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EXIGÊNCIA DE ZINCO PARA MATRIZES DE FRANGO DE CORTE

ANDRÉ NEVES MAYER
Mestre em Zootecnia/UFLA
Zootecnista/UFPR

Tese apresentada como um dos requisitos à obtenção do Grau de Doutor em Zootecnia Área de Concentração Produção Animal

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ANDRÉ NEVES MAYER
ZOOTECNISTA E MESTRE EM ZOOTECNIA

TESE

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para obtenção do Grau de
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Pela Banca Examinadora

SERGIO LUIZ VEIRA
PPG Zootecnia/UFRGS
Orientador

Homologado em: 26/09/2018
Por

DANILO PEDRO STREIT JR.
Coordenador do Programa de
Pós-Graduação em Zootecnia

INES ANDRETTA
PPG ZOOTECNIA/UFRGS

ALEX MAIORKA
UFPR

ANANDA PORTELA FELIX
UFPR

CARLOS ALBERTO BISSANI
Diretor da Faculdade de Agronomia

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EXIGÊNCIA DE ZINCO PARA MATRIZES DE FRANGO DE CORTE[1]

Autor: André Neves Mayer
Orientador: Sergio Luiz Vieira

RESUMO

Foram utilizadas 120 reprodutoras pesadas da linhagem comercial Cobb 500 SF. As aves foram alojadas aleatoriamente em gaiolas individuais as 20 semanas de idade. O estudo foi composto de três fases: adaptação as gaiolas (dieta basal), depleção (dieta deficiente contendo 18,7 ± 0,47 ppm Zn) e fase experimental. Após serem alimentadas com a dieta deficiente por 7 semanas, as aves receberam dietas com níveis crescentes de sulfato de Zn ($ZnSO_4$ $7H_2O$) por 12 semanas. Os níveis de zinco analisados nas dietas foram: 18,7 ± 0,47; 50,3 ± 10,6; 77,3 ± 11,0; 110,2 ± 12,8; 140 ± 12,2 e 170,6 ± 13,2 ppm. A exigência de Zn foi estimada utlizando os modelos polinomial quadratico (PQ), quadratico com plato (QP) e exponêncial assintótico (EA). A exigência de Zn estimada para produção diária de ovos e produção de ovos viáveis foi de 114,1; 83,3; 78,6 ppm e 112,6; 61,4; 65,4 ppm para o período de 33 a 36 semanas respectivamente. Para o período de 37 a 40 semanas a exigência estimada foi de 116,4; 63,3; 53,1 e 113,2; 60,4; 46,1 ppm respectivamente. Assim como, para o periodo de 41 a 44 semanas a exigência estimadas pelos modelos foi 116,3; 62,8; 52,8 e 120,0; 67,7; 62,1 ppm de Zn respectivamente, utilizando os modelos PQ, QP e EA. Já a estimativa da exigência de Zn para número total de ovos e o número total de ovos viáveis foi de 116,4; 75,7; 64,7 ppm, e 115,2; 56,5; 41,5 ppm para os modelos PQ, QP e EA. Enquanto que para concentração de fosfatase alcalina foi 161,8 e 124,9 ppm Zn utilizando os modelos PQ e QP. O peso do pintinho ao nascer e o peso do ovo reduziram com a suplementação de zinco. As variáveis hemoglobina, hematócrito, comprimento do pintinho, porcentagem de albumem e espessura da camada mamilar não foram afetadas pela nível de zinco na dieta. A maior concentração de Zn na gema para os períodos de 37 a 40 semanas e 41 a 44 semanas foi 127,9; 71,0; 78,1 e 124,9; 64,5; 59,6 ppm respectivamente utilizando os modelos QP, PQ e EA. A resistência da casca e porcentagem de casca apresentaram a estimativa de exigência de Zn de 161,6; 68,0; 96,7 ppm, e 126,1; 122,4; 147,8 ppm, enquanto a camada paliçada e a espessura da casca foram maximizadas com 126,0; 67,9; 67,9 ppm e 126,3; 67,7; 64,4 ppm respectivamente para os modelos PQ, QP e EA. A media de todas as estimativas de exigência de Zn obtidas no presente trabalho foi 91,4 ppm de zinco.

Palavras-chave: Nutrição mineral; Produção; Reprodutoras pesadas; Sulfato de zinco; Zinco.

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ZINC REQUIREMENTS OF BROILER BREEDER HENS[1]

Author: André Neves Mayer
Advisor: Sergio Luiz Vieira

ABSTRACT

One hundred and twenty Cobb 500 hens, 20 wks of age were randomly allocated into individual cages with the objective of estimating Zn requirements. The study was composed of 3 phases: adaptation to cages (basal diet), depletion (deficient diet containing 18.7 ± 0.47 ppm Zn) and experimental phases. After being fed a Zn deficient diet for 7 wks, hens were fed diets graded increments of Zn sulfate ($ZnSO_4$ $7H_2O$), totalizing 18.7 ± 0.47; 50.3 ± 10.6; 77.3.0 ± 11.0; 110.2 ± 12.8; 140 ± 12.2 and 170.6 ± 13.2 ppm analyzed Zn in feeds for 12 wks. Zn requirements were done using quadratic polynomial (QP) models, broken line quadratic (BLQ), and exponential asymptotic (EA). Zn requirements obtained for hen day egg production and settable egg production were 114.1, 83.3, 78.6 ppm and 112.6, 61.4, 65.4 ppm for period of 33 to 36 wks, and 116.4, 63.3, 53.1 and 113.2, 60.4, 46.1 ppm for period of 37 to 40 wks, and 116.3, 62.8, 52.8, and 120.0, 67.7, 62.1 ppm for period of 41 to 44 wks, respectively, using QP, BLQ and EA models. The total eggs, total settable egg per hen had Zn requirements estimated as 116.4, 75.7, 64.7 ppm, and 115.2, 56.5, 41.5 ppm, respectively for QP, BLQ and EA models, whereas for ALP were 161.8 and 124.9 ppm using QP and BLQ models. The hatching chick body and egg weight decreased with Zn supplementation. Hemoglobin, hematocrit, body length of hatching chick, albumen percentage and thickness of mammillary layer were not affected by dietary Zn. Maximum responses for Zn in yolk for periods of 37 to 40 and 41 to 44 wks were 127.9, 71.0, 78.1 and 124.9, 64.5, 59.6 ppm, respectively using QP, BLQ and EA models. Breaking strength and eggshell percentage had Zn requirements estimated at 161.6, 68.0, 96.7 ppm, and 126.1, 122.4, 147.8 ppm, whereas eggshell palisade layer and eggshell thickness were maximized with 126.0, 67.9, 67.9 ppm, and 126.3, 67.7, 64.4 ppm, respectively for QP, BLQ and EA models. The average of all Zn requirement estimates obtained in the present study was 91.4 ppm.

Key words: Mineral nutrition; Production; Broiler Breeder; Zinc sulfate; Zinc.

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RELAÇÃO DE ABREVIATURAS

ALP - Alkaline Phosphatase
AME_n - Apparent Metabolizable energy nitrogen corrected
BLQ - Broken line with quadratic
ºC - Graus Celsius
Cu - Cobre
DNA – Ácido Desoxirribonucleico
EC - European Commission
EXP - Exponential
g/cm^3 - grams per centimeters cubic
Hb - Hemoglobin
Ht - Hematocrit
kW - kilovolt
m - metros
ml - mililitros
NRC - National Research Council
PPM - parte por milhão
PROC - Procedures
QP - Quadratic Polynomial
Wks - Weeks
µm – Micrômetros
Zn- Zinc

CAPÍTULO I

INTRODUÇÃO

Os minerais essenciais ao organismo podem ser classificados em macrominerais e microminerais, de acordo com as suas concentrações nos tecidos, que indica sua necessidade no organismo (Bertechini, 2006). O enxofre, cálcio, fósforo, potássio, sódio, cloro e magnésio são classificados como macrominerais, pois são exigidos em maior quantidade na dieta, e estão envolvidos em funções estruturais e fisiológicas no organismo. O ferro, zinco, cobre, manganês, iodo, selênio, entre outros são classificados como microminerais, pois são exigidos em menor quantidade na dieta, no entanto, são extremamente importantes para o metabolismo (Vieira, 2003).

O zinco (Zn) é o segundo elemento traço mais abundante essencial para todos os organismos vivos, logo após o ferro (Kambe et al, 2015). O Zn é um micromineral essencial ao organismo, e está incluído na tabela periódica no grupo 2B, com o número atômico 30, massa atômica 65,4. Possui o orbital d completo (10 elétrons), podendo funcionar como um receptor de elétrons estável, que o torna capaz de participar de centemas de enzimas (Georgievskii et al., 1982). As funções do Zn podem ser catalíticas, estruturais e reguladoras no organismo animal (Sakomura et al., 2014). Este micromineral participa na formação de cerca de 300 enzimas no organismo animal (Henriques et al., 2003). As metaloenzimas dependentes de zinco estão distribuídas em todos os tecidos do organismo, exercendo funções importantes, com destaque para resposta imune, ação antioxidante, espermatogênese, armazenamento e liberação de insulina, síntese proteica, regulação da transcrição do DNA e divisão celular (Salgueiro, 2000). Dentre várias metaloenzimas Zn-dependente a fosfatase alcalina, que tem a função de realizar a hidrólise de ésteres, e a anidrase carbônica, que participa na formação de CO_2 e fornecimento de bicarbonato (Fischer et al., 1955; Luecke et al., 1968; Yamaguchi & Yamaguchi, 1986; Supuran et al., 2003). Sua atuação interfere diretamente na formação óssea, e no caso de poedeiras, também afeta a qualidade da casca do ovo (Dale & Strong Jr., 1998).

Em situações de deficiência de Zn nas dietas para matrizes de frangos de corte, pode ocorrer diminuição dos parâmetros produtivos e reprodutivos, como produção de ovos e eclodibilidade reduzida e anomalias embrionárias, afetando diretamente a progênie com desenvolvimento esquelético prejudicado (Blamberg et al., 1960; Kienholz et al, 1961; Sun et al., 2018). O teor de zinco depositado na gema pode ser alterado pelo conteúdo do mineral na dieta consumida pela reprodutora (Kienholz et al., 1964). Embora o conteúdo mineral da gema possa ser melhorado ao aumentar a sua concentração na dieta das galinhas, estudos demonstraram que, além de certo limite, não existe ou existe pouco efeito no teor mineral de ovos (Naber, 1979; Angel, 2007). Portanto o atendimento correto da exigência de zinco, sem deficiências ou excessos, para a matriz de frangos de corte é essencial para o desenvolvimento do embrião (Favero et al., 2013). O zinco é amplamente utilizado pelo embrião do 11º ao 17º dia de incubação (Yair & Uni, 2011).

Tendo em vista sua grande importância para o organismo, o zinco deve ser incluído na dieta dos animais. No entanto, a simples presença de microminerais na composição dos ingredientes das rações, como no milho e no

farelo de soja, não garante que o mesmo esteja sendo aproveitado pelo animal (Mafra & Cozzolino, 2004). Muitos fatores intrínsecos e extrínsecos ao alimento afetam a disponibilidade dos microminerais (Underwood & Suttle, 1999). Compostos como ácido fítico e oxalato reduzem drasticamente a disponibilidade do zinco (Hempe & Cousins, 1989; Leeson & Summers, 2001; Richards et al., 2010). Além disso, a concentração de cátions metálicos com semelhança química (por exemplo, Zn^{2+}, Cu^{2+} e Cd^{2+}), também interfere na capacidade do organismo em absorvê-los, uma vez que seu transporte para dentro do enterócito é realizado por transportadores de membrana específicos e não específicos (Evans et al., 1975; Vieira, 2008).

Adicionalmente às interações antagonistas, que interferem na absorção de Zn, a concentração de microminerais nos ingredientes utilizados na fabricação de rações é relativamente baixa. Deste modo, a suplementação de Zn, através de fontes mais concentradas e com maior disponibilidade se faz necessária para fornecer quantidade suficiente do elemento para o animal. As fontes de microminerais disponíveis para serem utilizadas na suplementação de rações são nas formas de sais ou minerais quelatados à compostos orgânicos. Mais de 90% da suplementação das rações para aves é feita com óxidos e sulfatos, com alta biodisponibilidade (Bertechini, 2012).

Os manuais de linhagens comerciais apresentam recomendações de suplementação para vários micromineiras incluindo o zinco. No entanto, os níveis de suplementação recomendados não contabilizam o zinco inerente ao alimento. A recomendação de suplementação de zinco para matrizes pesadas na fase de produção segundo o manual da linhagem Cobb (Cobb-Vantress, 2013) é de 110 mg Zn/kg de ração. Já a Tabela Brasileira para Aves e Suínos recomenda a suplementação 65 mg Zn/kg (Rostagno et al., 2017). A exigência de Zn descrita no NRC (1994) para reprodutoras é 4,5 mg por galinha por dia. É importante ressaltar que valores de recomendação de suplementação e valores de exigência nutricional são diferentes, todavia ambos podem ser utilizados na formulação de dietas animais.

A suplementação de microminerais em rações para aves frequentemente é feita em quantidades superiores às exigidas na tentativa de assegurar o bom desempenho das aves (Gomes et al., 2009), principalmente devido o desconhecimento do nutricionista da real exigência do animal. Esses excessos na suplementação podem gerar maior excreção do nutriente, causando um desequilíbrio ambiental, e adicionalmente onerar os custos da ração (Sakomura et al. 2014).

O objetivo deste trabalho foi avaliar o efeito dos níveis de zinco sobre o desempenho, análises sanguíneas, e qualidade dos ovos e progênie de reprodutoras pesadas, e posteriormente estimar os valores de exigência de zinco para as reprodutoras, o que possibilitará a atualização dos valores recomendados.

