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SUPLEMENTAÇÃO DE DIFERENTES NÍVEIS DE COLINA E AMINOÁCIDOS SULFURADOS DIGESTÍVEIS SOBRE O DESEMPENHO DE FRANGOS DE CORTE

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Dissertação apresentada como um dos requisitos à obtenção do Grau de Mestre em Zootecnia

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Muito obrigada.

SUPLEMENTAÇÃO DE DIFERENTES NÍVEIS DE COLINA E AMINOÁCIDOS SULFURADOS DIGESTÍVEIS SOBRE O DESEMPENHO DE FRANGOS DE CORTE[1]

Autor: Gabriela de Oliveira Santiago
Orientador: Sergio Luiz Vieira

Resumo – O objetivo desse estudo foi avaliar o efeito da suplementação de níveis crescentes de colina e de aminoácidos sulfurados totais (AST) digestíveis em uma dieta de milho e proteína isolada de soja, sobre o desempenho produtivo, bem como a ocorrência de deformidades e desvios nas patas e fígado gorduroso de frangos de corte. Foram alojados 525 frangos de corte, machos Cobb 500, de um dia de idade, em 75 gaiolas experimentais. As aves foram distribuídas em um delineamento inteiramente casualizado com 15 tratamentos, 5 repetições e 7 aves por unidade experimental. Foi utilizada uma dieta basal semipurificada com 74% de milho (736 ppm de colina) e esta foi suplementada utilizando um arranjo fatorial 3 x 5 com 3 níveis de AST digestíveis em relação à lisina digestível (70, 75 e 80%) e 5 níveis de suplementação de colina (0, 700, 1.400, 2.100 e 2.800 ppm). Foi utilizado um programa alimentar de 2 fases: pré-inicial (1 a 7 d) e inicial (8 a 21 d). Ganho de peso (GP), consumo de ração e conversão alimentar (CA) foram avaliados aos 7, 14 e 21 d. Aos 21 d, as aves foram avaliadas para perose, valgus, varus e tibia rotada na articulação tibio matatarsal, os fígados foram avaliados macroscopicamente (coloração, tamanho e consistência) e quanto ao seu teor de extrato etéreo. Os dados foram submetidos à análise de variância e as médias foram comparadas pelo teste de Tukey. Regressões lineares e quadráticas foram estimadas para as variáveis de desempenho produtivo e a resposta máximada de suplementação de colina foi estimada. Não houve interação entre AST digestíveis e colina, também não houve diferença entre os níveis de AST digestíveis (P>0,05). O GP dos frangos alimentados com níveis crescentes de colina aumentou quadraticamente (P<0,05) de 1 a 7 d, 8 a 14 d, 1 a 14 d, 15 a 21 d e 1 a 21 d e a CA diminuiu quadraticamente (P<0,05) 1 a 14 d e 1 a 21 d. No período de 1 a 7 d, 8 a 14 d e 15 a 21d foram estimadas exigências de 2.700, 2.907 e 3.105 ppm para GP. De 1 a 14 d e 1 a 21 d, as exigências foram de 2.875 ppm e 2.925 ppm para GP, e 2.938 ppm e 2.849 ppm para CA. Os tratamentos que não receberam suplementação de colina apresentaram maior ocorrência de varus e tibia rotada (*P*<0,05), quando comparados aos outros níveis de colina, não houve diferença entre os níveis de colina para valgus. Os níveis de colina em que houve o melhor GP e CA para frangos foram determinados como 2.925 ppm e 2.849 ppm para as fases iniciais. Considerando-se uma ração de milho e farelo de soja (1.500 ppm de colina), 1.425 e 1.349 ppm de suplementação de colina são apropriados para melhorar a performance de frangos de corte de 1 a 21 d. Esses valores são superiores às recomendações prévias para as fases iniciais (500 ppm).

Palavras chave: aminoácido sulfurado digestível, colina, exigência, frango de corte, performance.

[1]Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (85p.),Março, 2018.

SUPPLEMENTATION OF DIFFERENT LEVELS OF CHOLINE AND DIGESTIBLE SULFUR AMINO ACID FOR BROILER CHICKENS[1]

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Abstract – The objective of this study was to evaluate growth performance, the occurrence of leg deformity and deviations and fatty liver of broilers fed a corn and soy protein isolate diet supplemented with increasing levels of choline and digestible total sulfur amino acid (TSAA). A total of 525 one-day-old Cobb 500 chicks were distributed in a completely randomized design in 75 battery cages, 15 treatments, and 7 birds per cage. A 74% corn semi-purified basal diet (736 ppm of choline) was supplemented using a 3 x 5 factorial arrangement with 3 levels of digestible TSAA ratio to digestible lysine (70, 75 and 80%) and 5 levels of choline supplementation (0; 700; 1,400; 2,100, and 2,800 ppm). A 2-phases feeding program was used: pre-initial (1 to 7 d) and initial (8 to 21 d). Body weight gain (BWG), feed intake and feed conversion ratio (FCR) were evaluated at 7, 14 and 21 d. At 21 d all birds were evaluated for perosis, valgus, varus and rotated tibia in tibiometatarsal joint, livers were evaluated macroscopically (color, size, and consistency) and for fat content. Data were submitted to analysis of variance and means were compared by the Tukey test. Performance data were fitted to linear and quadratic polynomial regressions and the maximum response of choline supplementation was estimated. No interactions between digestible TSAA and choline were observed, also no differences among digestible TSAA levels ($P>0.05$). The BWG of broilers fed diets with increasing levels of choline increased quadratically ($P<0.05$) from 1 to 7 d, 8 to 14 d, 1 to 14 d, 15 to 21 d, and 1 to 21 d, also FCR decreased quadratically ($P<0.05$) from 1 to 14 d and 1 to 21 d. From 1 to 7 d, 8 to 14 d, and 15 to 21d quadratic regression estimated requirements as 2,700, 2,907, and 3,105 ppm for BWG. From 1 to 14 d and 1 to 21 d, quadratic regression estimates were 2,875 ppm and 2,925 ppm for BWG, and 2,938 ppm and 2,849 ppm for FCR. Treatments with no supplementation of choline had higher occurrence of varus and rotated tibia ($P<0.05$) compared to the other levels of choline. There was no difference for valgus deviation. Choline level that provided better BWG and FCR responses were determined as 2,925 and 2,849 ppm for starter phases, respectively. Considering a corn-soybean meal common diet (1,500 ppm of choline), 1,425 and 1,349 ppm of choline inclusions are appropriate to improve BWG and FCR response for broilers from 1 to 21 d, which are above previous recommendation for starter phases (500 ppm of choline).

Key words: broiler, choline, digestible sulfur amino acid, performance, requirement.

[1]Master of Science dissertation in Animal Science– Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (85p.),March,2018.

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RELAÇÃO DE ABREVIATURAS

AAG	Alquil-acil-glicerol
ADP	Adenosina difosfato
ATP	Adenosina trifosfato
CMP	Citidina monofosfato
CDP	Citidina difosfato
CTP	Citidina trifosfato
DAG	Diacilglicerol
FC	Fosfatidilcolina
PPi	Pirofosfato inorgânico
NRC	National Research Council
SAH	S-adenosil-homocisteina
SAM	S-adenosilmetionina

CAPÍTULO I

INTRODUÇÃO

A produção avícola industrial no Brasil obteve enormes avanços nos últimos anos, destacando-se nesse processo as áreas de melhoramento genético e nutrição, também técnicas de manejo e sanidade. Com a contínua evolução das linhagens de frangos de corte há necessidade de constantes atualizações quanto às exigências nutricionais. E como a alimentação participa de 60 a 70% dos custos dentro do sistema produtivo (CARVALHO et al., 2008; GRUNOW et al., 2009), é fundamental que as exigências nutricionais dos animais sejam adequadas, para evitar excessos ou faltas na ração. Permitindo, assim, a melhor expressão da potencialidade genética dos frangos, possibilitando o máximo desempenho, com menores custos de produção.

As pesquisas em alimentação e nutrição animal focam na digestibilidade dos ingredientes, exigências nutricionais dos animais e na resposta animal em termos de retenção e excreção de nutrientes, além do desempenho (SAKOMURA & ROSTAGNO, 2007). Diversos experimentos são conduzidos a fim de determinar exigências nutricionais. No entanto alguns nutrientes essenciais aos frangos de corte, como por exemplo, a colina, apresentam poucos e antigos estudos com resultados controversos, uma vez que apresentam diferenças na composição das dietas, nas linhagens de animais, nas metodologias utilizadas na avaliação da deficiência.

A colina está presente nos alimentos na forma de fosfatidilcolina, ou lecitina, que é um componente das gorduras, pode ser encontrada em diferentes tipos de alimentos que naturalmente contêm gorduras. A gema do ovo, as carnes glandulares, o cérebro e a carne de peixe são as fontes de origem animal mais ricas em colina, já as fontes vegetais são o germe de cereais, as leguminosas e oleaginosas (MCDOWELL, 1989). Dos ingredientes utilizados nas rações de aves, o milho apresenta pouca colina, comparado com o trigo e a cevada, que apresentam o dobro de colina, já o farelo de soja é rico nesse nutriente (NRC, 1994).

A quantificação dos ingredientes quanto ao teor de lecitina não é uma análise corriqueira. No Brasil, não há laboratórios que quantifiquem a colina presente nos fosfolipídios. Ainda podem ocorrer alterações no conteúdo de colina dos ingredientes, por variações de crescimento das culturas. E com relação ao farelo de soja, no seu processamento, a goma, que é composta de 50% de fosfolipídios e 25% de óleo (BELLAVER, 1999), pode ser utilizada para aumentar o teor de gordura do farelo (OLIVEIRA, 1995). Outra questão importante sobre essas fontes naturais de colina é a biodisponibilidade, não existem muitos estudos sobre esse assunto. Sabe-se que a biodisponibilidade de colina no farelo de soja para frangos é de 60 a 75% (MCDOWELL, 1989). Portanto, pela escassez de informações sobre a quantidade de colina presente nos alimentos, bem como sua biodisponibilidade e a dificuldade de análise, se torna importante a sua suplementação nas dietas para aves.

A colina é considerada um nutriente essencial, pois constitui a membrana celular de todas as células, é importante na calcificação endocondral dos ossos, no transporte de gorduras do fígado para o corpo, é componente da acetilcolina, que é um importante neurotransmissor, além de ser primordial para a funcionalidade cerebral, por ser um nutriente doador de grupos metil. Sua deficiência causa menor desempenho produtivo, fígado

gorduroso, problemas de aprumos, como perose (MCDOWELL, 1989), desvios angulares (valgus e varus) e rotacional (tíbia rotada) (JULIAN, 2005).

A maioria dos animais consegue formar colina endógena, porém as aves até 8 semanas de vida não conseguem formar em quantidade suficiente para evitar a deficiência desse nutriente, mesmo com aporte de grupamentos metil (MCDOWELL, 1989). E como o comprometimento dos primeiros 21 dias do frango de corte pode afetar negativamente o desempenho final do lote (CASTRO, 1998), a suplementação de colina na dieta é imprescindível para o desempenho e a lucratividade do lote, principalmente nessa fase inicial.

A colina tem um papel relevante na transmetilação, uma reação química, na qual o grupamento metil de um composto é transferido para outro. As duas principais fontes de grupamentos metil provenientes da dieta são a colina e a metionina (PINOTTI et al., 2002). A concentração dietética de metionina é importante devido à síntese endógena de colina, que usa grupamentos metil advindos da metionina combinados com etanolamina (LI & VANCE, 2008). A metionina também pode ser formada a partir de grupamentos metil provenientes da colina associados com a homocisteína (NICULESCU & ZEISEL, 2002). Consequentemente os níveis de metionina e colina interferem no requerimento um do outro (ZEISEL, 1990).

A presente dissertação foi conduzida para avaliar o efeito da suplementação de níveis crescentes de colina e de aminoácidos sulfurados digestíveis e seus efeitos sobre o desempenho produtivo de frangos de corte. Bem como avaliar os efeitos da deficiência de colina, com uma dieta semipurificada, nas deformidades e desvios nas patas das aves, nas características macroscópicas e extrato etéreo dos fígados de frangos de corte de 1 a 21 d.

REVISÃO BIBLIOGRÁFICA

Colina: histórico e definição

A colina ($C_5H_{14}NO$) é quimicamente denominada β-hidroxietil-trimetilamônio hidróxido (Figura 1), é uma amina quaternária, que se encontra no organismo das plantas e dos animais na forma de colina livre, acetilcolina e fosfolipídios. Os principais fosfolipídeos são a fosfatidilcolina (FC), ou lecitina, que representa 95% da forma da colina presente nos tecidos de mamíferos (UELAND, 2011), alisofosfatidilcolina, os plasmógenos de colina e a esfingomielina (ZEISEL, 1981). A colina pode ser oxidada em betaína no rim e no fígado, em uma reação irreversível, ou sintetizar FC, através da citidina difosfatocolina (Figura 2A), ou através da metilação de outro fosfolipídio, a fosfatidiletanolamina (Figura 2B). Já a esfingomielina é sintetizada pela transferência de um resíduo de fosfocolina proveniente da FC para uma ceramida (LI & VANCE, 2008).

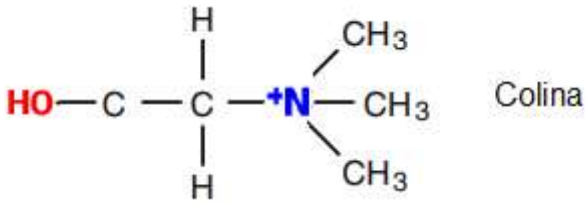

Figura 1 - Estrutura química da colina.
Fonte: adaptado de Mcdowell (1989).

