a crew of industry-trained personnel into bone-in drumsticks, thighs, wings as well as deboned breast fillets and tenders. Abdominal fat was weighed separately. Carcass yield was expressed relative to live weight while commercial cuts and abdominal fat were expressed as percentage of the eviscerated carcass.

2.4. Statistical analysis

Data were submitted to ANOVA using the GLM procedure (SAS, 2001). Pens were considered as experimental units for the evaluation of live performance and the average of 6 processed birds per pen for carcass yield assessment. Means were compared by

Tukey test when effects of dietary treatments were significant at 5% (Tukey, 1991). Estimations of HMTBA inclusion that maximized responses were done using quadratic polynomial regression (Robbins et al., 1979; Pesti et al., 2009) as follows: $Y = \beta 1 + \beta 2 \times$ HMTBA + $\beta 3 \times$ HMTBA^2) had Y as the dependent variable as a function of dietary level of HMTBA; $\beta 1$ as the intercept; $\beta 2$ as the linear coefficient and $\beta 3$ as the quadratic coefficient. The maximum response to HMTBA was defined as HMTBA = - $\beta 2 \div (2 \times \beta 3)$.

3. Results

Formulated diets with increasing levels of HMTBA are presented in Table 1. Slight deviations between analyzed and formulated crude protein (CP), AA and HMTBA existed. These were considered acceptable since analyzed values demonstrated trends as expected in the formulated diets and, therefore, allowed adequate interpretation of results.

Broiler chickens from 28 to 42d fed corn-soy diets supplemented with increasing levels of a HMTBA varying from 0 to 0.56% had increased BWG and breast meat yield and decreased FCR ($P < 0.001$). Live performance responses demonstrated that birds benefited from the increasing levels of HMTBA to response points that flattened out, which, therefore, allowed quadratic adjustments ($P < 0.001$). Maximum responses for BWG of broilers from 28 to 35 d were estimated to be 0.36% of HMTBA and from 36 to 42 d were 0.35% HMTBA (Table 2). The best result for FCR from 28 to 42 d was estimated when broilers were fed diets supplemented with 0.37% HMTBA. In parallel, carcass data presented significant quadratic adjustments ($P < 0.001$) with values that maximized the percentage of the different commercial products at 0.35%, 0.36%, and 0.27%, respectively for the eviscerated carcass as a proportion of the live bird, as well as the proportions of breast fillets, drumsticks and breast tender as respective proportions of the eviscerated carcass. A level of 0.33% HMTBA was needed to minimize abdominal fat.

4. Discussion

Reevaluations of nutrient requirements are needed along with the consistent changes in the proportions of body components of broilers due to genetic selection. Proportions of breast muscles are the ones that have presented the most noticeable increases with time, and, therefore, AA levels that maximize breast meat yield have been shown increasing trends (Garcia and Batal, 2005; Corzo et al., 2006; Corzo et al., 2007; Lumpkins et al., 2007; Dozier et al., 2008a; Dozier et al., 2008b). Breast muscle proportions of broilers within a similar market age has increased around 20% in the last 20 years (Havenstein, et al., 2003). On the other hand, studies on TSAA requirements of broilers conducted in the last 20 years were few, but with quite similar results. A scenario with increases in dietary concentrations of AA in broiler feeds to allow the expression of higher proportions of breast meat is likely to follow further increases of breast muscle proportions. This has been well established for lysine (Lys) because of its importance in muscle synthesis. In terms of TSAA, their increased proportional dietary demand is expected to derive mainly because of their importance for the maintenance of birds that have increased muscle as proportion of the overall live broiler protein.

Eventually the information user will relate these answers with the values obtained in other studies with Met. In addition, it does not seem appropriate to use data from animal performance to increasing levels of an analog to estimate the Met requirements without know exactly the actual bioequivalence of the Met analogue. To date, there is a great range of diversity in what is said about the bioequivalence between HMTBA and DL-Met. These differences may have originated in the experimental design, the statistical analysis, as well as the diversity of variables. Just to illustrate the magnitude of bioequivalence studies, it have been indicated values between 60 and 88% (Wallis, 1999; Lemme et al., 2002; Mandal et al., 2004). Anyway, HMTBA being a product widely used around the world as Met precursor, the presentation of live performance responses and product processed at increasing levels allows the reader adding that information to their most efficient use especially when their main MET source comes from HMTBA.

