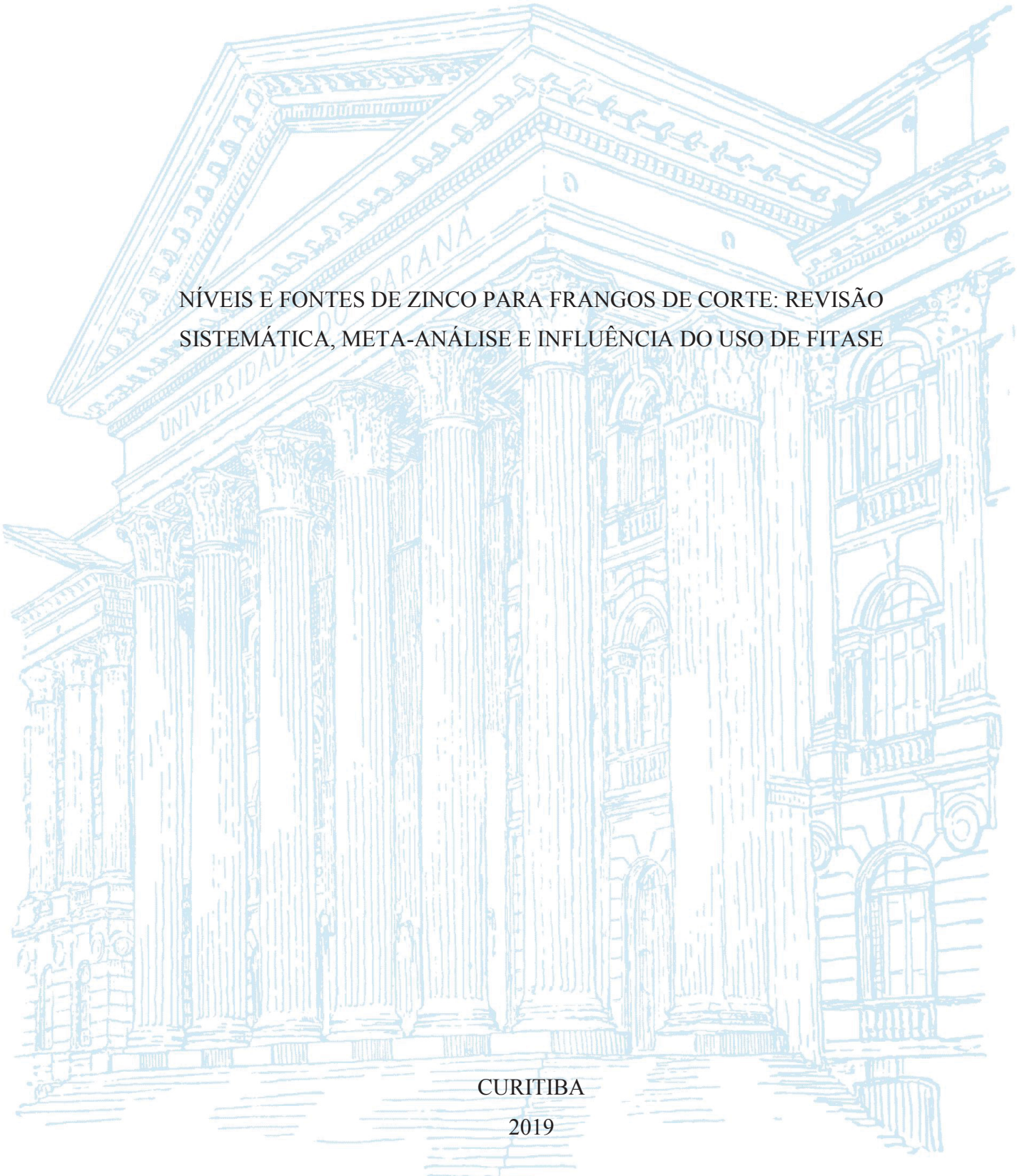
UNIVERSIDADE FEDERAL DO PARANÁ

EDUARDO TREVISOL



NÍVEIS E FONTES DE ZINCO PARA FRANGOS DE CORTE: REVISÃO SISTEMÁTICA, META-ANÁLISE E INFLUÊNCIA DO USO DE FITASE

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2019

EDUARDO TREVISOL

NÍVEIS E FONTES DE ZINCO PARA FRANGOS DE CORTE: REVISÃO SISTEMÁTICA, META-ANÁLISE E INFLUÊNCIA DO USO DE FITASE

Dissertação apresentada ao curso de Pós-Graduação em Zootecnia, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Zootecnia.

Orientador: Prof. Dr. Alex Maiorka

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ZOOTECNIA da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **EDUARDO TREVISOL** intitulada: **Níveis e fontes de zinco para frangos de corte: revisão sistemática, meta-análise e influência do uso de fitase**, sob orientação do Prof. Dr. ALEX MAIORKA, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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RESUMO

Em geral, atribui-se aos minerais complexados a compostos orgânicos uma maior biodisponibilidade relativa perante fontes inorgânicas, porém os resultados mostram-se ainda inconsistentes. Para estudar de forma mais aprofundada este tema, foi realizada uma revisão de literatura e também duas avaliações, sendo uma meta-análise e um teste em granja experimental. Na primeira avaliação foi realizada uma revisão sistemática dos trabalhos já publicados sobre o uso de zinco para frangos de corte e posteriormente executada uma meta-análise, buscando entender quais fatores poderiam estar influenciando nos resultados quando comparadas fontes orgânicas a inorgânicas, avaliando o consumo médio diário de ração (CMDR), ganho de peso diário (GPD), conversão alimentar (CA) e concentração de zinco nos principais tecidos. Da base de dados final para a meta-análise, constatou-se que 91% dos tratamentos não continham fitase, 70% possuíam níveis de Ca total na dieta superiores a 0,9% e 28% de todas as fontes inorgânicas usadas para comparação eram ZnO. A CA de aves recebendo zinco orgânico foi melhor que a de aves consumindo ZnO ($P<0,001$), porém igual ao de aves consumindo $ZnSO_4$ ($P=0,10$). Em dietas contendo fitase, não houve diferença entre as fontes para nenhuma das variáveis em estudo. Já em dietas sem fitase, aves consumindo zinco orgânico apresentaram maior CMDR ($P<0,001$), maior GPD ($P<0,001$) e maior concentração de zinco na tíbia ($P<0,001$), porém a mesma CA ($P=0,15$) que aves consumindo $ZnSO_4$. Não houve diferença na concentração de zinco no fígado entre as fontes ($P=0,48$), porém no plasma, aves que consumiram zinco orgânico apresentaram maior teor de zinco que aves consumindo $ZnSO_4$ ($P=0,02$), porém com a ressalva de que dos 10 comparativos desta análise, apenas 1 apresentava fitase na dieta. Quanto aos níveis de Ca utilizados, nos tratamentos com teores menores que 0,8%, não houve diferença para CA ($P=0,26$), GPD ($P=0,80$) e CMDR ($P=0,49$). Já para dietas com nível de Ca superior a 1%, as aves consumindo zinco orgânico apresentaram maior CMDR ($P<0,001$), maior GPD ($P=0,02$), porém a mesma CA ($P=0,79$). Com relação ao experimento *in vivo*, 3.600 pintos de 1 dia foram distribuídos dentre 10 tratamentos, utilizando-se um delineamento inteiramente casualizado. Foram testadas 2 fontes de zinco ($ZnSO_4$ e zinco complexado a aminoácido), 2 níveis de fitase (500 e 1500 FTU/kg) e 3 níveis de zinco suplementar (0, 30 e 60 mg/kg). Aves recebendo dietas sem zinco suplementar apresentaram maior consumo que aves recebendo 30 ou 60 mg/kg ($P<0,001$), porém resultando em uma pior CA ($P<0,001$). Dietas contendo 30 e 60 mg/kg de zinco, não diferiram entre si para as variáveis estudas. O uso de complexo zinco aminoácido melhorou a conversão alimentar, mas não o GPD e consumo quando comparado à dietas com sulfato de zinco. Não houve diferença na concentração de Zn no fígado e tíbia perante os tratamentos.

Palavras-chave: Elementos traço. Microminerais. Fitato. Cálcio. Calcário.

ABSTRACT

In general, minerals that are complexed to organic compounds show a higher relative bioavailability, although these results are still inconsistents. In order to deeply evaluate this topic, a literature review and two studies were performed, a meta-analysis and a broiler trial. In the first topic, a systematic review of published papers about zinc for broilers was done, with a further meta-analysis carried out, aiming to understand which factors could be influencing the final results when comparing organic to inorganic zinc sources, using the variables average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR) and tissue zinc concentration. From the final database for the meta-analysis, 91% of treatments didn't contain phytase, 70% had Ca levels above 0.9% and 28% of all inorganic sources used for comparison were ZnO. Birds fed with organic zinc had better FCR than the ones fed with ZnO ($P<0.001$), but the same as broilers fed with $ZnSO_4$ ($P=0.10$). When phytase was dosed, there was no difference for any of the variables in evaluation. On the other hand, without phytase, broilers consuming organic zinc had higher ADFI ($P<0.001$), higher ADG ($P<0.001$) and higher tibia zinc content ($P<0.001$), although the same FCR ($P=0.15$) than birds fed with $ZnSO_4$. In liver, no zinc concentration difference was found ($P=0.48$), but in plasma, birds fed with organic zinc showed higher Zn content than the ones fed with $ZnSO_4$ ($P=0.02$). Although it is important to mention that from the 10 comparisons made for this analysis, in just one of them phytase was dosed. Regarding diet's Ca levels, no difference was found for FCR ($P=0.26$), ADG ($P=0.80$) and ADFI ($P=0.49$) when Ca was below 0.80%. On the other hand, at diet's Ca above 1%, birds fed with organic zinc presented higher ADFI ($P<0.001$), higher ADG ($P<0.001$), but the same FCR ($P=0.79$). Regarding the *in vivo* experiment, 3,600 one day old chicks were distributed along 10 treatments, in a completely randomized design, comprehending 2 sources of zinc ($ZnSO_4$ or zinc aminoacid complex), 2 phytase levels (500 or 1,500 FTU/kg) and 3 levels of supplemental zinc (0, 30 or 60 mg/kg). Birds fed with diets without supplemental zinc showed higher feed intake than the ones fed with 30 or 60 mg/kg ($P<0,001$), but resulting in a worse FCR ($P<0,001$). Diets containing 30 or 60 mg/kg of zinc did not differ between each other for the studied variables. Zinc amino acid complex improved FCR, but not ADG and feed intake when compared to zinc sulfate. Tibia and liver zinc concentration didn't change in any of the treatments.

Keywords: Trace elements. Microminerals. Phytate. Calcium. Limestone.

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LISTA DE ABREVIATURAS OU SIGLAS

SINDIRAÇÕES – Sindicato Nacional da Indústria de Alimentação Animal

BDR – Biodisponibilidade Relativa

SOD – Superóxido Dismutase

ROS – Espécies Reativas de Oxigênio

AC – Anidrase Carbônica

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1 REVISÃO DE LITERATURA

1.1 INTRODUÇÃO

Os microminerais ou elementos traço, denominados assim por serem necessários aos animais apenas em miligramas ou microgramas diariamente, têm sido suplementados nas dietas ainda com grande margem de segurança, baseado no conceito de que representam pouco do custo total da ração (GOFF, 2018). Entretanto, considerando o volume de 31,7 milhões de toneladas de ração produzidas para frangos de corte no Brasil em 2018 (SINDIRAÇÕES, 2019), a um custo médio de R$1.130 por tonelada (EMBRAPA, 2018), somente com microminerais temos um dispêndio aproximado de R$71,6 milhões por ano.

Um destes microminerais suplementados, o zinco, tem atuação como cofator em diversas enzimas do organismo, influenciando na resposta imunitária celular e humoral, crescimento e manutenção das penas e atuando como agente antioxidante (AO; PIERCE, 2013; NAZ et al., 2016; ABD EL-HACK et al., 2017; NYS, 2018). Desta forma, diferentes fontes de zinco para frangos de corte têm sido objeto de estudo, em especial as fontes complexadas a moléculas orgânicas, como aminoácidos, proteínas, carboidratos, entre outros (MANANGI et al., 2012; STAR et al., 2012; LIU et al., 2013), comumente denominadas de minerais orgânicos.

Alguns trabalhos mostram que as fontes orgânicas são mais biodisponíveis que as fontes inorgânicas em virtude de serem moléculas menos reativas com os demais componentes da digesta e possuírem mecanismos de absorção intestinal distintos e com menor competição por sítios de absorção (STAR et al., 2012). Entretanto, em relação à biodisponibilidade entre as fontes, os resultados encontrados na literatura são controversos. Por meio de meta-análise, Schlegel, Sauvant e Jondreville (2012) concluíram que,

dependendo da variável considerada para o cálculo da biodisponibilidade relativa (BDR), o resultado pode variar de 93 a 113% em frangos de corte. Já para suínos, considerando variáveis como zinco plasmático, zinco no fígado e zinco ósseo, a biodisponibilidade oscilou entre 85 e 117%.

