UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

EFEITOS DE UMA DIETA SUPLEMENTADA COM FONTE NATURAL DE COLINA NO DESEMPENHO DE FRANGOS DE CORTE

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

Área de Concentração: Nutrição e metabolismo animal

Porto Alegre (RS), Brasil

Novembro de 2023

CIP - Catalogação na Publicação

Maria, Douglas Drebes Brunhaus
Efeitos de uma dieta suplementada com fonte natural de colina no desempenho de frangos de corte / Douglas Drebes Brunhaus Maria. -- 2023.
79 f.
Orientador: Sergio Luiz Vieira.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, BR-RS, 2023.

1. Colina. 2. Desempenho. 3. Nutrição animal. 4. Frango de corte. 5. Indicadores sanguíneos. I. Luiz Vieira, Sergio, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

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DISSERTAÇÃO

Submetida como parte dos requisitos
para obtenção do Grau de
MESTRE EM ZOOTECNIA
Programa de Pós-Graduação em Zootecnia
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Porto Alegre (RS), Brasil

Aprovada em: 27.11.2023
Pela Banca Examinadora

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“Aqueles que se sentem satisfeitos sentam-se e nada fazem. Os insatisfeitos são os únicos benfeitores do mundo.”

– Walter S. Landor.

AGRADECIMENTOS

Primeiramente, gostaria de expressar minha gratidão a todos aqueles que estiveram ao meu lado durante essa jornada. À minha amada família, meus pais, Janice e Marco Antônio, quero agradecer do fundo do meu coração pelo apoio incondicional que me proporcionaram ao longo desse processo desafiador. À minha família, meu maior porto seguro, que sempre esteve presente e torceu pelo meu sucesso, dedico minha conquista. À minha noiva incrível, Raquel Horn, quero expressar minha gratidão por sua presença constante nos momentos mais difíceis desta jornada. Seu apoio e amor foram fundamentais para que eu pudesse superar os obstáculos e seguir em frente com determinação.

Ao meu estimado professor e orientador, Sergio Luiz Vieira, não tenho palavras suficientes para agradecer. Suas oportunidades, ensinamentos e conselhos valiosos foram essenciais para o meu crescimento acadêmico e pessoal. A magnífica estrutura que você proporcionou para a realização do trabalho e da pesquisa foi fundamental para o meu sucesso. Aos colegas que compartilharam comigo a experiência do Aviário de Ensino e Pesquisa ao longo desses 9 anos, quero expressar minha sincera admiração pela dedicação e comprometimento com as atividades. Os aprendizados que adquirimos juntos e a amizade que construímos são tesouros que levarei para o resto da minha vida. Aos professores e funcionários do Programa de Pós-Graduação em Zootecnia e à Estação Experimental Agronômica da UFRGS, meu profundo agradecimento pelo apoio constante e pela infraestrutura impecável que tornaram possível minha pesquisa e estudos. À CAPES, por conceder a bolsa de Mestrado que viabilizou minha educação, e às diversas empresas que apoiaram financeiramente minhas pesquisas, quero expressar minha sincera gratidão.

Em resumo, agradeço a cada pessoa que contribuiu de alguma forma para minha jornada acadêmica e pessoal. Vocês foram pilares fundamentais nessa conquista. Muito obrigado.

EFEITOS DE UMA DIETA SUPLEMENTADA COM FONTE NATURAL DE COLINA NO DESEMPENHO DE FRANGOS DE CORTE.

Autor: Douglas Drebes Brunhaus Maria

Orientador: Sergio Luiz Vieira

Resumo - A colina é essencial para desempenho dos frangos de corte, impactando diretamente na função hepática e sistema nervoso. Objetivou-se nesse estudo avaliar a substituição do cloreto de colina (CC) por uma fonte natural de colina no desempenho, rendimento de carcaça e indicadores sanguíneos de frangos de corte. Foram distribuídos 1,050 pintos de corte machos Cobb 500, em 7 tratamentos e 10 repetições. Foram aplicadas duas fases nas dietas, inicial (1 a 14 dias) e crescimento (15 a 28 dias). A dieta basal foi formulada com colina total de 700 mg/kg e 600 mg/kg para as fases inicial e de crescimento, respectivamente. Os demais tratamentos receberam suplementação de colina em níveis de 444, 900 e 1,333 mg/kg a partir de cloreto de colina 60%, ou de fonte natural (FN) de 111, 222 e 333 mg/kg. O desempenho zootécnico (GP, CR, CA) foi avaliado semanalmente. Aos 28 dias, quatro aves por box foram selecionadas para rendimento de carcaça, coleta de sangue e fígado para análise de gordura, colesterol total (CT), lipoproteína de alta densidade (HDL), lipoproteína de baixa densidade (LDL), triglicerídeos (TG), Aspartato aminotransferase (AST), Alanina aminotransferase (ALT) e heterófilo:linfócito (H/L). As análises estatísticas foram realizadas utilizando ANOVA, modelos de regressão polinomial linear e quadrática. Os resultados mostraram que os rendimentos de CA, HDL, LDL, H/L e rendimento de carcaça não foram afetados pelas fontes suplementadas (P ≤0,05), GP e CR mostraram um efeito linear quando ambas as fontes de colina foram suplementadas. AST, ALT, TC, TG e gordura no fígado reduziram quando os níveis foram suplementados com ambas as fontes de colina (P ≤0,05). Os resultados de CC 52,2% e NS tiveram efeito quadrático sobre CT, AST e TG em 1.055 mg/kg para CC 60% e resposta máxima e 260 mg/kg para suplementação de FN. A FN demonstrou ser uma fonte viável de colina para uso em dietas comerciais, sendo necessária a suplementação de 4,1 vezes menor que a dose de CC 60%.

Palavras-chave: frangos de corte, colina, indicadores sanguíneos, desempenho.

[1]Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (79 p.), Novembro, 2023

EFFECTS OF A NATURAL CHOLINE SOURCE SUPPLEMENTED DIET ON BROILER PERFORMANCE

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Abstract - Choline is essential for the performance of broiler chickens, directly impacting liver function and the nervous system. The objective of this study was to evaluate the replacement of choline chloride (CC) with a natural source of choline on the performance, carcass yield and blood parameters of broiler chickens. 1,050 male Cobb 500 broiler chicks were distributed in 7 treatments and 10 replications. Two phases were applied to the diets, initial (1 to 14 days) and growth (15 to 28 days). The basal diet was formulated with total choline of 700 mg/kg and 600 mg/kg for the starter and growth phases, respectively. The other treatments received choline supplementation at levels of 444, 900 and 1,333 mg/kg from choline chloride 60%, or from a natural source (NS) at 111, 222 and 333 mg/kg. Zootechnical performance (GP, CR, CA) was evaluated weekly. At 28 days, four birds per box were selected for carcass yield, blood, and liver collection for analysis of fat, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and heterophil:lymphocyte (H/L). Statistics were performed using ANOVA, linear and quadratic polynomial regression models. The results showed that CA, HDL, LDL, H/L yields and carcass yield were not affected by the supplemented sources ($P \leq 0.05$), GP and CR showed a linear effect when both choline sources were supplemented. AST, ALT, TC, TG, and liver fat levels were reduced when supplemented with both choline sources ($P \leq 0.05$). The results of CC 60% and NS had a quadratic effect on CT, AST, and TG at 1,055 mg/kg for CC 60% and maximum response and 260 mg/kg for NS supplementation. FN was demonstrated to be a viable source of choline for use in commercial diets, requiring supplementation 4.1 times lower than the 60% CC dose.

Keywords: broilers, choline sources, blood indicators, performance.

[1]Master of Science Dissertation in Animal Science – Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (79 p.), November 2023.

SUMÁRIO

LISTA DE TABELAS

LISTA DE ABREVIATURAS

ALT	Alanina transaminase.
AST	Aspartato transaminase
CA	Conversão alimentar.
CC	Cloreto de colina.
CR	Consumo de ração.
FC	Fosfatidilcolina.
GP	Ganho de peso.
HDL	Lipoproteínas de alta densidade.
LDL	Lipoproteínas de baixa densidade.