A colina foi descrita pela primeira vez, indiretamente, através da lecitina, isolada de tecido cerebral e ovos de carpas por Theodore Gobley em 1850, e seu nome veio do grego “lekithos”, que significa gema de ovo (ZEISEL, 2012). Em 1864, Adolph Strecker, ao ferver a bile suína e bovina, isolou a colina, por isso sua nomenclatura é derivada da palavra grega “chole”, que significa bile. Também foi isolada de uma semente de mostarda branca (*Sinapis alba*) por Balb e Hirschbrunn em 1852. A estutura química da colina foi estabelecida por Bayer em 1867 (MCDOWELL, 1989).

Otto Loewi e Henry Dale ganharam um prêmio Nobel por descobrirem a acetilcolina como componente químico da neurotransmissão em 1936. Estudos posteriores, incentivados por essa descoberta, de Edith Cohen e Dean Haubrich, em trabalhos distintos, descobriram que a síntese de acetilcolina poderia ser modulada pela ingestão de colina na dieta. Sugerindo que somente a síntese endógena de colina não era suficiente para suprir as exigências de colina (ZEISEL, 2012).

A sua aceitação como um nutriente essencial foi estabelecida em 1929, através de estudos que comprovaram a prevenção de fígado gorduroso em cachorros (BEST & HUNTSMAN, 1932) e ratos (BEST & HUNTSMAN, 1935). As formas de sintetização da colina pela citidina difosfatocolina e pela metilação da fosfatidiletanolamina foram descobertas por Eugene Kennedy (KENNEDY, 1954), e Jon Bremer e David Greenberg (BREMER & GREENBERG, 1960), respectivamente. E somente em 1998, a colina foi

reconhecida oficialmente como um nutriente essencial pelo Instituto de Medicina (ZEISEL & DA COSTA, 2009).

A colina é um nutriente hidrossolúvel, comumente classificado como pertencente ao complexo vitamínico B. No entanto, diversos trabalhos já não a classificam mais como pertencente a esse complexo vitamínico (JAIN et al., 2005; BELLOWS & MOORE, 2012). Uma vez que ela não participa do metabolismo como uma coenzima, e sim é componente estrutural das células (INSTITUTE OF MEDICINE, 1998), e também é exigida em quantidades superiores às outras vitaminas (BERTECHINI, 2006).

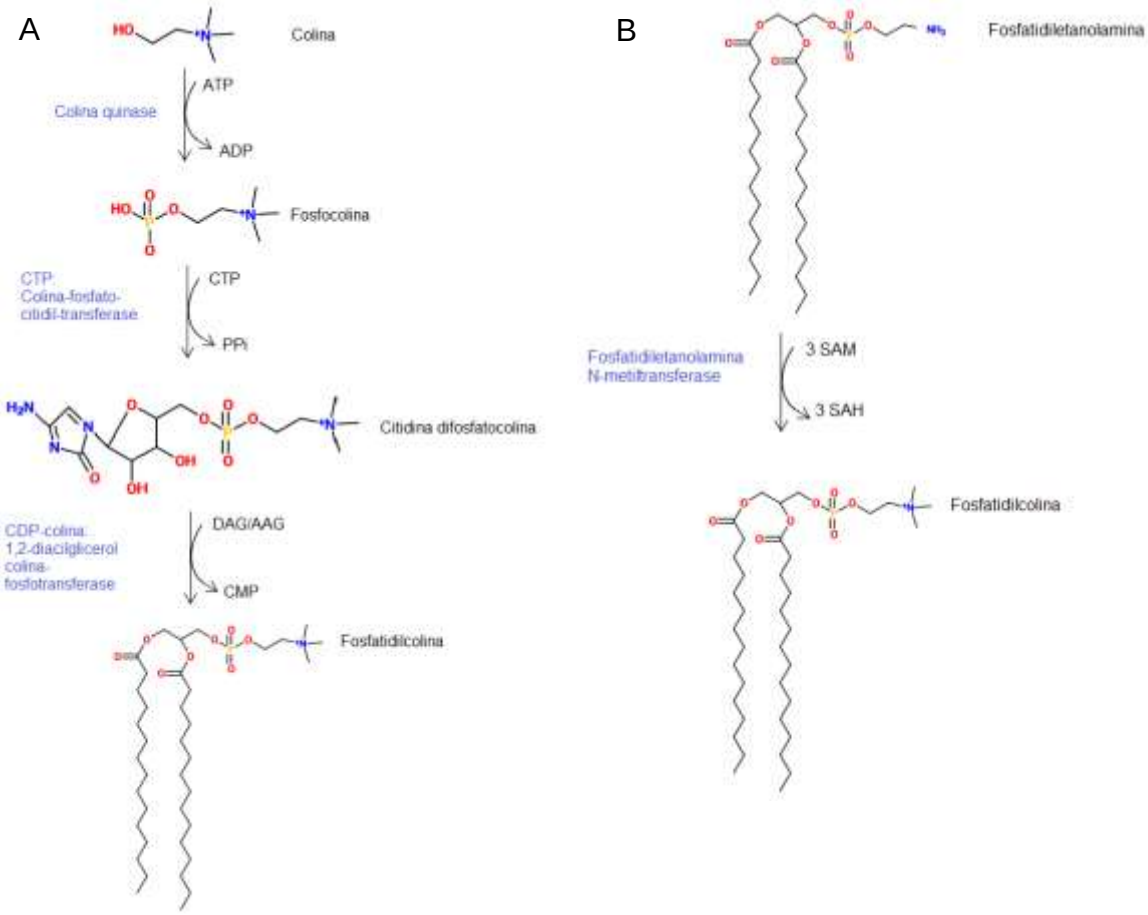

Figura 2 – Sintese da fosfatidilcolina. A) Fosfatidilcolina é sintetizada via citidina difosfatocolina (CDP-colina); B) Fosfatidilcolina é sintetizada através da metilação de outro fosfolipídio (fosfatidiletanolamina).
Fonte: adaptado de Higdon (2003).
(AAG: alquil-acil-glicerol; ADP/ATP: adenosinadi/trifosfato; CMP/CDP/CTP: citidinamono/di/trifosfato; DAG: diacilglicerol; PPi: pirofosfato inorgânico; SAH: S-adenosil-homocisteina; SAM: S-adenosilmetionina).

Interação entre colina, metionina e betaína

Colina e metionina são os principais doadores de grupos metil (MCDOWELL, 1989). A metilação é importante para a regulação da expressão de certos genes, especialmente durante a embriogênese (REIK et al., 2001),

também para a formação da creatina (DU VIGNEAUD et al., 1941), carnitina (REBOUCHE, 1991), além da metionina, da colina e de seus metabólitos (LI & VANCE, 2008).

A metionina é precursora da S-adenosilmetionina, enquanto a colina é precursora da betaína. Os animais podem sintetizar colina endogenamente, através da síntese *de novo* da colina, na qual a fosfatidiletanolamina é convertida em FC e é necessário que ocorra 3 metilações, usando a S-adenosinametionina como doadora dos grupamentos metil. Posteriormente, a FC é catabolizada por uma fosfolipase, resultando em colina. Após a S-adenosinametionina doar seu grupo metil, ocorre a formação da S-adenosil-homocisteína, que é metabolizada em homocisteína.

A homocisteína é formada somente a partir da desmetilação da metionina proveniente da dieta ou de seu catabolismo (FONSECA, 1999), e ela pode ser convertida em metionina através da doação do grupo metil pela betaína, ou pelo 5-metiltetrahidrofolato, reação dependente da vitamina B12. A homocisteina também pode ser metabolizada em cisteína via vitamina B6, através da transulfuração (MCDOWELL, 1989; LI & VANCE, 2008), que é uma reação irreversível (LEESON & SUMMERS, 2001). Todas essas reações entram em um ciclo metabólico chamado de ciclo do metil ou SAM (Figura 3).

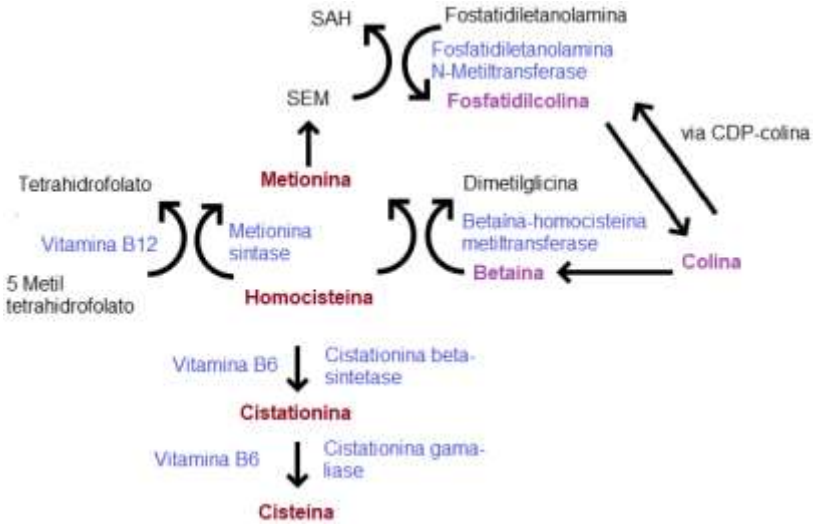

Figura 3 – Ciclo do Metil ou SAM.
Fonte: adaptado de Higdon (2003).

Funções da Colina

A colina é fundamental para a formação e a estrutura das células, uma vez que esta compõe a membrana das células de origem animal, na forma de fosfolipídios (ZEISEL, 2012). Atuando também na formação e liberação das lipoproteínas no fígado, a FC é o único fosfolipídio comprovadamente fundamental para tal ação (COLE et al., 2012). Desse modo, a colina atua como agente lipotrófico, evitando o acúmulo de triglicerídeos nos hepatócitos, portanto níveis adequados de colina atuam prevenindo o aparecimento de fígado gorduroso ou esteatose hepática nos animais (BERTECHINI, 2006).

A função da colina como agente lipotrófico é crucial para as aves, devido ao seu sistema linfático pobremente desenvolvido e pelos lipídios serem transportados do intestino até o fígado, via sistema porta mesentérico, por

portomicrons, antes de atingirem os tecidos periféricos (ANNISON, 1983). O fígado gorduroso também está associado a dietas deficientes em doadores de grupo metil, além da colina, como a metionina (COOK et al., 1989), betaína, vitamina B12 e ácido fólico (CORDERO et al., 2013) em ratos. Outro agravante é a capacidade reduzida das aves de sintetizar FC até 8 semanas de idade (MCDOWELL, 1989). Ambos os processos estão relacionadas com a síntese *de novo* de colina insuficiente.

A FC atua na ossificação endocondral, possibilitando a proliferação adequada de condrócitos e osteócitos. Estudos anteriores relatam que a enzima colina quinase é necessária para a ossificação endocondral e formação óssea, uma vez que essa enzima tua na biossíntese da FC a partir da colina. Além disso, a deficiência de colina está relacionada com a diminuição da funcionalidade dessa enzima e também de seu substrato (LI et al., 2005; LI et al., 2014).

Outra função associada à colina é compor a acetilcolina, um neurotransmissor importantíssimo, que age no sistema cardiovascular, excretor, respiratório, muscular e no cérebro. Também forma a bainha de mielina, que é fundamental para a condução do impulso nervoso, composta majoritariamente por esfingomielina e FC. Atua no fechamento do tubo neural e no desenvolvimento cerebral em neonatos (ZEISEL, 2012).

Deficiência de colina e sua exigência

Níveis baixos de colina na dieta estão correlacionados com o aparecimento de esteatose hepática (COLE et al., 2012). Caracterizada pelo aumento de volume, bordos arredondados, textura friável e coloração amarelada nos fígados, já as lesões microscópicas são vacúolos bem delimitadas, com o núcleo dos hepatócitos localizados na periferia da célula (CRAWFORD & LIU, 2010). Segudo Rama Rao et al. (2001), houve redução significativa do conteúdo de gordura hepático em matrizes pesadas suplementadas com 760 ppm de colina, em dietas com diferentes fontes de energia e 1.100 ppm de colina, em média, nas dietas sem suplementação.

Diversas deformidades e desvios nas patas de animais são relacionadas com a deficiência de colina, como a perose, valgus e varus (MCDOWELL, 1989) e tíbia rotada (JULIAN, 2005). A deformidade perose é caracterizada pelo crescimento anormal de ossos longos, presença de edema na articulação tíbio metatarsal e deslizamento do tendão de Aquiles de seus côndilos. Já o desvio valgus apresenta uma angulação lateral no segmento distal da articulação, para varus a angulação é medial. Em caso de tíbia rotada, há uma rotação externa da articulação tíbio metatarsal, fazendo com que o animal não consiga manter a pata afetada em estação (JULIAN, 2005).

Há algumas décadas não havia uma definição exata de perose e por ser muito parecida com valgus e varus, por vezes, ambas eram consideradas iguais. Jukes (1940) relata a perose em perus como um desvio lateral, medial ou anterior, e também encurtamento e engrossamento do osso na articulação da pata, principalmente na articulação tíbio metatarsal, que não corresponde à definição atual de perose e pode caracterizar todas as deformidades e desvios relatadas anteriormente. Nos experimentos de Fritz et al. (1967) e Lipstein et al. (1977) o escore de severidade de lesão nas patas variava de 0 (patas normais) a 4 (grau severo de perose), os escores intermediários não eram bem

descritos, nem a perose. Trinta anos após a descoberta da colina, Julian (1984) lança um compêndio para delimitar bem as diferenças entre essas deformidades e desvios nas patas de frangos. Alguns estudos com frangos de corte relatam a diminuição da ocorrência de perose com níveis crescentes de colina total na dieta (de 150 a 600 ppm) e prevenção com níveis de 300 e 600 ppm (PESTI et al. 1981) com 14 dias, e 700 ppm de colina total tanto para perose, quanto valgus e varus (LIPSTEIN et al. 1977) com 21 dias.