De acordo com Schlegel, Nys e Jondreville (2010), o fitato não influencia na biodisponibilidade de fontes orgânicas ante inorgânicas, sugerindo que sua interação antagonística ocorre apenas com o zinco naturalmente presente nos ingredientes vegetais, e não com o zinco suplementado. De forma oposta, Swiatkiewicz, Koreleski e Zhong (2001) mostraram que a BDR de fontes orgâncias ante inorgâncias foi influenciada negativamente com a adição de fitase às dietas, indicando assim que pode haver superestimação neste indicador quando não adicionada fitase às dietas experimentais. Corroborando com estes resultados, Huang et al. (2013) verificaram que a adição de fitato às dietas resulta em aumento da BDR de fontes orgânicas quando comparadas ao sulfato de zinco.

Dito isso, o uso de fitase nas dietas de frangos de corte pode elevar o aproveitamento do zinco presente nos ingredientes vegetais, embora em menor grau quando comparado à espécie suína. Acredita-se que a menor eficiência da fitase nas aves esteja atrelada a maior acidez gástrica, que resulta em aumento da solubilidade e consequentemente disponibilização do zinco à absorção (SCHLEGEL, NYS; JONDREVILLE, 2010). Neste trabalho, entretanto, estudou-se apenas a influência do uso de dose padrão de fitase produzida por *Aspergillus niger* nas dietas, portanto não se sabe o efeito que o uso de doses elevadas e também que outras fitases possam exercer.

Para as agroindústrias é essencial que se tenham respostas assertivas quanto ao uso ou não de fontes de zinco complexadas a moléculas orgânicas, para isso buscando resposta de variáveis que tragam retorno à cadeia de produção. Desta forma, este trabalho tem por objetivo realizar uma revisão sistemática e meta-análise dos experimentos publicados na

literatura científica sobre fontes de zinco para frangos de corte, comparando os dados de desempenho zootécnico e concentração tecidual de zinco, bem como avaliar em granja experimental os níveis nutricionais de zinco na forma de sulfato ou complexo zinco aminoácido e uso de fitase de *Buttiauxela spp* expressa em *Trichoderma reesei* em dose padrão e *super-dosing* nas dietas para frangos de corte sobre variáveis de desempenho e concentração óssea e hepática de zinco.

1.2 ZINCO PARA FRANGOS DE CORTE

Apresentações do zinco e características químicas

O zinco é o segundo microelemento mais abundante nos vertebrados, atrás apenas do elemento ferro. Encontra-se classificado na tabela periódica no grupo dos metais de transição, possuindo dois elétrons em sua camada de valência e assim sendo classificado como um metal bivalente (EUROPEAN FOOD SAFETY AUTHORITY - EFSA, 2012). Esta característica determina o número de ligações químicas que o elemento pode realizar com outros átomos, buscando uma configuração eletrônica estável (MORTIMER, MOL; DUARTE, 1994).

Para uso como suplemento nas dietas de animais de produção, encontra-se zinco disponível no mercado sob diversas formas, algumas denominadas inorgânicas, como por exemplo o óxido de zinco, sulfato de zinco, carbonato de zinco e cloreto de zinco, e outras chamadas de fontes orgânicas, como o acetato de zinco, proteinato de zinco, zinco polissacarídeo, propionato de zinco, gluconato de zinco, picolinato de zinco e quelatos de aminoácido e zinco (CANO-SANCHO, 2014).

Até a década de 90, em torno de 80% das agroindústrias utilizavam óxido de zinco como fonte de suplementação de zinco para frangos de corte. Entretanto dados experimentais mostram que sua BDR é inferior à forma de sulfato (WEDEKIND, 1990).

Já em relação às fontes orgânicas, acredita-se que sejam mais biodisponíveis que fontes inorgânicas, atribuindo isso a fatores como solubilidade elevada, estabilidade química e neutralidade elétrica, desta forma não possuindo íons metálicos livres para formação de complexos insolúveis indesejáveis e por chegarem aos sítios de absorção de forma intacta, sendo absorvidos em sua forma original (BROWN; ZERINGUE, 1994).

Funções metabólicas

O zinco é um dos nutrientes essenciais suplementados nas dietas para aves comerciais. Ele atua como cofator em mais de 200 enzimas do organismo, influenciando por exemplo a resposta imunitária celular e humoral, o crescimento e a manutenção das penas, atuando como agente antioxidante e na função hormonal, como detalhado em revisões de literatura previamente publicadas (AO; PIERCE, 2013; NAZ et al., 2016; ABD EL-HACK et al., 2017; NYS et al., 2018).

Em relação às reações antioxidantes, o seu papel está ligado ao fato de ser um dos componentes principais da enzima superóxido dismutase (SOD), fundamental na neutralização das espécies reativas de oxigênio (ROS) (NAZ et al., 2016). As ROS são geradas por células inflamatórias, atuando na destruição de DNA e proteínas, processo importante no mecanismo de defesa contra bactérias. Em reações exacerbadas, a mesma destruição de DNA pode se estender aos tecidos corporais, sendo, entretanto, prevenida pela ação de antioxidantes como a SOD (SIMON, HAJ-YEHIA; LEVI-SCHAFFER, 2000). A SOD atua catalizando a reação $O_2^- + O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$, neutralizando radicais

superóxido, e assim sendo essencial a sobrevivência de qualquer organismo em ambiente aeróbico (MCCORD, KEELE JR; FRIDOVICH, 1971). Tem, portanto, uma função defensiva à célula, sendo abundante em organismos aeróbicos e ausentes ou presentes em quantidades muito pequenas em anaeróbicos obrigatórios (FRIDOVICH, 1986).

Além da SOD, estudos elementares de purificação da enzima anidrase carbônica (AC) apontam que se trata de um composto zinco-proteico, haja vista a concentração de 0,31-0,33% de zinco, a qual é semelhante à concentração de ferro presente na hemoglobina, por exemplo (O'DELL, 1992). A AC atua catalisando a hidratação reversível do dióxido de carbono $CO_2 + H_2O \leftrightarrows, HCO_3^- + H^+$ (LINDSKOG, 1997), envolvida em diversos mecanismos metabólicos, dentre eles o transporte de CO_2 para eliminação via pulmões, equilíbrio ácido base e solubilização da matriz óssea para reabsorção via osteoclastos (BOYLE; SIMONET; LACEY, 2003; TAKAHASHI et al., 2007) A remoção do íon zinco da anidrase carbônica resulta em uma apoenzima inativa, retomando sua atividade completa com a reincorporação do metal à sua estrutura (LINDSKOG, 1997).

A carboxipeptidase, enzima responsável pela clivagem de peptídeos na terminação carboxil, possui zinco em sua constituição. Estudos realizados removendo o átomo de zinco da estrutura enzimática resultaram em uma apoenzima inativa (LIPSCOMB, 1970), embora a substituição por outros íons metálicos, como manganês e ferro por exemplo, resulte em uma enzima com funcionalidade (SCHEINER; LIPSCOMB, 1977). Os dados apresentados por Bao et al. (2009) mostram que dietas suplementadas com proteinatos de zinco e manganês resultaram em aumento da digestibilidade proteica, podendo ter relação com as metaloenzimas que contem estes minerais em sua composição.

Atribui-se ao zinco também a manutenção e melhoria na resistência da pele por sua ligação com a síntese de ácido nucléico e colágeno, impactando positivamente no número de camadas de células epiteliais e aumento da flexibilidade e resiliência da pele, refletindo em

melhoria das carcaças de frango ao abate (ROSSI et al., 2007). Ainda, atribui-se ao zinco a melhora em rendimento de cortes no frigorífico. Fontes orgânicas de zinco resultam em incremento no rendimento de peito, fato relacionado a melhora na saúde das aves em geral, maior biodisponibilidade do zinco orgânico e consequentemente uso para deposição muscular e não defesa do sistema imune (MARCO et al., 2017).

Exigências nutricionais

Considerando os ingredientes tradicionalmente com maior participação em dietas para frangos de corte, como o milho, farelo de soja e trigo, é possível verificar que a concentração nestes é relativamente baixa, sendo de aproximadamente 21, 46 e 53 mg/kg, respectivamente (ROSTAGNO, 2017). Além da baixa concentração, encontra-se grande variação nutricional nos ingredientes por diversos fatores, dentre eles a genética, tipo de solo, pluviometria e origem, como visto no farelo de soja, o qual pode apresentar concentração de zinco com variações de 42 até 84 mg/kg dependendo de sua origem (GARCÍA-REBOLLAR et al., 2016).

Dados de diversos experimentos publicados mostram que aves recebendo dietas sem suplementação de zinco apresentam redução de desempenho, confirmando assim a necessidade da suplementação para a expressão de todo o potencial genético (HUANG et al., 2007; BAO et al., 2009; SALIM et al., 2012). As dietas basais (sem suplementação) para tais experimentos apresentavam dentre 21 e 32 mg/kg, indicando que estes níveis de zinco são insuficientes para atender o requerimento de frangos de corte.

Se analisarmos as diferentes fontes bibliográficas sobre o tema de níveis nutricionais de zinco para frangos de corte, vê-se ainda uma grande variação na recomendação de suplementação (Tabela 1), que pode ser explicada por diversos fatores como: variação na

concentração de zinco nos ingredientes da dieta, presença de fatores anti qualitativos e antagonísticos à absorção de zinco e variáveis dependentes estudadas para determinação do nível ótimo de suplementação (NYS et al., 2018).

Tabela 1. Recomendações de suplementação de zinco (mg/kg) na dieta de frangos de corte na primeira semana de vida.

COBB, 2015	ROSS, 2014	ROSTAGNO, 2017	NRC, 1994
100,00	110,00	76,15	40,00

Fontes: *Cobb Broiler Performance and Nutrition Supplement* (2015); *Ross 308 Nutrition Specifications* (2014); Rostagno (2017); NRC (1994).

Somado à questão da baixa concentração nos ingredientes, cabe ressaltar que a presença de fitato nas dietas pode atuar de forma antagonística na disponibilização do zinco à digestão e absorção, como mostrou Yu et al. (2010), cuja avaliação com níveis crescentes de fitato adicionados à dieta resultaram em redução na absorção intestinal de zinco. De forma oposta, Schlegel, Nys e Jondreville (2010) afirmam que o fitato afeta apenas a disponiblização do zinco naturalmente presente nos ingredientes, assim não exercendo influência sobre o zinco suplementado, independente da fonte utilizada. De uma forma ou de outra, estes resultados reforçam a necessidade e importância da suplementação de fitase às dietas, a qual resulta na quebra da molécula de fitato, um forte agente quelante que complexa as moléculas de zinco e as torna indisponíveis à absorção intestinal.

De acordo com Erdman (1979), o ácido fítico tem a capacidade de formar quelatos com outros minerais quando em pH neutro, como ilustrado na Figura 1. Entretanto, Sebastian et al. (1996) mostram que a retenção relativa de zinco torna-se aumentada quando a fitase é suplementada à dieta, atribuindo à maior disponibilidade do zinco do complexo fitato-mineral.

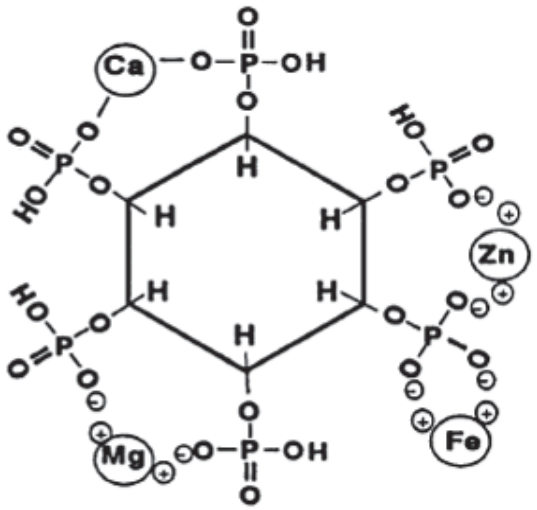

Figura 1. Ácido fítico e interação com minerais em pH neutro.

Além do fitato, outras moléculas podem exercer este mesmo efeito quelante sobre os íons de zinco no intestinal, reagindo entre si e interferindo na absorção. Um exemplo clássico são as interações antagonísticas entre diferentes minerais, principalmente com cobre, molibdênio, cobalto, ferro, potássio, cálcio, fósforo e sódio. A carga iônica presente no átomo de zinco é o que o torna tão reativo com outras moléculas, podendo haver compartilhamento de elétrons e formação de outros complexos que se tornam indisponíveis aos animais (GEORGIEVSKII, ANNENKOV; SAMOKHIN, 2013). Além desta interação, alguns minerais compartilham dos mesmos canais de absorção intestinal, como é o caso do ferro, absorvido através das proteínas transportadoras de metais bivalentes, DMT1 (do termo em inglês, *Divalent Metal Transporter 1*), as quais também atuam na absorção do zinco. Desta forma, níveis elevados de ferro podem retroalimentar negativamente a expressão de DMT1, podendo reduzir a absorção de zinco (GOFF, 2018).