CAPÍTULO I

1. INTRODUÇÃO

A colina é conhecida como um elemento essencial para o organismo das aves, desempenhando um papel ativo na preservação de diversas membranas e organelas. Além disso, ela representa um componente crucial para a síntese da acetilcolina, um neurotransmissor fundamental na transmissão de impulsos nervosos através das sinapses (WAUBEN et al., 1999, CALDERANO et al., 2015). A sua absorção ocorre por meio de micelas que são absorvidas pelos enterócitos localizados no intestino delgado através de um processo de transporte passivo. Uma vez absorvida, a colina é transportada pelas lipoproteínas de alta densidade (HDL) até o fígado, que representa seu principal sítio de ação (COMBS, 2008). Na avicultura, a colina é encontrada em diversas matérias-primas empregadas na formulação de rações. Tipicamente, manifesta-se na forma de colina livre ou em complexos, tais como fosfocolina, esfingomielina ou fosfatidilcolina (FC) (ZEIZEL et al., 2003). A FC faz parte dos fosfolipídios presentes na membrana celular, podendo fazer parte em até 35% de sua composição, além disso, exerce um papel vital na retirada de lipídios do fígado, sendo indispensável para a síntese de lipídios de baixa densidade (LDL). Essas moléculas são responsáveis por transportar gordura para os diferentes tecidos, desempenhando um papel essencial no equilíbrio do metabolismo lipídico (YAO, 1988, BATTAGLIA, 1997).

As aves possuem baixa eficiência na síntese endógena de colina em quantidades que supram seu metabolismo. Assim, torna-se vital a suplementação de colina nas formulações dietéticas, visando assegurar um aporte adequado desse nutriente para atender às exigências metabólicas das aves (MCDOWELL, 1989). O fígado das aves é suscetível a uma variedade de alterações nutricionais, sendo possível avaliar sua saúde e funcionalidade por meio dos níveis séricos de determinadas enzimas, a exemplo da Alanina Transaminase (ALT) e Aspartato Transaminase (AST). A elevação na concentração dessas enzimas é indicativa de danos celulares (CORDUK et al. 2007). Em um estudo conduzido por Selvam et al. (2018), observou-se que a uma dieta deficiente em colina (531 mg/kg) resultou em um aumento no conteúdo da enzima AST de 4 IU/L para 8 IU/L. Esse acréscimo representou um aumento de 50%, quando comparado com uma dieta suplementada com cloreto de colina (CC). Além disso, ao suplementar a dieta com colina natural, os valores de AST foram de 5 IU/L em comparação com a dieta contendo CC. Esses resultados corroboram a função

hepatoprotetora da colina, evidenciando sua capacidade de mitigar os efeitos adversos induzidos pela deficiência dietética.

A prática comum na avicultura para suplementação de colina é por meio do CC. Contudo, a propriedade higroscópica desse composto pode contribuir para a perda de outras vitaminas presentes na dieta, o que representa um desafio em sua aplicação nas fábricas de rações. (ALBERS et al., 2002). As dietas a base de milho e farelo de soja possuem um teor de colina estimado em aproximadamente de 1.300 mg/kg na dieta, e as recomendações de suplementação para dietas de frangos de corte a base de milho e farelo de soja variam na faixa de 400 a 550 mg/kg, respectivamente. (ROSTAGNO et al., 2017, NRC, 1994). Em um estudo conduzido por POMPEU et al. (2013), a suplementação de níveis crescentes de 0, 400, 800, 1.200 e 1.600 mg/kg de CC 60% revelou uma melhoria no consumo de ração (CR) em frangos aos 42 dias, em comparação com o grupo de controle sem inclusão de CC. No trabalho de SANTIAGO et al. (2020), a suplementação de níveis crescentes de 736, 1.443, 2.143, 2.846 e 3.546 mg/kg de CC 60%, os resultados demonstraram uma resposta quadrática para ganho de peso (GP) em 2.516 mg/kg e para conversão alimentar (CA) em 2.533 mg/kg.

Atualmente, existem diversos produtos naturais caracterizados por uma elevada concentração de colina, os quais demonstram uma biodisponibilidade que pode variar até 90%. Tais produtos emergem como uma potencial alternativa ao cloreto de colina, apresentando viabilidade para consideração em diferentes contextos nutricionais (CHATTERJEE, 2004, SANTOS, 2010). Uma pesquisa realizada por D'SOUZA (2022) ao realizar a suplementação de uma fonte natural de FC a base de *Acacia nilotica* e *Curcuma longa* nos níveis de 400, 500 e 750 mg/kg, as respostas de gordura no fígado e gordura abdominal foram afetadas, enquanto o desempenho zootécnico manteve valores similares a fonte de CC 60%. Outro estudo realizado por DIAS et al. (2022) com colina natural derivada de *Ocimum sanctum*, *Andrographis paniculata*, *Silybum marianum*, *Glycine max* e *Azadirachta indica* e realizou substituições estratégicas de 25, 50 e 100% de CC por colina natural, durante o ensaio não foram encontradas diferenças para GP, CR e CA, O tratamento com 100% de inclusão de colina natural diminuiu a concentração sérica de lipoproteína de LDL aos dias 21 e 42 de idade, respectivamente.

Com fundamentação nestas pesquisas, surge a conjectura de que a fonte natural tem o potencial de substituir a fonte convencional de CC sem prejudicar a

performance dos frangos de corte. Dessa forma, o propósito deste estudo foi avaliar os efeitos da fonte natural de colina no desempenho, rendimento de carcaça e parâmetros sanguíneos em frangos de corte.

2. REVISÃO BIBLIOGRÁFICA

2.1. Colina

Quimicamente denominada β-hidroxietil-trimetilamônio hidróxido a colina, é considerada uma amina quaternária presente nas plantas e nos animais. No organismo, é encontrada na forma de colina livre, acetilcolina e diversos fosfolipídios. A acetilcolina é responsável pela mediação da atividade nervosa, sendo sintetizada a partir da colina e também pelo ácido acético, sendo responsável pela transmissão dos estímulos nervosos pelo animal (FERREIRA E NETO, 2003). Os principais fosfolipídios são a FC ou lecitina, alisofosfatidilcolina, plasmógenos de colina e esfingomielina (ZEISEL, 1981). A FC é um fosfolípidio considerado essencial, pois é produzido através da colina via todas células nucleadas do organismo. E envolve-se no transporte, mobilização e absorção de lípideos para o fígado, além do mais, também é um componente essencial das mitocôndrias, estando presente nas membranas celulares e nas organelas. A sua metilação ocorre na bíossintese endógena de colina, possibilitando que a S-adenosil-metionina doe um grupo metil, fazendo com que mamíferos demandem menos colina no organismo (KROENING et al 1967). Em contrapartida, as aves tem uma limitação na sua capacidade de biossíntese, fazendo que aves mais jovens necessitem maior aporte nutricional de colina na dieta (MOLITORIS et al., 1976, BAKER et al., 1970).

A colina pode ser oxidada em betaína, que é um processo irreversível que ocorre no rim e no fígado. Além disso, a síntese de FC pode ocorrer através da citidina difosfatocolina ou da metilação de fosfatidiletanolamina. A esfingomielina, por sua vez, é produzida através transferência de fosfocolina, proveniente da FC, para um esfingolípideo ceramida. Esses processos de síntese e transformação da colina desempenham papéis fundamentais na formação e manutenção de diversos componentes celulares, incluindo membranas celulares e neurotransmissores (LI & VANCE, 2008).

Seu reconhecimento aconteceu quando Theodore Gobley em 1850, através do isolamento da lecitina do tecido cerebral e ovos de carpas, seu nome deriva do grego "lekithos", que seu significado é gema de ovo (ZEISEL, 2012). Já Adolph Strecker, no ano de 1864, isolou a molécula da colina ao ferver a bile suína e bovina, originando sua nomenclatura a partir da palavra grega "chole", que seu significado é bile (MCDOWELL, 1989). A aceitação da colina como nutriente essencial só ocorreu

em 1929, com estudos que demonstraram seu efeito na prevenção de fígado gorduroso em cães e ratos (BEST & HUNTSMAN, 1932; BEST & HUNTSMAN, 1935). Somente em 1998, a colina foi oficialmente reconhecida como nutriente essencial pelo Instituto de Medicina (ZEISEL & DA COSTA, 2009). Otto Loewi e Henry Dale receberam o Prêmio Nobel em 1936 por mapearem a acetilcolina como componente químico da neurotransmissão. Edith Cohen e Dean Haubrich, em alguns estudos subsequentes, descobriram que a modulação da síntese de acetilcolina ocorria através da ingestão dietética de colina, sugerindo que a síntese endógena não era o suficiente (ZEISEL, 2012). As vias de síntese da colina podem ser a via citidina difosfatocolina e metilação da fosfatidiletanolamina, que foram mapeadas por Eugene Kennedy (1954) e Jon Bremer & David Greenberg (1960), respectivamente.