REVISÃO BIBLIOGRÁFICA

1. Absorção e Metabolismo do zinco

A absorção dos microminerais é feita por mecanismos complexos, e depende principalmente das características físico-químicas do intestino e da solubilidade do mineral (Mohanna & Nys, 1999). O Zn é absorvido principalmente no duodeno, primeira porção do intestino delgado (Davies, 1980; Cousins, 1985; Ashmead, 1993), pois é solubilizado em pH ácido (Oberleas et al., 1999). A taxa de absorção é dependente de fatores como pH, forma física e química do mineral, presença de outros minerais, agentes quelantes e carreadores (Hill & Matrone, 1970).

O zinco pode ser absorvido por transporte ativo ou passivo. O transporte passivo não é alterado pela concentração de zinco da dieta, pois ocorre por difusão e sua eficiência é proporcional à ingestão de zinco. Por outro lado, o transporte ativo é saturável, ou seja, quando a dieta contém alto nível de zinco, esse transporte é reduzido, e quando as dietas contém baixo nível de zinco, ocorre o aumento deste transporte (Suttle, 2010). Assim, o Zn pode ser transportado para o interior da membrana celular através de carreadores (Underwood & Suttle, 1999).

Minerais como o cálcio, ferro, manganês e zinco são absorvidos conforme a necessidade fisiológica do animal. A quantidade aparentemente absorvida pode ser menor do que a fração disponível por consequência da regulação do transporte ativo. Os animais respondem ao baixo nível nutricional de zinco aumentando vários transportadores intestinais (Cousins et al., 2006). Dentro do enterócito, a transferência do zinco é realizada pela metalotioneína (McDowell, 1992).

O zinco absorvido é transportado pelo sistema porta hepático ligado à albumina, que corresponde a 2/3 do zinco plasmático. O zinco também aparece no plasma como alpha-2-macroglobulina e como traços de metalotioneína. A indução da síntese de metalotioneína decorrente da chegada do Zn no fígado tem um papel importante na remoção do Zn do sistema porta hepático e no particionamento do mesmo nas diversas vias (Bremner, 1993; Lee et al., 1994). Em situações de excesso, o Zn pode ser armazenado no fígado na forma de metalotioneína, na qual sua produção é induzida pela presença desse elemento no fígado, ou ainda ser excretado (McDowell, 1992). A partir do fígado, o Zn é transportado para a circulação periférica com objetivo de suprir diferentes órgãos e tecidos em taxas determinadas por transportadores localizados na membrana celular e organelas até atingirem a necessidade intracelular. Na circulação periférica o Zn pode estar ligado principalmente á albumina, e uma menor parcela á alfa2-macroglobulina, transferrina, cisteína e histidina (Suttle et al.,2010).

As proteínas transportadoras específicas de zinco encontradas no organismo são o: ZIP1, ZnT-1 (transportador de zinco 1), ZnT-2, ZnT-3 e ZnT-4. O ZIP 1 participa da absorção de Zn no intestino. O transportador DCT1 (transportador dimetalvalente) não é específico para o Zn, mas também tem a capacidade de auxiliar na sua absorção (Oberleas et al., 1999). O transportador ZnT-1 regula diretamente a quantidade de zinco ingerida e está associada ao efluxo do metal, e localiza-se na membrana basolateral do enterócito e das

células tubulares renais. O ZnT-2 também está envolvida na exportação ou captação do zinco dentro de vesículas em diversos tipos celulares no intestino, nos rins e nos testículos. O ZnT-3 regula a captação do zinco em vesículas neuronais e possivelmente nos testículos. O ZnT-4 além de apresentar localização neuronal, também é responsável pela captação do zinco nas glândulas mamárias (Tapiero &Tew, 2003). Os transportadores de zinco, ZnT e ZIP, controlam a absorção, sequestro e liberação de zinco entre membranas biológicas (Jeong & Eide, 2013; Kambe et al., 2015). Trabalhos sobre transportadores de Zn dentro das espécies aviárias são relativamente escassos (Troche et al., 2015). No entanto, a função transportadora de Zn é altamente conservada entre as espécies (Cousins et al., 2006; Wang & Zhou, 2010). Além dos transportadores ZnT e ZIP, mais de 30 proteínas de transporte de membrana, como os canais de cálcio demonstraram estar envolvidas na homeostase de zinco em mamíferos (Kambe et al., 2015). Há evidências crescentes de que as proteínas da membrana envolvidas no transporte de Zn são cruciais para uma variedade de processos biológicos (Hara et al., 2017). A homeostase do zinco é mantida principalmente pelo trato gastrointestinal, por mecanismos de alta regulação do processo mediado por transportador e aumento ou diminuição da excreção de Zn (King et al., 2000).

A absorção intestinal de Zn pode ser diminuída por fatores antagonistas na alimentação, como o fitato e o oxalato (Cruz & Soares, 2011), assim como por competição de transportadores com outros minerais (Vieira, 2008). Alguns minerais dividem o mesmo transportador metal divalente. O ferro e o manganês, por exemplo, são transportados pela mesma proteína, a transferrina. Desta forma os minerais podem afetar a absorção um do outro (Garrick et al., 2003; Bai et al. 2008).

A excreção de minerais pode ser feita por secreções, por exemplo, pelo suco digestivo, ovo (nas galinhas), bile ou urina. Aqueles secretados no intestino via bile, antes dos sítios de absorção, podem ser reabsorvidos, e assim a reciclagem resultante atrasa o início da deficiência mineral (Suttle, 2010). A absorção e reabsorção de zinco no intestino variam de 15-40% (Whitney & Rolfes, 1993). A excreção do zinco ocorre principalmente via suco pancreático e fezes, sendo pouco expressivo na urina (Schryver et al., 1980). Com pouca regulação da excreção, a retenção de zinco esta fortemente associada a sua absorção. Outra forma de excreção deste mineral em galinhas,é através do ovo, sendo que a gema é o principal compartimento de armazenamento de nutrientes para embriões (Yair & Uni, 2011). No entanto, Rutz et al. (2005) descreveram que altos níveis de zinco na dieta não aumentaram o conteúdo desse mineral nos ovos.

2. Funções do zinco

A primeira evidência que o Zn era necessário para o crescimento e saúde do organismo foi obtida em animais de laboratório (Todd et al., 1934). Em 1940 foi comprovado que o Zn é necessário para a formação da enzima anidrase carbônica (Keilin & Mann, 1940). Em animais domésticos, experimentos com privação de zinco foram feitos inicialmente com suínos (Stevenson & Earle, 1956) e frangos (O'Dell & Savage, 1958). Em ambas as

espécies a deficiência foi caracterizada por perda de apetite, redução no crescimento, anormalidades de pele e anexos e falhas reprodutivas.

Existem mais de 300 enzimas dependentes de Zn com diversas estruturas e funções (Vallee & Falchuk, 1993), assim como milhares de proteínas funcionais (Coleman, 1992). Além disso, o Zn é necessário para integridade estrutural e funcional de mais de 2000 fatores de transcrição (Menezo et al., 2011). Portanto o Zn participa de quase todas as vias metabólicas (Beattie & Kwun, 2004; Cousins et al. 2006). De forma geral, o Zn desempenha funções na resposta imune, ação antioxidante, espermatogênese, armazenamento e liberação de hormônios, síntese proteica, regulação da transcrição do DNA, divisão celular entre outras funções (Salgueiro, 2000). Segundo Zago & Oteiza (2001) o zinco desempenha um papel antioxidante importante no organismo, e pode proteger as membranas celulares da oxidação lipídica.

A versatilidade das características físico-químicas do zinco constitui a base de sua extensa participação no metabolismo de proteínas, ácidos nucléicos, carboidratos, lipídios e, mais recentemente, tornou-se uma importante via de investigação na elucidação do processo de controle da expressão gênica e de outros mecanismos biológicos fundamentais (Henriques et al., 2003).

O Zn possui funções catalíticas e estruturais nas enzimas zinco-dependentes, enquanto que nos dedos de zinco (“Fingers zinc”) ou estruturas semelhantes, tem a função de proporcionar uma estrutura de suporte que organiza subdomínios de proteína para a interação com o DNA ou outras proteínas (Maret, 2000). Assim, além da participação nas enzimas, o Zn no organismo pode ainda desempenhar funções de regulação hormonal e construção. A expressão gênica de muitas proteínas, a síntese de DNA e o metabolismo de ácidos nucleicos são dependentes de Zn. Este micromineral regula genes envolvidos na transdução de sinais, resposta ao estresse, crescimento e utilização de energia, entre outros (Cousins et al., 2003). A enzima RNA polimerase é Zn dependente e catalisa a transcrição do DNA (Keilin & Mann, 1940). Alguns defeitos teratogênicos podem estar relacionados ou serem desencadeados pela deficiência de zinco, devido ao seu efeito sobre a expressão gênica (Cousins, 1998). Deste modo, o Zn desempenha papel importante na formação do embrião.

2.1 Enzimas zinco-dependentes

O Zn é requerido em cerca de 300 enzimas no organismo, podendo ter função catalítica, co-catalítica e estrutural. O zinco participa diretamente da catálise enzimática, e sua remoção ocasiona a inativação da enzima. Os sítios catalíticos têm como característica única uma esfera de coordenação aberta, ou seja, o polímero que constitui o sítio de coordenação do metal possui uma molécula de água ligada a outras 3 ou 4 moléculas de aminoácidos. Essa característica única permite diferenciar um típico sítio catalítico dos demais sítios co-catalíticos e estruturais, nos quais toda esfera de coordenação é preenchida por cadeias laterais de aminoácidos. A molécula de água ligada ao zinco é um componente critico do sítio catalítico, pois é a partir dela que o zinco pode ser ionizado a hidróxido de zinco (como acontece na anidrase carbônica),

polarizado por uma base (como acontece na carboxipeptidase A) gerando um nucleófilo para a catálise, ou ainda ser deslocado pelo substrato (Henriques et al., 2003).

Em locais catalíticos, os íons de zinco são geralmente expostos e ligados a uma molécula solvente. O seu ligante mais comum é a histidina, seguido pela glutamina, asparagina e cistina, e a geometria predominante é tetraédrica (Alberts et al., 1998). Alguns exemplos de metaloenzimas nas quais o zinco atua como fator catalítico são: a enzima conversora de angiotensina (germinal), fosfolipase A_2, fosfatase alcalina, a anidrase carbônica II, a carboxipeptidase A, e a álcool desidrogenase, entre centenas de enzimas (Valee & Auld, 1990). Segundo Hagaman et al. (1998) o zinco é fundamental na ativação da enzima ECA (Enzima Conversora de Angiotensina), que afeta diretamente a fertilidade.

O Zn também é essencial no metabolismo lipídico. Ele faz parte do centro catalítico da enzima fosfolipase A_2 (PLA_2). As fosfolipases são uma classe de enzimas que catalisam a hidrólise de fosfolipídios de membrana para liberar ácidos graxos livres (Chang et al., 1987). A PLA_2 enzima, que é secretada pelo pâncreas (Kim et al., 1998), hidrolisa a fosfatidilcolina, e facilita a formação de quilimícrons, essenciais para absorção das micelas (Noh & Koo, 2001). Essa enzima hidrolisa o ácido graxo (AG), geralmente poli-insaturado, na posição sn-2 dos fosfolipídios da membrana, e quando liberados, estes podem ser metabolizados para formar vários eicosanoides e mediadores de lipídios bioativos relacionados (Burke & Dennis, 2009).

A fosfatase alcalina (ALP) é uma enzima proteica, que contém cinco resíduos de cisteína, dois átomos de zinco e um átomo de magnésio, cruciais para sua função catalítica (Sharma et al., 2014). A fosfatase alcalina cliva o pirofosfato, removendo sua influência estabilizadora na matriz óssea, ao mesmo tempo em que aumenta o fosfato local para a cristalização. Deste modo essa enzima é fundamental para a mineralização óssea (Moss, 1984).

A anidrase de carbônica catalisa a interconversão entre CO2 e bicarbonato, bem como outras reações hidrolíticas. Assim possui um papel importante no controle do pH do sangue (Supuran, 2016). Também é considerada uma enzima vital na deposição de carbonato de cálcio durante a formação da casca em galinhas, pois o HCO_3 é o precursor do carbonato da casca de ovo (Lundholm, 1990). A suplementação de zinco na dieta de galinhas pode melhorar a qualidade da casca do ovo, devido ao aumento da atividade enzimática da anidrase carbônica, e no caso de matrizes, pode consequentemente aumentar o número de ovos incubáveis (Mao & Lien, 2017; Zhang et al., 2017a).

Segundo Underwood & Suttle (1999), o Zn também está na constituição da carboxipeptidase A, que é responsável pela hidrólise de aminoácidos C-terminal de peptídeos, portanto essencial para a digestão de proteínas e consequentemente na deposição proteica para o crescimento.

Além da função catalítica nas enzimas, o átomo de Zn também pode ter função estrutural ou co-catalítica, que aumenta ou diminui a catálise, quando associado a outro átomo de zinco ou a um átomo de outro metal no sítio ativo da enzima, no entanto sua remoção não condiciona a perda da atividade ou estabilidade desta (Henriques et al., 2003). Alguns exemplos de

metaloenzimas nas quais o zinco atua como fator co-catalítico são: fosfolipase C, nuclease P1 e leucina aminopeptidase (Valee & Auld, 1990).

O zinco estrutural é necessário apenas para manutenção da estabilidade conformacional das proteínas, pois contribui para estabilização da estrutura quaternária de holoenzimas oligoméricas. O íon metálico é geralmente coordenado por 4 cadeias laterais de aminoácidos, dispostos em geometria tetraédrica, e a água é excluída da esfera interna da coordenação, diminuindo drasticamente a capacidade reacional do sistema. Ao contrário dos sítios catalíticos de zinco, estes não apresentam um padrão regular de espaçamento entre as proteínas ligantes e apresenta uma maior flexibilidade nos parâmetros de acomodação do íon, diminuindo a rigidez das estruturas secundárias e permitindo às proteínas com átomos estruturais de zinco desempenhar uma gama diversificada de funções (Henriques et al., 2003).