Data obtained in the present study estimated the need of higher dig. TSAA requirements to maximize performance of 42 d male broilers higher when compared to those presented above, which may indicate higher demands for those AA for the present Cobb 500 slow feathering males. Small differences when it comes to comparison of research done in different settings, using different sex and genetics, however, can not be disregarded, as much as different bio equivalences between different supplemental Met sources. However, these are very difficult to assess in comparison terms, because of the impact size as well as of the added impact size of the different variables.

In conclusion, the present study provided regression data for the use of 2-hydroxy-4-(methylthio)-butanoic acid as a methionine dietary supplement in diets formulated with 0.95% digestible lysine and 13.40 MJ/kg. Growth performance and breast meat yield of broilers were increased as the only source of supplemental methionine precursor included in corn-soy diets was HMTBA.

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Table 1
Diets supplemented with increasing levels of HMTBA for broiler chickens from 28 to 42 d.

Item	HMTBA supplementation, %								
	0.00	0.07	0.14	0.21	0.28	0.35	0.42	0.49	0.56
Ingredients, g/kg									
Corn, 7.8%	648	647	645	644	642	641	640	638	637
Soybean meal, 45%	263	263	264	264	264	265	265	265	265
Soybean oil	26	27	27	28	28	29	29	30	30
Meat and bone meal, 48%					50				
Calcium carbonate					3.8				
Dicalcium phosphate					6.3				
Sodium bicarbonate					2.3				
Salt					2.3				
HMTBA[a], 88%	-	0.7	1.4	2.1	2.8	3.5	4.2	4.9	5.6
L-Lysine HCl, 78%	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.7	0.7
L-Threonine, 98.5%					0.2				
Choline chloride					0.9				
Vitamin and mineral mix[b]					1.5				
Nutrient composition, g/kg or as shown[c]									
AME_n, MJ/kg					13.20				
CP					197				
Calcium					9.0				
Available phosphorus					4.5				
Sodium					2.0				
Digestible Lys					9.5				
Digestible TSAA	5.20	5.82	6.43	7.05	7.66	8.28	8.90	9.51	10.13
Digestible Thr					6.4				
Digestible Val					7.8				
Digestible Ile					6.9				
Total His					4.9				
Total Lys	10.5 (10.6)	10.5 (10.6)	10.5 (10.8)	10.5 (10.7)	10.5 (10.4)	10.5 (10.5)	10.5 (10.7)	10.5 (10.4)	10.5 (10.7)
Total TSAA	6.1 (6.0)	6.6 (6.3)	7.1 (7.4)	7.6 (7.5)	8.1 (8.0)	8.6 (8.8)	9.1 (9.2)	9.6 (9.6)	10.1 (10.5)
Total Thr	7.4 (7.5)	7.4 (7.6)	7.4 (7.7)	7.4 (7.8)	7.4 (7.3)	7.4 (7.3)	7.4 (7.8)	7.4 (7.3)	7.4 (7.8)
Total Val	9.1 (9.3)	9.1 (9.0)	9.1 (9.5)	9.1 (8.9)	9.1 (9.5)	9.1 (9.5)	9.1 (9.5)	9.1 (9.4)	9.1 (9.5)

Total Ile	7.9 (8.1)	7.9 (8.3)	7.9 (8.2)	7.9 (8.3)	7.9 (8.0)	7.9 (7.9)	7.9 (8.2)	7.9 (7.8)	7.9 (7.7)
Total His	5.2 (4.9)	5.2 (4.7)	5.2 (5.2)	5.2 (4.7)	5.2 (5.1)	5.2 (5.1)	5.2 (5.1)	5.2 (5.4)	5.2 (5.4)

[a]2-hydroxy-4-(methylthio)-butanoic acid formulated at 88% Met equivalency.