Considerada um nutriente hidrossolúvel do complexo B, alguns estudos desafiam essa classificação, pois a colina não atua como coenzima, mas sim como componente estrutural celular (INSTITUTE OF MEDICINE, 1998). Considerada fundamental para a formação e estrutura celular, a colina compõe a membrana das células animais na forma de fosfolipídios (ZEISEL, 2012). Atuando na formação e liberação de lipoproteínas hepáticas, a FC é essencial para evitar o acúmulo de triglicerídeos nos hepatócitos, prevenindo o fígado gorduroso (BERTECHINI, 2006). A colina desempenha papel crucial como agente lipotrófico, especialmente em aves devido ao subdesenvolvimento do sistema linfático (ANNISON, 1983). Alguns estudos demonstram que níveis inadequados de colina na dieta estão correlacionados com o aparecimento de esteatose hepática, caracterizada por alterações macroscópicas e microscópicas no fígado (COLE et al., 2012). Deformidades nas patas, como perose, valgus e varus, são relacionadas à deficiência de colina (MCDOWELL, 1989; JULIAN, 2005). A colina também desempenha participa da formação de colágeno, influenciando a proliferação de condrócitos e osteócitos. Além disso, a colina é crucial na formação da bainha de mielina, essencial para a condução do impulso nervoso (ZEISEL, 2012).

Classificado como um nutriente essencial e precursor da FC, tem sido reconhecida por seu papel crítico no metabolismo lipídico, especialmente no fígado (WATANABE et al., 2006). Pesquisas apontam para os efeitos benéficos da colina na modulação dos níveis de HDL e LDL colesterol em frangos. A adequada suplementação de colina na dieta está associada a um aumento nos níveis de HDL, e à redução dos níveis de LDL (SOKUNBI et al., 2023). Os triglicerídeos em relação a

colina também desempenha um papel crucial na sua regulação. Estudos sugerem que a presença adequada de colina na dieta está associada a uma redução nos níveis de triglicerídeos (GANGANE et al., 2010). Os triglicerídeos são sintetizados na mucosa intestinal e no fígado a partir dos componentes da digestão e absorção de ácidos graxos. Suas concentrações podem variar em função de vários fatores e o seu valor diagnóstico não tem sido bem estudado em aves (HOCHLEITHNER, 1994). A colina demonstrou influenciar positivamente a redução do colesterol e gordura acumulada no fígado, sugerindo um potencial papel preventivo contra o acúmulo excessivo de lipídios nesse órgão (SANTIAGO et al., 2020, SOKUNBI et al., 2023). O colesterol plasmático origina-se tanto da dieta quanto da síntese hepática, sendo excretado na forma de ácidos biliares, e seus valores normais em aves situam-se entre 100 e 250 g/L (HOCHLEITHNER, 1994; GRUNKEMEYER, 2010).

Contudo, é fundamental ressaltar que a natureza dessas relações é complexa e dependente de vários fatores, incluindo a dosagem de colina na dieta, a composição geral da alimentação das aves e a genética do frangos. Pesquisas mais aprofundadas são necessárias para elucidar completamente os mecanismos subjacentes a essas interações e para otimizar as condições nutricionais visando à promoção da saúde metabólica e hepática das aves.

2.2. Fontes de colina

A colina é um componente presente em diversos ingredientes empregados na elaboração de rações destinadas a aves, particularmente em fontes proteicas que exibem concentrações significativas, variando de 2.700mg/kg a 3.500mg/kg, como observado no farelo de soja e na farinha de carne (BERTECHINI, 2006). A suplementação de colina é realizada mediante o uso do CC, sendo essencial em dietas destinadas a frangos de corte. Entretanto, a utilização do cloreto de colina apresenta desafios quando incorporado na formulação de rações, devido à sua propensão à higroscopicidade, o que pode resultar na oxidação de outras vitaminas presentes na dieta (KHOSE et al., 2018). Além disso, o cloreto de colina é corrosivo, demandando equipamentos específicos para armazenamento e manuseio, e não é adequado para inclusão em pré-misturas vitamínicas concentradas. Adicionalmente, apenas um terço do CC é absorvido no lúmen intestinal, enquanto os dois terços restantes são convertidos em trimetilamina, a qual é absorvida pela corrente sanguínea e pode impactar o metabolismo hepático, inibindo a atividade da enzima flavina monooxigenase 3 (HUERGA et al., 1952; AI WAIZ et al., 1987).

Em busca de atenuar as desvantagens inerentes ao CC sintético, diversos estudos estão sendo conduzidos para explorar alternativas. Estas pesquisas estão fundamentadas na avaliação dos efeitos lipotrópicos e hepatoprotetores (NARAYANANKUTTY et al., 2018). A colina presente em vegetais se apresenta nas formas de colina livre, esfingomielina e FC (GANGANE et al., 2010). A FC passa por uma degradação intestinal mínima, não sofrendo conversão em trimetilamina, preservando sua eficácia e utilização no organismo do animal (HUERGA, 1952; MCDOWELL, 2000). Além disso, a biodisponibilidade e a eficácia variam entre diferentes ésteres de colina, destacando-se a FC como a mais eficiente (CHENG et al., 1996). A colina natural contém FC em sua estrutura, e possui biodisponibilidade superior quando contrastada com o CC. Essa característica possibilita a utilização de quantidades menores de suplementação, aprimorando a formulação e reduzindo as demandas de armazenamento. Segundo Calderano et al. (2015), ao incorporar 100 mg/kg de colina proveniente de fonte natural à dieta de frangos de corte, observaram resultados equiparáveis no ganho de peso aos 42 dias em comparação com a suplementação de 800 mg/kg de CC. Adicionalmente, Sharma et al. (2015) obtiveram resultados similares ao adicionar 500 mg/kg de colina natural às dietas das aves.

Onde, os animais apresentaram ganhos de peso superiores, além de melhorias significativas nos parâmetros de colesterol e triglicerídeos, resultando em uma redução de 18% e 36%, respectivamente.

2.3. Fonte natural

As pesquisas frequentes sobre fontes vegetais de colina se deve à facilidade de acesso e à simplicidade de preservação dessas fontes. Além disso, esses recursos apresentam níveis de biodisponibilidade comparáveis aos observados em produtos de origem animal, com variações que podem situar-se entre 60% e 90% (SANTOS, PEREIRA, 2010). Considerando sua origem natural, a colina natural pode ser empregada como suplemento alternativo em alimentos destinados ao mercado de produtos orgânicos, ao contrário do CC obtido por síntese química (NASCIMENTO, 2020).