Outro fator que pode interferir na absorção de zinco é o nível de cálcio da dieta, o qual em níveis elevados levam a uma menor absorção de zinco, sendo de forma mais acentuada com fontes inorgânicas do que com orgânicas (WEDEKIND et al., 1994). De acordo com Angel et al. (2002), normalmente no pH encontrado no trato gastrointestinal, a

fitina possui uma forte carga negativa que favorece a complexação de cátions di e trivalentes, como é o caso do Zn^{2+}, tornando-os indisponíveis à absorção.

Maiores inclusões de calcário à dieta mostraram afetar negativamente a degradação do fitato, também relacionado ao aumento do pH na porção proximal do trato gastrointestinal (AMERAH et al., 2014). Desta forma, a disponibilidade de zinco reduzida com níveis de cálcio elevados nas dietas pode ser explicada pela maior presença de fitato e também pelo aumento do pH, o qual favorece a complexação de cátions à molécula (ERDMAN, 1979).

Digestão, absorção e metabolismo

A absorção de minerais em geral ocorre de duas maneiras: através de transporte transcelular, meio em que proteínas especializadas localizadas na membrana apical das células são responsáveis por captar os minerais do lúmen intestinal e conduzi-los ao citosol; ou via transporte paracelular, isto é, através dos poros das junções intercelulares. Este tipo de transporte é possível quando há um gradiente eletroquímico no lúmen intestinal que permita que os íons sejam empurrados em direção ao espaço intersticial ou também quando os minerais se solubilizam no meio aquoso do lúmen e assim sejam carreados quando da absorção de água (GOFF, 2018).

Embora a absorção paracelular possa ser considerável, principalmente quando solubilizadas à água, o influxo de zinco através da membrana apical ocorre no intestino delgado principalmente por processo transcelular (GOFF, 2018) que neste caso é mediado por um grupo de proteínas denominadas ZIP (LIUZZI; COUSINS, 2004). A expressão destas proteínas é regulada pela concentração de zinco no citosol do enterócito, elevando-se quando há baixo zinco intracelular, para que assim possa aumentar a absorção do lúmen intestinal ou também fazendo o processo inverso, na qual a expressão de ZIP é reduzida para evitar

intoxicação dos animais que possuem concentração citosólica suficiente ao metabolismo (MAO et al., 2007).

Além do transporte através das proteínas ZIP, sabe-se que a absorção pode ocorrer por meio da DMT1 (MACARI, MAIORKA; MASSUQUETTO, 2017), embora tenha que competir por sítios de ligação com o ferro e manganês (GOFF, 2018). No caso específico da DMT1, sua expressão é regulada pelo status de ferro no organismo, desta forma quando há repleção de ferro, há menor expressão de DMT1 e em caso de excesso de ferro na dieta, haverá menor absorção de zinco devido a competição por sítios de absorção (GOFF, 2018).

Os minerais complexados a moléculas orgânicas por outro lado possuem mecanismos distintos de absorção, como por exemplo os complexados a aminoácidos, que chegam ao intestino e são absorvidos por sítios de absorção de aminoácidos, por isso resultando em maior biodisponibilidade (STAR et al., 2012). Para que isso ocorra, o complexo mineral-aminoácido deve chegar ao sítio de absorção sem que ocorra sua dissociação, desmembrando a molécula orgânica do mineral, o que está ligado diretamente a constante de estabilidade do complexo, conforme demonstrado em trabalhos recentes (LIU et al., 2013; LIU et al., 2011). Esta constante de estabilidade fornece informações sobre a força com que ligantes permanecem unidos ao metal sob condições de pH fisiológicas, embora poucos estudos ainda tenham sido realizados correlacionando análises *in vitro* e *in vivo* (CAO et al., 2000).

Outros trabalhos têm sido publicados abordando o uso de diferentes fontes e níveis de zinco para frangos de corte, sendo os resultados controversos. Star et al. (2012) por exemplo, avaliando o nível de zinco na tíbia, encontraram maior biodisponibilidade do zinco complexado a aminoácido quando comparado ao sulfato de zinco. Por outro lado, neste mesmo experimento, quando o sulfato de zinco foi suplementado a 40 mg/kg na ração, não houve diferença em relação à forma complexada, levando a crer que a fonte inorgânica

poderia ser utilizada ainda com segurança. Além disso, Schlegel, Sauvant e Jondreville (2012), através de meta-análise, concluíram que a BDR das fontes orgânicas em frangos de corte pode variar entre 93 e 113% dependendo da variável escolhida para cálculo.

Ao serem incorporados ao citoplasma, os átomos de zinco ligam-se à molécula de metalotioneína, servindo como fonte de reserva e também exercendo retroalimentação negativa sobre a expressão de sítios de absorção (LIUZZI; COUSINS, 2004; DAVIS; COUSINS, 2000). Por outro lado, para sair da célula em direção ao espaço intersticial, o zinco é carreado através de proteínas transportadoras denominas Znt presentes na membrana basolateral das células (LIUZZI; COUSINS, 2004), sendo então transportadas na corrente sanguínea ligadas principalmente à albumina até o sítio de ação (GOFF, 2018).

1.3 REVISÃO SISTEMÁTICA E META-ANÁLISE

A revisão sistemática tem como objetivo agrupar as informações de publicações científicas ou outras bases de dados que atendam critérios de eligibilidade pré-definidos, e assim responder à questão pesquisada (HIGGINS; GREEN, 2011). A meta-análise por outro lado compreende o uso de métodos estatísticos para sumarizar os resultados de estudos independentes, fornecendo estimativas mais precisas do que qualquer estudo individual incluso em uma revisão (GLASS, 1976). Devido à análise estatística dos dados, a meta-análise também é descrita como uma revisão sistemática quantitativa, enquanto a revisão sistemática *per se* restringe-se a análise qualitativa dos dados (COOK, MULROW; HAYNES, 1997). Dito isso, revisões sistemáticas podem não conter uma meta-análise, entretanto meta-análises devem ser realizadas em dados previamente revisados de forma sistemática, reduzindo assim o risco de viés nas conclusões (HIGGINS; GREEN, 2011).

A revisão sistemática tem como características principais uma definição clara dos objetivos e critérios de inclusão e exclusão de estudos na base de dados; metodologia explícita e reproduzível; compreende uma busca sistemática por dados para identificar todos os estudos que atendem os critérios de eligibilidade; avaliação da validade dos dados dos estudos publicados, identificando o risco de viés de cada um; apresentação e síntese das características e achados dos estudos incluídos na base de dados (HIGGINS; GREEN, 2011).

Os testes de significância da hipótese nula permitem apenas uma decisão de forma dicótoma, isto é, rejeição ou aceite da hipótese de nulidade. Entretanto, carecem de informações importantes para a inferência estatística, sendo elas a estimativa de tamanho do efeito de interesse e a precisão da estimativa, neste caso o intervalo de confiança do tamanho do efeito (NAKAGAWA; CUTHILL, 2007). Com a utilização de técnicas meta-analíticas, podemos determinar se dentre as variáveis estudadas há algum efeito, se este é positivo ou negativo e obter uma sumarização da estimativa do tamanho do efeito (LEAN et al., 2009).

Definição da questão e critérios de eligibilidade

Uma boa revisão sistemática inicia com uma questão ou objetivo bem definido, a qual deve englobar o tipo de população, de intervenção e de variáveis a serem estudadas. O termo PICO (do inglês, “Population”, “Interest”, “Context” and “Outcomes”) relembra quais detalhes a questão deve abranger, tendo, portanto, os participantes bem definidos, quais intervenções serão estudadas e quais variáveis serão posteriormente comparadas (HIGGINS; GREEN, 2011).

Os critérios de eligibilidade de estudos são os que definem quais estudos serão incluídos ou excluídos na base de dados, sendo esta a principal diferença em relação a revisões de literatura (HIGGINS; GREEN, 2011).

Busca por estudos e coleta de dados

Idealmente uma revisão sistemática deve abranger todos os dados referente ao assunto a ser pesquisado, visto que a perda de potenciais estudos pode elevar o risco de viés. Para isso, recomenda-se a busca em bases de dados eletrônicas (como por exemplo *Scholar Google*, *Science Direct* e *Scielo*), busca em bibliotecas, e de preferência abrangendo vários idiomas. O uso de dados não publicados (provenientes de empresas, por exemplo) é possível, embora a decisão de incluí-los seja controversa (LEAN et al., 2009).

Os resultados de uma meta-análise dependem criticamente dos estudos ou publicações que são incluídas na base de dados, e para isso os métodos de decisão devem ser claros e transparentes, reduzindo o risco de viés ou conclusões tendenciosas (HIGGINS; GREEN, 2011).

Análise de dados

Para análise de dados dicótomos, o método estatístico mais comum é o de relação de risco ou risco relativo, enquanto que para dados contínuos o método mais prevalente é o de tamanho de efeito, também chamado de diferença média padronizada (LEAN et al., 2009).

Todos os métodos analíticos devem possibilitar a determinação de pesos para cada estudo, sendo este reflexo do número de animais analisados (para dados dicotomizados) ou da combinação do número de animais e da variância de cada estudo (em caso de dados contínuos). Desta forma, estudos mais precisos em termos de número de repetições e com menor variabilidade de dados (indicado por menor variância) têm maior contribuição com o resultado meta-analítico final, que estudos pequenos e com muita variabilidade (LEAN et al., 2009).

Uma decisão importante é de quando analisar os dados por modelo de efeito-fixo ou efeito-aleatório. Quando se assume que os dados são heterogêneos, isto é, há outras fontes de

variação no estudo do que somente a intervenção em questão, costuma-se usar o método de efeito-aleatório, que engloba no cálculo de tamanho de efeito geral a variabilidade entre experimentos.

Desta forma, o uso das técnicas meta-analíticas permite que toda uma base de dados distinta, decorrente de estudos independentes e até mesmo com resultados conflitantes, sejam agrupados e assim analisados de forma a tirar conclusões sobre o assunto investigado. Em relação ao uso de diferentes fontes de zinco para frango de corte, encontram-se na literatura diversas publicações e com resultados variados. Desta forma, o uso de técnicas de meta-análise é justificado, buscando assim entender os fatores de variação inerentes a cada trabalho.

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2 DIETARY SOURCES OF ZINC FOR BROILERS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT: A systematic review and meta-analysis was carried out to evaluate the effect of different sources of zinc on animal performance and tissue zinc concentration in broilers. Through web searching tools (Scholar Google, Science Direct and Scielo), publications with the keywords "zinc" AND "broiler" OR "poultry" OR "chicken" were selected. A screening criterion was applied, and only those fulfilling the requirements were maintained. Data from each trial were plotted in a data sheet of excel, each line corresponding to one of the treatments for each experimental phase, and columns were used to include the experimental characteristics, diet and nutrient composition, animal performance and tissue zinc concentration. Standardized Mean Difference (**SMD**) and SMD 95% Confidence Intervals (CI) for each treatment and overall were calculated following a randomized effect size. Forest plot type graphics were generated using the software RevMan5. Phytase was not used in 91% of treatments. From the inorganic zinc sources, 28% were zinc oxide (**ZnO**) and the others were zinc sulfate (**$ZnSO_4$**). Average diets total calcium was found to be 0.93%. In comparison to ZnO, broilers fed with organic sources showed improvement in feed conversion ratio (FCR), but the same average daily gain (ADG) and average daily feed intake (ADFI). Compared to $ZnSO_4$, organic group showed higher ADFI and ADG, but the same FCR. In diets with Ca below 0.8%, no difference was observed for any variable, but when above 1%, broilers fed with organic zinc showed higher ADFI and ADG than the ones fed with $ZnSO_4$, but the same FCR. No difference was found for ADFI, ADG, FCR and tibia zinc content when phytase was used. Oppositely, without phytase, ADFI, ADG and tibia zinc content were higher in broilers receiving organic zinc, but FCR had no difference compared to $ZnSO_4$. The source of inorganic zinc, the nutritional calcium levels and the presence of phytase in the diets, are some of the experimental characteristics that may interfere in the results when testing different zinc sources.

Key words: poultry, trace element, phytase, calcium, effect size.

INTRODUCTION

Zinc (**Zn**) is one of the essential nutrients supplemented in commercial poultry diets. It acts as a cofactor in many enzymes of the body, influencing the cellular and humoral immunity response, feathers growing and taking part as an antioxidant agent, as reviewed in previously published papers (Ao and Pierce, 2013; Naz et al., 2016; Abd El-Hack, et al., 2017; Nys et al., 2018). Due to its importance in metabolism and necessity of supplementation in poultry diets, several Zn sources have been the subject of study in broiler experiments, especially those complexed to organic molecules as aminoacids, proteins and carbohydrates (Manangi et al., 2012; Star et al., 2012; Liu et al., 2013).