Diferentes trabalhos mostram que há melhora no desempenho produtivo de frangos suplementados com colina. Menten et al. (1997) obteve maior ganho de peso em frangos de corte de 4 a 18 d, suplementados com níveis crescentes de colina, que atingiram um platô com 1.352 ppm de colina total na dieta. Já Waldroup et al. (2006) obteve menores valores de conversão alimentar e ganho de peso numericamente maior com suplementação de 1.000 ppm de colina, ou betaína, ou com a combinação de 500 ppm de colina e 500 ppm de betaína. As dietas sem suplementação continham 1.060 ppm de colina, em média, para frangos de corte de 35 a 56 dias. Pompeu et al. (2011) obteve melhora na conversão alimentar de frangos de corte de 1 a 21 d, alimentados com dietas com 400 ppm de suplementação de colina e 1.367 ppm de colina nas dietas sem suplementação.

Segundo o NRC (1994) é recomendado 1.300 ppm de colina total na ração de frangos de corte de 1 a 21 d, as recomendações de Rostagno et al. (2011) são de suplementação de colina em rações comerciais de 550 ppm e 496 ppm de colina para frangos de corte de 1 a 7 d e 8 a 21 d, respectivamente.

Função e exigência de aminoácidos sulfurados

Os aminoácidos sulfurados (metionina e cisteína) são importantes para a síntese proteica e, consequentemente, adequado desempenho produtivo animal, são constituintes de tecidos como pele, penas, ligamentos, músculos e órgãos (NRC, 1994).

A metionina é o primeiro aminoácido limitante para frangos, alimentados com dietas à base de milho e farelo de soja (BAKER, 2006). Também é um importante doador de grupo metil, necessária para a síntese de espermina e espermidina, que atuam como mediadores do crescimento, multiplicação e divisão celular (PEGG & MCCANC, 1982). É precursora de outros aminoácidos sulfurados, como a cisteína, em uma reação irreversível, na qual a homocisteína é convertida em cistationina e, posteriormente, em cisteína (FINKELSTEIN et al., 1988). A cisteína é importante para o sistema de defesa antioxidante, pois é precursora da glutationa peroxidase, que protege os tecidos contra os danos de peróxidos de hidrogênio, produzidos durante o metabolismo (MEISTER et al., 1986).

As recomendações de aminoácidos sulfurados totais em relação à lisina total são de 74% (NRC, 1994) e segundo Rostagno et al. (2011) são de 72 a 74% de aminoácido sulfurado totais digestível, com relação à lisina digestível, ambos para frangos de corte de 1 a 21 d.

HIPÓTESES E OBJETIVOS

Hipóteses

Níveis crescentes de colina e aminoácidos sulfurados totais digestíveis podem melhorar o desempenho produtivo, diminuir a ocorrência de deformidades, como perose, desvios valgus, varus e tibia rotada na articulação tibio matatarsal de frangos de corte.

Dietas formuladas com colina com ajuste dos aminoácidos sulfurados totais digestíveis podem prevenir o aparecimento de características relacionadas a fígado gorduroso, como fígado aumentado, friável, com coloração amarelada e maior deposição de gordura.

Objetivos

Determinar a exigênciade colina para frangos de corte de 1 a 21 dias.

Avaliar os efeitos da suplementação de níveis crescentes de colina e aminoácidos sulfurados totais digestíveis sobre o desempenho produtivo, a ocorrência da deformidade perose e desvios valgus, varus e tibia rotada na articulação tibio metatarsal, além de fígado gorduroso.

CAPÍTULO II

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Artigo elaborado conforme as normas da revistaPoultry Science (Apêndice1).

METABOLISM AND NUTRITION

CHOLINE AND SULFUR AMINO ACIDS FOR BROILERS

Requirements of choline for broilers under low and high ratios of dietary sulfur aminoacids

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ABSTRACT An experiment was conducted to evaluate growth performance of broilers fed corn and isolate soy protein based diets supplemented with increasing levels of choline from choline chloride. A total of 525 one-day-old Cobb 500 chicks were distributed in a completely randomized design with 15 treatments, 5 replicates and 7 birds each. A 3 x 5 factorial arrangement was used with 3 digestible TSAA ratios to digestible Lys (70, 75 and 80%) and 5 increasing levels of choline supplementation (0; 700; 1,400; 2,100, and 2,800 ppm). Choline was supplemented in a semipurified basal diet with 736 ppm of choline. A 2-phase feeding program was used and growth performance was evaluated from 1 to 21 d. At 21 d all birds were evaluated for valgus, varus, and rotated tibia deviations. All birds were also slaughtered to collect livers and determine ether extract concentration. Growth performance were fitted to linear and quadratic polynomial regressions and the maximum response of total choline was estimated. No interactions between digestible TSAA and choline were observed for all evaluated parameters ($P > 0.05$). The BW gain of broilers increased quadratically fed diets with increasing levels of total choline. The FCR decreased quadratically ($P < 0.05$) with choline supplementation from 1 to 14 d and 1 to 21 d. Maximum responses for broiler performance from 1 to 14 d were 2,875 ppm of total choline for BW gain, and 2,938 ppm for FCR, respectively ($P < 0.05$). From 1 to 21 d, choline requirement was estimated at 2,925 ppm and 2,849 ppm for BW gain and FCR, respectively ($P < 0.05$). Birds fed diets without choline supplementation had the lowest normal and the highest varus and rotated tibia scores ($P < 0.05$). In conclusion, considering a corn-soybean meal common diet (1,500 ppm of choline), 1,425 and 1,349 ppm of choline inclusions are appropriate to improve BW gain and FCR in starter phases, which are above previous recommendation.

Key words: broiler, choline, digestible total sulfur amino acid, performance, requirement

INTRODUCTION

Choline is an essential nutrient for animals which has a complex function in the body. It is a structural component of all cell membranes in the form of phospholipids, such as phosphatidylcholine (lecithin), lysophosphatidylcholine, choline plasmalogen, and sphingomyelin (Zeisel, 1991). It also plays an important role in fat metabolism in the liver, where triglycerides are transported by very-low-density lipoproteins and lecithin is known to be required for lipoprotein assembly and secretion. Without adequate lecithin, triglycerides accumulate in the liver, leading to fatty liver or hepatic steatosis (Cole et al., 2012). The macroscopic lesions characteristics of this process are liver increased in size, friable, with rounded edges, with a light-yellow color and microscopic are well-delimited vacuoles in hepatocytes, moving the cell nucleus to the periphery (Crawford and Liu, 2010).

Choline is also important in the endochondral bone formation, allowing adequate chondrocyte proliferation and bone elongation, therefore preventing leg disorders as perosis (slipped tendon) or chondrodystrophy (Wen et al., 2016), which is characterized by the abnormal growth of long bones, widening of the tibiometatarsal joint and slipping of Achilles tendon from its condyles (Julian, 1984). This disorder is also related to diets deficient in manganese, biotin, folic acid, and pyridoxine (Pierson and Hester, 1982). The valgus and varus deviations (**VVD**) are linked with choline deficiency (Ryu et al., 1995), they refer to the direction of the joint, valgus is a outwar and varus is a medial angulation of the distal segmento of a bone or joint (Julian, 1984). They may occur separately or together with rotated tibia (**RT**), which is a rotation of the shaft of the tibiotarsus (Julian, 2005). They occur in normal broilers flock and are related to rapid growth (Thorp, 1994), restricted mobility (Cole and Haresing, 1989), and Le Bihan-Duval et al. (1996) suggested a genetic basis for VVD in a study analyzing genetic parameters for VVD in 2 commercial broiler strains and the model of

analysis considered the effects of the hatch, the sex as well as the random effects of the sire, maternal grandsire and dam within maternal grandsire.

Choline has a relevant function in the transmethylation, a chemical reaction in which a methyl group is transferred from one compound to another. The two major dietary sources of methyl groups are choline and methionine (**Met**) (Pinotti et al., 2002). The dietary concentration of Met are important because of the endogenous synthesis of choline that uses labile methyl groups combine with ethanolamine (Li and Vance, 2008). On the other hand, methyl groups from choline can associate with homocysteine to form Met (Niculescu and Zeisel, 2002). Consequently, levels of Met and choline affect requirements of each other (Zeisel, 1990). Rostagno et al. (2011) suggests from 72 to 74% of digestible (**dig.**) TSAA in relation to dig. lysine (**Lys**) in feed from 1 to 21 d. Data from the NRC (1994) indicates a ratio of 74%.

Choline can be synthesized by most animals (Leeson and Summers, 2001). However, it seems that birds of up to approximately 8 wks are unable to synthesize choline in sufficient amounts to support their needs (Mcdowell, 1989). Therefore choline supplementation is necessary in most practical broiler diets, being choline chloride the most used supplemental source. The recommendation for choline supplementation of broiler diets in starter phase is 550 ppm from 1 to 7 d and 496 ppm from 8 to 21 d (Rostagno et al., 2011). The NRC (1994) suggest 1,300 ppm of total choline for broilers from 1 to 21 d, which are in agreement with the research from Menten et al. (1997). Another study suggested levels of choline supplementation at 400 ppm at the same period (Pompeu et al., 2011).

The present study was conducted to evaluate the effects of choline supplementation using dietary dig. TSAA from defficient to excessive (70, 75 and 80% of TSAA : Lys) on growth performance, leg abnormalities, and liver fat contents of male broilers from 1 to 21 d.

MATERIAL AND METHODS

All procedures used in this study were approved by the Ethics and Research Committee of the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Bird Husbandry

A total of 525 slow-feathering, Cobb × Cobb 500 one-day-old male chicks, with an average BW of 45 ± 1.5 g, vaccinated for Marek's disease at a commercial hatchery were randomly placed into 75 wire cages (0.9 × 0.4 m^2). Each cage was equipped with 1 trough feeder and 1 drinker. Birds had ad libitum access to water, mash feeds and lighting was continuous during all the study. The average temperature was 32ºC at placement being reduced by 1ºC every 2 d targeting comfort throughout the study with the use of a central air-conditioning system.

Experimental Diets

Analyses of choline in ingredients and feeds were done following the choline enzymatic method 999.14 (AOAC International, 2000). A 2-phase feeding program was used as follow: pre-starter (1 to 7 d) and started (8 to 21 d) (Table 1). All diets were a 74% corn semi-purified diet with isolated soy protein based. Birds were allocated to 15 experimental diets with 5 replications of 7 birds each in a completely randomized design. A 3 × 5 factorial arrangement of 3 levels of dig. TSAA ratio to dig. Lys (70, 75 and 80%) and 5 supplementation levels of choline (0; 700; 1,400; 2,100, and 2,800 ppm) was used. Levels of total choline (supplemented + basal diet) calculated were 727; 1,427; 2,127; 2,827, and 3,527, respectively. The choline source used was choline chloride with the minimum of 52.2% of choline. Analyzed choline in the 5 treatments were 736; 1,427; 2,143; 2,846, and 3,546 ppm.

Growth Performance

Chicks were individually weighed and placed into groups of 7 per cages. Bird weights were averaged by pen and recorded at 1, 7, 14 and 21 d. BW gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) corrected for the weight of dead birds were calculated from 1 to 7 d, 8 to 14 d, 1 to 14 d, 15 to 21 d and 1 to 21d on a pen-basis. Mortality was recorded immediately after noticed. At 21 d, all birds from each cage were euthanized by cervical dislocation following electrical stunning at 45 V for 3 s.

Liver Collection and Analyses

Livers from all birds per cage were evaluated macroscopically considering size, color, and consistency at 21 d and conditioned into plastic containers, pooled by pen, immediately frozen in liquid nitrogen, and stored in a freezer at −20ºC. The pooled livers per cage were evaluated for ether extract. Previous acid hydrolysis with hydrochloric acid was performed, samples were defatted using ethyl ether as solvent (method 920.39, AOAC International, 1995).

Leg Abnormalities

At 21 d, valgus, varus, and rotated tibia deviations in the tibiometatarsal joint were evaluated in all birds per cage mantaining animals in the normal anatomic position (Figure 1). The animals were classified by the presence or absence of each deformity by 3 evaluators. The tibiometatarsal joint considered with valgus deviation was the joints that presented an outward angulation of the distal segment of tibiometatarsal joint. For varus was the joints that presented a medial deviation of the distal tibiometatarsal joint (Julian, 1984). Rotated tibia above 90º was considered abnormal, and were characterized as a torsional rotation of the shaft

of the tibiotarsus of 1 or both legs causing the metatarsus to point laterally and the bird to assume a spraddle leg posture (Thorp, 1994).

In addition, leg abnormalities were scored: 0 - normal leg; 1 - slipping of the calcaneus tendon of the tibiometatarsal joint; 2 - swelling of the tibiometatarsal joint. The thickness of the tibiometatarsal joint was measured with a digital pachymeter (IP65, Mitutoyo, Kawasaky, Japan) from the right leg of 7 birds per cage. Tibiometatarsal joint width - the distance between the medial and lateral borders of tibiometatarsal joint-, and tibiometatarsal joint length - the distance between malleolus tibiotarsal and trochlea tarsometatarsal.