Alguns exemplos de metaloenzimas em que o zinco atua como fator estrutural são: aspartato carbamoiltransferase, Cu-Zn superóxido dismutase, proteínas dedos de zinco (Zif268), ferredoxin (Valee & Auld, 1990). Segundo Alberts et al. (1998) na enzima superóxido dismutase, os íons de zinco não participam diretamente na reação catalítica da enzima, mas confere estabilidade a enzima, portanto, são categorizados como estruturais.

3. Distribuição do zinco no tecido animal

O tecido mais responsivo à alta suplementação de Zn são os ossos (Wedekind et al., 1992) apesar de estar presente em todos os tecidos. A taxa de renovação mineral varia de acordo com o tecido, mas geralmente são altos na mucosa e no fígado, intermediário em outros tecidos moles e devagar nos ossos, e é influenciada pelo estado fisiológico e nutricional (deficiência ou toxicidade).

Aves alimentadas com dietas contendo entre 50 e 70 ppm de Zn apresentam concentrações médias de 0,8 a 1,4 mg Zn/l no soro, 20 a 30 ppm de Zn no pâncreas e 100 a 160 ppm de Zn na tíbia (Mohanna & Nys, 1999).

Em animais privados de um mineral, o declínio na concentração nos eritrócitos é normalmente mais lenta do que no plasma, uma vez que o status mineral do eritrócito permanece praticamente constante ao longo de sua ‘vida’ (120 dias). Minerais são comumente incorporados como unidades funcionais no eritrócito imaturo antes de ser liberado na corrente sanguínea, e o conteúdo mineral do eritrócito jovem sozinho reflete precisamente um aporte mineral recente (Suttle, 2010). Resultados de amostras de plasma geralmente refletem o ‘status do mineral em transporte’, e valores baixos indicam o início da deficiência.

A concentração de Zn no plasma é um parâmetro melhor do que a concentração do mesmo no fígado devido a baixa reserva e taxa de mobilização do mineral. Os principais parâmetros para determinação do status de Zn no animal são: Zn na membrana plasmática durante a depleção, deficiência de fosfatase alcalina, mudanças no conteúdo de proteínas e lipídios no quadro de deficiência, mudança na concentração de íons de cálcio intracelular e enzimas dependentes no início da disfunção.

4. Fontes alimentares de zinco

Os grãos e cereais utilizados para fabricação de rações apresentam concentrações variáveis de zinco, por exemplo, o glúten de milho tem em média 80 ppm de Zn, já o trigo e o milho grão por volta de 20 ppm de Zn, o farelo de soja tem em média 49 ppm, o sorgo somente 14 ppm, enquanto os produtos de origem animal apresentam níveis muito superiores, a farinha de carne com 130 ppm de Zn e a farinha de penas com 152 ppm (Givens & Moss, 1990; Rostagno et al., 2011).

No entanto, a simples presença do mineral nos ingredientes, como grãos e cereais, não garante que o animal esteja sendo suprido (Mafra & Cozzolino, 2004), o que requer suplementação do mineral via premix na dieta para atender a exigência. Existem fatores antinutricionais presentes na dieta podem interagir com o zinco, complexando-o e afetando sua biodisponibilidade, quando na forma de sais. A presença de fitatos e outros íons no alimento limitam a utilização de zinco, devido à formação de quelatos indigeríveis (Wise, 1995).

As fontes de microminerais disponíveis para serem utilizadas na suplementação de rações são nas formas de sais ou minerais quelatados a compostos orgânicos (Brito et al., 2006). Mais de 90% da suplementação das rações para aves é feita com óxidos e sulfatos, com alta biodisponibilidade (Bertechini, 2012). O percentual de zinco nas fontes e sua biodisponibilidade relativa podem ser observados na Tabela 1.

As formas de Zn comumente utilizadas para suplementar as rações são: oxido (ZnO) e sulfato ($ZnSO_4.7H_2O$). A biodisponibilidade relativa do ZnO para frangos em relação ao sulfato ($ZnSO_4$) chega a ser 44 a 61% (Wedekind & Baker, 1990; Wedekind et al., 1992). Enquanto o cloreto de Zn tetrabásico tem uma biodisponibilidade similar a do sulfato para frangos (Batal et al.,2001).

Tabela 1. Percentual e fontes de zinco e sua biodisponibilidade relativa.

Elemento	Fonte	% do elemento na fonte	Biodisponibilidade
Zinco	Carbonato de zinco	52,0	Alta
	Cloreto de zinco	48,0	Intermediária
	Sulfato de zinco	22,0 - 36,0	Alta
	Oxido de zinco	46,0 - 73,0	Alta

Fonte: Bertechini, 2012.

5. Interações com outros elementos

A simples presença do mineral no alimento não garante que o animal esteja sendo suprido do mesmo (Mafra & Cozzolino, 2004). Fatores antinutricionais presentes na dieta podem interagir com o zinco, complexando-o e afetando sua biodisponibilidade. A presença de fitatos e outros íons no alimento limitam a utilização de Zn, devido à formação de quelatos indigeríveis, o que ocorre em condições normais de alimentação.

O fitato é o principal agente quelador do Zn. Ele pode ser facilmente complexado com o fitato da dieta, assim altos níveis de zinco tem sido suplementados para evitar problemas com a deficiência desse micromineral.

Frangos de corte conseguem reter 65% do zinco de cereais com baixo teor de fitato, enquanto cereais com alto teor de fitato permitem uma retenção de apenas 45% (Linares et al., 2007). No entanto, estudos de balanços de minerais apresentam algumas dificuldades, principalmente pela excreção que ocorre ao nível de lúmen através do suco pancreático, como resposta a homeostase orgânica desses microelementos.

As interações com fontes proteicas, cálcio e fósforo também prejudicam a absorção deste micronutriente. De acordo com Shoulten et al. (2002), quando se elevam os níveis de cálcio nas rações de frango de corte, ocorre uma redução na retenção de Zn intestinal. As dietas normalmente utilizadas para aves no Brasil são à base de milho e farelo de soja, com alto teor de ácido fítico, e possuem altos níveis de garantia de cálcio. Essa combinação pode prejudicar a absorção de Zn, devido a uma interação tripla, onde o alto nível de Ca, o Zn e o fitato. O excesso de cálcio é mais comum, principalmente nas regiões calcárias, onde as fontes de água podem conter níveis de cálcio. O Zn pode ainda interagir com outros microminerais, através da competição por transportadores, um exemplo é o cobre (Cu), que na presença de altas concentrações de Zn tem sua absorção diminuída (Hall et al., 1979). Por sua vez, a diminuição na absorção de Cu pode levar a diminuição nos valores de hemoglobina e hematócrito no organismo (Baumgartner et al., 1978).

6. Deficiência e toxidez de zinco

As anormalidades clínicas e patológicas associadas à deficiência da maioria dos minerais não são específicas e se confundem com outras desordens nutricionais ou infecções. A falta de apetite ocasionada pela deficiência de Zn reduz o consumo de ração ao ponto que os sinais apresentados passam a ser consequência da carência de diversos nutrientes e não somente do Zn (Veiga, 2006). Com o menor consumo de alimento, os animais apresentam retardo no crescimento (Emmert & Baker, 1995).

O consumo de alimento é afetado pela deficiência de zinco (O'Dell & Reeves, 1989), pois a privação de zinco aumenta a expressão do gene para o hormônio colecistoquinina (Cousins et al., 2003). No entanto o papel do zinco parece ser multifatorial, pois também ocorre aumento na expressão da leptina, o hormônio da saciedade (Kwun et al., 2007), e a redução da expressão da piruvato-quinase (Beattie et al., 2008) que é altamente regulado pela insulina.

Na deficiência de zinco pode ocorrer diminuição da síntese e da secreção dos hormônios luteinizante (LH) e folículo-estimulante (FSH), e problemas associados a diferenciação das gônadas, crescimento testicular, formação e maturação dos espermatozóides, esteroidegênese e manutenção de parâmetros normais de fertilidade (Henriques et al., 2003).

Problemas de pele (paraqueratose) são sinais tardios de deficiência de Zn. Nas aves é visível nos pés e penas (Sunde, 1978). A cicatrização da pele é mais lenta em animais deficientes (Miller et al., 1965). Engrossamento e encurtamento de ossos longos foram reportados em frangos deficientes em zinco (O'Dell & Savage, 1958). Falta de zinco durante o desenvolvimento embrionário de pintinhos afeta a formação do esqueleto (membros, vértebras e

cabeça) provenientes de poedeiras deficientes (Kienholz et al., 1961) assim como redução na eclodibilidade.

Animais de produção apresentam uma considerável tolerância a altos níveis de Zn. A extensão da tolerância depende inicialmente da espécie, mas principalmente da natureza da dieta. Leitões a desmama podem ser alimentados com dietas contendo 2-3g Zn/kg, contudo o crescimento e consumo de ração são reduzidos quando a suplementação chega a 4-8g Zn/kg com alta mortalidade. No entanto, ao aumentar o nível de cálcio de 7g para 11 g /kg o efeito tóxico do zinco é reduzido em função do antagonismo desses minerais (Underwood & Suttle, 1999).

Frangos de corte e poedeiras toleram 1-2g Zn/kg e demonstram uma pequena queda no desempenho e consumo de ração quando suplementado 4g Zn/kg de ração (Oh et al., 1979). Em poedeiras, níveis muito altos de Zn (20g/kg) tem sido utilizados por curto período de tempo (14 dias) para induzir a muda forçada sem consequências negativas em longo prazo (Stevenson & Jackson, 1984).

Segundo Jose et al. (2017) a ocorrência de toxicidade por zinco é incomum em animais de produção, no entanto relatam que a administração de níveis mais elevados de zinco deve ser fornecida com cautela, pois pode ser tóxico para os embriões em desenvolvimento ou prejudicar o posterior desenvolvimento dos pintinhos comerciais.

7. Exigência Nutricional

7.1 Métodos de avaliação da exigência nutricional

O requisito ou exigência de um dado nutriente pode ser definido como "a quantidade mínima do nutriente necessário para produzir o melhor ganho de peso, eficiência alimentar, etc., assim como a falta de sinais de deficiência nutricional" (Leeson & Summers, 2001).

Os valores de exigência nutricional podem ser estimados utilizando o método empírico (dose-resposta) ou o método fatorial. No método fatorial as exigências são estimadas pela soma dos nutrientes utilizados para cada função (mantença, acréscimo de proteína, engorda e produção). Esse método tem sido base para elaboração de modelos que estimam as exigências nutricionais levando em conta as diferenças de pesos, composição corporal, potencial de crescimento e de produção dos animas, assim como do ambiente na definição da exigência (Sakomura et al., 2014). O método fatorial fraciona a exigência total em manutenção e crescimento/produção, sendo expresso por: consumo do nutriente = nutriente para manutenção + nutriente para produção. O NRC (2012) para suínos, por exemplo, está baseado em modelos para crescimento e reprodução. No entanto, o método mais utilizado para definir as exigências dos monogástricos tem sido o dose-resposta, por apresentar maior facilidade e praticidade de aplicação.

O NRC (1994) e as Tabelas Brasileiras para aves e suínos (2005) têm estabelecido às recomendações nutricionais na maioria dos estudos realizados com base no método dose-resposta. No entanto alguns fatores podem interferir na determinação da exigência por este método, como por exemplo, o ambiente,

clima e genética, o que torna necessário a repetição dos experimentos em várias condições (Sakomura & Rostagno, 2007). O método dose-resposta é baseado na resposta do animal ao aumento na ingestão de um determinado nutriente. Segundo Euclydes & Rostagno (2001), a adição de um nutriente limitante na ração, mantendo níveis adequados dos demais nutrientes, promove crescimento do animal até que sua exigência seja atendida, onde posteriormente existirá uma faixa de estabilização no crescimento e, em seguida, dependendo do nutriente e da quantidade, poderá ocorrer uma perda de peso do animal, ou seja, causar toxidez no animal. Portanto, esse método estima a exigência nutricional de determinado nutriente pela avaliação de uma resposta a parâmetros pré-definidos, como o ganho de peso, a conversão alimentar, a deposição de carne magra, a produção de ovos no período, através do oferecimento de quantidades crescentes do nutriente (níveis) nas dietas (Rostagno et al., 2007).

Uma análise mais simplista, embora equivocada, das respostas aos níveis é apenas para comparar pontos usando contrastes ortogonais ou testes de comparação de médias (Teste T, Scheffe, Duncan, Student-Newman-Keuls, e Tukey). Esses testes levam a conclusões de que o requisito é entre duas concentrações do nutriente que foram utilizadas no experimento, e não há como dizer exatamente qual é o requisito ou como dizer o nível de confiança na estimativa do requisito (Pesti et al., 2009). Assim, os dados devem ser submetidos a análises de regressão, utilizando modelos lineares, por exemplo, linear e exponencial, ou modelos não lineares, por exemplo, resposta linear com platô, reposta linear com quadrática e exponencial.

Os resultados (exigência) podem ser diferentes segundo o critério de resposta ou modelos de regressão utilizados (modelo descontínuo - Linear Response Plateau (LRP), modelo quadrático e modelo exponencial) para estimar o nível ótimo do nutriente (Sakomura & Rostagno, 2007). Segundo Lerman & Bie (1975) é difícil selecionar curvas realistas que relacionam a resposta animal com a composição de alimentação, e reforçam a importância de escolher o modelo correto para a resposta na obtenção do lucro máximo em formulações.