Some researchers showed that complexed Zn sources have higher relative bioavailability (**RBV**) than traditional inorganic ones, explaining that it occurs due to the lower reactivity with other digesta components and by the different intestinal absorption mechanisms, these providing a path with lower absorption sites competition (Światkiewicz et al., 2001; Star et al., 2012; Sahraei et al., 2013). On the other hand, there is an inconsistency of results when supplementing different sources available in the market for broilers (Cao et al., 2000; Huang et al., 2009), so just being complexed to an organic ligand is not a guarantee of improvement in the studied variables. This issue was the subject of an extensive literature search to evaluate the bioavailability, incompatibilities and interactions of different mineral sources for many species of animals (Cano-Sancho, 2014).

Some of this variation could be attributed to the variables included in each trial, which was addressed in the systematic review and meta-analysis conducted by Schlegel et al. (2012). The authors showed that changing the dependent variable to calculate RBV in broilers can result in final values from 93 to 113%, and for swine species, from 85 to 117%.

Besides, the use or not of phytase in the diets can also influence the outcomes, showing that phytate can be playing as a chelating agent, making Zn from ingredients to turn

unavailable for absorption (Światkiewicz et al., 2001). The addition of phytate to the diets has also been proved to negatively influence the absorption of supplemented zinc sources, more pronounced in the case of sulfates instead of organic ones (Huang et al., 2013). Although, at the same research, sources of organic zinc with a strong chelation strength had lower RBV compared to sulfate, suggesting that part of the zinc was so tightly bound to the organic molecule that could not be dissociated and utilized by the animal.

The number of replicates in each trial is also an important issue. The null hypothesis significance test alone can lack of measurement precision, so depending on the sample size, even the null hypothesis estimate could be not representative of the population (Nakagawa et al., 2007), leading to statistic errors of type I or II. Conducting a systematic review and performing a meta-analysis can quantitatively and formally provide information of data from previous research, being possible then to draw conclusions about the whole body of studies (Lean et al., 2009). This type of analysis move away from just dichotomous outcomes (reject or fail to reject null hypothesis) and focus on determining the magnitude of an effect and the estimate precision (Nakagawa et al., 2007).

The aim of this study is to combine previously published papers about Zn sources for broilers and to evaluate the effect on animal performance and tissue Zn concentration through a systematic review and meta-analysis.

MATERIALS AND METHODS

Database

A search for indexed publications was performed using Google Scholar (http://scholar.google.com), Science Direct (https://www.sciencedirect.com/) and Scielo (http://www.scielo.br/), aiming to find studies published between the years of 2000 and 2018 with the keywords (“zinc”) AND (“broiler” OR “poultry” OR “chicken”).

From all the papers found, a systematic literature review was conducted, only selecting studies fulfilling the following criteria: a) experiment was performed with broilers; b) zinc was one of the nutrients in evaluation, comparing organic and inorganic sources of supplementation in iso-nutritional diets; c) it was not a literature review, short communication or case report; d) details of the diet composition and nutritional levels were informed; e) dependent variables studied were animal performance and/or tissue Zn concentration. The study flow diagram in Figure 1 describes the studies inclusion/exclusion process, and the final list of the ones included in the database can be found in Table 1.

Once defined the studies included in the database, information from each treatment found in Materials and Methods and Results sections were tabulated in a worksheet of Excel 2013 (Microsoft Office). Each line corresponded to one of the treatments in each experimental phase, and columns were used to include the experimental characteristics (age, sex, breed, number of replicates, birds per replicate), diet and nutrient composition (percentage of each ingredient in the diet, use or not of phytase, source and level of supplemental Zn, total dietary Zn level, crude protein, metabolizable energy, calcium and phosphorus level, aminoacids levels), animal performance (average daily gain, average daily feed intake, feed conversion ratio), and tissue zinc concentration (tibia, liver and plasma zinc).

Zinc sources were compared in each trial just between isonutritional diets, so basal treatments (with no supplementation) or treatments that didn't match zinc levels between organic and inorganic sources were excluded from the database. Zinc oxide (**ZnO**) and zinc sulfate (**$ZnSO_4$**) were compared separately to the organic forms. Nano zinc, tribasic zinc chloride, zinc acetate and zinc propionate sources were used in only one experiment each, being excluded from the database due to the impossibility of a meta-analytical comparison.

In order to confirm the information of previously published studies, which show that zinc bioavailability can be affected by calcium (**Ca**) level and phytate content of the diet,

database was segmented by Ca level and also by use or not of phytase, performing then subgroup statistical analysis.

Statistical analyses

For sources comparison, Standardized Mean Difference (SMD) was calculated using the formula of Hedges's adjusted g, which comprises an adjustment for small sample bias. SMD Confidence Intervals (CI) for each treatment were calculated and a summary was determined following a randomized effect size, which corrects for heterogeneity of data. Forest Plot type graphics were generated using the software RevMan5 (free software provided by the Cochrane Organization).

For all the calculations, sample size (defined by the number of replicates of each treatment) and pooled standard deviation (pooled SD) was inputted in the software. When pooled SD was not provided in the publication, it was obtained by calculating through Standard Error of the Mean (SEM), as cited by Lean et al. (2009).

Heterogeneity was calculated by I^2 statistics, according to the formula described by Lean et al. (2009):

$$I^2(\%) = \frac{Q-(k-1)}{Q} \times 100$$

Where Q is the X^2 heterogeneity statistic and k is the number of trials.

RESULTS AND DISCUSSION

The final database comprised a total of 23 publications in 13 different journals, mainly Journal of Applied Poultry Research, Poultry Science, The Journal of Poultry Science and British Poultry Science, totaling 333 lines (experimental phases of each treatment in each study). The breeds under evaluation were Ross (52%), Cobb (18%), Arbor Acres (16%), Hubbard x Cobb (10%) and Starbro (4%). The sex of the birds were males in 70% of treatments, being the other 30% composed by mixed flocks. The phases under analysis were

151 starter (from day 0 to 21 days of age) in 57% of treatments, followed by grower (from day 21
152 to 49 days of age) in 23% and total (0-49 days) on the other 20%. The mean number of
153 replicates was 6.57 (mode 4), with 88% of treatments comprising 8 or less replicates. The
154 average number of birds per replicate was 15.90 (mode 6) with 77% of treatments having 10
155 birds or less. In 76% of the treatments, there was no information about the physical form of
156 the diet (mashed/pelleted/crumbled), being the rest pelleted (15%) and mashed (9%). Corn-
157 soybean diets comprised 82% of the treatments, followed by corn-wheat-soybean (13%) and
158 others (5%).

159 Phytase was not used in 91% of treatments, being the rest distributed in doses of 500
160 FTU/kg (3% of treatments), 750 FTU/kg (2%) and 1,500 FTU/kg (4% of treatments). This
161 finding was a surprise in the systematic review, since the enzyme is used worldwide in
162 probably all the industries. The negative effect that phytate plays over the availability of
163 different zinc sources, varying in chelation strengths, has been shown by Huang et al (2013).

164 The mode of supplemental zinc was 80 mg/kg, with an average of 83.70 mg/kg.
165 Complexed zinc sources were used in 43% of the treatments, followed by inorganic sources
166 (41%) and association of complexed and inorganic (16%). From the inorganic sources used
167 for comparison, 28% were ZnO and the others were $ZnSO_4$. The average total Ca level of the
168 diets was 0.93%, ranging from 0.56% to 1.40%, as showed in Figure 2. There was no
169 information about particle size and solubility of the limestones used for feed composition.

170 The forest plot presenting the standardized mean difference (**SMD**) and confidence
171 intervals (CI) for each study, the overall summarization and the heterogeneity coefficient (I^2)
172 between ZnO and organic zinc sources for feed conversion ratio (**FCR**) is presented in the
173 Figure 3. The summary effect size was -0.52 with a confidence interval ranging from -0.83
174 and -0.22 ($p < 0.001$). According to Cohen (1988), a SMD of 0.2 represent a small effect, 0.5
175 a moderate effect and 0.8 a large effect. Therefore, there was a moderate effect over FCR,

176 showing that the birds consuming organic zinc converted better than the ones being fed with
177 ZnO. The I^2 for this variable was 58% ($p < 0.001$), meaning that there is still a high variation
178 in the results due to other factors than just the zinc source.

179 For the average daily gain (**ADG**) and average daily feed intake (**ADFI**), no
180 differences were detected between organic zinc sources and ZnO ($p = 0.29$; $I^2 = 73\%$, and $p =$
181 0.51; $I^2 = 75\%$, respectively).

182 Zinc oxide was reported to have lower bioavailability when compared to $ZnSO_4$,
183 ranging from 36 to 105% (Jongbloed et al., 2002), which may be in part explained by the high
184 temperature used in the production process of the oxides (Edwards and Baker, 1999). In other
185 words, the difference between organic and inorganic may be more pronounced when ZnO is
186 the inorganic choice for comparison.

187 On the other hand, when comparing organic Zn sources with $ZnSO_4$, the results were
188 different. Forest plot graphics in this case were not shown here due to the high number of
189 studies included. By this way, the summarization for each variable is available in Table 2.
190 According to the data, birds fed with the organic sources had higher feed intake than the ones
191 fed with $ZnSO_4$ ($p < 0.001$; $I^2 = 59\%$), showing also a higher ADG ($p = 0.004$; $I^2 = 48\%$), but
192 that didn't turn in improvement of FCR ($p = 0.10$; $I^2 = 51\%$). It is important to cite that all
193 comparisons presented a high heterogeneity coefficient, ranging from 48 to 59%, which
194 indicates that other factors than the Zn sources may be posing variability to the results.

195 Higgins and Green (2011) described thresholds for heterogeneity, informing that
196 values above 50% may be substantial, being then necessary to take some caution when
197 interpreting the data, considering the magnitude and direction of the effect, and p-value of the
198 chi square for correct analysis. In order to deal with heterogeneity, database was further
199 explored through data segmentation and running the analysis through random effect instead of
200 fixed effect.

Besides the sources of variation that were explored in this meta-analysis (use or not of phytase and diet's Ca level), we know that the response of animals may vary when fed with different organic zinc sources, e.g., proteinates, glycinates, inespecific aminoacid complexes or any other source available in the market, but we didn't intend to enter to this level of analysis and rather just compare in a broad way (organic vs inorganic). Many examples of comparisons of organic compounds can be found in the literature, and just to cite some of them we have the studies of Marco et al. (2017), Foltz et al. (2017) and Mohammadi et al. (2015). Also, to include this factor in the analysis (type of organic zinc), more studies would be necessary in order to increase the database.

The first data segmentation was done aiming to investigate the results found by Świątkievicz et al. (2001). The authors concluded that the relative bioavailability of organic sources was reduced when phytase was present, correlating it to phytate degradation, which avoid its negative interaction with the inorganic source of comparison.

In treatments with phytase, no difference between organic source and $ZnSO_4$ was found for FCR ($p = 0.20$; $I^2 = 72\%$), ADG ($p = 0.83$; $I^2 = 14\%$) and ADFI ($p = 0.55$; $I^2 = 68\%$). On the other hand, when phytase was not added, a higher ADFI ($p < 0.001$; $I^2 = 59\%$) was seen in broilers consuming organic Zn, also showing a higher ADG ($p < 0.001$; $I^2 = 49\%$), but resulting in the same FCR ($p = 0.15$; $I^2 = 48\%$) as birds consuming $ZnSO_4$. Despite the importance of these results, just 4 trials selected for this meta-analysis had phytase dosed in the diets, and the one performed by Marco et al. (2017) used ZnO as control group, being then excluded of this analysis. It definitely represents an important source of bias of the body of studies, as 91% of the treatments of this meta-analysis had no phytase, an enzyme that is probably used in all diets or close to that in the industry, and so important to avoid the anti qualitative effects of phytate.

225 Liver Zn content was analyzed in 3 of all trials, and one of them used ZnO as control
226 group, then being not considered in the comparison. No difference was seen between $ZnSO_4$
227 and the organic group ($p = 0.48$; $I^2 = 79\%$), although it would be desirable a higher number of
228 studies to increase the power of this summarization. The major influence was placed by the
229 study of Liu et al. (2015), which no difference was found between $ZnSO_4$, zinc aminoacid
230 complex and 2 forms of zinc proteinate. In their study, the only difference in liver Zn was
231 found for levels of supplementation in the diet, which no supplementation led to the lower
232 content, and 60 mg/kg of zinc in the diet reached a plato of response.
233 Plasma Zn concentration was accessed in 4 of the trials of this database, and birds fed
234 with organic zinc had higher concentration when compared to $ZnSO_4$ ($p = 0.02$; $I^2 = 39\%$), as
235 shown in Figure 4. It is fundamental to mention that for this comparison, phytase was not
236 used in any of the treatments, with the exception of one of the comparisons in Schlegel et al.
237 (2010) trial, which is the one that animals showed higher zinc concentration in plasma when
238 fed with $ZnSO_4$ instead of organic zinc.
239 Regarding tibia Zn content, as more results were found in the database, it was possible
240 to run a subgroup analysis of trials using or not phytase (Figure 5). On those which had no
241 phytase in the diets, it was clearly seen a difference, showing that birds that consumed organic
242 zinc sources had higher tibia zinc content than those being fed with $ZnSO_4$ ($p < 0.001$, $I^2 =$
243 10%). On the opposite situation, i.e., with phytase supplemented in the diets, no difference
244 was observed between sources ($p = 0.36$; $I^2 = 0\%$). There was no statistical difference
245 between the 2 subgroups ($p = 0.09$; $I^2 = 64\%$), although it is clearly seen at the forest plot
246 graphic through an eye ball test that both groups showed distinct patterns of response, tending
247 to observe higher tibia zinc concentration in the organic zinc group when phytase is not
248 supplemented only.