All right tibias from 4 birds per cage were conditioned into plastic containers, pooled by pen, immediately frozen in liquid nitrogen, and stored in a freezer at −20ºC. The ash content was measured (method 984.27; AOAC International, 1995) and samples were defatted using petroleum ether as the solvent, according to method 945.16 (AOAC International, 1995).

Statistical Analysis

Data were analyzed using the GLM procedures of SAS Institute (SAS Inst. Inc., Cary, NC, 2009) and when significant, means were compared by Tukey test (Tukey, 1991) at 5% significance. A factorial arrangement was used (3 levels of dig. TSAA ratio to dig. Lys x 5 levels of supplemental choline). Linear and quadratic regressions were estimated for BWG, FCR, and FI as dependent variables and total choline levels as independent variables.

RESULTS

Effects of dietary treatments on broiler performance are presented in Table 2 and 3. There were no interactions between different levels of dig. TSAA and total choline ($P > 0.05$). Mortality was not affected ($P > 0.05$) by dietary treatments (0.57 ± 3.6%). Different ratios of dig. TSAA did not affect ($P > 0.05$) BWG, FCR, and FI for all experimental period.

Increasing dietary choline levels resulted in higher BWG, FI and lower FCR ($P < 0.01$) from 1 to 7 d, 8 to 14 d, 1 to 14 d, and 1 to 21 d. Broilers fed diets without choline supplementation had the lowest BWG, FI and the highest FCR from 1 to 7 d (110 g, 156 g and 1.416, respectively), 8 to 14 d (212 g, 258 g and 1.218, respectively), 1 to 14 d (309 g, 415 g and 1.344, respectively), 15 to 21 d (377 g, 497 g and 1.319, respectively), and 1 to 21 d (687 g, 97 3g and 1.416, respectively).

A linear response ($P < 0.0001$) of BWG and FCR was observed in birds fed diets with increasing levels of choline for all periods. Body weight gain increased quadratically ($P < 0.0001$) from all periods and FCR decreased quadratically ($P < 0.01$) when broilers were fed diets with increasing levels of choline from 1 to 14, and 1 to 21 d, as shown in Figures 2, 3, 4 5 and Table 4. Maximum responses from 1 to 7 d, 8 to 14 d, and 15 to 21 d for BWG were 2,700, 2,907, and 3,108 ppm, respectively. Also from 1 to 14 d for BWG and FCR were obtained using 2,875 and 2,938 ppm of total choline, respectively. From 1 to 21 d, maximum responses for BWG and FCR were obtained using 2,925 and 2,849 ppm of total choline, respectively.

Livers evaluated by size, color, consistency were not affected by dietary treatments, also fat content were not influenced by increasing levels of choline (Table 5). Liver fat content was different for dig. TSAA ($P < 0.10$) with birds fed 70% of dig. TSAA having higher means when compared with the ones fed with 80% of dig. TSAA (20.3 *vs*. 19.0%). In addition, tibiometatarsal joints were all scored as normal. There was no evidence of slipping tendons or swelling joints and average width was 22.5± 0.1 mm, length was 33.9 ± 0.3 mm. Ash content on a dry matter basis was 51.7 ± 1.6%.

The occurrence of valgus, varus, and RT deviations are shown in Table 6. There was no interaction between different levels of dig. TSAA and choline ($P > 0.05$). Also, no differences among dig. TSAA levels ($P > 0.05$). No effect of treatments on valgus deviation was

observed in the present study, (average was 27.8%) ($P > 0.05$). Birds fed diets without choline supplementation had the lowest normal scores (44.6%) and the highest varus and RT scores (5.7 and 19.3%, respectively) ($P < 0.01$).

DISCUSSION

In the present study, dietary TSAA and choline did not show interaction. Using lower contents of TSAA, Derilo and Balnave (1980) found an interaction between TSAA : Lys ratios (53 and 70%) and total choline (dietary plus supplemented: 1,300 and 2,300 ppm) for BW in broilers from 1 to 28 d, in experiment 4. In spite of, in experiment 1, Derilo and Balnave (1980) reported no interaction between total choline (from 571 to 1,771 ppm) and the same levels of TSAA for BW and FCR from 1 to 35 d. Pesti et al. (1979) did not detect improvements for 3 wk broilers BW and FCR in treatments when supplementing both choline (0.23%) and Met (0.46%) at 0.75% of TSAA and 1,500 ppm of dietary choline.

Addicionally, Waldroup et al. (2006) did not find interaction between Met reductions (0, 0.05, 0.10, 0.15, and 0.20%) and choline supplementation (0 choline or betaine, 1,000 ppm of choline or betaine, and 500 ppm of choline + 500 ppm of betaine) for BWG and FCR, except for FCR at 14 d, in 8 wk broilers at 77 to 88 % of TSAA : Lys and 1,200 to 920 of dietary choline. The absence of interaction between TSAA and choline may be explained by the methyl groups from Met failure to supply the choline deficiency, or the birds were not able to synthesize enough choline to show enhancement for both TSAA and choline supplementation, considering their age under 8 wk (Mcdowell, 1989).

The different levels of dig. TSAA did not show enhancement for all variables evaluated, except liver fat content. Our results are in agreement with several studies that presented an average of optimal ratio of dig. TSAA : Lys according to our range from 70 to 80% or even above. Baker and Han (1994) estimated ideal requirements of 72% dig. TSAA : Lys for

broilers until 3 wk for BWG, FCR and FI. In addition, Dozier and Mercier (2013) which reported an optimal ratio of dig. TSAA : Lys for broiler of 74% in one experiment (8 levels of dig. TSAA, avarage from 0.66 to 0.99%) fitted with a linear broken line regression for FCR from 1 to 14 d. And in other (8 levels of dig. TSAA, avarage from 0.66 to 0.95%). These authors found 78 and 77% of optimal ratio of dig. TSAA : Lys fitted with a quadratic broken-line model for FCR from 1 to 7 d and 1 to 14 d, respectively. Garcia and Batal (2005) founded the requierement of dig. TSAA : LYS as 74% in one experiment (6 levels of dig. TSAA, ranging from 0.68 to 1.08%) and 63% in other (6 levels of dig. TSAA, ranging from 0.61 to 1.01%) for FCR of broiler with 3 wk, both data were fitted a quadratic broken-line model.

A significant improvement in BWG, FCR and increased FI was recorded in the present study when choline was supplementedat the level of 2,827 and 3,527 ppm of total choline. The BWG and FCR were maximized using a quadratic polynomial model at 2,875 and 2,938 ppm of total choline from 1 to 14, and 2,925 and 2,849 ppm of total choline from 1 to 21 d, respectively. In contrast to the results of the present study, Swain and Johri (2000) did not find improvements in BWG, FCR, and FI with different total choline levels (1,300, 2,300, and 3,300 ppm), in spite of exhibited numerical improvement for FCR with 3,300 ppm of total choline. This finding was in accordance with the observations of Pompeu et al. (2013) who found no effect in supplementation levels of choline, except for FI of male broilers, which presented higher FI when fed with a total choline of 2,657 ppm, from 22 to 42 d. Previosuly, Pompeu et al. (2011) had observed a significant improvement in FCR for male broilers from 1 to 21 d with 1,767 ppm of total choline. This observation is in agreement with the study conducted by Waldroup et al. (2006) who reported lowest FCR, also highest numerical BW at 35, 42, 49 and 56 d birds with total choline analyzed ranging from 1,681 to 1,941 ppm.

In the present study, the supplementation of choline did not cause differences in macroscopic characteristics (size, color, and consistency) and fat composition of the liver.

Similar findings were recorded by Pompeu et al. (2011 and 2013) who did not find the effect of supplementation levels of choline in macroscopic characteristics (size, color, and consistency) and ether extract from the liver of broilers, varying 19,8 and 13,1% on dry matter, respectively. Furthermore, Lipstein et al. (1977) did not find differences in liver ether extract (averages were between 19.6 and 22.3% in the dry matter) for male chicks fed with a semi-purified basal diet with total choline varying between 468 and 868 ppm, from 8 to 20 d. Although among dig. TSAA levels presented differences, the highest levels of dig. TSAA presented lowest ether extract content in livers. This is in agreement with other studies that presented higher fat content in livers with diets deficient in others methyl donor besides the choline in rats (Cook et al., 1989; Cordero et el., 2013). Presumably, the choline deficiency in this experiment was not so severe and young birds could use the amount of dietary choline and synthesized choline using methyl groups from the Met, ensuring lipoprotein formation and assembly in the liver, allowing the triglycerides to be transported from the liver without limitations that could cause accumulation.

No symptoms of perosis over the course of the present study occurred. In contrast to our findings, Pesti et al. (1981) reported perosis in chick fed a diet with total choline from 150 to 600 ppm, which were lower than those used in the presente study. In one experimente by Pesti (1981), increased levels of choline decreased the perosis and in other 2,300 and 600 ppm of choline did not lead to perosis, respectively, from 1 to 14 d. Also, for a basal diet containing 200 ppm of choline, 0.42% of supplemental choline did not present perosis at the same age. Lipstein et al. (1977) founded that about 40% of broilers with approximately 400 ppm of total choline presented slight symptoms of perosis. The symptoms are not well described, however, the scores ranged from 0 (normal appearance of legs) to 4 (severe degree of perosis). Probably they presented abnormal appearance of legs and this finding is in agreement with this experiment. Besides, Ryu et al. (1995) reported no perosis and decrease

in VVD for increasing levels of choline for male broilers fed increased total choline (750, 1,250, and 1,750 ppm) until 18 d.

Different levels of choline did not show differences for valgus deviation in the present study. However, previous studies suggested a genetic effect (Le Bihan-Duval et al.,1996) which are also in agreement with Julian (1984) that reported 0.5 to 2% in a normal broiler flock, and 5 to 25% for VVD in a problem flock. Leterrier and Nys (1992) observed that valgus occurence was 30 to 40% and varus was 1 to 3% in a normal broiler flock. Cole and Haresing (1989) also suggested that 5% of occurrence of RT can be natural in a regular broiler flock . In this study, increasing levels of choline reduced varus and RT leg deviations and the treatment with the highest level of choline presented no RT. Gorustovish et al. (2003) reported problems of mandible remodeling and lower bone density in rats fed with choline deficiency. Therefore adequate endochondral bone formation (Wen et al., 2016) and remodeling (Julian, 2005) in animals with no choline deficiency, can prevent leg disorders as the bone presents higher quality and density.

In the present study, results for bone ash content was not different for increasing levels of choline, other studies presented 51% (Onyango et al., 2003) and 56% (Han et al., 2015) of ash content in tibias from 1 to 21 d for normal broilers. Perhaps there was insufficient deficiency to present differences in bone ash, but capable to interfere in the remodeling of the bones, increasing the amount of some leg deviations in broilers.

In conclusion, considering a corn-soybean meal common diet (1,500 ppm of choline), 1,425 and 1,349 of choline supplementation are appropriate to improve BWG and FCR, decrease varus and RT leg deviations in starter phases, which are above previous recommendation (500 ppm of choline for starter phase).

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Table 1. Ingredient and nutrient composition of the pre-starter and starter experimental diets

	Pre-starter (1 to 7 days)			Starter (8 to 21 days)		
Item	70%[1] TSAA	75%[1] TSAA	80%[1] TSAA	70%[1] TSAA	75%[1] TSAA	80%[1] TSAA
Ingredients %						
Corn	73.88	73.83	73.77	74.00	73.95	73.90
Isolated soy protein, 88%		20.79			19.70	
Inert[2]		0.54			0.54	
Soybean oil	0.61	0.59	0.58	1.97	1.95	1.94
Dicalcium phosphate		2.11			1.86	
Limestone		1.23			1.12	
Salt		0.01			0.04	
Sodium Bicarbonate		0.04			-	
L-Lysine HCl, 78%		0.28			0.27	
DL-Methionine, 99%	0.25	0.32	0.39	0.23	0.30	0.37
L-Threonine, 98.5%		0.07			0.07	
Vit. Premix[3]		0.10			0.10	
Min. Premix[4]		0.05			0.05	
Monensin Sodium, 26%[5]		0.12			0.03	
Sangrovit, 1.5%[6]		0.005			0.005	
Calculated nutrient composition, % unless noted						
AME_n, kcal/kg		3,000			3,100	
CP		24.58			23.61	
Ca		1.01			0.91	
Av. P		0.48			0.43	
Na		0.23			0.66	
K		0.25			0.22	
Cl		0.12			0.12	
DEB, mEq/kg		152			142	
Choline, mg/kg[7]		727 (736)			727 (736)	
Dig. Lys[8]		1.36			1.30	
Dig. Met	0.60	0.67	0.74	0.57	0.64	0.70
Dig. Met+Cys	0.95	1.02	1.09	0.91	0.98	1.04
Dig. Thr		0.90			0.86	

Dig Trp		0.24			0.23	
Dig. Arg		1.71			1.64	
Dig. Val		1.04			1.00	
Dig. Ile		0.93			0.89	
Dig. Leu		1.92			1.85	
Dig. Met+Cys / Dig. Lys	0.70	0.75	0.80	0.70	0.75	0.80

[1]Ratio of digestible TSAA to digestible Lys.

[2]Inert amou nt will be replaced by choline chloride for each TSAA to Lys ratio.

[3]Composition per kg of feed: vitamin A, 9,000 UI; vitamin D_3, 2,500 UI; vitamin E, 20 UI; vitamin K_3, 2,5 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 3.8 mg; cyanocobalamin, 0.015 mg, pantothenic acid, 12 mg; niacin, 35 mg; folic acid, 1,5 mg; biotin, 0.1 mg.

[4]Composition per kg of feed: iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.25 mg.

[5]Coban with minimum of 26% monensin sodium (Elanco, Greenfield, IN).