A *Acacia nilotica* (*Mimosaceae*) têm demonstrado efeitos positivos devido às suas propriedades antioxidantes, enquanto espécie, destaca-se como uma fonte proeminente de compostos bioativos, incluindo flavonoides, alcaloides, fenólicos, saponinas, polissacarídeos, taninos e terpenoides também sendo condiderada lipotrópica e hepatoprotetora. O gênero *Acacia* pertence a família *Leguminosae*, subfamília *Mimosoideae*. A família *Leguminosae* é uma das maiores dentre as dicotiledôneas, compreendendo mais de 13.000 espécies reunidas em mais de 600 gêneros distribuídos mundialmente, principalmente nas regiões tropicais e subtropicais (JOLY, 1998). *Acacia nilotica* é geralmente utilizada em tratamentos do trato respiratório e diarréias devido as suas propriedades tônicas, adstringentes e estimulantes (NABI, 1982). Alguns estudos demonstram seu efeito positivo na ação contra bactérias gram positivas e negativas, seu etanólico combate ambas bactérias, além disso, seu extrato hexânico demonstra mais atividade contra *candida albicans* (SOTOHOY et al., 1995, MUSTAFA et al., 1999, KAMBIZI et al., 2001). A alta presença de taninos na sua composição exibiu em alguns ensaios um efeito antimicrobiano sobre o *Clostridium perfringens,* (SOTOHOY et al., 1995). Efeitos antiflamátorios também foram encontrandos quando utilizada essa espécie, outrossim, o uso desse extrato natural demonstrou efeito hepatoprotetor e intenso atividade hipoglicêmica quando utilizada (DAFALLASH et al., 1996, JAYASEKHAR et al., 1997, WASSEL et al., 1992). Segundo KHAN et al. (2012), a suplementação de colina natural com extrato de *Acacia nilotica e Curcuma longa* em quantidades de 250, 350 e 500g melhorou o desempenho dos frangos, além de reduzir a gordura hepática e melhorar as caracteristicas da carcaça na dose de 350 e 500g.

A *Curcuma longa*, é conhecida como açafrão-da-terra, é uma planta herbácea perene rizomatosa pertencente à família *Zingiberaceae*. Seu extrato é um polifenol amarelo-laranja, geralmente encontrado na forma de um pó amarelo seco, solúvel em óleo em seu estado natural (KHAN et al., 2012). Outra parte muito utilizada dessa planta é o seu rizoma, pois contém ativos como tetrahidrocurcuminóides, curcumina, desmetoxicurcumina e bisdemetoxicurcumina (OSAWA et al., 1995; AL-SULTAN, 2003). A curcumina, um diferuloil metano, presente nos rizomas da cúrcuma. Alguns estudos utilizam a cúrcuma em dietas para aves, demonstrando melhorias no ganho de peso, consumo de ração, conversão alimentar, como também na atividade enzimática (AL-KASSIE et al., 2011). Além disso, os óleos essenciais da cúrcuma estão associado a propriedades antimicrobianas, antifúngicas e antioxidantes, contribuindo para uma melhor utilização dos nutrientes dietéticos pelas aves (AL-KASSIE et al., 2011; RADWAN ET AL., 2008).

2.4. Marcadores enzimáticos

As aminotransferases são enzimas de extrema importância, integrando um grupo de catalisadores que facilitam a transferência de um grupo amino de um aminoácido para um cetoácido. A ALT e a AST destacam-se como as transaminases mais cruciais para o diagnóstico clínico de diversas alterações metabólicas (COLES, 1974). A ALT apresenta uma forma citoplasmática (c-AST) com massa molecular de 93.000 e uma forma mitocondrial (m-AST) com massa molecular enzimática de 90.400; ambas são codificadas e sintetizadas no citosol (SWENSON & REECE, 1996). A cadeia de aminoácidos dessas enzimas foi identificada e está presente em vários tecidos, como o estriado, miocárdio e hepático, sendo considerada a principal fonte de AST e ALT no organismo (TELANG, 1975). A descoberta dessas enzimas foram atribuídas a Hird e Rowsell, cujos pesquisas destacaram uma atividade elevada nas porções citosólicas e mitocondriais das células hepáticas. Além disso, inúmeras pesquisas consideram a atividade dessa enzima em nível sérico como um indicador significativo de lesão hepática, tanto aguda quanto crônica (FRANSON et al., 1985).

A ALT está localizada no citosol dos hepatócitos e células musculares (HARR, 2002; JAENSCH, 2000). Sua função consiste na catalisação da transaminação reversível da L-alanina e do 2-oxoglutarato para formar piruvato e L-glutamato. Assim como outras transaminases, a ALT desempenha um papel no catabolismo de aminoácidos e na transferência de nitrogênio entre os órgãos (KANEKO et al., 2008). Frequentemente, o aumento na atividade da enzima ALT está associado a lesões musculares e hepáticas (HARR et al., 2002; GRUNKEMEYER, 2010). Em aves, esse aumento ocorre em resposta a danos em diversos tecidos, o que pode complicar a interpretação dessa análise (JAENSCH, 2000). Em determinadas situações, foi observado que aves com lesões hepáticas graves exibem níveis normais de ALT na corrente sanguínea. É relevante ressaltar que a atividade desta enzima pode ser reduzida em algumas espécies. Os resultados na literatura são divergentes, uma vez que alguns pesquisadores argumentam que o nível de sensibilidade é muito baixo, enquanto outros afirmam que a ALT é um indicador altamente sensível para a avaliação de distúrbios hepatocelulares, indicando lesão ou inflamação (‘HOCHLEITHNER, 1994, ARAÚJO et al., 2001, CAMPBELL, 2007).

3. HIPÓTESES E OBJETIVOS

HIPÓTESES

Frangos de corte suplementados com uma fonte de colina natural podem melhorar o desempenho zootécnico.

A suplementação de colina natural melhora o desempenho quando comparadas a aves alimentadas com a fonte de cloreto de colina 60%.

OBJETIVO GERAL

Avaliar os impactos da inclusão de colina natural no desempenho frangos de corte, analisando seus efeitos no desempenho das aves

OBJETIVO ESPECÍFICO

Avaliar as respostas das suplementação de colina natural no rendimento de carçaça e indicaroes sanguíneos.

CAPÍTULO II

Effects of a natural choline source supplemented diet on broiler performance.

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Summary

Choline is essential for the health and performance of broiler chickens, directly impacting liver function. The study aimed to evaluate substitution of choline chloride (CC) by a natural choline source on the performance, carcass yield, and blood variables of broiler chickens. A total of 1,050 male Cobb 500 broilers chicks were distributed, 7 treatments and 10 replicates. Two-phases, starter (1 to 14 d), and grower (15 to 28 d) were applied. The basal group diet formulated choline were 700 mg/kg, and 600 mg/kg for starter and grower phases, respectively. The other treatments received choline supplementation of 444, 900, and 1,333 mg/kg from choline chloride 60%, or from a natural source (NS) by, 111, 222, and 333 mg/kg. Broiler performance (BWG, FI, FCR) were evaluated weekly. At 28 d, four birds per pen were selected for carcass yields, blood, and liver collection to analyze fat, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and heterophile:lymphocyte (H/L). Statistics were conducted using ANOVA, linear and quadratic polynomial regression models. Results showed that FCR, HDL, LDL, HL, and carcass yields were not affected by the sources ($P \leq 0.05$), BWG and FI showed a linear effect when both sources of Choline were supplemented. AST, ALT, TC, TG, and fat in the liver reduced when the feeds were supplemented with both choline sources ($P \leq 0.05$). Results of CC 60% and NS had a quadratic effect on TC, AST, and TG at 1,055 mg/kg for maximum response average, and 260 mg/kg to NS supplementation. The NS has proven to be a viable source of choline, and it is necessary to supplement 4.1 times the dose of CC 60% in relation to NS.

Keywords: broilers, blood, choline sources, performance.

Description of Problem

Choline is an essential nutrient for the poultry organism, playing a fundamental role in several physiological functions. The choline serves as a structural component in cellular organelles, such as mitochondria, playing a significant role in the formation of the cartilaginous matrix of bones and in the composition of cell membranes in the form of phospholipids, notably phosphatidylcholine (Zeisel, 1991). In parallel, choline is recognized as a lipotropic agent, playing a role in preventing anomalous lipid accumulation and inhibiting the development of hepatic steatosis (Halver, 2002). Furthermore, choline is endowed with active methyl groups, playing a crucial role as a donor of labile methyl groups, a requirement for the transformation of homocysteine into methionine after its oxidation to betaine (Zhang et al., 2013). The absence of choline in the feeds may have adverse implications on the metabolism and growth of birds. It is important to note that, although many animals could synthesize choline in their bodies, birds, until the eighth day of life, lack the ability to produce it in sufficient quantities to meet their nutritional needs (McDowell, 1989). Adequate choline supplementation is essential for the health and proper development of young birds, as the deficiency of this nutrient can result in stunted growth and, in more severe cases, lead to pathogenic conditions such as perosis (Taconi, 1988).