As indicated by Sebastian et al. (1996), zinc relative retention is increased when phytase is supplemented, attributed to the higher availability of zinc from the phytate-mineral complex. According to Angel et al. (2002), normally at the pH found in the GIT, phytin carries a strong negative charge that may bind with di and trivalent cations, such as Zn^{2+}, turning them unavailable for absorption.

Another source of variation of results was the Ca level of the diets. Excessive calcium was first shown by Zeigler et al. (1961) to induce zinc deficiency disorders, and later corroborated by Wedekind et al. (1994) to affect zinc RBV. As described in material and methods, there is a huge variability in diet's calcium from the trials included in this database, ranging from 0.56 to 1.18%, and with 70% of treatments containing levels above 0.9%.

Herein we summarized the results obtained when treatments were segmented by Ca level and analyzed separately (Table 3). For FCR, no differences were seen between groups, except when calcium ranged from 0.90 and 0.99%, showing better results for the birds fed with $ZnSO_4$. For ADFI and ADG, no difference between groups were seen when Ca was below 0.80%, and also for ADG, when it ranged from 0.90 and 0.99%. On all the other subgroups, an improvement in consumption and weight gain was seen on birds fed with organic zinc.

Interestingly, through an eye ball test, it was possible to see that the results found by Jahanian et al. (2008) had the major influence over the summarization of ADFI and ADG results due to its wide SMD, as we can see in Figure 6, which brings the data of feed consumption at Ca level between 0.8 and 0.89%. The causes of the pronounced effect in this study are unknown, but if these results were not considered in the evaluation, there would be no difference between groups for ADFI at Ca level between 0.8 and 0.89% ($p = 0.34$; $I^2 = 0\%$) and 0.9 and 0.99% ($p = 0.14$; $I = 22\%$), having results more homogeneous also, as indicated by the reduction in the I^2 coefficient. For the ADG, there would also be no

274 difference between groups at Ca between 0.8 and 0.89% ($p = 0.12$; $I^2 = 0\%$) and at Ca
275 between 0.9 and 0.99% ($p = 0.91$; $I^2 = 21\%$).

276 Lai et al. (2010) found differences in feather coverage score with a 2-fold increase in
277 calcium level, indicating it may be an important source of variation when zinc
278 supplementation is under evaluation. Birds fed with diets at 1.6% of calcium had worse
279 feather coverage in tail, under-wing and breast when compared to birds receiving diets with
280 0.8% of calcium. The authors believe calcium may inhibits zinc utilization, which drives to a
281 lower keratin synthesis. These results are in agreement with Wedekind et al. (1994), who
282 found that the absorption of Zn is dependent on the Ca level of the diet, especially when
283 inorganic sources are used. In their study, an increase of calcium from 0.60 to 0.74% resulted
284 in 3.8-fold reduction in $ZnSO_4$ utilization and only 2.1-fold reduction for zinc methionine.

285 The increase of the diet's calcium levels was shown to negatively affect the
286 degradation of phytate, also related to an increase in the proximal GIT pH (Amerah et al.,
287 2014). The formation of calcium-phytate complexes takes place in the gut, favoured by the
288 addition of limestone due to its high acid binding capacity and therefore its relationship with
289 pH rise (Selle et al., 2009).

290 Finally, no information about the solubility and granulometry of limestones used in the
291 trials was found in published papers, which may be an important detail when evaluating Zn
292 absorption. That is not a surprise, as a new method of limestone solubility had just been
293 published and correlated to calcium digestibility (Kim et al., 2019). As cited before, calcium
294 may interfere in phytate degradation, therefore it is relevant that future publications bring
295 these data, and also there is a need for further evaluation about the impact that limestones
296 varying in solubility can cause over zinc absorption in animals.

297

CONCLUSIONS

From this meta-analysis we were able to explore some of the sources of variation of results when testing zinc for broilers. The experimental design of published trials can increase the risk of bias, favoring complexed minerals when comparing to inorganic sources. The use or not of phytase, nutritional calcium levels and the source of inorganic zinc are some of the characteristics that may interfere in the results.

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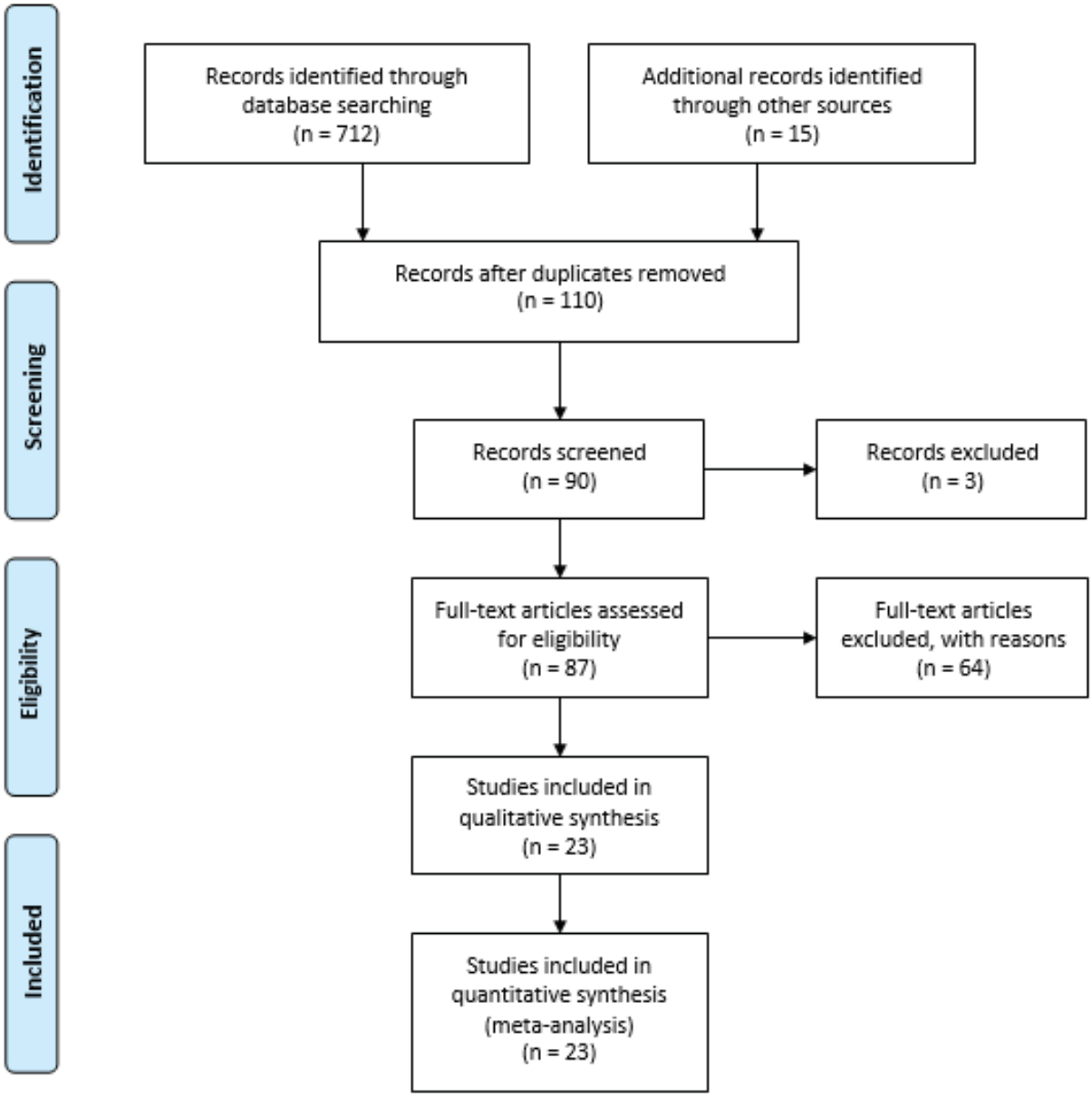

443

444 Figure 1. Study flow diagram.

445

446 Table 1. List of included studies in the database.

Author	Year	Journal
Ao et al	2009	Poultry Science
Burrell et al	2004	British Poultry Science
Dozier et al	2003	British Poultry Science
Feng et al	2010	Biological Trace Element Research
Foltz et al	2017	Journal of Applied Poultry Research
Huang et al	2009	Journal of Animal Science
Hudson et al	2004	Journal of Applied Poultry Research
Hudson et al	2005	Animal Feed Science and Technology
Jahanian et al	2008	Asian-Australasian Journal of Animal Sciences
Kamran Azad et al	2017	Animal Production Science
Kwiecień et al	2016	Livestock Science
Liu et al	2011	Poultry Science
Liu et al	2013	Journal of Applied Poultry Research
Liu et al	2015	Poultry Science
Marco et al	2017	Animal Feed Science and Technology
Mohammadi et al	2015	British Poultry Science
Mwangi et el	2017	Poultry Science
Pacheco et al	2017	Brazilian Journal of Poultry Science
Saenmahayak et al	2010	Journal of Applied Poultry Research
Sahraei et al	2013	The Journal of Poultry Science
Schelegel et al	2010	Animal
Star et al.	2012	Poultry Science
Świątkiewicz et al	2001	Journal of Animal and Feed Sciences

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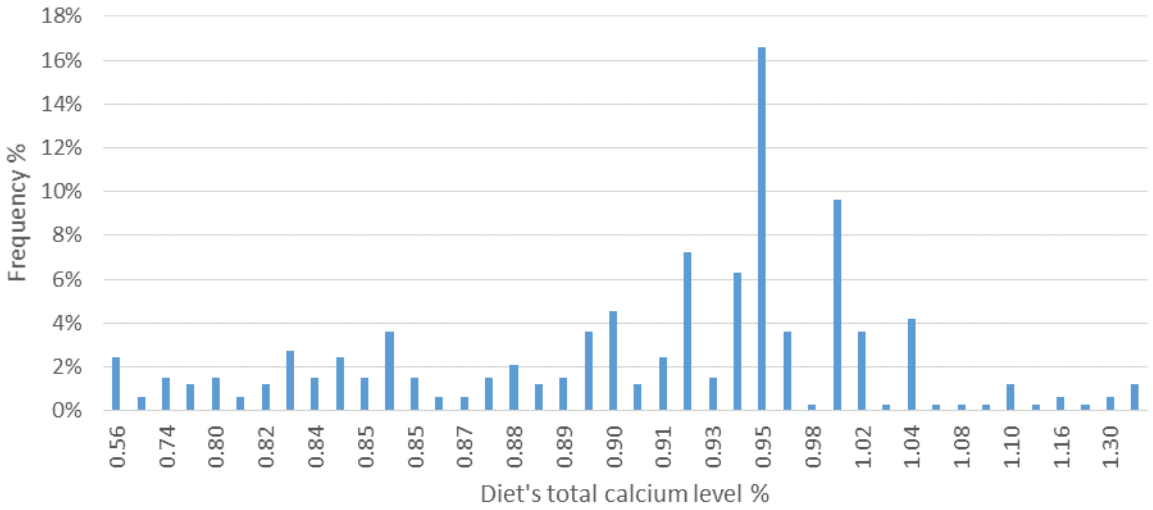

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450 Figure 2. Frequency of treatments in the database according to total calcium level of the diets.