[6]Sangrovit with minimum of 1.5% sanguinarine (Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany).

[7]Values between parentheses were analyzed.

[8]Ratios of digestible amino acids to digestible Lys were maintained at Thr: 0.66; Trp: 0.18; Arg: 1.26; Val: 0.75; Ile: 0.67; Leu: 1.36.

Table 2. Growth performance of broilers fed diets supplemented with increasing levels of total sulfur amino acid and choline from 1 to 7, 8 to 14, and 15 to 21 d[1]

	1 to 7 d			8 to 14 d			15 to 21 d		
Item	BWG, g[2]	FCR[2,3]	FI, g[2]	BWG, g	FCR	FI, g	BWG, g	FCR	FI, g
TSAA, %[4]									
70	124	1.364	169	275	1.193	327	444	1.304	577
75	122	1.351	165	281	1.191	334	455	1.300	591
80	122	1.358	166	281	1.186	333	457	1.300	594
Total choline, ppm[5,6]									
727 (736)	110[b]	1.416[a]	156[b]	212[c]	1.218[a]	258[c]	377[c]	1.319[a]	497[c]
1,427 (1,443)	125[a]	1.362[ab]	170[a]	279[b]	1.202[ab]	335[b]	452[b]	1.312[ab]	592[b]
2,127 (2,143)	125[a]	1.359[ab]	170[a]	293[a]	1.187[bc]	348[ab]	462[ab]	1.300[ab]	600[ab]
2,827 (2,846)	128[a]	1.324[b]	170[a]	306[a]	1.171[c]	358[a]	482[ab]	1.285[b]	622[ab]
3,527 (3,546)	126[a]	1.328[b]	167[ab]	305[a]	1.173[c]	358[a]	488[a]	1.289[b]	627[a]
SEM	1.050	0.009	1.591	4.312	0.003	4.721	5.649	0.003	6.712
Main effect *P*-value									
TSAA	0.5782	0.7478	0.4857	0.1486	0.5681	0.2259	0.2565	0.7485	0.2574
Total choline	0.0001	0.0096	0.0115	0.0001	0.0001	0.0001	0.0001	0.0118	0.0001
TSAA x Total choline	0.2909	0.9424	0.1693	0.2238	0.6832	0.6008	0.8772	0.9991	0.8587

[a-c]Means with different superscript letters differ (P < 0.05) based on Tukey's honestly significant difference test.

[1]Means were obtained from 5 replicate cages of 7 birds per replicate cage at the start of the experiment.

[2]BWG = BW gain, FCR = feed conversion ratio, and FI = feed intake.

[3]Feed conversion ratio corrected for the weight of dead birds.

[4]Ratio of digestible TSAA to digestible Lys.

[5]Choline from ingredients calculated in the treatment with no supplementation (727 ppm of choline), with added values of choline supplementation levels: 0, 700, 1,400, 2,100, and 2,800 ppm, respectively.

[6]Values between parentheses were analyzed.

Table 3. Growth performance of broilers fed diets supplemented with increasing levels of total sulfur amino acid and choline from 1 to 14 and 1 to 21 d[1]

Item	1 to 14 d			1 to 21 d		
	BW gain, g	Feed conversion ratio[2]	Feed intake, g	BW gain, g	Feed conversion ratio	Feed intake,g
TSAA, %[3]						
70	394	1.257	493	842	1.330	1,114
75	400	1.259	502	853	1.322	1,125
80	403	1.254	503	862	1.292	1,110
Total choline, ppm[4,5]						
727 (736)	309[c]	1.344[a]	415[b]	687[c]	1.416[a]	973[b]
1,427 (1,443)	404[b]	1.265[b]	511[a]	864[b]	1.310[b]	1,130[a]
2,127 (2,143)	416[b]	1.233[b]	513[a]	880[b]	1.297[b]	1,141[a]
2,827 (2,846)	433[a]	1.218[b]	528[a]	914[a]	1.271[b]	1,162[a]
3,527 (3,546)	432[a]	1.225[b]	529[a]	918[a]	1.281[b]	1,176[a]
SEM	5.614	0.007	5.849	10.553	0.012	12.567
Main effect *P*-value						
TSAA	0.6560	0.9504	0.3704	0.3864	0.3407	0.8156
Total choline	0.0001	0.0001	0.0001	0.0001	0.0007	0.0001
TSAA x Total choline	0.6075	0.9679	0.3410	0.3598	0.8614	0.9055

[a-c]Means with different superscript letters differ (P < 0.05) based on Tukey's honestly significant difference test.

[1]Means were obtained from 5 replicate cages of 7 birds per replicate cage at the start of the experiment.

[2]Feed conversion ratio corrected for the weight of dead birds.

[3]Ratio of digestible TSAA to digestible Lys.

[4]Choline from ingredients calculated in the treatment with no supplementation (727 ppm of choline), with added values of choline supplementation levels: 0, 700, 1,400, 2,100, and 2,800 ppm, respectively.

[5]Values between parentheses were analyzed.

Table 4. Regression equations for performance of broilers fed diets supplemented with increasing levels of choline[1]

Item	Regression equations[2]	Effect[3]	r^2	*P*-value	Maximum response, ppm
BW gain 1 to 14 d, g	$Y= 37.35x + 318.85$	L	0.5901	0.0001	
	$Y= -25.46x^2 + 146.36x + 227.25$	QP	0.7797	0.0001	2,875
FC 1 to 14 d	$Y= -0.036x + 1.33$	L	0.2758	0.0001	
	$Y= 0.023x^2 - 0.13x + 1.41$	QP	0.3510	0.0051	2,938
BW gain 1 to 21 d, g	$Y= 70.86x + 700.74$	L	0.6011	0.0001	
	$Y= -45.18x^2 + 264.34x + 538.18$	QP	0.7701	0.0001	2,925
FC 1 to 21 d	$Y= -0.042x + 1.40$	L	0.1613	0.0004	
	$Y= 0.030x^2 - 0.169x + 1.51$	QP	0.2169	0.0267	2,849

[1]Regression equations considering total choline levels: 736, 1,443, 2,143, 2,846 and 3,546 ppm from ingredients and choline chloride source.

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

[3]Linear (L) or Quadratic Polynomial (QP) effect ($P < 0.05$).

Table 5. Macroscopical liver evaluation (size, color, and consistency) and ether extract content of broilers at 21 d, %

Item	Consistence[1]	Color[2]	Size[3]	Ether extract[4]
TSAA, %[5]				
70	0.09	0.08	0.04	20.3[a]
75	0.10	0.08	0.04	19.8[ab]
80	0.05	0.05	0.05	19.0[b]
Total choline, ppm[6]				
727 (736)	0.07	0.08	0.04	20.2
1,427 (1,443)	0.09	0.07	0.04	20.1
2,127 (2,143)	0.10	0.09	0.04	19.9
2,827 (2,846)	0.05	0.07	0.08	18.8
3,527 (3,546)	0.08	0.04	0.01	19.3
SEM	0.0141	0.0132	0.0010	0.2236
Main effect *P*-value				
TSAA	0.2524	0.5275	0.9377	0.0661
Total choline	0.7759	0.7799	0.3918	0.3632
TSAA x Total choline	0.7541	0.2036	0.4099	0.9324

[a-b]Means with different superscript letters differ (P < 0.05) based on Tukey's honestly significant difference test.

[1]Means of consistence, where 0 = normal and 1= friable.

[2]Means of color, where 0 = normal and 1 = yellowish.

[3]Means of size, where 0 = normal and 1 = enlarged.

[4]Values on dry matter basis.

[5]Ratio of digestible TSAA to digestible Lys.

[6]Values between parentheses were analyzed.

Table 6. Normal, valgus, varus and rotated tibia frequency[1] of broilers at 21 d, %

Item	Normal	Valgus	Varus	Rotated tibia
TSAA, %[2]				
70	64.3	29.1	1.1	5.5
75	65.0	28.9	2.4	3.7
80	66.2	25.5	2.8	5.5
Total choline, ppm[3]				
727 (736)	44.6[b]	30.4	5.7[a]	19.3[a]
1,427 (1,443)	68.9[a]	28.1	1.3[ab]	1.7[b]
2,127 (2,143)	70.4[a]	27.1	1.2[ab]	1.3[b]
2,827 (2,846)	71.4[a]	27.0	1.2[ab]	0.4[b]
3,527 (3,546)	73.1[a]	26.1	0.8[b]	0.0[b]
SEM	2.1319	1.6370	0.5454	1.2093
Main effect *P*-value				
TSAA	0.9135	0.6250	0.3782	0.7071
Total choline	0.0001	0.9438	0.0151	0.0001
TSAA x Total choline	0.4674	0.6207	0.7941	0.9905

[a-b]Means with different superscript letters differ (P < 0.05) based on Tukey's honestly significant difference test.

[1]Deformities in tibiometatarsal joint avaliations.

[2]Ratio of digestible TSAA to digestible Lys.

[3]Values between parentheses were analyzed.

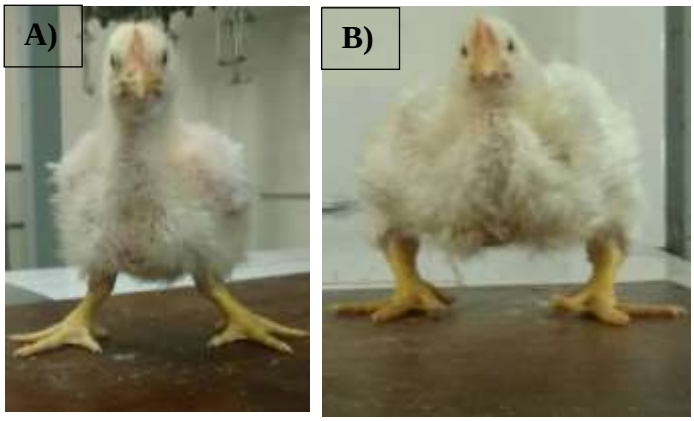

Figure 1. Broiler with leg deformity at 21 d: A) valgus, and B) varus.

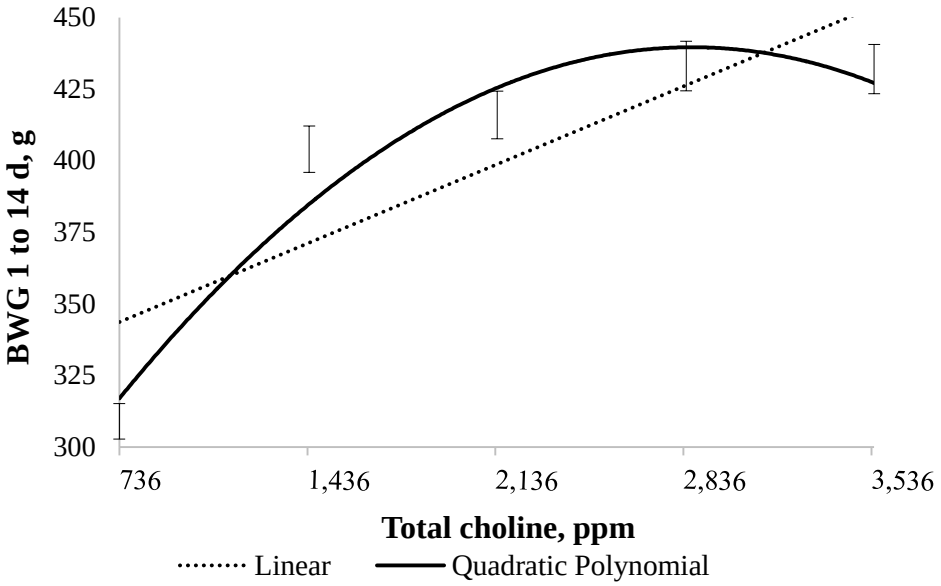

Figure 2. Body weight gain (Y, in g) vs. choline supplementation (X, in ppm) fed from 1 to 14 d of age[1]. Regression equations considering total choline levels: 736, 1,443, 2,143, 2,846 and 3,546 ppm from ingredients and choline chloride source. Linear, Y= 37.35x + 318.85, r2 =0.5901; quadratic polynomial, Y= -25.46x² + 146.36x + 227.25, r2 = 0.7797, maximum response at 2,875 ppm choline.

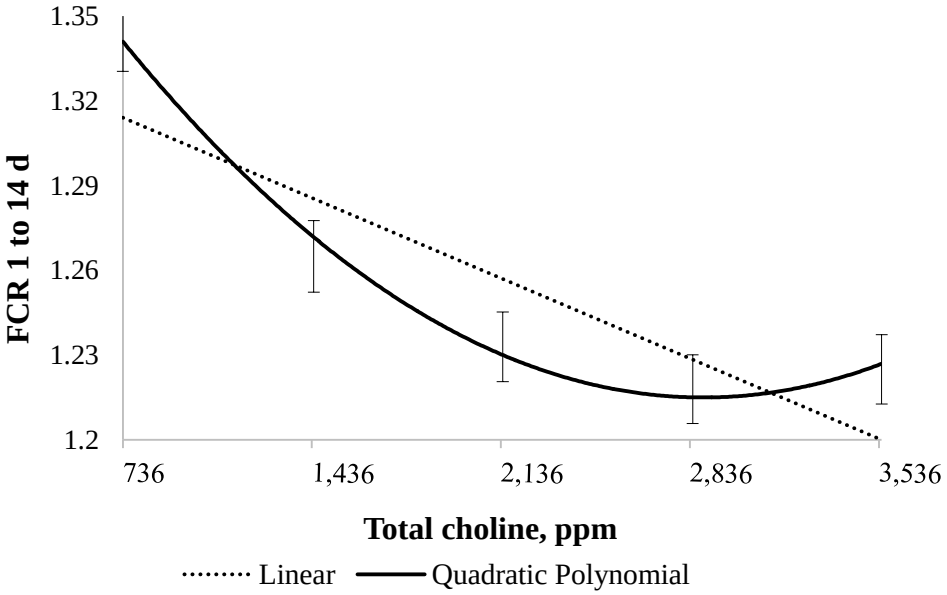

Figure 3. Feed conversion ratio (Y, in g:g) vs. choline supplementation (X, in ppm) fed from 1 to 14 d of age[1]. [1]Regression equations considering total choline levels: 736, 1,443, 2,143, 2,846 and 3,546 ppm from ingredients and choline chloride source.Linear, $Y= -0.036x + 1.33$, r2 =0.2758; quadratic polynomial, $Y= 0.023x^2 - 0.13x + 1.41$, r2 = 0.3510, maximum response at 2,938 ppm choline.