A aplicação de cada um dos modelos de regressão dependerá da relação entre os níveis do nutriente em estudo e a resposta aos mesmos (Euclydes & Rostagno, 2001). O LRP, linear response com platô pode proporcionar subestimação do nível ótimo, já a quadrática, que aparentemente apresenta vantagem em determinar a exigência no máximo desempenho possível, tem a desvantagem de apresentar simetria bilateral (queda na mesma proporção que o acréscimo) e alta sensibilidade às diferenças entre os níveis estudados (Samokura & Rostagno, 2007). Os modelos não lineares, como reposta linear com quadrática e exponencial, foram considerados vantajosos em comparação com modelos lineares, pois os sistemas biológicos raramente funcionam de forma perfeitamente linear (Pesti et al., 2009).

O modelo quadrático polinomial (QP) determina o nível ótimo pelo ponto de máxima da equação, no entanto esse modelo tem sido criticado pelo fato de superestimar os valores de requerimento. Alguns pesquisadores têm utilizado 95% do valor encontrado como sendo o resultado. O modelo LRP tem comportamento ascendente até certo ponto, quando o crescimento se

estabiliza abruptamente, representado por uma paralela ao eixo das abscissas (x). O modelo assintótico (exponencial) baseia-se no conceito de que a resposta animal reduz à medida que se aproxima do máximo desempenho, e proporciona um excelente ajuste às respostas biológicas, no entanto não prevê o efeito do excesso dos nutrientes, sendo importante considerar a amplitude dos níveis estudados (Sakomura & Rostagno, 2007). Outro procedimento utilizado para estudar a exigência de um nutriente é a utilização combinada do molelo broken line com a equação quadrática (QRP ou BLQ) (Euclydes & Rostagno, 2001; Baker et al.,2002). Neste modelo o ponto onde o platô da broken line cruza a curva quadrática é tido como ponto de máxima. A partir de todas essas opções, o modelo que melhor se ajusta ao conjunto de dados experimentais deve ser escolhido, observando o comportamento dos dados, valor de probabilidade e o coeficiente de determinação (R^2) (Lerman & Bie, 1975).

7.2 Exigência de zinco

Os manuais de linhagens comerciais apresentam recomendações de suplementação para vários micromineiras incluindo o zinco. No entanto, os níveis de suplementação recomendados não contabilizam o zinco inerente ao alimento. A recomendação de suplementação de zinco para matrizes pesadas na fase de produção segundo o manual da linhagem Cobb (Cobb-Vantress, 2013) é de 110 mg Zn/kg de ração. Já a Tabela Brasileira para Aves e Suínos recomenda a suplementação 65 mg Zn/kg (Rostagno et al., 2017). Segundo o NRC (1994) a exigência de Zn é de apenas 4,5 mg por galinha diariamente. É importante ressaltar que valores de recomendação de suplementação e valores de exigência nutricional são diferentes, todavia ambos podem ser utilizados na formulação de dietas animais.

A estimativa da exigência nutricional pode ser maior ou menor dependendo do critério escolhido. A diferença entre as recomendações de suplementação mineral para animais acontece por causa dos diferentes parâmetros analisados em cada experimento. Dependendo do parâmetro ou modelo escolhido para avaliação dos dados, a exigência pode ser superestimada ou subestimada (Pesti et al., 2009).

Alguns estudos realizados com frangos até 21 dias de idade indicam uma exigência de cerca de 40 a 50 mg Zn/kg de ração para melhor desempenho (Mohanna & Nys, 1999; Huang et al.,2007; Linares et al., 2007). Poucos trabalhos avaliaram a exigência de zinco para reprodutoras. De acordo com Baião & Lúcio (2005), níveis de 70 a 100 ppm nas rações para matrizes pesadas são suficientes para garantir a produção de ovos com boa qualidade. Altas recomendações de Zn para poedeiras e reprodutoras pesadas (NRC, 1994) são provavelmente justificadas pela baixa disponibilidade do Zn em dietas com alto nível de cálcio.

Nos últimos anos, as pesquisas com microminerais têm sido realizada testando a biodisponibilidade entre diferentes fontes (Cheng et al., 1998; Hudson et al., 2004; Yenice et al., 2015; Sirri et al., 2016; Zhang et al., 2017b). No entanto, pesquisas relacionadas a exigência de microminerais para reprodutoras têm sido uma preocupação secundária. Existe uma escassez na literatura científica a respeito deste assunto. A falta destes trabalhos pode estar

promovendo o uso inadequado deste micromineral nas rações, trazendo como consequências possíveis problemas nutricionais, devido a interação com outros compostos, além de oneração do custo da ração e aumento na sua concentração nas excretas, aumentando sua excreção no meio ambiente (Schmidt et al., 2005).

HIPÓTESES E OBJETIVOS

Hipóteses

Existe diferença entre os níveis de zinco dietético para reprodutoras pesadas.

Existe interação entre períodos e níveis de zinco dietético para reprodutoras pesadas.

Objetivo Geral

Determinar a exigência de zinco para reprodutoras pesadas da linhagem comercial Cobb 500 SF utilizando o Sulfato de Zn.

Objetivos Específicos

Avaliar a interação entre os níveis de suplementação de zinco e períodos de produção.

Determinar o melhor nível de zinco dietético para produção de ovos, qualidade de ovos, deposição de zinco no ovo, fertilidade, eclodibilidade e parâmetros sanguíneos da matriz e da progênie.

CAPÍTULO II[1]

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METABOLISM AND NUTRITION

ZINC AND BROILER BREEDERS

Zinc requirements of broiler breeder hens

A.N. Mayer*, S.L. Vieira*,[1], E. Berwanger*, C.R. Angel†, L. Kindlein‡, I. França*, T.L. Noetzold*

*Department of Animal Sciences, Federal University of Rio Grande do Sul, Av. Bento Gonçalves, 7712, Porto Alegre, RS, Brazil, 91540-000

†Department of Animal and Avian Sciences, University of Maryland, College Park, 20742, USA

‡Department of Preventive Veterinary Medicine, Federal University of Rio Grande do Sul, Av. Bento Gonçalves, 8834, Porto Alegre, RS, Brazil , 91540-000

[1]Corresponding author: slvieira@ufrgs.br

S. L. Vieira

Department of Animal Sciences, Federal University of Rio Grande do Sul

Avenida Bento Gonçalves, 7712, Porto Alegre, RS, Brazil, 91540-000

Phone/FAX: +55-51-3308-6048

ABSTRACT One hundred and twenty Cobb 500 hens, 20 wks of age, were randomly allocated into individual cages with the objective of estimating their Zn requirements. The study was composed of 3 phases: adaptation to cages (basal diet), depletion (deficient diet containing 18.7 $\pm$ 0.47 ppm Zn) for 7 wks, and experimental phases. Hens were fed diets with graded increments of Zn sulfate ($ZnSO_4$ $7H_2O$), totaling 18.7 $\pm$ 0.47; 50.3 $\pm$ 10.6; 77.3.0 $\pm$ 11.0; 110.2 $\pm$ 12.8; 140 $\pm$ 12.2 and 170.6 $\pm$ 13.2 ppm analyzed Zn in feeds for 12 wks (experimental phase). Requirements of Zn were done using quadratic polynomial (QP), broken line quadratic (BLQ), and exponential asymptotic models (EA). In general, the non-linear statistical models were the ones that best fit the results in this study. Requirements obtained for hen day egg production and settable egg production were 83.3, 78.6 ppm and 61.4, 65.4 ppm for period of 33 to 36 wks, and 63.3, 53.1 and 60.4, 46.1 ppm for period of 37 to 40 wks, and 62.8, 52.8, and 67.7, 62.1 ppm for period of 41 to 44 wks, respectively, using BLQ and EA models. Total eggs and total settable eggs produced per hen had Zn requirements estimated as 75.7, 64.7 ppm, and 56.5, 41.5 ppm, respectively for BLQ and EA models, whereas for ALP and eggshell percentage were 161.8, 124.9 ppm and 126.1, 122.4 ppm, using QP and BLQ models. Egg and hatching chick weights decreased with Zn supplementation. Maximum responses for Zn in yolk for periods of 37 to 40 and 41 to 44 wks were 71.0, 78.1 and 64.5, 59.6 ppm, respectively using BLQ and EA models. Breaking strength had Zn requirements estimated at 68.0 and 96.7 ppm, whereas eggshell palisade layer and eggshell thickness were maximized with 67.9, 67.9 ppm, and 67.7, 64.4 ppm, respectively for BLQ and EA models. The average of all Zn requirement estimates obtained by EA and BLQ models this present study was 72.28 ppm or 11.1 mg/ hen/d.

Key words: breeders, chick hatching, mineral, zinc.

INTRODUCTION

Zinc (Zn) is an essential trace mineral with several roles in animal metabolism as part of structural components, signaling mediators, and catalytic factors (Maret, 2000; Parkin, 2004; Jurowski et al., 2014; Kambe, et al. 2015). More than 200 enzymes require Zn as a functional component, conferring widest involvement in enzyme functioning in animals (MacDonald, 2000). The main processes mediated by Zn affect protein synthesis, cell proliferation, gene expression, endocrine hormone synthesis, immunity and reproduction (Shankar and Prasad, 1998; Liu et al., 2015; Naz et al., 2016; Perez et al., 2017; Sloup et al., 2017). In chickens, Zn is largely required for eggshell deposition since it is a structural component of the carbonic anhydrase enzyme (Guimarães et al., 2013). This enzyme has its main activity in the shell gland (Zhang et al., 2017) and is involved in egg shell synthesis via the catalysis and interconversion of carbon dioxide and water to bicarbonate (Roberts, 2004). The appropriate formation of eggshell is essential to maintain an acceptable production of settable and hatchable eggs since the shell provides structure and mechanical protection to the egg while serving as a source of calcium and other minerals to the embryo (Hunton, 1995; Vieira, 2007).

It has been reported that Zn absorption can occur in the duodenum and jejunum by a saturable carrier-mediated process, whereas Zn absorption in the ileum can occur with a nonsaturable diffusion process (Antonson et al., 1979; Sorensen et al., 1998; Krebs, 2000; Wang et al., 2001; Yu et al., 2008). Transporter functioning of Zn is highly conserved between species (Cousins et al., 2006; Wang and Zhou, 2010), but it seems that the ZnT and ZIP transporters

control most of the uptake, sequestration and release of Zn between biological membranes (Jeong and Eide, 2013; Kambe et al., 2015). The ZIP family transporters are responsible for the influx Zn from the extracellular space into the cytoplasm, while those from the ZnT family function by extruding Zn from the cytoplasm into the lumen of intracellular organelles or to the outside of the cell (Eide, 2006; Fukada et al., 2011). Zinc homeostasis is mainly maintained by the gastrointestinal tract, by mechanisms of up-regulation of the carrier-mediated process and with increased or decreased Zn excretion (King et al., 2000).

Inadequacies in dietary Zn for broiler breeders lead to low hatchability as well as impaired growth and abnormal development of the entire major organ systems and embryo death follows in extreme deficiencies (Blamberg et al., 1960; Amen and Al-Daraji, 2011; Zhu et al., 2017). Dietary Zn fed to hens is mostly deposited in the yolk with a much lower concentration in the albumen and eggshell (Richards, 1997). The developing oocyte accumulates circulating vitellogenin, which is further hydrolyzed to phosvitin and lipovitellin (Vieira, 2007). Most of the Zn present in the yolk is bound in lipovitellin (Tupper et al., 1954). Dietary Zn concentration affects Zn deposition in egg yolk (Kienholz et al., 1961).

Zinc concentration in feedstuffs is low (NRC, 1994). In parallel, Zn present in plant feedstuffs is poorly utilized by chickens due to its chelation to phytic acid (O´Dell and Savage, 1960). Thus, supplementation of poultry feeds with Zn is usual ant this frequently includes a considerable safety margin (Li et al., 2015, Sridhar et al., 2014), which may be promoting the excessive use of this trace mineral in addition to the requirement. The Zn sources commonly used in poultry diets are oxide and sulfate, being the latter more bioavailable (Sandoval et al., 1997).

There have been many published reports on Zn supplementation in broilers, laying hens, and breeder feeds (Bartlett and Smith, 2003; Burrell et al., 2003; Hudson et al., 2004a; Wang et al., 2016; Zakaria et al., 2017; Tsai et al., 2016; Sharideh et al., 2016; Pacheco et al., 2017; Qin et al., 2017; Abd El-Hack et al., 2017; Zhu et al., 2017). Most of them, however, have not investigated the actual Zn requirements of breeder hens. Therefore, references for Zn supplementation in these types of diets are largely based on suggestions. Zinc recommendations published in the NRC (1994) for commercial laying hens is 45 mg Zn/kg feed, whereas these vary from 65 to 110 mg Zn/kg feed in other publications (FEDNA, 2008; Cobb-Vantress, 2013; Aviagen, 2017; Rostagno et al., 2017).

The lack of updates in Zn recommendations for commercial poultry based on published work contrasts with the progress in their performance traits (Navidshad et al., 2016). On the other hand, there is a current concern over the high excretion of micro minerals used to supplement animal diets into the environment (Leeson, 2009). Thus, the European Commission has recently established a maximum limit for the total Zn content, including the supplemental premix, of poultry diets at 100 ppm (EFSA, 2014). The knowledge of the requirements may enable the reduction in the use of Zn in poultry diets, without affecting animal health and welfare and productivity.

The objective of this study was to assess the Zn requirements of broiler breeder hens using Zn sulfate as the source of the added Zn. The responses used to determine the requirements were related with the productive performance, egg quality, blood constituents, and quality of the hatching chicks.

MATERIALS AND METHODS

Birds

All procedures utilized in the present study were approved by the Ethics and Research Committee of the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil. One hundred and twenty Cobb 500 broiler breeder hens, 20 wks old, were individually placed in cages (0.33 m length x 0.46 m deep x 0.40 m height) whereas 30 Cobb breeder males were placed in 3 collective floor pens (2.0 x 1.5 m) for semen collection. Each cage was electrostatic painted and provided with one stainless steel nipple drinker and a plastic trough feeder. Overall breeder management followed Cobb-Vantress (2016) guidelines. Semen collection, and hen insemination was done as described by Taschetto et al. (2017).