451

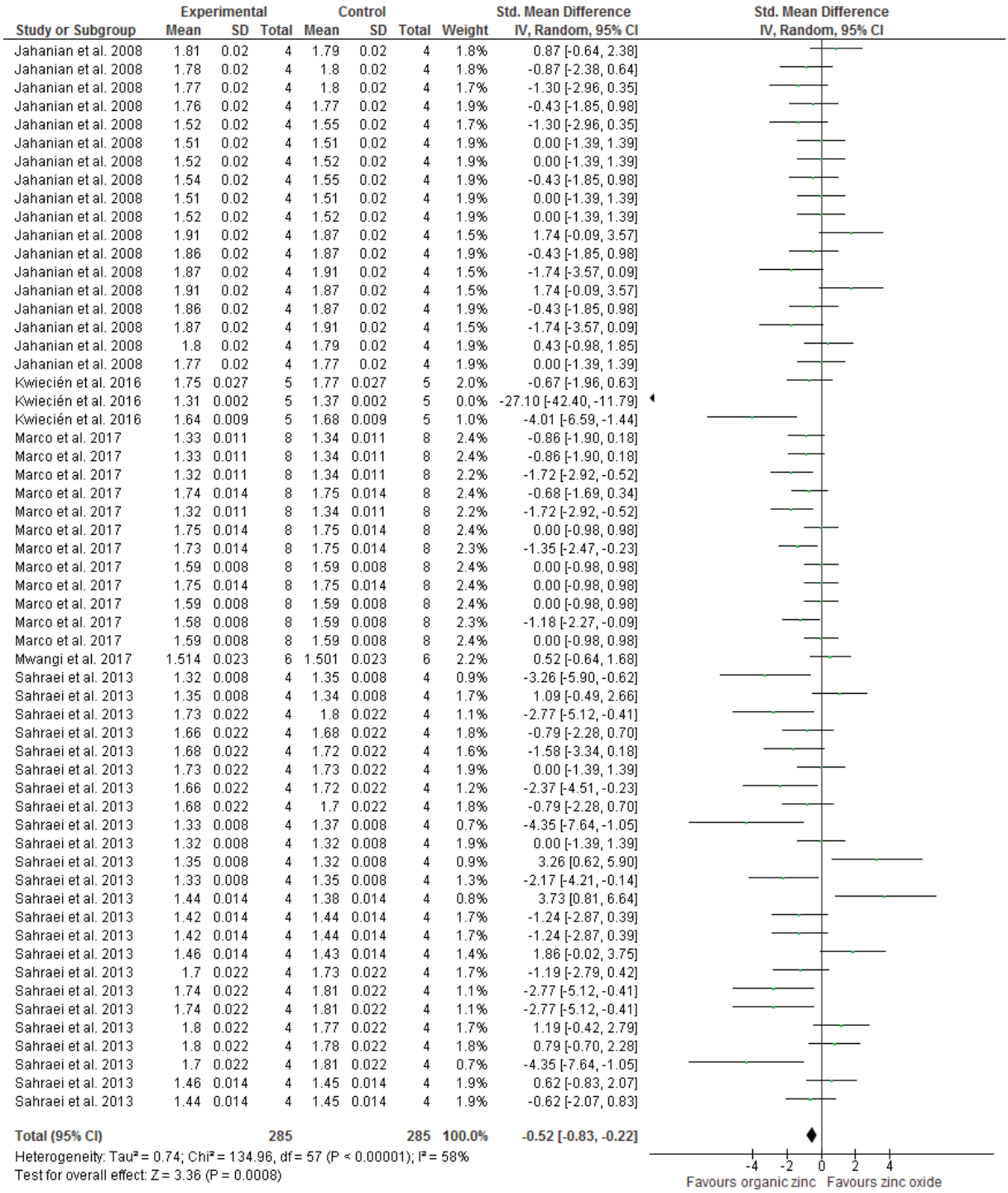

Figure 3. Standardized Mean Difference (SMD) for feed conversion ratio (FCR) between organic zinc sources and zinc oxide.

456 Table 2. Feed conversion ratio (FCR), average daily gain (ADG) and average daily feed
457 intake (ADFI) between zinc sulfate and organic zinc sources.*

Outcome	SMD (CI)	P-Value	I^2 %	Observation
FCR	0.12 (-0.02; 0.26)	0.10	51	Not different
ADG	0.25 (0.11; 0.38)	<0.001	48	Favours organic zinc
ADFI	0.54 (0.33; 0.75)	<0.001	59	Favours organic zinc

458 * The number of comparisons in each variable was 140, 143 and 98 for FCR, ADG and
459 ADFI, respectively.
460

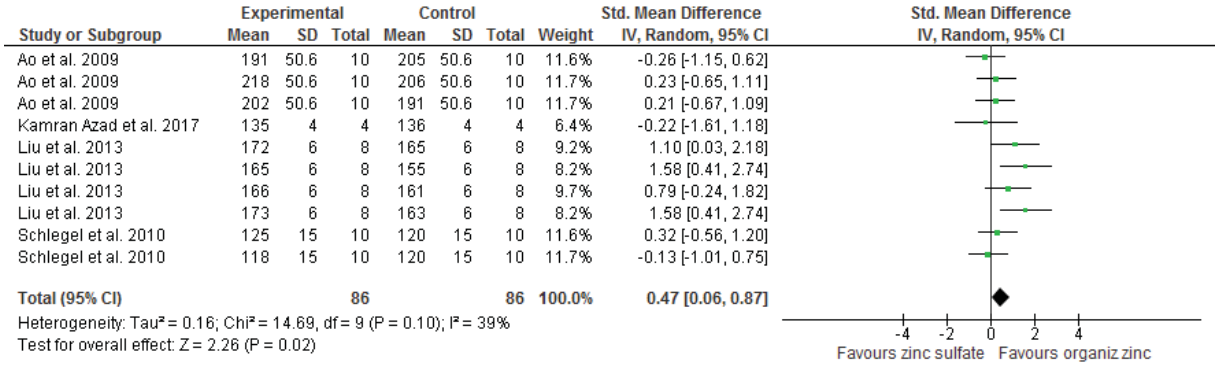

Figure 4. Standardized Mean Difference (SMD) for plasma zinc content between organic zinc sources and zinc sulfate.

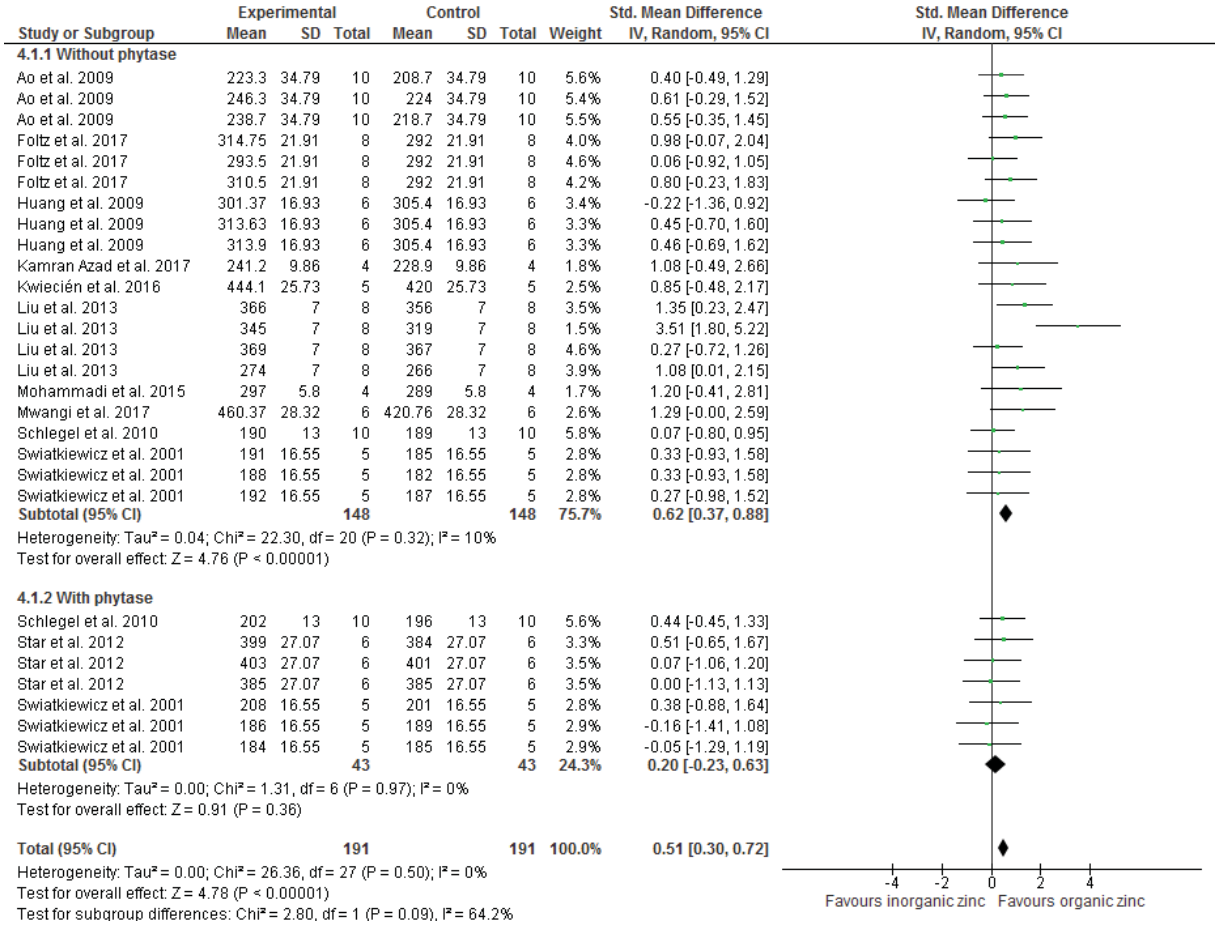

Figure 5. Standardized Mean Difference (SMD) for tibia zinc between inorganic and organic zinc sources in trials without or with phytase.

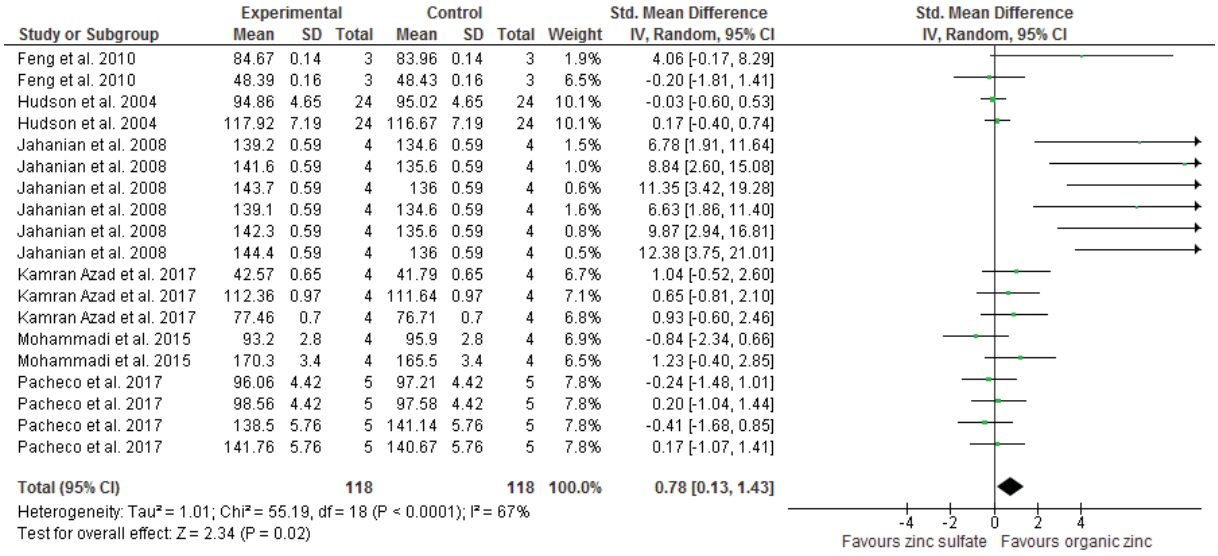

Figure 6. Standardized Mean Difference (SMD) for ADFI between organic zinc sources and zinc sulfate in trials with calcium level ranging from 0.8 and 0.89%.

473 Table 3. Feed conversion ratio (FCR), average daily gain (ADG) and average daily feed
474 intake (ADFI) between zinc sulfate and organic zinc sources, according to the diet total
475 calcium level (%).