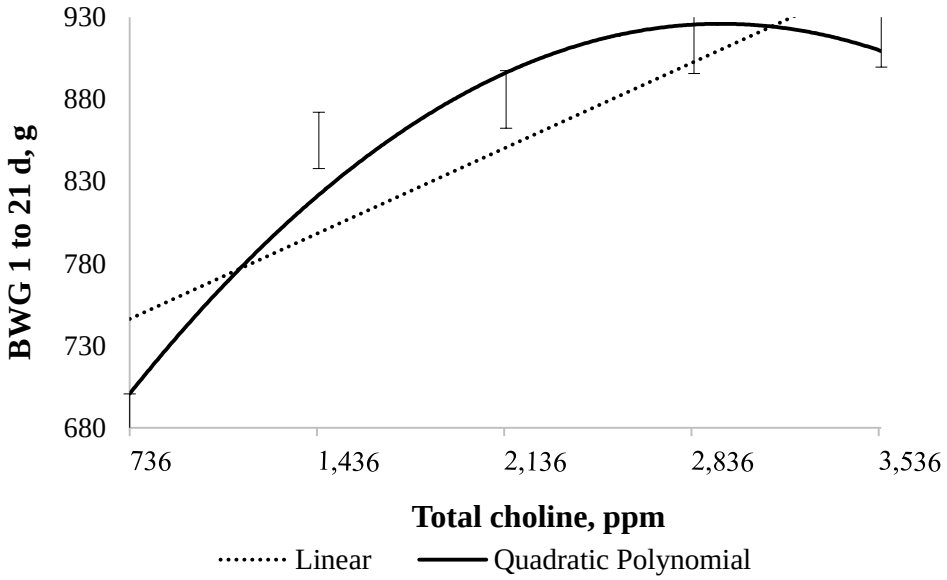

Figure 4. Body weight gain (Y, in g) vs. choline supplementation (X, in ppm) fed from 1 to 21 d of age[1]. [1]Regression equations considering total choline levels: 736, 1,443, 2,143, 2,846 and 3,546 ppm from ingredients and choline chloride source.Linear, Y= 70.86x + 700.74, r2 =0.6011; quadratic polynomial, Y= -45.18x² + 264.34x + 538.18, r2 = 0.7701, maximum response at 2,925 ppm choline.

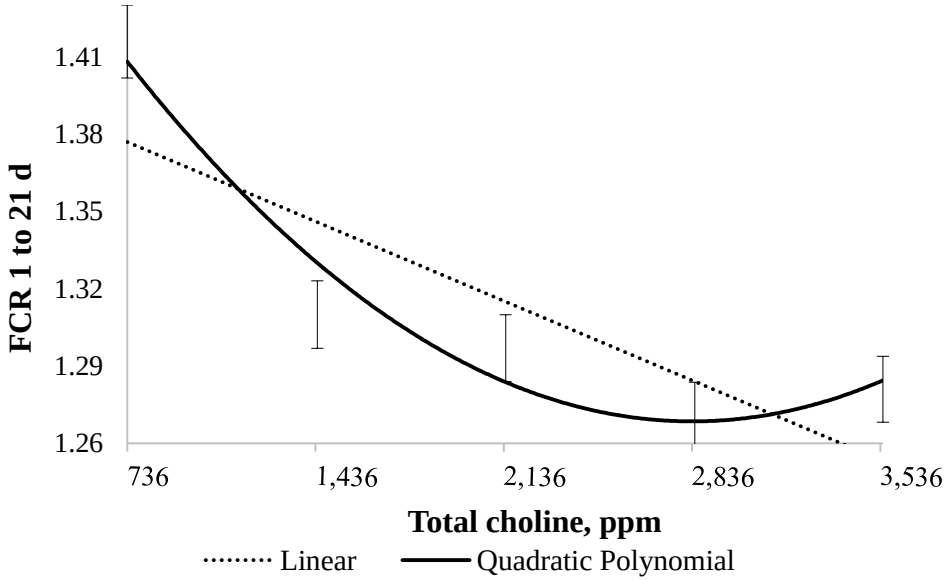

Figure 5. Feed conversion ratio (Y, in g:g) vs. choline supplementation (X, in ppm) fed from 1 to 21 d of age[1]. [1]Regression equations considering total choline levels: 736, 1,443, 2,143, 2,846 and 3,546 ppm from ingredients and choline chloride source.Linear, $Y= -0.042x + 1.40$, r2 =0.1613; quadratic polynomial, $Y= 0.030x^2 - 0.169x + 1.51$, r2 = 0.2169, maximum response at 2,849 ppm choline.

CAPÍTULO III

CONSIDERAÇÕES FINAIS

O presente trabalho reporta que a utilização de níveis crescentes de colina melhoraram o desempenho zootécnico de frangos de corte, suplementados com cloreto de colina. Os níveis de máxima resposta de colina tanto para ganho de peso e conversão alimentar foram maiores nesse experimento do que os indicados pela literatura.

Foi possível observar que lesões associadas à deficiência de colina, como desvios nas patas do tipo varus e tíbia rotada, apresentaram-se mais graves nos frangos que não receberam suplementação. Não houve indício de fígado gorduroso; no entato, houve uma melhora no percentual de gordura dos fígados das aves que receberam maiores níveis de aminoácidos sulfurados, relacionando esse fato com a síntese *de novo* de colina, pelo maior aporte de grupamentos metil.

Como as dietas eram deficientes em colina, comparando-as com dietas de milho e farelo de soja, houve uma exacerbação das deficiências. Seria interessante realizar outro experimento com níveis de colina total mais próximos do nível máximo de performance que encontramos nesse experimento, afim de estabelecer essas mesmas relações de nível máximo de colina total na ração de milho e farelo de soja, com maiores desafios microbiológicos para os frangos de corte, aproximando-os mais da realidade da indústria. Além de realizar o experimento repetido no tempo para ter uma amostra maior.

Outra questão importante a ser abordada, é a importância de ser realizado a análise dos ingredientes previamente ao experimento, quanto ao nível de colina. Nós analisamos os ingredientes posteriormente a fabricação das rações, mas usamos o valor analizado nas tabelas das dietas. A princípio esperávamos ter o nível sem suplementação com menos colina, com uma deficiência maior, pois o isolado deveria ter menos de 10 ppm de colina. No entanto, este apresentou por volta de 1,500 ppm de colina, provavelmente foi utilizado a goma para diluição do produto, pois não deveria haver tanta colina no produto.

Pela escassez de experimentos abordando dietas deficientes em colina e seus efeitos, além de diferentes níveis de aminoácidos sulfurados, podendo relacionar a deficiência de colina com a presença ou não de grupamentos metil, de ter resultados divergentes da literatura, esse trabalho se torna relevante.

Os resultados observados neste estudo contribuem para um maior conhecimento sobre a suplementação de colina e lesões ocasionadas pela sua deficiência em frangos de corte de 1 a 21 d, também fornecem dados que poderão ser utilizados para a realização de outros estudos.

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APÊNDICES

Apêndice 1: Normas para publicação de artigos no periódico Poultry Science
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A. Scope

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Papers must be written in English. The text and all supporting materials must use American spelling and usage as given in The American Heritage Dictionary, Webster's Third New International Dictionary, or the Oxford American English Dictionary. Authors should follow the style and form recommended in Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers. 2006. 7th ed. Style Manual Committee, Council of Science Editors, Reston, VA.

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Manuscripts should be typed double-spaced, with lines and pages numbered consecutively, using Times New Roman font at 12 points.

All special characters (e.g., Greek, math, symbols) should be inserted using the symbols palette available in this font. Complex math should be entered using MathType from Design Science (www.dessci.com). Tables and figures should be placed in separate sections at the end of the manuscript (not placed within the text).

iii.) Headings

- Major headings: Major headings are centered (except ABSTRACT), all capitals, boldface, and consist of ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), ACKNOWLEDGMENTS (optional), APPENDIX (optional), and REFERENCES.
- First subheadings: First subheadings are placed on a separate line, begin at the left margin, the first letter of all important words is capitalized, and the headings are boldface and italic. Text that follows a first subheading should be in a new paragraph.
- Second subheadings: Second subheadings begin the first line of a paragraph. They are indented, boldface, italic, and followed by a period. The first letter of each important word should be capitalized. The text follows immediately after the final period of the subheading.

iv.) Title page

The title page shall begin with a running head (short title) of not more than 45 characters. The running head is centered, is in all capital letters, and shall appear on the top of the title page. No abbreviations should be used.

The title of the paper must be in boldface; the first letter of the article title and proper names are capitalized, and the remainder of the title is lowercase. The title must not have abbreviations. Under the title, names of authors should be typed (first name or initial, middle initial, last name). Affiliations will be footnoted using the following symbols: *, †, ‡, §, #, ||‖, and be placed below the author names. Do not give authors' titles, positions, or degrees. Numbered footnotes may be used to provide supplementary information, such as present address, acknowledgment of grants, and experiment station or journal series number. The corresponding author should be indicated with a numbered footnote (e.g., Corresponding author: name@university.edu).

Note that there is no period after the corresponding author's e-mail address. The title page shall include the name and full address of the corresponding author. Telephone numbers and e-mail address must also be provided. The title page must indicate the appropriate scientific section for the paper (i.e., Animal Well-Being and Behavior; Genetics and Genomics; Immunology, Health and Disease; Metabolism and Nutrition; Molecular and Cellular Biology; Physiology and Reproduction; Processing and Products; Microbiology and Food Safety; Management and Production).

v.) Abbreviations

Author-derived abbreviations should be defined at first use in the abstract and again in the body of the manuscript. The abbreviation will be shown in bold type at first use in the body of the manuscript. Refer to the Miscellaneous Usage Notes for more information on abbreviations.

vi.) Abstract

The Abstract disseminates scientific information through abstracting journals and through convenience for the readers. The Abstract, consisting of not more than 325 words, appears at the beginning of the manuscript with the word ABSTRACT without a following period. It must summarize the major objectives, methods, results, conclusions, and practical applications of the research. The Abstract must consist of complete sentences and use of abbreviations should be limited. References to other work and footnotes are not permitted. The Abstract and Key Words must be on a separate sheet of paper.

vii.) Key words

The Abstract shall be followed by a maximum of five key words or phrases to be used for subject indexing. These should include important words from the title and the running head and should be singular, not plural, terms (e.g., broiler, not broilers). Key words should be formatted as follows: Key words: . . .

viii.) Introduction

The Introduction, while brief, should provide the reader with information necessary for understanding research presented in the paper. Previous work on the topic should be summarized, and the objectives of the current research must be clearly stated.

ix.) Materials and methods

All sources of products, equipment, and chemicals used in the experiments must be specified parenthetically at first mention in text, tables, and figures [i.e., (model 123, ABC Corp., Provo, UT)]. Model and catalog numbers should be included. Information shall include the full corporate name (including division, branch, or other subordinate part of the corporation, if applicable), city, and state (country if outside the United States), or Web address. Street addresses need not be given unless the reader would not be able to determine the full address for mailing purposes easily by consulting standard references. Age, sex, breed, and strain or genetic stock of animals used in the experiments shall be specified. Animal care guidelines should be referenced if appropriate.

Papers must contain analyzed values for those dietary ingredients that are crucial to the experiment. Papers dealing with the effects of feed additives or graded levels of a specific nutrient must give analyzed values for the relevant additive or nutrient in the diet(s). If products were used that contain different potentially active compounds, then analyzed values for these compounds must be given for the diet(s). Exceptions can only be made if appropriate methods are not available. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as

appropriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phosphorus contents should also be specified. When describing the composition of diets and vitamin premixes, the concentration of vitamins A and E should be expressed as IU/kg on the basis of the following equivalents:

Vitamin A

1 IU = 0.3 µg of all-trans retinol
1 IU = 0.344 µg of retinyl acetate
1 IU = 0.552 µg of retinyl palmitate
1 IU = 0.60 µg of β-carotene

Vitamin E

1 IU = 1 mg of dl-α-tocopheryl acetate
1 IU = 0.91 mg of dl-α-tocopherol
1 IU = 0.67 mg of d-α-tocopherol

In the instance of vitamin D3, cholecalciferol is the acceptable term on the basis that 1 IU of vitamin D3 = 0.025 µg of cholecalciferol.

The sources of vitamins A and E must be specified in parentheses immediately following the stated concentrations.

- Statistical analysis:Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately.

  The experimental unit is the smallest unit to which an individual treatment is imposed. For group-fed animals, the group of animals in the pen is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use time-sequence analysis.

- Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. For example, it is recommended that percentage data between 0 and 20 and between 80 and 100 be

subjected to arc sin transformation prior to analysis. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random.