In conventional poultry nutrition practices the predominant form of supplementation is through choline chloride (CC) 60%. Choline supplementation levels in broiler feeds during periods of 1 to 7 days and 8 to 21 days are typically set at 550 mg/kg and 496 mg/kg (Rostagno et al. 2017), respectively. However, the use of synthetic choline in factories presents some disadvantages due to its high hygroscopicity and the possibility of oxidation of vitamins, which creates space for the exploration of viable alternatives (Zeisel et al., 1983, Selvam et al., 2018). Choline is naturally found in plants in the form of phosphatidylcholine (Botella et al., 2017). The search for natural products based on specific plants, characterized by high bioavailability, significant choline content

and in its esterified form, appears as a viable alternative to replace CC (Chatterje et al., 2004, Gangane et al., 2010).

Investigations regarding a natural source (NS) composed of the conjunction of *Acacia nilotica (Mimosaceae*) and *Curcuma longa (Zingiberaceae*) have shown positive effects due to their antioxidant, lipotropic and hepatoprotective properties, as documented in some studies (Yarru et al., 2009, Narayanan et al., 2013). A study aimed at evaluating the effectiveness of NS compared to CC in broiler chickens with choline deficiency revealed that NS and CC had similar performances in relation to body weight gain, feed conversion, liver function and body composition. Furthermore, NS at a dose of 400 g/ton was equivalent to CC in restoring serum aspartate aminotransferase activity and reducing abdominal fat (Selvan et al., 2018). Another study of broiler chickens using NS in the high-energy diet showed dose-dependent improvements in body weight, feed conversion ratio, reduced carcass fat and increased blood L-carnitine levels, suggesting similar benefits to CC (D'Souza et al., 2022).

Based on this knowledge, it's hypothesized that the natural source may replace the traditional source without any issue in broiler health and performance. Thus, the objective of the current study was to investigate the impacts of the natural choline source on the performance, carcass yield and blood variables of broiler chickens.

Material and methods

The Ethics and Research Committee of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil, approved all procedures used in the present study.

Bird Husbandry

A total of 1,050 d old male Cobb x Cobb 500 broilers were placed in 70 floor pens (1.65 x 1.65 m). Therefore, a total of 7 treatments with 10 replications of 15 birds each were utilized. Each pen had new rice hulls bedding and was equipped with one 15-kg capacity tube feeder and 3 nipple drinkers. Lighting was continuous until 7 d of age, with a 14L:10D cycle used afterward. Birds had *ad libitum* access to water and mash feed. The average temperature was 32°C at placement, being reduced by 1°C every 2d until 23°C to provide comfort throughout the study.

Dietary Treatments

Choline content in the dietary ingredients and feed formulations was determined using the choline enzymatic method 999.14 (AOAC International, 2000). The feed was structured around a two-phase feeding program, starter phase (1-14d), grower phase (15-28d). In the treatment group without supplementation, the dietary choline levels were formulated to contain 700 ppm of choline up to the 14th day, followed by a reduction to 600 ppm until the 21st day. Conversely, the other treatment groups received dietary supplementation alongside the base diet. Specifically, treatments 2, 3 and 4 featured an additional 444, 900 and 1,333 mg/kg of CC 60%, totaling 931, 1,170, 1,396 ppm of choline provided on starter feed, and 831, 1,070 and 1296 mg/kg of choline on grower feed, respectively. For treatment 5, 6 and 7 was 111, 222 and 333 ppm of choline derived from a NS (Kolin Plus®, M/s. Natural Remedies Pvt. Ltd., Bengaluru, India, *Acacia nilotica* (*Mimosaceae*) and *Curcuma longa* (*Zingiberaceae*) product dosage can be variated with 25 g/kg to 65 g/kg) totaling, 702 to 707, 705 to 714 and 708 to 722 mg/kg of phosphatidylcholine provided

on starter feed, and 602 to 607, 605 to 614 and 608 to 622 mg/kg off phosphatidylcholine on grower feed, respectively.

Performance and Carcass Yield

Performance was evaluated by measuring body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) corrected for the weight of dead broiler chickens at 1, 7, 21 and 28 d of age. At 28 d of age, four birds were selected from average weight (±5%) of each pen and processed for carcass yields and abdominal fat. Carcass yield was expressed as a percentage of live weight, and abdominal fat was expressed as a percentage of carcass weight.

Liver and blood analysis

Livers were collected from two birds per pen after euthanasia by neck dislocation. All collected samples were weighed and stored in plastic bags by cage at −20 °C until analysis. Livers were later submitted to ethyl ether extraction following previous acid hydrolysis with hydrochloric acid (method 920.39, AOAC International, 1995) to determine the percentual of fat. Blood samples were taken through heart punctures collection from two broilers randomly selected from each treatment on day 28. Blood was transferred into 5 mL test tubes containing a clot activator. Serum from centrifuged blood (3 mL) was used for total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and heterophil/lymphocyte (H/L), were determined using commercially kits (Labtest, Belo Horizonte, MG, Brazil).

Statistical Analysis

The study was designed in a completely randomized design. Data were tested for homoscedasticity and normality of the variance prior to statistical analyses. (Levene, 1960; Shapiro and Wilk, 1965). Data were transformed using the arcsine square root percentage (z = asin

(sqrt (y +0.5))) whenever not normally distributed, however, real means are presented as results. Data were submitted to analysis of variance (ANOVA) using GLM procedures of SAS (2009) when significant, means were compared by Tukey test at $P \leq 0.05$. Estimations of maximum responses to supplementation choline source were done using linear (L) and quadratic polynomial (QP) regression models. The L model ($Y = \beta1 + \beta2 \times X$) has Y as the dependent variable, X as the dietary level of choline source, β1 as the intercept, and β2 as the linear coefficient. The QP model ($Y = \beta1 + \beta2 \times Fe + \beta3 \times (\text{Choline source})^2$) has Y as the dependent variable as a function of dietary level of choline; β1 as the intercept; β2 as the linear coefficient and β3 as the quadratic coefficient. The maximum response for choline supplementation was defined as Choline source = $-\beta2 \div (2 \times \beta3)$.

Results and discussion

The choline in the experimental feeds were close to what had been formulated and, therefore, responses from broilers obtained were considered real effects of the treatments, the total choline concentrations in the basal feeds were found to be 721 mg/kg and 613 mg/kg (Table 1).

Performance

The performance is presented in Table 2 and the results show that broilers fed without choline supplementation had the lowest BWG and FI, as well as the highest CA. CC 60% and NS supplementation improved BWG and FI in all periods ($P < 0.05$), supplementation from both sources caused reductions in CA during all periods, however, when compared with a non-supplemented group, both supplements were not significant ($P > 0.05$). Similar results for FCR were found by Waldroup et al. (2005) observed that the supplementation of two levels of choline 0 and 1,000 ppm, associated or not with betaine also did not impair the FCR of broiler chickens aged 49 d.

The results of the regressions on the type of supplemented source are presented in Tables 5 and 6. The BWG showed linear growth at days 7, 14, 21 and 28 d from both sources. The responses for each CC 60% increment were 5.5, 14.1, 24.9 and 42.8g. Whereas, for NS the responses were 4.4, 12.0, 21.7 and 47.1g ($P < 0.05$), respectively. FCR responses demonstrated a linear decrease when choline sources were supplemented. For CC 60% supplementation there was a reduction of 0.003g, and NS the reduction was 0.04g, ($P < 0.05$), respectively. FI responses have demonstrated a linear increase at days 7, 14, 21 and 28 when the choline was supplemented. FI responses for CC 60% were 5.5, 14.7, 27.7 and 52.7g for each supplementation. For NS the linear increases correspond to 4.6, 12.7, 24.5 and 59.9g with each increasing supplementation, ($P < 0.05$), respectively. Lipstein et al. (1977) evaluated the supplementation of 0, 900, 1,800 and 2,700 of CC on broiler chickens in the accumulated period of 1 to 35 days with total choline concentrations of 268 to 1,942 ppm supplementing CC and did not observe significant effects on performance. In contrast, Santiago et al. (2020) evaluated broilers fed with 0, 700, 1,400, 2,100 and 2,800 mg/kg of CC and improvements were found in BWG and FI, on the other hand, there was also an improvement in leg deviation responses. Such controversial results may explain that the exact requirement of choline is not well established yet.