Outcome	Diet Ca level %	SMD (CI)	P-Value	I^2 %	Observation
FCR	Under 0.80%	0.97 (-0.74; 2.68)	0.26	87	Not different
	From 0.80 to 0.89%	0.00 (-0.22; 0.22)	0.98	0	Not different
	From 0.90 to 0.99%	0.22 (0.02; 0.42)	0.03	52	Favours zinc sulfate
	Above 1.00%	0.03 (-0.22; 0.29)	0.79	54	Not different
ADG	Under 0.80%	0.09 (-0.60; 0.78)	0.80	48	Not different
	From 0.80 to 0.89%	0.56 (0.20; 0.92)	0.002	46	Favours organic zinc
	From 0.90 to 0.99%	0.17 (-0.04; 0.37)	0.12	52	Not different
	Above 1.00%	0.25 (0.03; 0.47)	0.02	43	Favours organic zinc
ADFI	Under 0.80%	0.52 (-0.98; 2.02)	0.49	85	Not different
	From 0.80 to 0.89%	0.78 (0.13; 1.43)	0.02	67	Favours organic zinc
	From 0.90 to 0.99%	0.46 (0.16; 0.75)	<0.001	55	Favours organic zinc
	Above 1.00%	0.66 (0.33; 0.98)	<0.001	46	Favours organic zinc

3 INTERACTION BETWEEN DIETARY ZINC LEVELS AND SOURCES AND PHYTASE ON THE PERFORMANCE AND TISSUE ZINC CONCENTRATION OF BROILERS

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Scientific section: Metabolism and Nutrition

ABSTRACT: An experiment was carried out to evaluate performance and tissue zinc concentration of broilers fed a corn/soy-based diet with the inclusion of 500 or 1,500 FTU/kg of phytase and two different sources of supplemental zinc at different rates. In the trial, 3,600 one-day old male chicks were distributed in a completely randomized experimental design, consisting of 10 treatments, with 9 replicates of 40 birds each. Treatments consisted of three supplemental zinc levels (0, 30 or 60 mg/kg), two phytase levels (500 or 1,500 phytase units/kg; FTU) and two sources of zinc (zinc sulfate monohydrate or zinc aminoacid complex). Birds were fed only one diet from 1 to 21 days, following the nutritional specification of each treatment. Birds and feed were weighted at 7, 14 and 21 days. At placement, 20 chicks were randomly chosen and euthanized for tibia and liver collection. Samples were pooled by tissue and zinc content was determined. At the end of the experiment, 2 birds per replicate were euthanized for tibia and liver collection and zinc content determination. Feed intake and FCR were influenced by diet zinc levels ($p < 0.0001$), but no difference was seen for BWG. Maximum response was achieved at the inclusion of 30 mg of Zn per kg of the diet (66.36 mg/kg, total dietary Zn), regardless of source. Regarding tissue zinc concentration, there was no difference between treatments for tibia and liver zinc content. Zinc amino acid complex improved FCR, but not ADG and feed intake when compared to zinc sulfate. Maximum performance was reached at an inclusion of 30 mg of supplemental Zn per kg of the diet, regardless of source or phytase inclusion.

Key words: enzyme, phytate, poultry.

INTRODUCTION

The importance of zinc in poultry nutrition has been widely reviewed by researchers worldwide, all them addressing about the benefits it may bring for the animals when attending

the nutritional requirements (Ao and Pierce, 2013; Naz *et al*., 2016; Abd El-Hack, et al., 2017; Nys et al., 2018). For that reason, and also knowing that minerals represent a small part of the diet costs, most of the animal nutritionists have been over-supplementing diets in zinc, leading to unabsorbed mineral by the intestine which is later excreted in feaces (Nys et al., 2018).

On the other hand, there is an increased concern about the polution caused by the animal production waste, specially coming from the excretion of minerals in the environment through feces (Cano-Sancho et al., 2014). In order to mitigate that, organic zinc sources are cited to be more bioavailable than traditional inorganic ones, so they could be supplemented in lower levels without compromising animal performance. It is believed that the higher bioavailability is due to the different absorption paths in the intestine and also the lower reactivity with other digesta components (Sahraei et al., 2013; Star et al., 2012; Świątkiewicz et al., 2001).

Probably 100% of the industry diets around the world or close from that percentage have phytase being supplemented, and this tool has been proved to be determinant when comparing organic to inorganic zinc sources. Świątkiewicz et al. (2001) showed that relative bioavailability was negatively influenced by the addition of phytase to the diets in all variables under evaluation, indicating that bioavailability may be over estimated in those trials that have diets not supplemented with the enzyme.

Another important factor is the calcium content of the diet. In a trial conducted with broilers, an increase from 0.60 to 0.74% of dietary calcium resulted in a 3.8-fold reduction in $ZnSO_4$ utilization and just 2.1-fold decrease for zinc methionine, indicating that the differences between sources may be increased if calcium levels are high (Wedekind et al., 1994).

Last but not least important is the inorganic source used for comparison. Some researchers have compared organic sources with ZnO (Marco et al., 2017; Kwiecień et al., 2016; Sahraei et al., 2013; Jahanian et al., 2008), demonstrating benefits in favour of organic. Zinc oxide was reported to have lower bioavailability when compared to zinc sulfate, ranging from 36 to 105% (Jongbloed et al., 2002), which may be in part explained by the high temperature used in the production process (Edwards and Baker, 1999). In other words, the difference between organic and inorganic may be exacerbated when zinc oxide is the choice for comparison.

This trial aimed to compare 2 sources of zinc (zinc sulfate and zinc aminoacid complex, 3 levels of supplemental zinc (0, 30 or 60 mg/kg) and 2 levels of phytase (500 or 1,500 FTU/kg) under diet total calcium levels used by the industry.

MATERIALS AND METHODS

The experiment was approved by the Committee of Ethics on Animal Use (CEUA) of the Sector of Agrarian Sciences of the Federal University of Paraná, Curitiba, PR, Brazil.

Birds and location

One-day-old male Ross® chicks (n = 3,600), vaccinated for Infectious Bursal Disease, Infectious Bronquitis and Marek's Disease at the hatchery (Seara, Araranguá, Brazil), obtained from the same broiler breeder parent flock (40 weeks old), were housed in 1.75m long x 1.75m wide experimental units (13.06 birds/m^2), equipped with feeder bins and nipple drinkers. The floor was covered with new litter made of rice hulls. Environmental temperature and light intensity were controlled during the experimental period according to breeder guidelines. Birds were offered feed and water *ad libitum* throughout the experimental period.

Experimental diets

The corn/soybean diets were formulated to meet the starter nutrient requirements of chicks, except for Zn (Table 1). Dietary treatments consisted of three supplemental zinc levels (0, 30 or 60 mg/kg), two zinc sources (feed-grade zinc sulfate monohydrate or zinc aminoacid complex) and two phytase levels (500 or 1,500 FTU/kg), totaling 10 treatments with 9 replicates of 40 birds each.

The phytase product consisted of a granulated phytase preparation (IUB No. 3.1.3.26), produced by a *Buttiauxella* species bacterium expressed in a *Trichoderma reesei* fungus, with minimal activity of 10,000 FTU per gram. The phytase unit is defined as the amount of enzyme to liberate 1μmol inorganic phosphorus per minute at pH 5.5, 15 μM solution of sodium phytate at 37 ° C. At both doses, nutritional matrix of phytase was considered the same, contributing in available phosphorus and calcium by 0.15% and 0.13%, respectively.

Ingredients and Feed analyses

Prior to feed manufacturing, a sample from corn and soybean meal were collected to determine phytate content through the K-Phyt assay procedure (Megazyme – Bray Business Park, Bray, Co. Eicklow, A98 YV29, Ireland).

Limestone was collected for geometrical mean diameter (GMD) determination and solubility analysis, following the multiple-time point dynamic solubility assay model at pH 3 buffer solution, as described by Kim et al. (2019). The sample solubilized at 37.5%, 66.06% and 88.47%, at 5, 15 and 30 min, respectively. Mean particle size of limestone was 951.79 microns. Other analyses were calcium (39.97%, AOAC 927.02) and phosphorus (0.04%, AOAC 965.17); and magnesium (0.51%), potassium (0.01%), sodium (0.01%), iron (298.49

123 mg/kg), manganese (14.95 mg/kg), zinc (8.55 mg/kg), and copper (0.01%), all determined by
124 atomic absorption spectrophotometry.

125 Feed from each treatment was sampled following the procedures described in the
126 Regulation CE 152/2009, and analyzed for moisture (AOCS Ca2c-25), ash (AOAC 942.05),
127 crude protein (AOAC 984.13), crude fat (AOAC 920.39), crude fiber (AOCS Ba 6-84),
128 calcium (AOAC 927.02) and phosphorus (AOAC 965.17) in Seara laboratory (Montenegro,
129 Brazil); FTU/kg of phytase by Dupont laboratory, using the colorimetric procedure MoV
130 Assay; and zinc concentration (mg/kg) in CBO laboratory by atomic absorption
131 spectrophotometry. The analyzed zinc content and FTU of phytase from each diet are
132 presented in Table 2.

133

134 ***Growth performance measurement***

135 Body weight gain (BWG), feed intake and feed conversion ratio (FCR) were
136 determined weekly and for the overall experiment.

137

138 ***Tibia and Liver analysis***

139 At the first day of the experiment, right before housing the birds, 20 baby chicks were
140 randomly chosen and euthanized by cervical dislocation for liver and right tibia extraction.
141 The material was pooled by tissue. At the 21st day of trial, two birds per replicate were
142 euthanized also by cervical dislocation for liver and right tibia collection, pooling each tissue
143 separately by replicate.

144 Tibias were cleaned of soft tissues, fat extracted in petroleum ether and ashed. Liver
145 was lyophilized. The samples were analyzed for zinc concentration by atomic absorption
146 spectrophotometry as previously described.

147

148 ***Statistical analyses***

149 Data was tested for normality by the Shapiro-Wilk test and transformed by box-cox in
150 case of being non-parametric. In case of being non-parametric even after transformation,
151 Kruskal-Wallis test was applied for zinc level (multiple independent samples) and Mann-
152 Whitney test for zinc source or phytase level (two independent samples). For the normal
153 distributed data, one-way ANOVA was performed and statistically significant differences
154 were considered at $p < 0.05$. Comparison of means was done by Tukey test at 5%.

155

156 **RESULTS AND DISCUSSION**

157 In baby chicks, the average tibia Zn concentration was 357.57 mg/kg of ash, and the
158 average liver Zn content was 51.67 mg/kg of DM.

159 The broiler performance results are presented in Table 3. Feed intake and FCR were
160 influenced by diet zinc levels ($p < 0.0001$), but no difference was seen for BWG ($p = 0.1273$).
161 Maximum response was achieved at the inclusion of 30 mg of Zn per kg of the diet (66.36
162 mg/kg, total dietary Zn), regardless of source. This result is similar with the findings of Huang
163 et al. (2007), who found a plato response at a diet supplementation of 20 mg of Zn/kg (48.37
164 mg/kg, total dietary Zn).

165 There was a clear regulation in feed consumption due to the diet zinc level, where
166 birds receiving no supplemental zinc increased their feed intake in order to be not deficient in
167 this nutrient. Ferket and Gernat (2006) explain that small mineral deficiencies in the diets can
168 lead birds to increase their feed intake in order to achieve the mineral requirement. On severe
169 deficiencies although, metabolic disorders may cause adverse effects on feed consumption,
170 which is related to the reduction in circulating leptin levels, then acting in regulation of
171 hypothalamic factors (Shay and Mangian, 2000).

Cao et al. (2000) compared different organic zinc products and zinc sulfate for broilers, and the results showed no difference for animal performance between sources, even that phytase was not used and diet total calcium was 1%. In our trial no difference was observed for feed intake and BWG between zinc sources, but FCR was improved in treatments consuming zinc aminoacid complex instead of zinc sulfate.

This finding suggest that in this period the organic source may have showed higher bioavailability than the inorganic one, even under diets with phytase, which breaks down the phytate, one of the major antagonists for zinc absorption; and under lower levels of nutritional total calcium (0.67%). In our trial, it was intentionally used copper sulfate as growth promotor (200 mg of copper per kg of diet), which may have posed some challenge for zinc absorption, although the study of Van Campen (1969) showed in rats that this interference just occurs in a very high Cu:Zn relation (50:1).

The low solubility at 5 minutes of the limestone used in the diets should have contributed to maximize zinc absorption (even the sulfate source), as it is correlated with P digestibility and also with phytase efficacy (Kim et al., 2019). Even so, birds consuming the zinc aminoacid complex showed better FCR than the ones consuming zinc sulfate.

Lai et al. (2010) found differences in feather coverage score with a 2-fold increase in Ca level, indicating it may be an important source of variation when Zn supplementation is studied. Birds who consumed diets with 1.6% of calcium had worse feather coverage in tail, under-wing and breast when compared to birds receiving diets with 0.8% of calcium. The authors believe Ca may inhibits Zn utilization, which drives to a lower keratin synthesis. In another study conducted by Wedekind et al. (1994), researchers found that the absorption of zinc is dependent on the calcium level of the diet, especially when inorganic sources are used. In their study, an increase of calcium from 0.60 to 0.74% resulted in 3.8-fold reduction in $ZnSO_4$ utilization and only 2.1-fold reduction for zinc methionine.

Regarding tissue zinc concentration, there was no difference between treatments for tibia and liver zinc content (Table 4). Schlegel et al. (2010) found a difference for tibia zinc between 0 and 15 mg/kg of supplemental zinc in the diet, with no variation when the source was $ZnSO_4$ or zinc glycinate. At the same study, between these 2 levels of inclusion, plasma zinc was different just when phytase was not used. As stated by Jondreville (2007), maximum plasma and bone zinc concentrations are reached with 55 and 51 mg of total zinc per kg of diet. We were expecting to find a lower concentration at least for the basal diets (T1 and T2), as in our trial it had an average of 38 mg/kg of native zinc. We believe that it didn't occur in part due to the feed intake (that was higher in the groups without supplementation) and the weight of the birds, which were just numerically different.

In our trial, nor Ca or phytate were high in the diets, so it may have contributed to maximize Zn absorption. Oberleas et al. (1966) showed that excessive calcium can lead to the complexation of zinc in phytate, turning it less available for absorption by the animals.