Treatment Diets

The study was composed of 3 phases, each one having different diets as follow: adaptation to cages (basal diet), depletion (deficient diet) and experimental phases (treatment diets) (Table 1). Immediately after placement in cages, the 20 wk old hens were fed a basal diet for 6 wks (adaptation phase), based on the nutritional requirements recommended by Cobb-Vantress (2013) guidelines. From 26 to 32 wks of age, hens received a Zn deficient diet (15.9 ppm formulated, 18.7 $\pm$ 0.47 ppm analyzed), in order to deplete the hens body Zn storage. The Zn deficient diet was formulated to meet all nutritional requirements, except for Zn (Cobb-Vantress, 2013). At 33 wks of age, hens were fed the experimental diets, starting the experimental phase. At this point birds were individually weighed and randomly assigned to the experimental cages, resulting in similar average weight for all treatments. The experimental diets were composed of 6 different Zn concentrations (Table 2). Each treatment had 20 replicates and one hen was the

experimental unit. Supplementation was done using laboratory grade Zn sulfate heptahydrate ($ZnSO_4$ $7H_2O$) (Sigma Aldrich, St.Louis, USA). The supplemented levels were 0, 30, 60, 90, 120, and 150 mg Zn/kg of feed. Dietary Zn was analyzed in 4 samples per treatment of the 4 batches mixed throughout the study and averaged 18.7 $\pm$ 0.47; 50.3 $\pm$ 10.6; 77.3 $\pm$ 11.0; 110.2 $\pm$ 12.8, 140.0 $\pm$ 12.2, and 170.6 $\pm$ 13.2 mg Zn/kg (Table 2).

The experimental phase was divided into 3 periods of 28 d, from 33 to 44 wks of age. Thus, the study was a 6 x 3 factorial arrangement of 6 Zn supplementation levels and 3 periods. Feed was restricted and provided daily as recommended by Cobb-Vantress (2016). The amount of feed provided was equal for all hens, and can be observed in the Table 2. Males were fed corn-soy-wheat bran mash diet to meet Cobb-Vantress (2013) recommendations.

All ingredients and feeds were analyzed for Zn content, using inductive coupled plasma atomic emission spectroscopy (ICP - Spectro Flamme, Spectro Analytical Instruments, Kleve, Germany) (Anderson, 1999). Consumption of Zn in mg/kg/d per hen was calculated using daily feed consumption and feed concentration (Table 2). Water Zn content was analyzed using atomic absorption (ZEEnit 650 P, Analytik Jena, Jena, Germany). Averaged duplicate analysis of Zn in water was 0.195 $\pm$ 0.030 ppm, which was not considered a significant dietary source of the mineral.

Hen Performance Measurements

Eggs were classified daily as hatchable or not with broken and deformed eggs being considered not hatchable. The percentage of total and hatchable eggs in the period was calculated for each hen. In each period, the hatchable eggs were weighed and grouped into 3 replicates per treatment and incubated in a single-stage incubator (Avicomave, Iracemápolis, SP, Brazil) set at

37.5ºC and 65% RH until 18 d. Eggs were then transferred to a hatcher set to 36.6ºC and 80% RH. Total hatchability and hatchability of fertile eggs were calculated as percentage of hatching chicks to the total and fertile eggs set, respectively. All unhatched eggs were broken open to determine the approximate day of embryonic death as described by Favero et al (2013). Hatchling chicks were weighed and length measured, corresponding the distance from the tip of the beak to the end of the middle toe (third toe) (Molenaar et al., 2008).

Three broiler breeder hens from each treatment per period were randomly selected for blood collection. Hematocrit (Ht) and hemoglobin (Hb), and alkaline phosphatase (ALP) concentration were obtained from pooled blood samples. Blood obtained was partially transferred to 0.5 ml test tubes containing EDTA for Ht and Hb analyzes. Ht was determined using micro capillaries containing blood centrifuged for 5 min at 15,650 – 18,510 × g. The cyanmethemoglobin method was used to determine Hb concentration (Crosby et al., 1954). Blood left was centrifuged to obtain the serum. Analysis of ALP was performed as described by Roy et al. (1970), using a digital bench colorimeter (Model Labquest, Vernier Software & Technology, Beaverton, United States). Determination of Hb and Ht was done with 15 chicks hatched per treatment in each period. Chick blood samples were obtained from the jugular vein after euthanasia by cervical dislocation.

In addition to performance and blood analyzes, egg quality was evaluated. Eggs from 10 replicates were collected during of the last 5 days of each period, totaling 45 eggs per treatment. Eggs (n=25) were used to measure egg weight, specific gravity, yolk, albumen and eggshell percentage. Specific gravity was determined using saline solutions with concentrations ranging from 1.065 to 1.095 g/cm^3 in intervals of 0.005 units (Novikoff and Gutteridge, 1949). Shell weight was obtained after washing and drying at 105ºC overnight whereas shell thickness was

measured using a micrometer (Model IP65, Mitutoyo Corp., Kawasaky, Japan) in the apical, equatorial and basal regions with these values being averaged for statistical analysis. The other 20 eggs were used to determine eggshell breaking strength, using a texture analyzer (Model TA.XT.plus, Texture Technologies Corp., Hamilton, United States), with a 75-mm (P/75) breaking probe (Molino, et al., 2015). Three yolks from eggs from the same replicate hen were pooled and lyophilized to obtain a total of 10 replicates per treatment per period. Yolk Zn content was quantified using ICP as described for feedstuffs and rations.

Five eggs with the same average weight $^{\pm}$ 10% SD per treatment by period (age 36, 40, and 44 wks), were used in the analysis of eggshell ultrastructure using a scanning electron microscopy (King and Robinson, 1972). In preparation for this analysis, each eggshell was broken into 3 samples (0.5 cm^2) at the equatorial region. The membranes of the shell were removed by immersion of samples in a solution of 6% sodium hypochlorite, 4.12% sodium chloride, and 0.15% sodium hydroxide (Stefanello et al., 2014). Samples were mounted transversely and horizontally on aluminum stubs using carbon tape, to measure the thickness of eggshell layers and the number of mammillary buttons/mm^2, respectively. These were metallized with gold at 35 nm for 3 minutes (BAL-TEC SCD050 Sputter Coater, Capovani Brothers Inc., Scotia, New York, United States). A total of 1,080 images were obtained in the scanning electron microscope (JEOL JSM 6060, GenTech, Arcade, New York, United States) with an acceleration voltage from 10 to 20kw and at magnification of 300× for transversal samples (810 images) and 200× for horizontal samples (270 images). Eggshell layer identification was done according to the descriptions of Dennis et al. (1996). Microscopy images were analyzed in the Image-Pro Plus software (Media Cybernetics, Rockville, United States). Average of measurements (µm) were estimated from 3 different locations in each image.

Statistical Analysis

An homoscedasticity test was applied to the data set (Shapiro and Wilk, 1965). Data were transformed using the arcsine square root percentage ($z = \text{asin}\ (\text{sqrt}\ (y\ ^{+}\ 0.5))$) when not presenting residues with normal distribution (Ahrens et al., 1990). Then data were submitted to ANOVA using the MIXED procedure of SAS (2011), with periods as repeated measures, excepted for total egg production and settable egg production per hen at 44 wks, which were analyzed using the general linear models (PROC GLM). The choice of covariance structures of PROC MIXED was based on the Akaike criteria (Littell et al., 1998). Tukey-Kramer test was used for means comparison and differences were considered significant at $P < 0.05$ (Tukey, 1991).

Estimates of Zn requirements were obtained using quadratic polynomial (QP), broken line quadratic (BLQ), and exponential asymptotic (EA) models (Robbins et al., 1979). The goodness of fit of the different models was evaluated by the coefficient of determination (R^2) and Akaike information criteria (AIC). The QP model ($Y = a + b \times Zn + c \times (Zn)^2$) had Y as the dependent variable and as a function of dietary level of Zn; *a* as the intercept; *b* as the linear coefficient and *c* as the quadratic coefficient. The maximum response for Zn was defined as $Zn = - b \div (2 \times c)$. The EA model ($Y = a + b \times (1- \text{EXP}\ (- c \times (Zn - d)))$) had Y as the dependent variable as a function of dietary level of Zn; *a* estimated the relative response to the diet containing the lowest Zn (deficient diet); *b* estimated the difference between the minimum and the maximum response obtained with Zn supplementation; *c* was the curve slope coefficient; and *d* was the Zn level of the deficient diet. The Zn requirement was defined as $Zn = (ln\ (0.05) / - c) + d$, using 95% of the asymptotic response, since the exponential curve never reaches the asymptotic point. The BLQ

model ($Y = a + b \times (c - Zn)^2$) had ($c$ - Zn) = 0 for Zn > c had Y as the dependent variable as a function of the dietary level of Zn, a the value of the dependent variable at the plateau and b as the slope of the line. The Zn level at the break point (c) was considered the one providing maximum responses.

RESULTS

There were interactions between dietary Zn and period for egg production, settable egg production and Zn deposition in the egg yolk (Table 3 and 4). Significant interactions ($P < 0.05$) showed that in the first period (33 to 36 wks) egg production increased when the 77.3 ppm Zn treatment was fed, whereas egg production in the 2 and 3rd periods increases were seen from the 50.3 ppm Zn treatment and up. Hens receiving the Zn deficient diet had impaired egg production and settable egg production in the 2 and 3rd period as compared to those fed diets containing Zn supplementation. In addition, the deposition of Zn in the yolk increased as Zn was added at 33 to 36 wks with the highest supplemental Zn concentration ($P < 0.05$). However, the level of the 50.3 ppm dietary Zn was sufficient to stabilize the Zn yolk deposition from 37 to 44 wks Zn yolk ($P < 0.05$; Table 5).

Most of the evaluated responses were affected by period, except for the thickness of mammillary buttons ($P > 0.05$). Egg production, fertile egg hatchability, eggshell percentage, and number of mammillary buttons decreased according to hen age ($P < 0.05$). Egg hatchability, breeder Hb, ALP, and eggshell thickness were higher at period of 37 to 40 weeks ($P < 0.05$). Whereas Ht of hen, chick blood measurements, hatching chick measurements, egg weight, yolk percentage, albumen percentage, Zn concentration in the yolk, and palisade layer thickness increased according to the periods ($P < 0.05$) (Table 3 and 4).

Zn supplementation did not affect Ht, Hb, hatching chick length, and albumen percentage or mammillary layer thickness ($P > 0.05$). Total and settable egg production, palisade layer thickness and eggshell thickness increased, whereas hatching chick weight, egg yolk percentage, and egg weight decreased when hens were fed diets containing diets having from 50.3 to 170.6 ppm Zn ($P < 0.05$). The highest ALP value was obtained with dietary Zn level from 110.2 ppm of Zn, as well as the eggshell percentage was at 110.2 and 140.0 ppm ($P < 0.05$). Specific gravity increased up to 170.6 ppm of dietary Zn. On the other hand, the number of the mammillary buttons increased in eggs from hens fed the deficient diet ($P < 0.05$; Table 3 and 4).

Estimates of Zn requirements were determined using QP, BLQ and EA models of regression. The values were expressed as dietary concentration (ppm) as well as daily intake per hen (mg per hen day) (Tables 6 and 7). Overall, Zn requirements obtained for the period of 33 to 36 wks were higher than the values obtained from 37 to 40 and 41 to 44 wks. Maximum responses for egg production obtained by the QP model were 114.1 ppm (17.5 mg/hen/day), 116.4 ppm (17.8 mg/hen/day), and 116.3 ppm (17.8 mg/hen/day) of Zn in the hens diets for periods 33 to 36, 37 to 40 and 41 to 44 wks, respectively. The BLQ and EA models, on the other hand, produced lower requirements and data that were better fitted. Requirements of Zn obtained using the BLQ model for egg production were 83.3 ppm (12.7 mg/hen/day), 63.3 ppm (9.7 mg/hen/day), and 62.8 ppm (9.6 mg/hen/day) in the periods of 33 to 36, 37 to 40 and 41 to 44 wks, respectively. Requirement values obtained for the EA model were similar to those obtained by BLQ models. In the EA model, maximum egg production were obtained using 78.6 ppm (12.0 mg/hen/day), 53.1 ppm (8.1 mg/hen/day), and 52.8 ppm (8.1 mg/hen/day) Zn at 33 to 36, 37 to 40, and 41 to 44 wks, respectively (Table 6).

Settable egg production as well as Zn concentration in the yolk showed different behavior between periods with a significant interaction of Zn level and period. Requirements for settable egg production were 112.6 ppm, 113.2 ppm and 120.0 ppm (QP model) from 33 to 36 wks, and 61.4 ppm, 60.4 ppm and 67.7 ppm from 37 to 40 wks (BLQ model), and 65.4 ppm, 46.1 ppm and 62.1 ppm from 41 to 44 wks. On the other hand, yolk Zn contents from 33 to 36 wks increased linearly with Zn level in diets. From 37 to 40 wks, the maximum Zn concentration in the yolk was obtained using 127.9 ppm (19.6 mg/hen/day), 71.0 ppm (10.9 mg/hen/day), and 78.1 ppm (11.9 mg/hen/day) Zn, by QP, BLQ and EA models. In the last period, from 41 to 44 wks, the maximum response were estimated as 124.9 ppm (19.1 mg/hen/day), 64.5 ppm (9.9 mg/hen/day), and 59.6 ppm (9.1 mg/hen/day) Zn, by QP, BLQ and EA models (Table 7).