Carcass yield

The carcass yield of broilers slaughtered on day 28 are presented in Table 3. Results were not affected by supplementation of both choline sources ($P > 0.05$). Similar results were found by Alagawani et al. (2015) where increasing levels of CC 60% ranging between 1,000 and 2,500 mg/kg were supplemented in the Japanese quail feed, and the supplementation did not affect the carcass yield data. Carcass abdominal fat data were not affected by supplementation of both choline sources ($P > 0.05$). These results corroborate those of Rahnama et al. (2020) where two

levels of choline were supplemented, 0.1% and 0.2%, associated or not with lecithin, and choline supplementation did not affect the amount and percentage of abdominal fat in the carcass of broiler chickens. Contrary results were found by Hossain and Das. (2014), where Choline supplementation up to 3,000 mg/kg caused a reduction in abdominal fat in 35-day-old broiler chickens.

Blood and fat liver analysis

The blood parameters and fat liver are presented in Table 4. High high-density lipoprotein, LDL and H/F was not affected by the choline sources during this trial (P >0.05). These results agree with the study by Navidshad et al. (2019) where dietary choline levels (recommended level and 25% less than the recommendation of Ross 308) were evaluated and no significant differences were found in the relationship between H/L. Furthermore, other authors report in their studies reduced or unchanged levels of HDL and LDL in broiler chickens supplemented with other sources of choline (Guerreiro et al., 2011, Saleh et al., 2020). The AST data showed a quadratic response for both types of sources, which led to the lowest AST content in the blood estimated at 1,146 ppm from CC 60% and for NS 293 ppm from supplementation (P <0.05). Total cholesterol levels showed a quadratic effect for both supplemented sources, where the lowest serum TC levels were 1.022 mg/kg for CC 60% and 235 mg/kg for NS (P <0.05). For TG there was also a quadratic effect, where the lowest levels of TG on blood occurred at 999 mg/kg of CC 60% and 253 mg/kg of NS added to the feed (P <0.05). The ALT results show linear effects for both types of choline supplementation, where serum levels are reduced with CC 60% addition, causing a decay of 2.8 U/L. For NS supplementation, serum levels reduce by 4.3 U/L depending on the supplementation. These results were similar to Khose et al. (2019) fed broiler chickens with increasing levels of herbal choline of 250, 350 and 550 mg/kg and obtained reductions of 49% for ALT and 64% for AST when comparing the highest level of supplementation with the control treatment without

added NS at 42d. Rahnama et al. (2020), studies supplementation with increasing levels of 0.1% and 0,2% of choline decreased TC, TG, LDL, HDL by 11, 21, 20 and 20%. Furthermore, serum AST and ALT levels also decreased. Liver fat demonstrated a linear decreasing effect for CC 60% and NS supplementation, where with each dietary supplement there was a 0.24% decrease in abdominal fat. For NS supplementation, there was a 0.25% drop in liver fat when birds were fed increasing levels. The results of this study show, broiler chickens fed with both sources and increasing levels of choline had reduced liver injury parameters, as well as TG and TC and liver fat, when compared to the diet without choline supplementation.

Conclusions and applications

1. The primary outcome of this investigation revealed that choline derived from a natural source exhibited similar responses to choline chloride, thereby establishing itself as a viable alternative for incorporation into commercial feed formulations.
2. The concentration of AST and ALT in the blood decreased when supplemented with different sources of choline, TC and TG levels showed a quadratic effect. total cholesterol and triglyceride levels demonstrated quadratic effects for both types of choline sources, showing better responses in supplements of 1,022 and 999 mg/kg for CC 60% and 235 and 253 for NS.
3. Choline supplementation linearly reduced liver fat in broiler chickens, regardless of source, showing a clear benefit in reducing liver fat.

Acknowledgements

The authors acknowledge the partial funding from Conselho Nacional de Pesquisa (CNPq – Brasilia, DF, Brazil), Coordenacão de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Vidara for their integrated supported in this project.

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306 Table 1. Composition of the experimental feeds.

Ingredients, % as is	Starter 1-14 d	Grower 15-28 d
Corn	62.57	74.09
Isolated soy protein, 80%	17.81	16.78
Broken rice	10.06	5.00
Soybean meal	5.00	-
Soybean oil	0.50	0.50
Dicalcium Phosphate	1.72	1.56
Limestone	1.44	1.27
DL-Methionine 99%	0.36	0.29
L-Lysine 78%	0.23	0.23
L-Threonine 98.5%	0.08	0.07
Salt	0.02	0.01
Vitamin and mineral mix[1]	0.20	0.20
Total	100.00	100.00
Calculated nutrient composition, % unless noted		
EMA_n, kcal/kg	3,248	3,319
CP	22.8 (22.3)	20.1 (20.4)
Ca	0.95	0.84
Av. P	0.46	0.42
Na	0.22	0.20
Dig. Lys	1.28	1.12
Dig. Met	0.67	0.57
Dig. TSAA	0.97	0.85
Dig. Thr	0.83	0.72
Dig. Val	0.97	0.86
Dig. Arg	1.44	1.24
Choline[2], mg/kg	700 (721)	600 (613)

307 [1]Composition per kilogram of feed: vitamin A, 12,000 IU; vitamin D_3, 3,000 IU; vitamin E, 100 IU; vitamin K_3, 6 mg; vitamin B12, 35 µg; thiamin,
308 3 mg; riboflavin, 15 mg; vitamin B6, 6 mg; niacin, 40 mg; pantothenic acid, 25 mg; folic acid, 4 mg; biotin, 0.3 mg; Zn, 65 ppm; Mn, 65 ppm; Fe,
309 40 ppm; Cu, 10 ppm; Se, 0.45 mg and I, 2 mg.
310 [2]Analyzed values are in the parentheses.
311

Table 2. Performance of broilers varying choline sources, g.

Treatment[2]	Body weight gain, g[1]				Feed conversion ratio				Feed intake, g			
	1 – 7	1 - 14	1 - 21	1 - 28	1 - 7	1 - 14	1 - 21	1 - 28	1 - 7	1 - 14	1 - 21	1 - 28
Basal[3]	99^{d}	334^{c}	736^{b}	1,296^{c}	1.135	1.170	1.307	1.359	112^{c}	391^{c}	961^{b}	1,762^{c}
444 mg/kg CC 60%	105^{bcd}	373^{ab}	794^{ab}	1,396^{ab}	1.131	1.164	1.302	1.355	118^{bc}	433^{a}	1,033^{ab}	1,892^{ab}
900 mg/kg CC 60%	106^{bcd}	373^{ab}	804^{a}	1,412^{ab}	1.128	1.157	1.295	1.350	120^{abc}	430^{ab}	1,042^{a}	1,906^{ab}
1,333 mg/kg CC 60%	116^{a}	381^{a}	816^{a}	1,434^{a}	1.123	1.156	1.292	1.348	130^{a}	441^{a}	1,053^{a}	1,932^{a}
111 mg/kg Kolin Plus[4]	101^{cd}	338^{bc}	759^{ab}	1,320^{bc}	1.134	1.171	1.304	1.356	115^{bc}	395^{bc}	989^{ab}	1,790^{bc}
222 mg/kg Kolin Plus	109^{abc}	345^{bc}	771^{ab}	1,400^{ab}	1.129	1.171	1.302	1.355	123^{ab}	405^{abc}	1,003^{ab}	1,897^{ab}
333 mg/kg Kolin Plus	111^{ab}	372^{ab}	804^{a}	1,426^{a}	1.121	1.156	1.295	1.350	125^{ab}	430^{ab}	1,038^{ab}	1,926^{a}
Mean	106	360	783	1,384	1.129	1.164	1.300	1.353	121	418	1,017	1,872
SEM	0.994	3.665	6.308	9.965	0.002	0.003	0.003	0.002	1.086	3.876	7.480	13.209
Probability	<0.0001	<0.0001	0.0029	<0.0001	0.2573	0.6692	0.8874	0.4690	<0.0001	0.0001	0.0047	0.0002

[1] Chick body weight at placement was 47.6 g ± 0.88.