If animals are stratified by ancestry or weight or if some other initial measurement should be accounted for, they should include a blocking factor, or the initial measurement should be included as a covariate.

- A parameter [mean (μ), variance (σ2)], which defines or describes a population, is estimated by a statistic (x, s2). The term parameter is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment.
- Standard designs are adequately described by name and size (e.g., “a randomized complete block design with 6 treatments in 5 blocks”). For a factorial set of treatments, an adequate description might be as follows: “Total sulfur amino acids at 0.70 or 0.80% of the diet and Lys at 1.10, 1.20, or 1.30% of the diet were used in a 2 × 3 factorial arrangement in 5 randomized complete blocks consisting of initial BW.” Note that a factorial arrangement is not a design; the term “design” refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted).
- Standard deviation refers to the variability in a sample or a population. The standard error (calculated from error variance) is the estimated sampling error of a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not “statistically significant” is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by “±” to a number implies that the second value is its standard error (not its standard deviation). Adequate re-porting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of experimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of observations or the heterogeneity of the error variance is to be emphasized.

Presenting individual standard errors clutters the presentation and can mislead readers.

- For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator

of each F statistic. Unbalanced factorial data can present special problems. Accordingly, it is well to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases.

- Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by Bonferroni t statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged. Fixed-range, pairwise, multiple-comparison tests should be used only to compare means of treatments that are unstructured or not related. Least squares means are the correct means to use for all data, but arithmetic means are identical to least squares means unless the design is unbalanced or contains missing values or an adjustment is being made for a covariate. In factorial treatment arrangements, means for main effects should be presented when important interactions are not present.

However, means for individual treatment combinations also should be provided in table or text so that future researchers may combine data from several experiments to detect important interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

- The terms significant and highly significant traditionally have been reserved for $P < 0.05$ and $P < 0.01$, respectively; however, reporting the P-value is preferred to the use of these terms. For example, use ". . . there was a difference ($P < 0.05$) between control and treated samples" rather than ". . . there was a significant ($P < 0.05$) difference between control and treated samples." When available, the observed significance level (e.g., $P = 0.027$) should be presented rather than merely $P < 0.05$ or $P < 0.01$, thereby allowing the reader to decide what to reject. Other probability (α) levels may be discussed if properly qualified so that the reader is not misled. Do not report P-values to more than 3 places after the decimal. Regardless of the probability level used, failure to reject a hypothesis should be based on the relative consequences of type I and II errors. A "nonsignificant" relationship should not be interpreted to suggest the absence of a relationship. An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of $P > 0.05$ to declare nonsignificance, such as indicating that a difference is not significant at $P > 0.05$ and subsequently declaring another difference significant (or a tendency) at $P < 0.09$. In addition, readers may incorrectly interpret the use of $P > 0.05$ as the probability of a β error, not an α error.
- Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of

the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information contained in the data is sacrificed. Significant digits in data reported should be restricted to 3 beyond the decimal point, unless warranted by the use of specific methods.

iv.) Results and discussion

Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Results section shall contain only the results and summary of the author's experiments; there should be no literature comparisons. Those comparisons should appear in the Discussion section. Manuscripts reporting sequence data must have GenBank accession numbers prior to submitting. One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experiments are adequately replicated. The results of experiments must be replicated, either by replicating treatments within experiments or by repeating experiments.

v.) Acknowledgements

An Acknowledgments section, if desired, shall follow the Discussion section. Acknowledgments of individuals should include affiliations but not titles, such as Dr., Mr., or Ms. Affiliations shall include institution, city, and state.

vi.) Appendix

A technical Appendix, if desired, shall follow the Discussion section or Acknowledgments, if present. The Appendix may contain supplementary material, explanations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel computer programs or mathematical computations would be appropriate. The Appendix will not be a repository for raw data.

vii.) References

- Citations in text**:** In the body of the manuscript, refer to authors as follows: Smith and Jones (1992) or Smith and Jones (1990, 1992). If the sentence structure requires that the authors' names be included in parentheses, the proper format is (Smith and Jones, 1982; Jones, 1988a,b; Jones et al., 1993). Where there are more than two authors of one article, the first author's name is followed by the abbreviation et al. More than one article listed in the same sentence of text must be in chronological order first, and alphabetical order for two publications in the same year. Work that has not been accepted for publication shall be listed in the text as: "J. E. Jones (institution, city, and state, personal communication)." The author's own un- published work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and un-published data must not be included in the References section.
- References section: To be listed in the References section, papers must be published or accepted for publication. Manuscripts submitted for publication can be cited as "personal communication" or "unpublished data" in the text.

- In the References section, references shall first be listed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the authors' names. As with text citations, two or more publications by the same author or set of authors in the same year shall be differentiated by adding lowercase letters after the date. The dates for papers with the same first author that would be abbreviated in the text as et al., even though the second and subsequent authors differ, shall also be differentiated by letters. All authors' names must appear in the Reference section.

Journals shall be abbreviated according to the conventional ISO abbreviations given in journals database of the National Library of Medicine. One-word titles must be spelled out. Inclusive page numbers must be provided. Sample references are given below. Consult recent issues of Poultry Science for examples not included below.

- *Article*:

Bagley, L. G., and V. L. Christensen. 1991. Hatchability and physiology of turkey embryos incubated at sea level with increased eggshell permeability. Poult. Sci. 70:1412–1418. Bagley, L. G., V. L. Christensen, and R. P. Gildersleeve. 1990.

Hematological indices of turkey embryos incubated at high altitude as affected by oxygen and shell permeability. Poult. Sci. 69:2035–2039.

Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, with and without prior vaccination, to challenge with Marek's disease virus. Avian Dis. 50:354–365. doi:10.1637/7498-010306R.1

- *Book*:

Metcalfe, J., M. K. Stock, and R. L. Ingermann. 1984. The effects of oxygen on growth and development of the chick embryo. Pages 205-219 in Respiration and Metabolism of Embryonic Vertebrates. R. S.

Seymour, ed. Dr. W. Junk, Dordrecht, the Netherlands.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

- *Federal Register*:

Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Regis. 69:10137–10151.

- *Other*:

Choct, M., and R. J. Hughes. 1996. Long-chain hydrocarbons as a marker for digestibility studies in poultry. Proc. Aust. Poult. Sci. Symp. 8:186. (Abstr.)

Dyro, F. M. 2005. Arsenic. WebMD. Accessed Feb. 2006. http://www.emedicine.com/neuro/topic20.htm.

El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, as- signee. US Pat. No. 6,766,767.

Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium,
MD. Univ. Maryland, College Park.

Luzuriaga, D. A. 1999. Application of computer vision and electronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gainesville.

Peak, S. D., and J. Brake. 2000. The influence of feeding program on broiler breeder male mortality. Poult. Sci. 79(Suppl. 1):2. (Abstr.)

B. Tables

Tables must be created using the MS Word table feature and inserted in the manuscript after the references section. When possible, tables should be organized to fit across the page without running broadside. Be aware of the of the printed page when planning tables (use of more than 15 columns will create layout problems). Place the table number and title on the same line above the table. The table title does not require a period. Do not use vertical lines and use few horizontal lines. Use of bold and italic typefaces in the table should be done sparingly; you must define such use in a footnote. Each table must be on a separate page. To facilitate placement of all tables into the manuscript file (just after the references) authors should use “section breaks” rather than “page breaks” at the end of the manuscript (before the tables) and between tables.

Units of measure for each variable must be indicated. Papers with several tables must use consistent format. All columns must have appropriate headings. Abbreviations not found on the inside front cover of the journal must be defined in each table and must match those used in the text. Footnotes to tables should be marked by superscript numbers. Each footnote should begin a new line.

Superscript letters shall be used for the separation of means in the body of the table and explanatory footnotes must be provided [i.e., “Means within a row lacking a common superscript differ ($P < 0.05$).”]; other significant P-values may be specified. Comparison of means within rows and columns should be indicated by different series of superscripts (e.g., a,b, . . . in rows; x–z . . . in columns) The first alphabetical letter in the series (e.g., a or A) shall be used to indicate the largest mean. Lowercase super- scripts indicate $P \leq 0.05$. Uppercase letters indicate $P \leq 1.1$ or less.

Probability values may be indicated as follows: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, and †$P \leq 0.10$. Consult a recent issue of *Poultry Science* for examples of tables.

Generally, results should be presented to the significant figure of the instrument used to collect the data. For example, results should not be presented to 5 digits when the instrument used only reads to 2 digits.

C. Miscellaneous usage notes

i.) Abbreviations

- Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD). A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the scientific field. Abbreviations may be used in heads within the paper, if they have been first defined within the text. The inside back cover of every issue of the journal lists abbreviations that can be used without definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used less than three times must be spelled out in full rather than abbreviated. All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter, rather than the full term.
- The abstract, text, each table, and each figure must be understood independently of each other. Therefore, abbreviations shall be defined within each of these units of the manuscript.
- Plural abbreviations do not require “s.” Chemical symbols and three-letter abbreviations for amino acids do not need definition.
  Units of measure, except those in the standard *Poultry Science* abbreviation list, should be abbreviated as listed in the *CRC Handbook for Chemistry and Physics* (CRC Press, 2000 Corporate Blvd., Boca Raton, FL, 33431) and do not need to be defined.
- The following abbreviations may be used without definition in *Poultry Science*:
  A adenine
  ADG average daily gain
  ADFI average daily feed intake
  AME apparent metabolizable energy
  AMEn nitrogen-corrected apparent metabolizable energy
  ANOVA analysis of variance
  B cell bursal-derived, bursal-equivalent derived cell bp base pairs
  BSA bovine serum albumin
  BW body weight
  C cytosine
  cDNA complementary DNA
  cfu colony-forming units
  CI confidence interval

CP crude protein
cpm counts per minute
CV coefficient of variation
d day
df degrees of freedom
DM dry matter
DNA deoxyribonucleic acid
EDTA ethylenediaminetetraacetate
ELISA enzyme-linked immunosorbent antibody assay
EST expressed sequence tag
g gram
g gravity
G guanine
GAT glutamic acid-alanine-tyrosine
G:F gain-to-feed ratio
GLM general linear model
h hour
HEPES N-2-hydroxyethyl piperazine-N′-ethane-sulfonic acid
HPLC high-performance (high-pressure) liquid chromatography
ICU international chick units
Ig immunoglobulin
IL interleukin
IU international units
kb kilobase pairs
kDa kilodalton
L liter*
L:D hours light:hours darkness in a photoperiod (e.g., 23L:1D)
m meter
µ micro
M molar
MAS marker-assisted selection
ME metabolizable energy
MEn nitrogen-corrected metabolizable energy
MHC major histocompatibility complex
mRNA messenger ribonucleic acid
min minute
mo month
MS mean square
n number of observations
N normalNAD nicotinamide adenine dinucleotide
NADH reduced nicotinamide adenine dinucleotide
NRC National Research Council
NS not significant
PAGE polyacrylamide gel electrophoresis
PBS phosphate-buffered saline
PCR polymerasechain reactionpfu plaque-forming units
QTL quantitative trait loci
r correlation coefficient

r2 coefficient of determination, simple
R2 coefficient of determination, multiple
RH relative humidity
RIA radioimmunoassay
rpm revolutions per minutes
s second
SD standard deviation
SDS sodium dodecyl sulphate
SE standard error
SEM standard error of the mean
SRBC sheep red blood cells
SNP single nucleotidepolymorphism
T thymine
TBA thiobarbituric acidT cell thymic-derived cell
TME true metabolizable energy
TMEn nitrogen-corrected true metabolizable energy
Tris tris(hydroxymethyl)aminomethane
TSAA total sulfur amino acids
U uridine
USDA United States Department of Agriculture
UV ultraviolet
vol/vol volume to volume
vs. versus
wt/vol weight to volume
wt/wt weight to weight
wk week
yr year
*Also capitalized with any combination, e.g., mL.

ii.)International words and phrases:Non-English words in common usage (defined n recent editions of standard dictionaries) will not appear in italics(e.g., in vitro, in vivo, in situ, a priori). However, genus and species of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. German nouns shall begin with capital letters.

iii.)Capitalization: Breed and variety names are to be capitalized (e.g., Single Comb White Leghorn).

iv.)Number style: Numbers less than 1 shall be written with preceding zeros(e.g., 0.75). All numbers shall be written as digits. Measures must be in the metric system; however, US equivalents maybe given in parentheses.

Poultry Science requires that measures of energy be given in calories rather than joules, but the equivalent in joules may be shown in parentheses or in a footnote to tables. Units of measure not preceded by numbers must be writ-ten out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract

and in the body of the paper at first use. Units of measure for feed conversion or feed efficiency shall be provided (i.e., g:g).

v.)Nucleotide sequences: Nucleotide sequence data must relate to poultry or poultry pathogens and must complement biological data published in the same or a companion paper. If sequences are excessively long, it is suggested that the most relevant sections of the data be published in Poultry Science and the remaining sequences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submission of sequence data to one of the databases. The following statement should appear as a footnote to the title on the title page of the manuscript. “The nucleotide sequence data reported in this paper have been submitted to Embank Submission (Mail Stop K710, Los Alamos National Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN.” Publication of the description of molecular clones is assumed by the editors to place the min the public sector. Therefore, they shall be made available to other scientists for research purposes.