Breeder hen requirements of Zn for total and settable egg production were estimated as 116.4 ppm (17.8 mg/hen/day) and 115.2 ppm (17.6 mg/hen/day) by QP model, 75.7 ppm (11.6 mg/hen/day) and 56.5 ppm (8.6 mg/hen/day) by BLQ model, and 64.7 ppm (9.9 mg/hen/day) and 41.5 ppm (6.3 mg/hen/day) by EA model, respectively. The EA model did not have a good fit for ALP. Maximum ALP values were obtained using 161.8 ppm (24.8 mg/hen/day) and 124.9 ppm (19.1 mg/hen/day) Zn by QP and BLQ model. Requirements obtained for ALP, as well as for eggshell quality were higher than those for egg production measures.

Breaking strength and eggshell percentage were increased up to 161.6 ppm (24.7 mg/hen/day) and 126.1 ppm (19.3 mg/hen/day), 68.0 ppm (10.4 mg/hen/day) and 122.4 ppm (18.7 mg/hen/day), and 96.7 ppm (14.8 mg/hen/day) and 147.8 ppm (22.6 mg/hen/day), respectively, using the QP, BLQ and EA models. The Zn requirement was similar for eggshell and palisade thickness. The QP model estimated 126.3 ppm (19.3 mg/hen/day) and 126.0 ppm (19.3 mg/hen/day) as Zn requirement. BLQ model estimated 67.9 ppm (10.4 mg/hen/day) and

67.7 ppm (10.4 mg/hen/day) Zn, and EA model estimated 67.9 ppm (10.4 mg/hen/day) and 64.4 ppm (9.9 mg/hen/day) Zn as requirement for eggshell and palisade thickness, respectively. The number of mammillary button thickness decreased linearly with increasing dietary Zn, thus the deficient diet provided higher values of this variable.

DISCUSSION

In the present study Zn deficiency was clearly demonstrated with the diets not supplemented with Zn sulfate (18.7 ppm). The different evaluated responses demonstrated variable impacts due to Zn supplementation. As with other studies when more than one response is measured, determining a unique requirement value was not possible. In addition, dietary values that optimize the diverse responses vary depending on the statistical model utilized (Robbins et al., 1979). In the present study, EA and BLQ models provided better fits for most measurements and estimated lower requirements than the QP model. The quadratic model tended to overestimate the requirements, since the curvature is very sensitive to variations in the treatment intervals. In addition, this model is physiologically incorrect, since it presupposes symmetrical responses to deficiency and excess, whereas nonlinear models better explain the biological models (Runho et al., 2001; Pesti et al, 2009). EA and BLQ models reported requirements varied from 62.2 ppm (9.4 mg/hen/d) Zn for maximum egg production, whereas for egg quality it was 81.3 ppm (12.2 mg/hen/d) Zn.

The interaction observed between Zn supplementation and period indicated differences in the Zn requirements from 33 to 44 wks when compared to the other periods. Data showed that egg production increased as a function of Zn supplementation in the first period (33 to 36 wks) with a less pronounced response in the following periods (37 to 40 and 41 to 44 wks). Yolk Zn

responded linearly to increasing dietary Zn from 33 to 36 wks. The difference between the first period after the depletion phase and the other 2 periods were likely due to a lower Zn body content at the beginning of the study. Other authors also observed an increase in egg production of various bird species when Zn was supplemented at 30 ppm, 45 ppm and 160 ppm Zn in diets fed to laying hens, ducks and japanese quails, respectively (Kucuk et al., 2008; Aghaei et al., 2017; Chen et al., 2017). Supplemental Zn contents for broiler breeder hens from popular tables of recommendation vary from 65 to 110 ppm (Cobb-Vantress, 2013; Aviagen, 2017; Rostagno, 2017). The NRC (1994) recommends 4.5 mg of Zn per hen daily for breeders, but this was based on a few research reports. In the present study, the average Zn requirement for maximum total egg production from 33 to 44 wks was 85.6 ppm or 13.1 mg per hen per day. In contrast, the BLQ and EA models, which fitted better, estimated 75.7 and 64.7 ppm or 11.6 and 9.9 mg per hen per day, indicating higher values than the NRC (1994), but similar to the other sources cited above. The values reported in the present study refer to total Zn, which contrasts with the suggestion present in most references which refer to supplemental values.

It appears that Zn requirements for egg hatchability were lower than the deficient level tested in the present study (18.7 ppm Zn), since no significant differences between treatments were found. According to Stahl et al. (1986) 28 ppm dietary Zn is sufficient to prevent decreased egg hatchability, as well as egg production. However, Blamberg et al. (1960) showed a decrease in egg hatchability of hens supplemented with 6 ppm Zn in the diets. Kienholz et al. (1961) reported that dietary Zn fed to hens did not impact blood Hb and Ht, but Sahin et al. (2009) found that the activity of several enzymes in metabolic pathways decrease in Zn deficient poultry. In the present study, ALP increased with Zn addition to the deficient diet. Plasma ALP levels have been reported to increase when pullets begin to lay (Bell, 1960). There have been

numerous reports of the role of ALP activity in the efflux of Ca from the basolateral membrane into the interstitial space by the Ca^{2+}- ATPase dependent active process (Lawson and Davies, 1979). This implies an important role of Zn in the regulatory pathways of cartilage (Starcher et al., 1980) and bone synthesis (hydroxyapatite crystallization) (Sauer et al., 1997).

Nutrient transference into the egg is required for normal development of the embryo, which depends on a good nutritional status of the breeders (Wilson, 1997). The major mineral source for the embryo is the yolk, which contains most of the P, Zn, Cu, Mn, and Fe in eggs (Richards, 1997). In the present study, yolk Zn deposition increased as dietary Zn was gradually supplemented. It is necessary that each essential mineral be available at the appropriate time during the growth and development of the embryo within the egg to ensure its survival (Gilani and Alibhai, 1990). In this study there was no effect of Zn on most hatching chick measurements. However, hens consuming the Zn deficient diet produced eggs with higher egg yolk percentage and heavier eggs and chicks. As hen egg production decreased, their sequence length decreased leading to a greater mean inter-sequential interval. This may have led to the increased hatching chick body weight of the non supplemented diet (Shanawany, 1984) since a high correlation between egg weight and hatching chick weight exists, irrespective of the hens age (Pinchasov, 1991).

Supplementing Zn led to an increase in settable egg production, which may have occurred due to improvements in eggshell quality in this study, since cracked or broken eggs are not used for incubation. These results are in accordance with other published studies, which correlate Zn with improvement in eggshell quality (Hudson et al., 2004b; Stefanello et al., 2014; Manangi et al. 2015). One of the most important problems in the poultry industry is eggshell quality, influencing economic profitability of egg production and egg hatchability (Swiatkiewicz and

Koreleski, 2008). Zinc affects egg formation also through effects in oviduct epithelium due to the role of Zn in protein synthesis (Tabatabaie et al., 2007) as well as in the carbonic anhydrase enzyme, which plays a role in the synthesis of eggshell calcium carbonate (Christianson and Fierke, 1996). Carbonic anhydrase is a catalyst in the shell gland for the formation of carbonic acid, which can be dissociated into bicarbonate ions, thus allowing a greater number of carbonate ions to be produced, which can then be utilized for calcium carbonate deposition (Gutowska and Mitchell, 1945). It is likely that Zn levels tested in this study may have led to a higher activity of carbonic anhydrase and, then leading to improvements in eggshell thickness. This enzyme, however, was not analyzed in the present study. In addition to having increased the total thickness of the eggshell, increased dietary Zn led to improvements in egg breaking strength.

The eggshell contributes to a successful embryogenesis in many ways, such as through protection, gas and water exchange (Karlsson and Lilja, 2008). The eggshell is composed of several layers, which include the mammillary, palisade and the vertical or surface crystal layer, as well as the cuticle (Solomon, 2010). The mammillary layer is a regular array of cones or knobs, functioning as calcium storage that is eventually delivered to the embryo. It also forms the basis of the layer of palisades (Hincke et al., 2012). The palisade layer, the thickest of the whole shell, extends beyond the bases of the cones and ends in a thin vertical crystal layer where crystals are aligned perpendicularly to the shell surface (Nys et al., 2004, Solomon, 2010). Increases in dietary Zn (maximized at 67.9 ppm, using non-linear models) resulted in greater palisade layer thickness, as well as a decrease in the number of mammillary buttons (Figure 1). The density of mammillary knobs has a negative correlation with breaking strength and quality of the eggshell (Stefanello et al., 2014). With the increasing number of mammillary buttons it was possible to observe disorders and structural changes, which may act as nucleation sites of

breakage (Solomon, 1991). Thus, eggshells with a higher density of mammillary buttons, cracks, and scratches may present inordinate inner surfaces which are physically less resistant (Parsons, 1982; Van Toledo et al., 1982).

In summary, Zn requirements from the present study ranged from 41.5 to 161.6 ppm dietary Zn (6.3 to 24.7 mg/hen/d), depending on the response criteria. The average requirement estimates for egg and settable egg production was 84.0 ppm (12.9 mg/hen/d) and 74.9 ppm (11.4 mg/hen/d), whereas it was higher when averaged for egg quality responses (108.1 ppm Zn, 16.1 mg/hen/d). When requirements obtained using the three models and for all variables were averaged in the present study a value of 91.4 ppm Zn (14.1 mg/hen/d) was obtained. Averaged values separately for QP, BLQ and EA models, values obtained were 124.3, 76.3, and 69.9 ppm Zn (19.0, 11.7, and 10.7 mg/hen/day), respectively.

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Table 1. Experimental diets provided to breeder hens.

Ingredient, % as-is[1]	Basal diet 20 to 26 wks	Zn deficient diet 27 to 44 wks
	Adaptation phase	Pre-experimental and Experimental phases
Rice, polished and broken, 8.0% CP	-	30.00
Corn, 7.8% CP	52.51	35.59
Soy protein isolate, 89% CP	-	10.32
Oat hulls	-	11.63
Calcium carbonate	-	7.49
Soybean Meal, CP 45%	18.69	-
Wheat meal	16.87	-
Limestone	6.64	-
Dicalcium phosphate	1.67	-
Soybean oil	2.61	1.60
Phosphoric acid, 85% P	-	1.64
Potassium carbonate	-	0.78
Sodium bicarbonate	0.20	0.10
Sodium Chloride	0.20	0.06
Potassium chloride	-	0.18
Choline chloride	0.10	0.18
DL-methionine, 99%	0.17	0.16
L-Lysine, 98.5%	0.05	-
L-threonine 98.5%	0.03	0.05
Vitamin and mineral mix[2]	0.25	0.20
L-Tryptophan, 98%	0.01	0.02
Total	100.00	100.00
Calculated nutrient composition, % or as shown		
AME_n, kcal/kg	2,761	2,760
CP	15.4	15.42
Ca	2.99	3.00
Available P	0.43	0.45
Na	0.19	0.19
Zn, ppm		
Calculated	152.00	15.9
Analyzed[3]	-	18.7 ± 0.47
Choline, mg/kg	1,500	1,500

[1] Calcium carbonate, phosphoric acid, sodium bicarbonate and potassium chloride were Lab grade and had trace amounts of Zn (5.4; 0.6; 0.0; 8.1 ppm).

[2] Mineral and vitamin premix supplied the following per kilogram of diet: Cu, 15 mg; Mn, 120 mg, Fe, 50 mg; Se, 0.3 mg and I, 2 mg; and only basal diet contained Zn, 110 mg (all laboratory grade); vitamin A, 12,000 IU; vitamin D_3, 3,000 IU; vitamin E, 100 IU; vitamin C, 50 mg; vitamin K_3, 6 mg; vitamin B12, 40 µg; thiamine, 3.5 mg; riboflavin, 16 mg; vitamin B6, 6 mg; niacin, 40 mg; pantothenic acid, 25 mg; folic acid, 4 mg; biotin, 0.3 mg; BHT, 100 mg.

[3]Analyzed Zn was from one pooled sample from each batch (total of 4 batches).

Table 2. Supplemented, calculated and analyzed Zn concentrations in the experimental diets feed intake and Zn intake per hen day in each period.

Supplemented Zn, ppm[1]	Total dietary Zn, ppm		Period, wk			Average
	Calculated	Analyzed[2]	33 to 36	37 to 40	41 to 44	33 to 44
			Zn intake, mg/hen/d			
0	15.4	18.7 ± 0.47	2.9	2.9	2.8	2.9
30	45.4	50.3 ± 10.59	7.9	7.7	7.5	7.7
60	75.4	77.3 ± 11.01	12.1	11.8	11.6	11.8
90	105.4	110.2 ± 12.80	17.3	16.8	16.5	16.9
120	135.4	140.0 ± 12.18	22.0	21.4	20.9	21.4
150	165.4	170.6 ± 13.18	26.8	26.0	25.5	26.1
Zn intake, mg/hen/d			14.8	14.4	14.1	14.5
Feed intake, g/hen/d			157	152.5	149.5	153.0

[1]From Zn sulfate heptahydrate ($ZnSO_47H_2O$) Lab grade.

[2]Analyzed Zn was from one pooled sample from each batch (total of 4 batches).

Table 3. Response of broiler breeder hens to increased dietary Zn.