[2]Treatments: 444 mg/kg from CC 60% (522 g/kg) = 932 mg/kg of choline; 900 mg/kg from CC60% (522 g/kg) = 1,170 mg/kg of choline; 1333 mg/kg from CC60% (522 g/kg) = 1,396 mg/kg of choline.

[3] Basal = feed starter from 1 to 14 d (721 mg/kg choline) and feed grower from 15 to 28 d (613 mg/kg choline).

[4] Composition of Kolin Plus = between 25 g/kg and 65 g/kg of phosphatidylcholine, according to the product label.

$^{a>b>c}$ Means with different letters in the same column indicate significant differences ($P < 0.05$).

Table 3. Carcass and abdominal fat yield of broilers fed diets supplemented with Choline Chloride 60% and Kolin Plus at 28 d, %[1].

Treatment	Carcass[5]	Abdominal Fat
Basal[2]	73.1	1.34
444 mg/kg CC 60%[3]	73.5	1.41
900 mg/kg CC 60%	73.0	1.36
1333 mg/kg CC 60%	73.0	1.38
111 mg/kg Kolin Plus[4]	73.0	1.34
222 mg/kg Kolin Plus	73.0	1.38
333 mg/kg Kolin Plus	73.7	1.33
Mean	73.2	1.36
SEM[5]	0.099	0.019
Probability <	0.3794	0.9264

[1] Probabilities of carcass and abdominal fat are presented after arcsine transformation.

[2] Basal = feed starter from 1 to 14 d (721 mg/kg choline) and feed grower from 15 to 28 d (613 mg/kg choline).

[3] Treatments: 444 mg/kg from CC 60% (522 g/kg) = 932 mg/kg of choline; 900 mg/kg from CC60% (522 g/kg) = 1,170 mg/kg of choline; 1333 mg/kg from CC60% (522 g/kg) = 1,396 mg/kg of choline.

[4] Composition of Kolin Plus = between 25 g/kg and 65 g/kg of phosphatidylcholine, according to the product label.

[5] Eviscerated carcasses as a percentage of body weight (total per treatment = 40 broilers).

Table 4. Blood variables and liver fat of broilers varying choline sources at 28 d[1].

Treatment	AST, U/L	ALT, U/L	TG, mg/dL	HDL, mg/dL	LDL, mg/dL	TC, mg/dL	H/L	Liver fat, %
Basal[2]	309.8[a]	31.0[a]	131[a]	62	27	119[a]	0.54	6.34[a]
444 mg/kg CC 60%[3]	285.9[ab]	22.6[ab]	98[b]	58	26	105[b]	0.54	5.93[ab]
900 mg/kg CC 60%	276.8[b]	21.6[ab]	94[b]	57	27	104[b]	0.51	5.78[ab]
1333 mg/kg CC 60%	274.3[b]	21.8[ab]	92[b]	59	24	103[b]	0.51	5.59[ab]
111 mg/kg Kolin Plus[4]	287.9[ab]	20.4[ab]	99[ab]	60	29	104[ab]	0.53	5.61[ab]
222 mg/kg Kolin Plus	279.1[b]	19.9[ab]	98[b]	58	28	104[ab]	0.54	5.55[ab]
333 mg/kg Kolin Plus	276.4[b]	16.7[b]	94[b]	59	28	103[b]	0.55	5.49[b]
Mean	284	22.0	101	59	27	106	0.53	5.75
SEM	2.463	1.1136	3.121	1.323	0.737	1.259	0.0223	0.078
Probability	0.0005	0.0294	0.0090	0.9810	0.7264	0.0018	0.9947	0.0433

[1]AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, TG = Triglyceride, HDL= High-density lipoprotein, LDL= Low-density lipoprotein, TC= Total Cholesterol, H/L: heterophil/lymphocyte.

[2] Basal = feed starter from 1 to 14 d (721 mg/kg choline) and feed grower from 15 to 28 d (613 mg/kg choline).

[3] Treatments: 444 mg/kg from CC 60% (522 g/kg) = 932 mg/kg of choline; 900 mg/kg from CC60% (522 g/kg) = 1,170 mg/kg of choline; 1333 mg/kg from CC60% (522 g/kg) = 1,396 mg/kg of choline.

[4] Composition of Kolin Plus = between 25 g/kg and 65 g/kg of phosphatidylcholine, according to the product label.

[a>b] Means with different letters in the same column indicate significant differences ($P < 0.05$).

334 Table 5. Regression equations of broilers fed diets supplemented with choline chloride 60%.

Item	Regression equations[1]	Model[2]	R^2	*P*	mg/kg
BWG 7 d, g	$Y = 98.6352 + 0.0118x$	L	0.4028	<0.0001	-
BWG 14 d, g	$Y = 344.0469 + 0.0318x$	L	0.2171	0.0024	-
BGW 21 d, g	$Y = 749.8929 + 0.0562x$	L	0.1963	0.0042	-
BGW 28 d, g	$Y = 1{,}320.3763 + 0.0962x$	L	0.2618	0.0007	-
FCR 7 d	$Y = 1.1346 – 0.000008x$	L	0.1486	0.0140	-
FI 7 d, g	$Y = 111.9126 + 0.0124x$	L	0.3867	<0.0001	-
FI 14 d, g	$Y = 401.5792 + 0.0332x$	L	0.2181	0.0024	-
FI 21 d, g	$Y = 979.7477 + 0.0639x$	L	0.1783	0.0066	-
FI 28 d, g	$Y = 1{,}793.8697 + 0.1185x$	L	0.2273	0.0019	-
AST[3], U/L	$Y = 309.3644 – 0.0619x + 0.000027x^2$	Q	0.3497	0.1000	1,146
ALT[4], U/L	$Y = 28.5456 – 0.0064x$	L	0.0978	0.0495	-
TC[5], mg/dL	$Y = 118.6224 – 0.0327x + 0.000016x^2$	Q	0.3946	0.0254	1,022
TG[6], mg/dL	$Y = 129.5096 - 0.0795x + 0.00004x^2$	Q	0.2797	0.0636	999
Liver fat, %	$Y = 6.2685 – 0.00054x$	L	0.1617	0.0101	-

335 [1]Regression equations obtained using the increasing choline chloride 60% in the diets (0, 444, 900 and 1333 ppm CC60%).
336 [2] L = linear, Q = quadratic.
337 [3]Aspartate aminotransferase.
338 [4] Alanine aminotransferase.
339 [5] Total Cholesterol.
340 [6] Triglyceride.
341

342 Table 6. Regression equations of broilers fed diets supplemented with Kolin Plus[1].

Item	Regression equations[1]	Model[2]	R^2	*P*	mg/kg
BWG 7 d, g	$Y = 98.4592 + 0.0405x$	L	0.4631	<0.0001	-
BWG 14 d, g	$Y = 329.3895 + 0.1082x$	L	0.3296	0.0001	-
BGW 21 d, g	$Y = 734.8022 + 0.1954x$	L	0.3628	<0.0001	-
BGW 28 d, g	$Y = 1,290.0886 + 0.4249x$	L	0.5454	<0.0001	-
FCR 7 d	$Y = 1.1365 – 0.00004x$	L	0.0894	0.0609	-
FI 7 d, g	$Y = 111.9297 + 0.0416x$	L	0.3970	<0.0001	-
FI 14 d, g	$Y = 386.1344 + 0.1146x$	L	0.2983	0.0003	-
FI 21 d, g	$Y = 961.0831 + 0.2210x$	L	0.3747	<0.0001	-
FI 28 d, g	$Y = 1,753.5355 + 0.5401x$	L	0.5157	<0.0001	-
AST[3], U/L	$Y = 309,4570 – 0.2283x + 0.00039x^2$	Q	0.5198	0.0258	293
ALT[4], U/L	$Y = 28.5110 – 0.0391x$	L	0.3413	<0.0001	-
TC[5], mg/dL	$Y = 118.5421 – 0.1389x + 0.00028x^2$	Q	0.3388	0.0282	235
TG[6], mg/dL	$Y = 129.2993 – 0.2930x + 0.00058\ x^2$	Q	0.2553	0.0870	253
Liver fat, %	$Y = 6.1357 – 0.00234x$	L	0.1848	0.0056	-

343 [1] Regression equations obtained using the increasing Kolin plus in the diets (0, 111, 222 and 333 ppm Kolin Plus).
344 [2] L = linear, Q = quadratic
345 [3]Aspartate aminotransferase.
346 [4] Alanine aminotransferase.
347 [5]Total Cholesterol.
348 [6] Triglyceride.
349

CAPÍTULO III

4. CONSIDERAÇÕES FINAIS

O presente estudo demonstra o papel crucial na elucidação da aplicabilidade de uma fonte natural de colina em dietas destinadas a frangos de corte. Os resultados obtidos indicaram que o desempenho zootécnico foi significativamente superior em comparação ao tratamento sem suplementação de qualquer fonte de colina. Adicionalmente, a fonte natural de colina demonstrou respostas equiparáveis ao cloreto de colina, evidenciando uma correlação linear positiva entre o aumento dos níveis de colina e melhorias concomitantes no ganho de peso e no consumo de ração. Este achado sugere uma promissora alternativa de fonte natural de colina em substituição ao tradicional cloreto de colina em dietas para frangos de corte, proporcionando respostas para a otimização do desempenho zootécnico dessas aves.