Nucleotide sequences must be submitted as camera- ready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow Poultry Science guidelines.

vi.) Gene and protein nomenclature: Authors are required to use only approved gene and protein names and symbols. For poultry, full gene names should not be italicized. Gene symbols should be in uppercase letters and should be in italics. A protein symbol should be in the same format as its gene except the protein symbol should not be in italics.

vii.) General usage:

- Note that “and/or” is not permitted; choose the more appropriate meaning or use “x or y or both.”
- Use the slant line only when it means “per” with numbered units of measure or “divided by” in equations. Use only one slant line in a given expression (e.g., g/d per chick). The slant line may not be used to indicate ratios or mixtures.
- Use “to” instead of a hyphen to indicate a range. Insert spaces around all signs (except slant lines) of operation (=, –, +, ×, >, or <, etc.) when these signs occur between two items.
- Items in a series should be separated by commas (e.g., a, b, and c).
- Restrict the use of “while” and “since” to meanings related to time.
- Appropriate substitutes include “and,” “but,” or “whereas” for “while” and “because” or “al- though” for “since.”
- Leading (initial) zeros should be used with numbers less than 1 (e.g., 0.01).
- Commas should be used in numbers greater than 999.
- Registered (®) and trademark (™) symbols should not be used, unless as part of an article title in the References section. Trademarked product names should be capitalized.

D. Figures/illustrations For information on how to submit figure files, please see the Oxford Journals page on figures. You can also send queries about figure files to poultry.science@oup.com.

E. Supplementary data Submit all material to be considered as Supplementary Material online at the same time as the main manuscript. Ensure that the supplementary material is referred to in the main manuscript at an appropriate point in the text.

Supplementary material will be available online only and will not be copyedited, so ensure that it is clearly and succinctly presented, and that the style conforms to the rest of the paper. Also ensure that the presentation will work on any Internet browser. It is not recommended for the files to be more than 2 MB each, although exceptions can be made at the editorial office's discretion.

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E. CrossRef funding data registry

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Apêndice 2. Desempenho zootécnico, ganho de peso (GP), conversão alimentar (CA) e consumo de ração (CR) dos frangos de corte no período de 1 a 7 dias.

Item	GP, g	CA, g:g	CR, g
AST[1], %			
70	124	1,364	169
75	122	1,351	165
80	122	1,358	166
Colina total, ppm[2]			
727 (736)	110[b]	1,416[a]	156[b]
1.427 (1.443)	125[a]	1,362[ab]	170[a]
2.127 (2.143)	125[a]	1,359[ab]	170[a]
2.827 (2.846)	128[a]	1,324[b]	170[a]
3.527 (3.546)	126[a]	1,328[b]	167[ab]
Média	123	1,358	167
EPM	1,050	0,009	1,591
Valor de P			
AST	0,5782	0,7478	0,4857
Colina total	0,0001	0,0096	0,0115
AST x Colina total	0,2909	0,9424	0,1693

[1]AST= aminoácidos sulfurados totais digestíveis;[2]Os valores entre parênteses foram analisados.

Apêndice 3. Desempenho zootécnico, ganho de peso (GP), conversão alimentar (CA) e consumo de ração (CR) dos frangos de corte no período de 8 a 14 dias.

Item	GP, g	CA, g:g	CR, g
AST[1], %			
70	275	1,193	327
75	281	1,191	334
80	281	1,186	333
Colina total, ppm[2]			
727 (736)	212[c]	1,218[a]	258[c]
1.427 (1.443)	279[b]	1,202[ab]	335[b]
2.127 (2.143)	293[a]	1,187[bc]	348[ab]
2.827 (2.846)	306[a]	1,171[c]	358[a]
3.527 (3.546)	305[a]	1,173[c]	358[a]
Média	279	1,190	331
EPM	4,312	0,003	4,721
Valor de P			
AST	0,1486	0,5681	0,2259
Colina total	0,0001	0,0001	0,0001
AST x Colina total	0,2238	0,6832	0,6008

[1]AST= aminoácidos sulfurados totais digestíveis;[2]Os valores entre parênteses foram analisados.

Apêndice 4. Desempenho zootécnico, ganho de peso (GP), conversão alimentar (CA) e consumo de ração (CR) dos frangos de corte no período de 1 a 14 dias.

Item	GP, g	CA, g:g	CR, g
AST[1], %			
70	394	1,257	493
75	400	1,269	506
80	403	1,254	503
Colina total, ppm[2]			
727 (736)	309[c]	1,344[a]	415[b]
1.427 (1.443)	404[b]	1,282[ab]	518[a]
2.127 (2.143)	416[b]	1,233[bc]	513[a]
2.827 (2.846)	433[a]	1,218[c]	528[a]
3.527 (3.546)	432[a]	1,225[bc]	529[a]
Média	399	1,260	501
EPM	5,614	0,009	6,036
Valor de P			
AST	0,6562	0,6668	0,2855
Colina total	0,0001	0,0001	0,0001
AST x Colina total	0,6275	0,9396	0,4159

[1]AST= aminoácidos sulfurados totais digestíveis;[2]Os valores entre parênteses foram analisados.

Apêndice 5. Desempenho zootécnico, ganho de peso (GP), conversão alimentar (CA) e consumo de ração (CR) dos frangos de corte no período de 15 a 21 dias.

Item	GP, g	CA, g:g	CR, g
AST[1], %			
70	444	1,304	577
75	455	1,300	591
80	457	1,300	594
Colina total, ppm[2]			
727 (736)	377[c]	1,319[a]	497[c]
1.427 (1.443)	452[b]	1,312[ab]	592[b]
2.127 (2.143)	462[ab]	1,300[ab]	600[ab]
2.827 (2.846)	482[ab]	1,285[b]	622[ab]
3.527 (3.546)	488[a]	1,289[b]	627[a]
Média	452	1,302	587
EPM	5,649	0,003	6,712
Valor de P			
AST	0,2565	0,7485	0,2574
Colina total	0,0001	0,0118	0,0001
AST x Colina total	0,8772	0,9991	0,8587

[1]AST= aminoácidos sulfurados totais digestíveis;[2]Os valores entre parênteses foram analisados.

Apêndice 6. Desempenho zootécnico, ganho de peso (GP), conversão alimentar (CA) e consumo de ração (CR) dos frangos de corte no período de 1 a 21 dias.

Item	GP, g	CA, g:g	CR, g
AST[1], %			
70	837[b]	1,331	1,110
75	855[ab]	1,314	1,120
80	860[a]	1,300	1,114
Colina total, ppm[2]			
727 (736)	687[c]	1,416[a]	973[b]
1.427 (1.443)	855[b]	1,310[b]	1,119[a]
2.127 (2.143)	880[b]	1,297[b]	1,141[a]
2.827 (2.846)	914[a]	1,271[b]	1,162[a]
3.527 (3.546)	918[a]	1,281[b]	1,176[a]
Média	851	1,315	1,114
EPM	10,641	0,012	12,653
Valor de P			
AST	0,0464	0,5099	0,8894
Colina total	0,0001	0,0007	0,0001
AST x Colina total	0,3009	0,7878	0,9172

[1]AST= aminoácidos sulfurados totais digestíveis;[2]Os valores entre parênteses foram analisados.

Apêndice 7. Médias da largura e do comprimento da articulação tíbio matatarsal (TM) de frangos de corte de 21 d, mm.

Item	Largura da articulação TM	Comprimento da articulação TM
AST[1], %		
70	22,5	34,2
75	22,6	34,0
80	22,5	33,6
Colina total, ppm[2]		
727 (736)	22,4	34,0
1.427 (1.443)	22,5	33,9
2.127 (2.143)	22,5	34,2
2.827 (2.846)	22,6	34,2
3.527 (3.546)	22,6	33,4
Média	22,5	33,9
EPM	0,0721	0,1076
Valor de P		
AST	0,7028	0,3650
Colina total	0,8908	0,1887
AST x Colina total	0,3217	0,3619

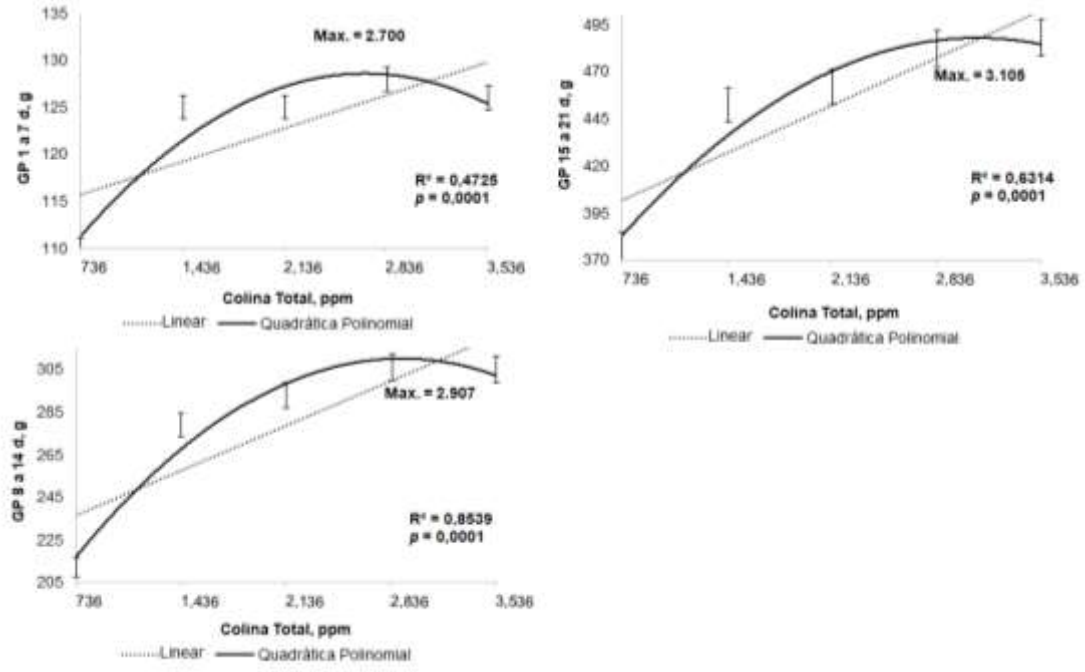
[1]AST= aminoácidos sulfurados totais digestíveis;[2]Os valores entre parênteses foram analisados.

Apêndice 8. Cinzas das tíbias dos frangos de corte aos 21 dias.

Item	Cinzas tíbias, %
AST[1], %	
70	51,76
75	51,93
80	51,37
Colina total, ppm[2]	
727 (736)	51,51
1.427 (1.443)	51,38
2.127 (2.143)	51,78
2.827 (2.846)	52,06
3.527 (3.546)	51,72
Média	51,70
EPM	0,1146
Valor de P	
AST	0,1145
Colina total	0,2655
AST x Colina total	0,2326

[1]AST= aminoácidos sulfurados totais digestíveis;[2]Os valores entre parênteses foram analisados.

Apêndice 9. Gráficos das regressões lineares e quadráticas estimadas para ganho de peso (GP), nos períodos de 1 a 7d, 8 a 14 d, e 15 a 21 d, de frangos de corte alimentados com níveis de colina total de 736, 1.443, 2.143, 2.846 e 3.546ppm.



Apêndice 10. Equações das regressões para ganho de peso (GP) e conversão alimentar (CA) de frangos de corte suplementados com níveis crescentes de colina[1].

Item	Equações de regressão[2]	Efeito[3]	r^2	Valor de P	Ponto de máxima, ppm
GP 1 a 7 d, g	Y= 5,02x + 112,07	L	0,3040	0,0001	
	Y= -4,48x² + 24,22x + 95,93	QP	0,4725	0,0001	2.700
CA 1 a 7 d, g:g	Y= -0,030x + 1,42	L	0,1582	0,0004	
GP 8 a 14 d, g	Y= 30,33x + 214,10	L	0,6593	0,0001	
	Y= -19,81x² +115,17x + 142,82	QP	0,8539	0,0001	2.907
CA 8 a 14 d, g:g	Y= -0,017x + 1,23	L	0,3717	0,0001	
GP 1 a 14 d, g	Y= 37,35x + 318,85	L	0,5901	0,0001	
	Y= -25,46x² + 146,36x + 227,25	QP	0,7797	0,0001	2.875
CA 1 a 14 d, g:g	Y= -0,036x + 1,33	L	0,2758	0,0001	
	Y= 0,023x² - 0,13x + 1,41	QP	0,3510	0,0051	2.938
GP 15 a 21 d, g	Y= 35,70x + 375,60	L	0,5324	0,0001	
	Y= -18,51x² + 114,96x + 309,00	QP	0,6314	0,0001	3.105
CA 15 a 21 d, g:g	Y= -0,011x + 1,33	L	0,1800	0,0001	
GP 1 a 21 d, g	Y= 70,86x + 700,74	L	0,6011	0,0001	
	Y= -45,18x² + 264,34x + 538,18	QP	0,7701	0,0001	2.925
CA 1 a 21 d, g:g	Y= -0,042x + 1,40	L	0,1613	0,0004	
	Y= 0,030x² - 0,169x + 1,51	QP	0,2169	0,0267	2.849

[1]Equações de regressão considerando os niveis de colina: 736, 1.443, 2.143, 2.846 e 3.546 ppm, suplementados através do cloreto de colina.
[2]Regressão linear: $Y = \beta1 + \beta2 \times X$; onde Y é a variável dependente, X é o nível de colina na dieta, $\beta1$ é o intercepto, e $\beta2$ é o coeficiente linear, respectivamente; Quadrática polinomial: $Y = \beta1 + \beta2 \times X + \beta3 \times X^2$; onde Y é a variável dependente, X é o nível de colina na dieta, $\beta1$ é o intercepto, $\beta2$ e $\beta3$ são o coeficiente linear e quadrático, respectivamente; a resposta máxima foi obtida através do cálculo: $-\beta2 \div (2 \times \beta3)$.
[3]EfeitoLinear (L) ou Quadrático Polinomial (QP) (P<0,05).

VITA

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