	Eggs						Breeder			Hatching chick			
	Hen day production[2], %	Settable egg/ hen, %[3]	Total/ hen [4]	Total settable eggs[5] / hen	Hatchability, %	Hatchability of fertile, %	Ht[6], %	Hb[7], g/dL	ALP[8], U/L	Hb, g/dL	Ht, %	Body Weight, g	Body Length, cm
Zn, ppm[1] (mg/ day)													
18.7 (2.9)	66.8[b]	58.0[b]	57[b]	49[b]	80.3	88.0	30.2	7.88	92.5[b]	7.83	30.4	48.4[a]	18.2
50.3 (7.7)	74.6[a]	69.0[a]	62[a]	58[a]	85.4	93.8	31.3	8.22	112.4[ab]	7.82	30.5	47.1[b]	18.3
77.3 (11.8)	76.4[a]	70.3[a]	64[a]	59[a]	86.1	94.3	31.1	8.08	123.1[ab]	7.66	29.7	46.9[b]	18.2
110.2 (16.9)	75.5[a]	68.8[a]	64[a]	58[a]	85.0	93.3	31.2	8.45	143.6[a]	7.82	30.6	47.2[b]	18.3
140.0 (21.4)	75.1[a]	68.6[a]	63[a]	58[a]	86.1	94.5	30.3	8.07	154.3[a]	7.85	30.2	47.4[b]	18.4
170.6 (26.1)	74.5[a]	68.4[a]	63[a]	58[a]	85.2	93.4	31.2	8.41	143.4[a]	7.91	30.8	46.7[b]	18.3
Period, wk													
33-36	78.8[a]	72.2[a]	-	-	85.6 [ab]	95.2 [a]	29.9[b]	7.97[b]	115.9[b]	7.78 [ab]	30.3[ab]	44.1[c]	17.4[c]
37-40	73.3[b]	67.6[b]	-	-	86.1 [a]	91.2 [b]	30.7[ab]	8.64[a]	148.4[a]	7,66 [b]	29.8 [b]	48.3[b]	18.6[b]
41- 44	69.4[c]	61.3[c]	-	-	82.1 [b]	92.4 [ab]	32.0[a]	7.94[b]	120.3[ab]	8.00 [a]	31.0 [a]	49.5[a]	18.8[a]
SEM	0.3780	0.4389	0.3849	0.4880	0.7274	0.6808	0.2927	0.1305	4.7370	0.0370	0.1195	0.1298	0.0259
Prob													
Level	<.0001	<.0001	<.0001	<.0001	0.4157	0.2157	0.7665	0.7776	0.0008	0.5806	0.1413	<.0001	0.1720
Period	<.0001	<.0001	-	-	0.0003	0.0017	0.0164	0.0424	0.0066	<.0001	0.0001	<.0001	<.0001
Level x Period	0.0016	0.0304	-	-	0.2365	0.2939	0.3273	0.1286	0.99846	0.0653	0.6339	0.5278	0.2443

[a>b>c>d] Means within a column without a common superscript differ significantly by Tukey test ($P < 0.05$).

[1]Values are analyzed and values between parentheses are Zn intake (mg/hen/day).

[2] Egg produced as a percentage of total live hens at the time of measurement.

[3] Settable eggs produced as a percentage of total live hens at the time of measurement.

[4] Total eggs at the end of the experiment.

[5] Total settable egg at the end of the experiment.

[6] Hematocrit.

[7] Hemoglobin.

[8] Serum alkaline phosphatase.

Table 4. Broiler breeder hen egg characteristics as affected by increased dietary Zn.

	Egg weight, g	Yolk, %[2]	Albumen, %[3]	Eggshell, %	Yolk Zn, ppm [4]	Specific gravity, g/cm[3]	Eggshell			
							Palisade layer Thickness, µm	Mammillary layer Thickness, µm	Thickness, µm	Number of mammillary buttons/ mm[2]
Zn, ppm[1] (mg/ day)										
18.7 (2.9)	68.3[a]	30.7[a]	60.7	8.6[c]	54.9[d]	1083.2[b]	227.8[b]	122.6	350.4[b]	169.6[a]
50.3 (7.7)	67.1[b]	29.9[b]	61.1	9.0[bc]	66.7[c]	1084.0[ab]	266.8[a]	125.8	392.6[a]	159.9[ab]
77.3 (11.8)	67.2[b]	29.9[b]	60.3	9.8[ab]	67.9[bc]	1084.4[ab]	271.1[a]	128.2	399.3[a]	158.3[ab]
110.2 (16.9)	67.0[b]	29.8[b]	60.2	10.0[a]	68.1[bc]	1084.7[ab]	269.6[a]	123.6	393.3[a]	145.5[b]
140.0 (21.4)	67.2[b]	29.8[b]	60.2	10.0[a]	70.1 [ab]	1085.0[ab]	275.2[a]	125.4	400.6[a]	144.6[b]
170.6 (26.1)	67.3[b]	29.9[b]	60.4	9.7[ab]	71.6[a]	1085.1[a]	274.2[a]	126.8	401.0[a]	142.5[b]
Period, wk										
33-36	66.2[c]	29.5[b]	59.7[b]	10.7[a]	62.3[c]	1084.4[b]	257.3[b]	122.3	379.6[b]	200.9[a]
37-40	67.5[b]	29.7[b]	61.0[a]	9.3[b]	67.3[b]	1085.9[a]	266.5[ab]	128.6	395.1[a]	126.6[b]
41- 44	68.3[a]	30.8[a]	60.7[a]	8.5[c]	70.1[a]	1082.9[c]	268.6[a]	125.3	393.9[ab]	132.7[b]
SEM	0.1028	0.0953	0.1366	0.1146	0.3993	0.298	2.5237	1.0389	3.1043	4.3093
Prob										
Level	0.0009	0.0188	0.2584	<.0001	<.0001	0.0378	<.0001	0.7035	<.0001	0.0003
Period	<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	0.0353	0.0661	0.0274	<.0001
Level x Period	0.4168	0.8290	0.5881	0.4517	<.0001	0.2138	0.4193	0.9885	0.7903	0.1960

a>b>c>d Means within a column without a common superscript differ significantly by Tukey test ($P < 0.05$).

[1]Values are analyzed and values between parentheses are Zn intake (mg/hen/day).

Table 5. Effects of dietary Zn *vs* periods on performance of broiler breeder hens.

	Hen day egg production, %				Settable egg production, %				Zn Yolk, ppm			
	Periods (wk)			*P*-value	Period, wk			*P*-value	Period, wk			*P*-value
Zn, ppm (mg/d)[1]	33-36	37-40	41- 44		33-36	37-40	41-44		33-36	37-40	41-44	
18.7 (2.9)	75.2^{Ab}	66.4^{Bb}	58.8^{Cb}	<.0001	66.8^{Ab}	58.0^{Bb}	49.2^{Cb}	<.0001	57.0^{Ac}	54.6^{Ab}	53.2^{Ab}	0.1040
50.3 (7.7)	78.2^{Aab}	74.4^{ABa}	70.9^{Ba}	<.0001	72.9^{Aa}	68.8^{ABa}	62.4^{Ba}	<.0001	60.4^{Bbc}	67.9^{Aa}	71.9^{Aa}	<.0001
77.3 (11.8)	80.7^{Aa}	75.8^{ABa}	72.8^{Ba}	<.0001	74.2^{Aa}	71.9^{Aa}	64.7^{Ba}	<.0001	60.8^{Bbc}	69.0^{Aa}	73.9^{Aa}	<.0001
110.2 (16.9)	79.8^{Aab}	75^{ABa}	71.8^{Ba}	<.0001	73.6^{Aa}	69.0^{ABa}	63.9^{Ba}	<.0001	61.3^{Bbc}	70.0^{Aa}	73.1^{Aa}	<.0001
140.0 (21.4)	79.5^{Aab}	74.8^{ABa}	70.9^{Ba}	<.0001	73.0^{Aa}	69.0^{ABa}	63.9^{Ba}	<.0001	66.1^{Bab}	71.0^{ABa}	73.1^{Aa}	0.0004
170.6 (26.1)	79.1^{Aab}	74.6^{Ba}	71.2^{Ba}	<.0001	72.7^{Aab}	68.8^{ABa}	63.9^{Ba}	<.0001	68.3^{Ba}	71.3^{ABa}	75.4^{Aa}	0.0003
P-value	0.0088	<.0001	<.0001		0.0002	<.0001	<.0001		<.0001	<.0001	<.0001	

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

$^{A>B>C>D}$ Means with different letters in the same line indicate significant differences ($P < 0.05$).

[1]Values are analyzed and values between parentheses are Zn intake (mg/hen/day).

Table 6. Regression equations of egg production of breeders fed with Zn supplementation**.**

	Model	Regression equations[1]	R^2	*P*-value	Requirement	Zn mg/d
Egg Production[2], %						
	QP	$y= 73.25663 + 0.12611x - 0.00055238x^2$	0.13	0.0002	114.1	17.5
33-36 wk	BLQ	$y= 79.7294 - 0.00111(83.2716 - x)^2$	0.14	0.0002	83.3	12.7
	EA	$y= 75.1319 + 4.5927(1-EXP(-0.05(x - 18.7)))$	0.14	0.0003	78.6	12.0
	QP	$y= 63.8034 + 0.215466x - 0.00092554x^2$	0.25	<.0001	116.4	17.8
37-40 wk	BLQ	$y= 75.048 - 0.00434(63.2685 - x)^2$	0.31	<.0001	63.3	9.7
	EA	$y= 66.4312+8.5973(1 – EXP(-0.0871(x - 18.7)))$	0.31	<.0001	53.1	8.1
	QP	$y= 56.26489 + 0.29996x - 0.001291x^2$	0.34	<.0001	116.3	17.8
41-44 wk	BLQ	$y= 71.753-0.00632(62.8582 - x)^2$	0.43	<.0001	62.8	9.6
	EA	$y= 59.4219 + 12.3025(1 – EXP(-0.0878(x - 18.7)))$	0.43	<.0001	52.8	8.1
Settable egg production [3]						
	QP	$y= 64.61493 + 0.17719x - 0.00078685x^2$	0.13	0.0001	112.6	17.2
33-36 wk	BLQ	$y= 73.3827 - 0.00362(61.3998 - x)^2$	0.17	<.0001	61.4	9.4
	EA	$y= 66.7826 + 6.5771(1 – EXP(-0.0969(x - 18.7)))$	0.17	<.0001	65.4	10.0
	QP	$y= 54.55188 + 0.29888x - 0.00132x^2$	0.36	<.0001	113.2	17.3
37-40 wk	BLQ	$y= 69.6707 - 0.0067(60.397 - x)^2$	0.44	<.0001	60.4	9.2
	EA	$y= 58.0169 + 11.6048(1 – EXP(-0.1085(x - 18.7)))$	0.44	<.0001	46.1	7.1
	QP	$y= 45.53743 + 0.34561x - 0.00144x^2$	0.41	<.0001	120.0	18.4
41-44 wk	BLQ	$y= 64.1773 - 0.00624(67.7559 - x)^2$	0.50	<.0001	67.7	10.4
	EA	$y= 49.1397 + 15.0572(1 - EXP(-0.0691(x-18.7)))$	0.50	<.0001	62.1	9.5
Egg Production[4]	QP	$y= 54.99477 + 0.16041x - 0.00068922x^2$	0.36	<.0001	116.4	17.8
	BLQ	$y= 63.4262 - 0.00196(75.6579 - x)^2$	0.42	<.0001	75.7	11.6
	EA	$y= 57.0506 + 6.3386(1 – EXP(-0.0651(x-18.7)))$	0.42	<.0001	64.7	9.9
Settable egg production[5]	QP	$y= 46.31342 + 0.23042x - 0.001x^2$	0.44	<.0001	115.2	17.6
	BLQ	$y= 58.0833 - 0.006590(56.4611 - x)^2$	0.57	<.0001	56.5	8.6
	EA	$y= 48.6917 + 9.3758(1 – EXP(-0.1311(x-18.7)))$	0.57	<.0001	41.5	6.3
ALP[6]	QP	$y= 74.72828 + 0.90597x - 0.00280x^2$	0.17	<.0001	161.8	24.8
	BLQ	$y= 148.9 - 0.5187(124.9 - x)^2$	0.19	<.0001	124.9	19.1

[1]Regression equations obtained using the increasing analyzed Zn in the diets (18.7; 50.3; 77.3; 110.2, 140, and 170.6 ppm).
[2]Eggs produced as a percentage of total live hens.
[3] Settable egg produced as a percentage of total live hens.
[4]Total eggs produced by live hens at the end of the experiment.
[5]Total settable egg produced by live hens at the end of the experiment.
[6]Alkaline Phosphatase.

Table 7. Regression equations of egg yolk Zn and eggshell breaking strength of breeders fed with Zn supplementation.

	Model	Regression equations[1]	R^2	*P*-value	Requirement	Zn mg/d
Yolk Zn[2]						
33-36 wk	L[5]	y= 55.79907 + 0.07023x	0.45	<.0001	-	-
	QP	y= 56.76219 + 0.04031x - 0.00015728x^2	0.44	<.0001	128.1	19.6
37-40 wk	L	y= 58.97527 + 0.0876x	0.47	<.0001	-	-
	QP	y= 50.64155 + 0.34017x - 0.00133x^2	0.67	<.0001	127.9	19.6
	BLQ	y= 70.3395 - 0.00574(71.0195-x)^2	0.76	<.0001	71.0	10.9
	EA	y= 54.6798 + 16.0281(1 - EXP (0.0504 (x - 18.7)))	0.76	<.0001	78.1	11.9
41-44 wk	L	y= 60.38094 + 0.10326x	0.40	<.0001	-	-
	QP	y= 49.15863 + 0.43711x-0.00175x^2	0.63	<.0001	124.9	19.1
	BLQ	y= 73.8955 - 0.00983(64.5542 - x)^2	0.79	<.0001	64.5	9.9
	EA	y= 53.2245 + 20.7419 (1 - EXP (0.0732(x - 18.7)))	0.79	<.0001	59.6	9.1
Eggshell, %	QP	y= 7.92702 + 0.0328x - 0.00013x^2	0.91	0.0122	126.1	19.3
	BLQ	y= 9.9074 - 0.00013(122.4 - x)^2	0.91	0.0266	122.4	18.7
	EA	y= 8.5291 + 1.4613(1- EXP(-0.0232(x - 18.7)))	0.86	0.0493	147.8	22.6
Breaking Strength (kg cm-2)	L	y= 4.00741 + 0.00339x	0.17	0.0006	-	-
	QP	y= 3.84705 + 0.00821x-0.0000254x^2	0.18	0.0013	161.6	24.7
	BLQ	y= 4.4272 - 0.00022(68.0381-x)^2	0.23	0.0007	68.0	10.4
	EA	y= 3.909 + 0.5518(1 - EXP (0.0384(x - 18.7)))	0.23	0.0006	96.7	14.8
Palisade layer[3], µm	L	y= 241.34341 + 0.24082x	0.43	<.0001		
	QP	y= 216.9903 + 0.97254x - 0.00386x^2	0.66	<.0001	126.0	19.3
	BLQ	y= 272.5 - 0.0185(67.8676 - x)^2	0.78	<.0001	67.9	10.4
	EA	y= 227.7 + 45.2528(1 - EXP(-0.0609(x - 18.7)))	0.79	<.0001	67.9	10.4
Thickness[4], µm	L	y= 365.89628 + 0.25128x	0.35	0.0003		
	QP	y= 340.62887 + 1.01047x - 0.004x^2	0.53	<.0001	126.3	19.3
	BLQ	y= 398.6 - 0.0198(67.67 - x)^2	0.66	<.0001	67.7	10.4
	EA	y= 351.1 + 47.6951(1-EXP(-0.0656(x - 18.7)))	0.66	<.0001	64.4	9.9
Number of mammillary bottons/ mm²	L	y= 170.72122 - 0.18489x	0.05	0.0271	-	-

[1]Regression equations obtained using the increasing analyzed Zn in the diets (18.7; 50.3; 77.3; 110.2, 140, and 170.6 ppm).
[2]Zn concentration in the egg yolk.
[3]Palisade layer thickness of the eggshell.
[4]Thickness of the eggshell.
[5] Linear model, used to demonstrate the behavior of the results.