Neste estudo, foi possível constatar que a suplementação de ambas as fontes de colina não exerceu efeito significativo sobre a quantidade de gordura abdominal e o rendimento de carcaça das aves. Esses achados corroboram com resultados de estudos anteriores que investigaram a influência de ambas as formas de colina nas dietas de frangos de corte.

Ao analisar as respostas hematológicas, foi possível identificar um padrão linear para a enzima ALT em ambas as fontes de colina suplementadas. Mais especificamente, ao aumentar gradativamente os níveis de suplementação de cloreto de colina, observou-se uma redução de 30% nos níveis dessa enzima no sangue das aves. Por outro lado, a fonte natural de colina demonstrou uma diminuição ainda mais pronunciada, alcançando uma redução de 46% nos níveis da referida enzima circulante. Esses resultados indicam que a suplementação de colina, independentemente de ser na forma de cloreto ou de uma fonte natural, exerce efeitos distintos nos parâmetros hematológicos. No que diz respeito ao colesterol total, triglicerídeos e AST (aspartato aminotransferase), as respostas foram quadráticas, revelando um ponto de suplementação médio ideal de cloreto de colina de 1.055 mg/kg na dieta de frangos de corte, e para a fonte natural de 260 mg/kg. Esta evidência sugere que há uma relação não linear entre a suplementação de colina e esses parâmetros específicos, destacando a importância da dosagem adequada para otimizar os benefícios desejados.

Na análise da gordura no fígado, foi observada uma resposta linear à suplementação de ambas as fontes de colina, indicando que os níveis de gordura

diminuíram proporcionalmente com a crescente suplementação de colina. Esses achados estão em consonância com dados encontrados na literatura, onde a colina é reconhecida como um agente hepatoprotetor.

Conduzindo uma análise de equivalência entre as duas fontes, observou-se que a colina proveniente de uma fonte natural pode ser suplementada em uma proporção 3,7 vezes menor do que o cloreto de colina para alcançar respostas superiores no desempenho zootécnico. Em relação aos indicadores hematológicos e à gordura no fígado, essa equivalência corresponde a uma redução de 4,4 vezes na quantidade de colina natural necessária para atingir resultados semelhantes. Essa constatação sugere uma eficiência significativamente maior na utilização da colina proveniente de fontes naturais em comparação ao cloreto de colina, destacando a viabilidade dessa abordagem como uma estratégia eficaz para otimizar a suplementação de colina em dietas para frangos de corte.

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6. APÊNDICES

Apêndice 1: Normas para publicação de artigos no periódico Journal of Applied Poultry Research

Journal Applied Poultry Research Guide to Authors[1]

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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 dimensions 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.

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MISCELLANEOUS USAGE NOTES

Abbreviations

The following abbreviations may be used without definition in JAPR. Plurals do not require "s." Chemical symbols and 3-letter abbreviations for amino acids do not need definition. Other abbreviations should be defined at first use in the summary and the main text, as well as in each table or figure in which they appear. Author-defined abbreviations are boldface at first use in the main text. Abbreviations should not be used in the manuscript title, running title, or to begin a paragraph or sentence. They can be used in section headings if previously defined. This list appears inside the back cover of each issue of the journal.

The following abbreviations may be used without definition in JAPR:

ADF acid detergent fiber

ADFI average daily feed intake

ADG average daily gain

AME apparent metabolizable energy

AMEn nitrogen-corrected apparent metabolizable energy

ANOVA analysis of variance AOAC Association of Official Analytical Chemists

BSA bovine serum albumin

BW body weight

°C Celsius

cDNA complementary DNA

CF crude fiber

cfu colony-forming units (following a numeral)
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
EE ether extract
ELISA enzyme-linked immunosorbent assay
°F Fahrenheit
FCR feed conversion ratio
FE feed efficiency
ft foot
g gram
gal gallon
G:F gain-to-feed ratio
GLM general linear model
h hour
HEPES N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid
HPLC high-performance (high-pressure) liquid chromatography
ICU international chick units
Ig immunoglobulin
IL interleukin
i.m. intramuscular
in. inch
i.p. intraperitoneal
IU international units
i.v. intravenous
kcal kilocalorie
L liter (also capitalized with any combination, e.g., mL)
lb pound

L:D hours of light:hours of darkness in a photoperiod
LSD least significant difference
m meter
µ micro
M molar
ME metabolizable energy
MEn nitrogen-corrected metabolizable energy
MHC major histocompatibility complex
mRNA messenger ribonucleic acid
min minute
mo month
MS mean squares
n number of observations
NADH reduced form of NAD
NDF neutral detergent fiber
NRC National Research Council
NS not significant
PBS phosphate-buffered saline
PCR polymerase chain reaction
mg/kg parts per million
r correlation coefficient
r2 coefficient of determination, simple
R2 coefficient of determination, multiple
RH relative humidity
RIA radioimmunoassay
RNA ribonucleic acid
rpm revolutions per minute
s second
SAS Statistical Analysis System
s.c. subcutaneous
SD standard deviation
SE standard error
SEM standard error of the mean
SNP single nucleotide polymorphism

SRBC sheep red blood cells
TBA thiobarbituric acid
T cell thymic-derived cell
TME true metabolizable energy
TMEn nitrogen-corrected true metabolizable energy
TSAA total sulfur amino acids
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

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Reporting sex- and gender-based analyses

Reporting guidance

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7. VITA

Douglas Drebes Brunhaus Maria, filho de Marco Antônio Brunhaus Maria e Janice Drebes Maria, nascido em 24 de dezembro de 1993, em Porto Alegre – RS. Realizou o ensino fundamental e médio no Instituto Estadual de Educação Isabel de Espanha, concluindo os estudos em dezembro de 2011. Em 2012 ingressou no curso técnico em informática na QI faculdades, cursou três semestres, mas não concluiu. Em agosto de 2014 iniciou a graduação em Zootecnia na Universidade Federal do Rio Grande do Sul. Fez parte do grupo de pesquisa Aviário de Ensino e Pesquisa, supervisionado pelo professor PhD. Sergio Luiz Vieira, desde agosto de 2014, totalizando 8 anos entre a graduação e o mestrado. No último semestre da faculdade, em 2020, foi estagiário na empresa Granja Santa Lívia, na cidade de Garibaldi – RS, tendo a oportunidade de conhecer a parte de produção de frangos de corte e na parte de coordenação de pesquisas científicas dentro da área de produção. Formou-se em janeiro de 2021. No primeiro semestre de 2021 ingressou como aluno de mestrado com dedicação exclusiva no Programa de Pós-Graduação em Zootecnia da UFRGS, sob orientação do professor Ph.D. Sergio Luiz Vieira. Além de ter se envolvido em diversos projetos de pesquisa ao longo do seu mestrado, teve a oportunidade de participar de eventos científicos nacionais e internacionais, onde em ambos realizou apresentações orais em inglês sobre trabalhos desenvolvidos no Aviário de Ensino e Pesquisa. No segundo semestre de 2022 realizou a troca de grau de mestrado para doutorado através de progressão, onde foi submetido à banca de defesa de Dissertação em 27 de novembro de 2023.