About the sources of supplemental zinc, our results corroborate with the findings of previous studies, as of Mwangi et al. (2017), who found no difference between ZnO and Zn proteinate for tibia ash zinc and liver zinc; Foltz et al. (2017), who tested $ZnSO_4$, Zn glycinate, Zn amino acid complex and Zn HMTBA, all resulting at the same Zn content in tibias.

CONCLUSIONS

Zinc amino acid complex improved FCR, but not ADG and feed intake when compared to zinc sulfate. Maximum performance was reached at an inclusion of 30 mg of supplemental Zn per kg of the diet, regardless of source or phytase inclusion.

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285 Table 1. Composition of the basal diet for broilers.

Ingredient[1]	%
Corn	57.904
Soybean meal 46%	36.388
Corn oil	1.896
Limestone	1.230
Monocalcium phosphate 22,7%	0.800
Methionine MHA	0.360
Salt	0.334
Lysine sulfate 70%	0.228
Bentonite	0.200
Sodium sulfate	0.162
Mineral premix[2]	0.116
Choline chloride 75%	0.086
Copper sulfate 25%	0.080
Threonine 98.5%	0.068
Anticoccidial[3]	0.063
Vitamin premix[4]	0.036
Flavouring additive [5]	0.030
Phytase[6]	0.015
Xylanase[7]	0.005
Composition	
DM[8]	89.04
ME, Kcal/kg	3,000
CP[8]	21.68
EE[8]	4.71
Total Ca[8]	0.67
Total P[8]	0.56
Phytate[8]	0.68

286 [1] Ingredient composition are reported on an as-fed basis.

[2] Provided per kg of diet: Mn, 90 mg; Fe, 45 mg; Cu, 10.8 mg; I, 1.08 mg; Se, 0.27 mg. Zn (as zinc sulfate or zinc aminoacid complex) was added according to each treatment at the expense of vehicle.

[3] Provided per kg of diet 50 mg of nicarbazin and 50 mg of narasine.

[4] Provided per kg of diet: vitamin A, 10,800 UI; vitamin D3, 4,050 UI; vitamin E, 63 UI; vitamin K3, 4.5 mg; vitamin B1, 2.7 mg; vitamin B2, 7.2 mg; vitamin B6, 4.5 mg; vitamin B12, 22.5 mcg; niacin, 54 mg; folic acid, 1.8 mg; panthotenic acid, 18 mg; biotine, 0.18 mg.

[5] Added as a substitute for growth promoter.

[6] Phytase (according to each treatment) added at the expense of vehicle.

[7] Xylanase nutritional matrix was considered to be of 30 kcal of ME per kg of diet.

[8] Analyzed.

298 Table 2. Supplemented and analyzed zinc (mg/kg) and phytase (FTU/kg) of the experimental diets.

Treatment	Supplemented Zn (mg/kg)	Analyzed dietary Zn (mg/kg)[1]	Supplemented phytase (FTU/kg)	Analyzed phytase (FTU/kg)[2]
Zn0 500P	0	41.41	500	823
Zn0 1500P	0	35.56	1,500	1971
ZnS30 500P	30	62.50	500	859
ZnS30 1500P	30	60.52	1,500	2016
ZnAA30 500P	30	64.87	500	1042
ZnAA30 1500P	30	77.58	1,500	1890
ZnS60 500P	60	90.07	500	1090
ZnS60 1500P	60	82.17	1,500	1867
ZnAA60 500P	60	86.40	500	950
ZnAA60 1500P	60	86.20	1,500	1940

299 [1] Samples analyzed by atomic absorption spectrophotometry (CBO Laboratory).

300 [2] Colorimetric procedure MoV Assay (Dupont Laboratory). FTU- phytase unit is defined as the amount of
301 enzyme to release 1μmol inorganic phosphorus per minute at pH 5.5, 15 μM solution of sodium phytate at 37°
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Table 3. Effect of different levels and sources of zinc on body weight gain (BWG), feed intake and feed conversion ratio (FCR) from 1 to 21 days.

Treatment	Zinc level (mg/kg)	Zinc source	Phytase level (FTU/kg)	BWG (g)	Feed Intake (g)	FCR (g/g)
Zn0 500P	0	-	500	1,023	1,320	1.290
Zn0 1500P	0	-	1,500	1,028	1,320	1.285
ZnS30 500P	30	Zinc sulfate	500	1,031	1,305	1.266
ZnS30 1500P	30	Zinc sulfate	1,500	1,031	1,303	1.264
ZnAA30 500P	30	Zinc aminoacid complex	500	1,032	1,300	1.260
ZnAA30 1500P	30	Zinc aminoacid complex	1,500	1,033	1,295	1.254
ZnS60 500P	60	Zinc sulfate	500	1,033	1,310	1.268
ZnS60 1500P	60	Zinc sulfate	1,500	1,032	1,303	1.263
ZnAA60 500P	60	Zinc aminoacid complex	500	1,029	1,302	1.265
ZnAA60 1500P	60	Zinc aminoacid complex	1,500	1,033	1,306	1.264
SEM				4.06	5.72	0.004
Zinc level (mg/kg)						
0				1,026	1,320 a	1.288 a
30				1,032	1,301 b	1.261 b
60				1,032	1,305 b	1.265 b
Zinc source						
Zinc sulfate				1,032	1,305	1.265 a
Zinc aminoacid complex				1,032	1,301	1.260 b
Phytase level (FTU/kg)						
500				1,030	1,307	1.270
1500				1,031	1,306	1.266
P-Value						
Zinc level				0.1273	0.0001	0.0001
Zinc source				0.9117	0.2178	0.0200
Phytase level				0.5311	0.6153	0.1719

317 [a-b] Values within a column with no common superscript are significantly different ($P < 0.05$).

Table 4. Effect of different levels and sources of zinc and phytase on tibia and liver zinc retention.

Treatment	Zinc level (mg/kg)	Zinc source	Phytase level (FTU/kg)	Tibia Zn (mg/kg ash)	Liver zinc (mg/kg DM)
Zn0 500P	0	-	500	410.45	76.42
Zn0 1500P	0	-	1500	407.93	85.30
ZnS30 500P	30	Zinc sulfate	500	399.63	79.79
ZnS30 1500P	30	Zinc sulfate	1500	392.75	76.59
ZnAA30 500P	30	Zinc aminoacid complex	500	393.06	81.64
ZnAA30 1500P	30	Zinc aminoacid complex	1500	423.14	78.78
ZnS60 500P	60	Zinc sulfate	500	418.24	81.10
ZnS60 1500P	60	Zinc sulfate	1500	403.90	84.74
ZnAA60 500P	60	Zinc aminoacid complex	500	418.08	80.42
ZnAA60 1500P	60	Zinc aminoacid complex	1500	422.31	83.22
SEM				5.007	1.384
Zinc level (mg/kg)					
0				409.19	80.86
30				402.14	79.20
60				415.63	82.37
Zinc source					
Zinc sulfate				403.63	80.56
Zinc aminoacid complex				414.15	81.02
Phytase level (FTU/kg)					
500				407.89	79.88
1500				410.01	81.73
P-Value					
Zinc level				0.4782	0.9305
Zinc source				0.3176	0.8791
Phytase level				0.8460	0.4035

318 [a-b] Values within a column with no common superscript are significantly different ($P < 0.05$).

4 CONSIDERAÇÕES FINAIS

A revisão de literatura sobre o uso de zinco para frangos de corte foi importante para conhecimento do tema, trazendo informações sobre as fontes disponíveis no mercado, níveis nutricionais de suplementação recomendados pelas diferentes instituições, funções bioquímicas do elemento no organismo, formas de absorção intestinal e aproveitamento pelos animais e fatores anti qualitativos que podem interferir no aproveitamento do zinco naturalmente presente nos ingredientes e suplementado.

A revisão sistemática mostrou-se como uma técnica interessante para análise das publicações sobre o tema de uso das diferentes formas de zinco para frangos de corte. Diferente da revisão de literatura, que traz uma visão qualitativa sobre o tema, com a revisão sistemática é possível também avaliar os dados de forma quantitativa, trazendo uma percepção geral da metodologia experimental praticada em cada teste, permitindo avaliar se a metodologia se aproxima da realidade da agroindústria, e também se contém algum viés interferindo nos resultados obtidos.

Os dados desta revisão sistemática mostraram que na metodologia empregada nas publicações existentes sobre o tema encontramos alguns pontos que acabam favorecendo a obtenção de diferença entre as fontes inorgânicas tradicionais e as fontes complexadas a moléculas orgânicas, mostrando assim superioridade das formas complexadas.

O primeiro ponto diz respeito a comparar complexos orgânicos de zinco a óxido de zinco, ao invés de sulfatos. O sulfato de zinco é amplamente utilizado como fonte inorgânica e tem se mostrado superior aos óxidos em termos de biodisponibilidade, vide trabalhos publicados sobre o tema. Desta forma, o uso deste tipo de comparativo acaba forçando mostrar diferenças que favoreçam as fontes orgânicas, mas que não representam a realidade das dietas.

O segundo fator importante diz respeito ao uso de fitase, responsável pela quebra do fitato, um dos principais fatores anti qualitativos das dietas, em especial por quelatar o zinco e torna-lo indisponível à absorção pelos animais. Foi possível verificar que mais de 90% da base de dados foi constituída de experimentos sem fitase, mesmo sendo publicações relativamente recentes sobre o tema (2000 a 2018). As fitases são amplamente empregadas em dietas para aves, e o não uso mostra prejudicar o aproveitamento do zinco principalmente na forma inorgânica, como mostrado nos trabalhos citados nesta dissertação.

A terceira característica dos experimentos, e não menos importante, diz respeito ao nível de cálcio praticado nas dietas. O efeito negativo sobre a biodisponibilidade de zinco que o calcário pode exercer já foi provado em publicações anteriores, e avaliando a base de dados da meta-análise, foi possível verificar que a grande maioria das dietas continham níveis elevados de cálcio se comparado às dietas das agroindústrias, a qual foi refletida no teste da granja experimental. O nível de cálcio total das dietas do experimento realizado para esta dissertação foi de 0,67%, enquanto que apenas 6% dos tratamentos na meta-análise foram formulados com níveis de cálcio inferiores a 0,8%.

Ainda sobre o tema de cálcio, é importante mencionar que em nenhum dos trabalhos foram detalhadas informações sobre a fonte de calcário utilizada. Sabe-se que o tamanho de partícula e a origem da mina pode influenciar na solubilidade da fonte, e esta consequentemente pode interferir negativamente no efeito da fitase, formando complexos com fitato e complexando também o zinco. O efeito tamponante deste ingrediente favorece a complexação do zinco ao fitato.

Os resultados analíticos obtidos no calcário utilizado nas dietas mostraram baixa solubilidade aos 5 minutos, que é desejável em termos de maximização de efeito da fitase e aproveitamento do fósforo fítico, e alta solubilidade aos 30 minutos, que tem correlação positiva com digestibilidade do cálcio. A baixa solubilidade inicial, por ter relação com a

atividade da fitase e consequentemente com a quebra do fitato, pode ter aumentado a biodisponibilidade do zinco.

Os resultados obtidos com a revisão sistemática e meta-análise permitiram corroborar os estudos anteriormente publicados, indicando que o uso de sulfato de zinco pode ser usado em dietas para frangos de corte com o objetivo de fornecer às aves o nutriente zinco com segurança. Para isso é importante que fitases eficientes sejam adicionadas às dietas, bem como os níveis de cálcio não sejam excessivos, haja vista que níveis elevados têm o poder de reduzir a degradação do fitato e elevar o pH do trato gastro intestinal, favorecendo a complexação de minerais ao ácido fítico.

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ANEXO 1 – TERMO DE APROVAÇÃO NA CEUA

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 041/2019, referente ao projeto **"Interação entre níveis nutricionais e fontes de zinco e fitase sobre o desempenho e concentração tecidual de zinco em frangos de corte",** sob a responsabilidade **Alex Maiorka** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 6.899, de 15 de julho de 2009,e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS
AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 2 de invasividade, em reunião de 07/08/2019.

Vigência do projeto	Setembro/2019 até Setembro/2019
Espécie/Linhagem	*Gallus gallus domesticus* (ave)/Cobb
Número de animais	3600
Peso/Idade	46 g/1 dia
Sexo	Macho
Origem	Incubatório comercial da Seara, Lages/SC, Brasil.

CERTIFICATE

We certify that the protocol number 041/2019, regarding the project **"Interaction between dietary zinc levels and sources and phytase on the performance and tissue zinc concentration of broilers"** under **Alex Maiorka** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October, 2008, of Decree nº 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE
UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 2 of invasiveness, in session of 07/08/2019.

Duration of the project	September/2019 until September/2019
Specie/Line	*Gallus gallus domesticus* (poultry)/Cobb
Number of animals	3600
Wheight/Age	46 g/1 day
Sex	Male
Origin	Seara's commercial farm, Lages/SC, Brazil.

Curitiba, 30 de agosto de 2019

Chayane da Rocha

Chayane da Rocha

Coordenadora CEUA-SCA