[b] Supplied the following per kilogram of diet: vitamin A, 8.000 UI; vitamin D_3, 2.000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg. pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.

[c] Values between parentheses are analyzed.

Table 2
Live performance of male broilers fed diets supplemented with a methionine analogue to provide increasing levels of HMTBA inclusion[a]

HMTBA supplementation, %	BW gain, g			FCR		
	28 to 35 d	35 to 42 d	28 to 42 d	28 to 35 d	35 to 42 d	28 to 42 d
0.00	630[b]	712	1,369[c]	1.916[a]	2.264[a]	2.051[a]
0.07	703[ab]	718	1,415[bc]	1.783[ab]	2.142[ab]	1.965[ab]
0.14	731[a]	754	1,499[abc]	1.729[b]	2.022[b]	1.862[b]
0.21	730[a]	763	1,474[abc]	1.727[b]	2.063[ab]	1.922[ab]
0.28	730[a]	788	1,531[ab]	1.730[b]	1.986[b]	1.847[b]
0.35	759[a]	785	1,542[ab]	1.709[b]	2.066[ab]	1.872[b]
0.42	768[a]	780	1,566[a]	1.720[b]	2.018[b]	1.852[b]
0.49	738[a]	790	1,528[ab]	1.742[b]	1.988[b]	1.867[b]
0.56	729[a]	744	1,473[abc]	1.720[b]	2.025[b]	1.893[b]
SEM	7.8	7.1	1.2	0.0139	0.0176	0.0128
P-value	< 0.001	0.161	< 0.001	0.008	< 0.001	< 0.001

Parameters	Regression equations[b]	*P*-value	r^2	Maximum response[c], %
BW gain, g				
28 to 35 d	$Y = 0.644 + 0.624X – 0.865X^2$	< 0.001	87.2	0.36
28 to 42 d	$Y = 1.361 + 1.016X – 1.424X^2$	< 0.001	88.0	0.35
FCR				
28 to 35 d	$Y = 1.881 - 1.046X + 1.397X^2$	< 0.001	79.3	0.37
35 to 42 d	$Y = 2.187 - 1.058X + 1.652X^2$	0.002	75.8	0.32
28 to 42 d	$Y = 2.022 - 0.962X + 1.296X^2$	< 0.001	87.5	0.37

[a–c] Values within a column not sharing a common superscript differ ($P < 0.05$) by Tukey test.

[a] 2-hydroxy-4-(methylthio)-butanoic acid.

[b] Quadratic polynomial: $Y = \beta1 + \beta2 \times X + \beta3 \times X^2$; where Y is the dependent variable, X is the dietary level of HMTBA, $\beta1$ is the intercept, $\beta2$ and $\beta3$ are the linear and quadratic coefficients, respectively; maximum response levels obtained by calculating $-\beta2 \div (2 \times \beta3)$.

[c] Not estimated when there was no significant fit ($P > 0.05$).

Table 3
Carcass and commercial cut yields from male broiler chickens fed diets supplemented with a methionine analogue.

HMTBA[a] supplementation, %	Carcass	Abdominal fat	Breast fillet	Breast tender	Drumstick	Thigh	Wing
0.00	77.3	2.1[a]	32.8[c]	5.4	13.3[a]	19.0[a]	9.8
0.07	77.6	1.9[ab]	33.8[bc]	5.6	13.1[a]	18.8[ab]	9.8
0.14	77.4	1.7[ab]	33.9[bc]	5.6	13.0[a]	18.7[ab]	9.8
0.21	77.6	1.6[b]	35.3[a]	5.7	12.1[b]	18.5[ab]	9.5
0.28	78.4	1.6[b]	35.1[ab]	5.6	12.7[ab]	18.8[ab]	9.6
0.35	79.0	1.6[b]	35.1[ab]	5.6	12.8[ab]	18.6[ab]	9.7
0.42	79.1	1.7[ab]	34.9[ab]	5.5	12.6[ab]	18.3[ab]	9.6
0.49	79.4	1.6[b]	34.9[ab]	5.5	12.6[ab]	18.3[b]	9.7
0.56	79.2	1.7[ab]	34.8[ab]	5.5	12.6[ab]	18.3[b]	9.7
SEM	0.18	0.04	0.13	0.03	0.07	0.06	0.03
P-value	0.064	0.004	< 0.001	0.104	< 0.001	< 0.001	0.195