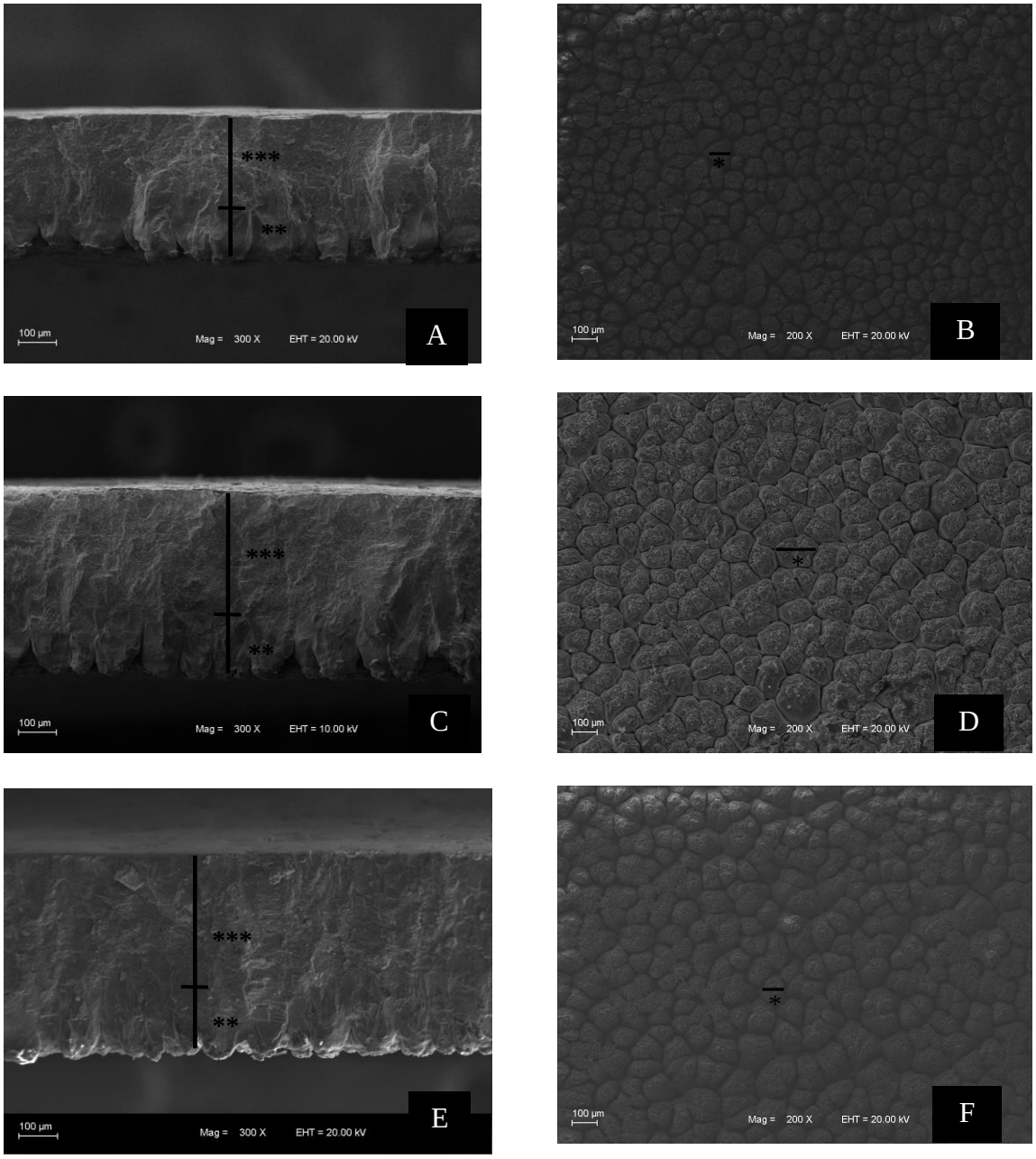

Figure 2. Scanning electron cross-sections and inner surface of the eggshell from broiler breeder hens fed a Zn deficient diet (18.7 ppm) (A;B), and diets with 110.2ppm (C;D) and 170.6 ppm (E; F) Zn (300x and 200x). Figures A, C, and E were used to mensure eggshell layers thickness, and figures B, D, and F used to mensure mammilary buttons density.

* Mammillary button.

** Mammillary layer thickness.

***Palisade layer thickness.

CAPÍTULO III

CONSIDERAÇÕES FINAIS

A essencialidade da suplementação de zinco na dieta dos animais para manter as condições fisiológicas e do metabolismo é imparcial e bem debatida, no entanto, há uma deficiência sobre a real exigência do mineral visando obter o melhor desempenho, que no caso de matrizes é a obtenção de pintinhos de qualidade, que também dependem da produção de ovos com alta qualidade.

O nível de 18,7 ppm de zinco na dietas das reprodutoras foi claramente deficiente, resultando em baixa produtividade do lote recebendo esta ração. O teor de zinco encontrado na água das matrizes foi irrelevante, por isso não foi contabilizado no consumo de zinco e consequente exigência do animal. O Zn propiciou melhores condições fisiológicas que possibilitou resultados excelentes de desempenho e qualidade de ovos. Além disso, foi essencial para deposição do micromineral na gema do ovo, o que pode refletir no futuro desempenho da progênie. Acredito que em próximos trabalhos a avaliação do efeito residual da nutrição mineral da reprodutora sobre o desempenho da progênie seria relevante, visto que os microminerais possuem inúmeras funções no metabolismo e desenvolvimento corporal.

Outro ponto que pode ser abordado futuramente, é o aproveitamento dos microminerais na matriz nutricional dos ingredientes, reduzindo a quantidade de mineral adicionado nas dietas via premix mineral na forma de sais, ou seja, incluindo a exigência do micromineral em si, ao invés de utilizá-lo na forma de suplementação. Atualmente o uso de enzimas exógenas nas rações tem melhorado a disponibilidade dos minerais presentes nos ingredientes, por exemplo a fitase, que pode liberar o zinco complexado ao ácido fítico. Entretanto, ainda se recomenda a utilização de margem de segurança na formulação das dietas.

O uso do proc mixed utilizando o período como medida repetida no tempo foi uma ferramenta importante para estas análises, uma vez que este procedimento faz o ajuste da melhor estrutura de covariância.

Através dos resultados deste trabalho podemos recomendar o uso de 41,5 á 161,6 ppm de Zn na dieta de matrizes de frangos de corte, conforme a resposta desejada. Considerando apenas os modelos não lineares, como o quadrático com platô ou o exponencial, que explicam melhor os modelos biológicos, a média para a exigência de zinco para reprodutoras é de 76,3 e 69,9 ppm na dieta, respectivamente. Entretanto, dependendo da variável a ser considerada no sistema produtivo, a exigência de zinco pode ser maior ou menor, conforme os valores encontrados neste trabalho. Assim, pode ser recomendado a atualização dos valores de exigência do mineral nos manuais de linhagem ou tabelas de recomendações nutricionais.

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APÊNDICES

Apêndice 1. Instruções para publicação na revista Poultry Science

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Scope

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• Article: Bagley, L. G., and V. L. Christensen. 1991. Hatchability and physiology of turkey embryos incubated at sea level with in- creased eggshell permeability. Poult. Sci. 70:1412–1418. Bagley, L. G., V. L. Christensen, and R. P. Gildersleeve. 1990. Hematological indices of turkey embryos incubated at high altitude as affected by oxygen and shell permeability. Poult. Sci. 69:2035–2039. Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, 23 with and without prior vaccination, to challenge with Marek's disease virus. Avian Dis. 50:354–365. doi:10.1637/7498-010306R.1

• Book: Metcalfe, J., M. K. Stock, and R. L. Ingermann. 1984. The effects of oxygen on growth and development of the chick embryo. Pages 205-219 in Respiration and Metabolism of Embryonic Vertebrates. R. S. Seymour, ed. Dr. W. Junk, Dordrecht, the Netherlands. National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

• Federal Register: Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and

rendering establishments, final rule. 9CFR part 71. Fed. Regis. 69:10137–10151.

• Other: Choct, M., and R. J. Hughes. 1996. Long-chain hydrocarbons as a marker for digestibility studies in poultry. Proc. Aust. Poult. Sci. Symp. 8:186. (Abstr.) Dyro, F. M. 2005. Arsenic. WebMD. Accessed Feb. 2006. http:// www.emedicine.com/neuro/topic20.htm. El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, as- signee. US Pat. No. 6,766,767. Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium, MD. Univ. Maryland, College Park. Luzuriaga, D. A. 1999. Application of computer vision and electronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gainesville. Peak, S. D., and J. Brake. 2000. The influence of feeding program on broiler breeder male mortality. Poult. Sci. 79(Suppl. 1):2. (Abstr.)

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A adenine
ADG average daily gain
ADFI average daily feed intake
AME apparent metabolizable energy
AMEn nitrogen-corrected apparent metabolizable energy
ANOVA analysis of variance
B cell bursal-derived, bursal-equivalent derived cell bp base pairs
BSA bovine serum albumin
BW body weight C cytosine
cDNA complementary DNA
cfu colony-forming units
CI confidence interval
CP crude protein
cpm counts per minute
CV coefficient of variation
d day

df degrees of freedom
DM dry matter
DNA deoxyribonucleic acid
EDTA ethylenediaminetetraacetate
ELISA enzyme-linked immunosorbent antibody assay
EST expressed sequence tag
g gram
g gravity
G guanine
GAT glutamic acid-alanine-tyrosine
G:F gain-to-feed ratio
GLM general linear model
h hour
HEPES N-2-hydroxyethyl piperazine-N′-ethane-sulfonic acid
HPLC high-performance (high-pressure) liquid chromatography
ICU international chick units
Ig immunoglobulin
IL interleukin
IU international units
kb kilobase pairs
kDa kilodalton
L liter*
L:D hours light:hours darkness in a photoperiod (e.g., 23L:1D)
m meter
µ micro
M molar
MAS marker-assisted selection
ME metabolizable energy
MEn nitrogen-corrected metabolizable energy
MHC major histocompatibility complex
mRNA messenger ribonucleic acid
min minute
mo month
MS mean square
n number of observations
N normal
NAD nicotinamide adenine dinucleotide
NADH reduced nicotinamide adenine dinucleotide
NRC National Research Council
NS not significant
PAGE polyacrylamide gel electrophoresis
PBS phosphate-buffered saline
PCR polymerase chain reaction pfu plaque-forming units
QTL quantitative trait loci
r correlation coefficient
r2 coefficient of determination, simple
R2 coefficient of determination, multiple
RH relative humidity

RIA radioimmunoassay
rpm revolutions per minute s second
SD standard deviation
SDS sodium dodecyl sulphate
SE standard error
SEM standard error of the mean
SRBC sheep red blood cells
SNP single nucleotide polymorphism T thymine
TBA thiobarbituric acid
T cell thymic-derived cell
TME true metabolizable energy
TMEn nitrogen-corrected true metabolizable energy
Tris tris(hydroxymethyl) aminomethane
TSAA total sulfur amino acids U uridine
USDA United States Department of Agriculture
UV ultraviolet
vol/vol volume to volume vs. versus
wt/vol weight to volume
wt/wt weight to weight
wk week yr year 28
*Also capitalized with any combination, e.g., mL.

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VITA

André Neves Mayer, filho de Werner Alberto Mayer e Marlene de Fátima Neves, nasceu em São Paulo, SP, em 19 de agosto de 1984. Em julho de 2011 obteve o grau de Zootecnista pela Universidade Federal do Paraná. Iniciou em março de 2012, o curso de mestrado na área de produção e nutrição animal, no Programa de Pós-graduação em Zootecnia da Universidade Federal de Lavras. No mestrado realizou seus estudos na área de produção e nutrição de frangos de corte, obtendo o título de mestre em março de 2014. Em abril de 2014 ingressou no curso de doutorado no Programa de Pós-graduação em Zootecnia da Universidade Federal do Rio Grande do Sul, permanecendo na área de pesquisa em produção e nutrição animal. Durante o doutorado participou do grupo de pesquisa em aves, coordenado pelo professor Sergio Luiz Vieira, e realizou seu projeto de doutorado na área de nutrição matrizes de frangos de corte, e no momento está finalizando seu doutorado pela Universidade Federal do Rio Grande do Sul, Porto Alegre, RS.