Parameters[b]	Regression equations[c]	*P*-value	r^2	Maximum response[d], %
Abdominal fat, %	$Y = 2.07 - 3.01X + 4.47X^2$	< 0.001	94.6	0.33
Breast fillets, %	$Y = 32.82 + 13.06X - 17.92X^2$	< 0.001	91.1	0.35
Drumsticks, %	$Y = 13.32 – 4.42X + 6.07X^2$	< 0.052	52.3	0.36
Breast tender, %	$Y = 5.40 + 1.71X - 3.13X^2$	<0.0011	79.7	0.27

[a–c] Values within a column not sharing a common superscript differ ($P < 0.05$) by Tukey test.
[a] 2-hydroxy-4-(methylthio)-butanoic acid.
[b] Observed means were calculated from 9 replicate pens of 6 birds each; carcass yield expressed as a percentage of live weight whereas commercial cuts and abdominal fat were expressed as percentage of the eviscerated carcass.
[c] Quadratic polynomial: $Y = \beta1 + \beta2 \times X + \beta3 \times X^2$; where Y is the dependent variable, X is the dietary level of HMTBA, $\beta1$ is the intercept, $\beta2$ and $\beta3$ are the linear and quadratic coefficients, respectively; maximum response levels obtained by calculating $-\beta2 \div (2 \times \beta3)$.
[d] Not estimated when there was no significant fit ($P < 0.05$).

CAPÍTULO III

CONSIDERAÇÕES FINAIS

A utilização de níveis adequados de aminoácidos sulfurados nas rações é fundamental para obter melhor ganho de peso, conversão alimentar e rendimento de cortes comerciais. A adição de HMTBA como suplementação de MET demonstrou melhorar o desempenho, rendimento de carcaça e peito em comparação a dieta sem adição de Met. Para maximizar a utilização deste análogo de Met, recomenda-se a inclusão de 0,35 e 0,37% para as variável GP e conversão alimentar. É recomendado a inclusão de 0,35% HMTBA 88% para obter-se melhor rendimento de carne de peito durante as fases de crescimento e final.

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APÊNDICES

Apêndice 1. Normas para publicação de artigos no periódico Animal Feed Science and Technology

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b. NDFom-NDF not assayed with a heat stable amylase and expressed exclusive of residual ash.
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e. ADFom-ADF expressed exclusive of residual ash.
f. ADF-ADF expressed inclusive of residual ash.
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Cesar Augusto Pontin, primogênito de Antônio Pontin e Marinês Scarsi Pontin, nasceu em Carlos Barbosa, RS, em 31 de março de 1989. Cursou o ensino fundamental na Escola Estudual de ensino fundamental São Roque, no bairro Ponte Seca, em Carlos Barbosa, RS. Concluiu o ensino médio na Escola Mutirão Objetivo em Bento Gonçalves, RS. Em 2008 ingressou no Curso de Medicina Veterinária da Universidade de Passo Fundo, RS, obtendo o Grau de Médico Veterinário em dezembro de 2012. No ano de 2014, ingressou no curso de Mestrado em Produção Animal pelo Programa de Pós-Graduação em Zootecnia pela Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, desenvolvendo o trabalho de dissertação sobre a dose resposta a níveis crescentes de metionina hidroxi análoga sobre zootécnico e rendimento de cortes comerciais de frangos de corte no período de 28 a 42 dias de idade. Foi submetido à banca de defesa de dissertação em março de 2016 pela Universidade Federal do Rio Grande do Sul em Porto Alegre, RS.