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EXIGÊNCIA DE MANGANÊS PARA MATRIZES DE FRANGO DE CORTE

THIAGO LUIZ NOETZOLD
Médico Veterinário/FAI

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Thiago Luiz Noetzold
Médico Veterinário

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Aprovada em: 12.03.2020
Pela Banca Examinadora

SÉRGIO LUIZ VIEIRA
PPG Zootecnia/UFRGS
Orientador

CATARINA STEFANELLO
UFSM

CARLOS AUGUSTO MALLMANN
UFSM

LIRIS KINDLEIN
PPG Zootecnia/UFRGS

Homologado em: 18/03/2020
Por

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

CARLOS ALBERTO BISSANI
Diretor da Faculdade de Agronomia

“A natureza fez o homem feliz e bom, mas a sociedade deprava-o e torna-o miserável.”

– Jean-Jacques Rousseau

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EXIGÊNCIA DE MANGANÊS PARA MATRIZES DE FRANGOS DE CORTE[1]

Autor: Thiago Luiz Noetzold
Orientador: Sergio Luiz Vieira

Resumo – O presente estudo foi conduzido com objetivo de determinar a exigência de manganês para matrizes de corte, avaliando a produção de ovos bem como outras características que são importantes para otimizar a produção de pintos saudáveis. Um total de 120 reprodutoras com 22 semanas de idade foram alojadas individualmente em gaiolas. Após serem alimentadas com uma dieta deficiente em Mn por 5 semanas, as galinhas foram divididas aleatoriamente e receberam dietas contendo 6 incrementos de 30 ppm de Mn, a partir do menor nível de Mn (22,2 ppm). A fonte de suplementação de Mn foi o sulfato de Mn (MnSO4 H2O). Os níveis de Mn analisados nas dietas foram: 22,2 ± 3,21; 48,5 ± 3,44; 77,9 ± 5,49; 103,1 ± 1,82; 140,0 ± 7,88 e 168,2 ± 3,57 ppm. As dietas experimentais foram fornecidas durante 4 períodos de 28 dias. As regressões foram estimadas usando modelos quadrático polinomial (QP) e *broken line* com quadrática (BLQ). As exigências de Mn para a produção de ovos e para produção de ovos incubáveis foram 115,8 e 56,6 ppm e 122,1 e 63,6 ppm (P <0,05), respectivamente, usando os modelos QP e BLQ, enquanto a produção de ovos totais e incubáveis ao final do experimento obtiveram exigência de Mn estimada em 121,8 e 61,7 ppm e 115,7 e 56,6 (P <0,05), respectivamente, para os modelos QP e BLQ. Ovos com casca quebrada bem como ovos defeituosos apresentaram 129,5, 66,4 ppm e 118,4 ppm Mn (P <0,05) usando os modelos QP, BLQ e QP, respectivamente. O número de ovos trincados, defeituosos e contaminados diminuiu, enquanto a eclodibilidade, eclodibilidade de ovos férteis, porcentagem de casca de ovo e camada de paliçada aumentaram quando as galinhas foram alimentadas com dietas contendo 48,5 a 168,2 ppm Mn (P <0,05). A resistência da casca e a gravidade específica dos ovos obtiveram requisitos de Mn estimados em 140,2 e 112,7 ppm, bem como 131,3, 68,5 ppm (P <0,05), enquanto a camada paliçada e espessura da casca de ovo foram maximizadas com 128,8 e 68,8 ppm e 140,2, 134,2 ppm, respectivamente para modelos QP e BLQ (P <0,05). Os valores máximos de conteúdo de Mn na gema foram obtidos usando 118,0 e 118,4 ppm de Mn pelos modelos QP e BLQ, respectivamente. A média de todos os requerimentos de Mn estimados para os modelos QP e BLQ foi 128,4 e 92,3 ppm Mn, respectivamente.

Palavras chave: Matriz de frango de corte, micromineral, manganês, exigência.

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MANGANESE REQUIREMENTS OF BROILER BREEDER HENS[1]

Author: Thiago Luiz Noetzold
Advisor: Sergio Luiz Vieira

Abstract – The present research has been conducted with the objective of determine the Mn requirement of broiler breeder hens, assessing egg production as well as other characteristics that are of importance to optimize healthy chick production. One hundred and twenty Cobb 500 hens, 22 wks of age, were allocated individually into cages. After being fed a Mn deficient diet for 5 wks, hens were randomly split in treatments having 6 graded increments of 30 ppm Mn starting with the lowest Mn content feed (22.2 ppm Mn). The supplemental incremental Mn source was lab grade Mn sulfate (MnSO4 H2O). Analyses were conducted in all mixing batches and had: 22.2 + 3.21; 48.5 + 3.44; 77.9 + 5.49; 103.1 + 1.82; 140.0 + 7.88 and 168.2 + 3.57 ppm. Requirements of Mn were estimated using quadratic polynomial (QP) and broken line quadratic (BLQ) models. Experimental feeds were fed during 4 periods of 28 d. Requirements of Mn for hen day egg production and settable egg production were 115.8, and 56.6 ppm and 122.1, and 63.6 ppm ($P < 0.05$), respectively, using QP, and BLQ models whereas total eggs and total settable eggs per hen had Mn requirements estimated as 121.8, and 61.7 ppm and 115.7, and 56.6 ($P < 0.05$), respectively for QP, and BLQ models. Eggs having cracked shells as well as defective eggs had 129.5, 66.4 ppm and 118.4 ppm Mn ($P < 0.05$) using QP, and BLQ models and QP model, respectively. Number of cracked, defective and contaminated eggs decreased, whereas hatchability, hatchability of fertile eggs, eggshell percentage and eggshell palisade layer increased when hens were fed diets having from 48.5 to 168.2 ppm Mn ($P < 0.05$). Breaking strength and egg specific gravity had Mn requirements estimated at 140.2 and 112.7 ppm as well as 131.3, 68.5 ppm ($P < 0.05$), whereas eggshell palisade layer and eggshell thickness were maximized with 128.8 and 68.8 ppm, and 140.2, 134.2 ppm, respectively for QP and BLQ models ($P < 0.05$). Maximum yolk Mn content values were obtained using 118.0, and 118.4 ppm Mn by QP and BLQ models, respectively. The average of all Mn requirements estimated for QP, and BLQ models are 128.4, and 92.3 ppm Mn (18.7 and 13.5 mg/hen/d), respectively.

Key words: broiler breeder hen, micromineral, manganese, requirement.

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RELAÇÃO DE ABREVIATURAS

Mn	Manganês
$MnSO_4$	Sulfato de Manganês
MnSOD	Superóxido Dismutase Mitocondrial
CuZnSOD	Superóxido Dismutase Citosólica e Extracelular
DMT1	*Divalent Metal Transporter-1*

CAPÍTULO I

INTRODUÇÃO

Os minerais são nutrientes importantes para o crescimento e desenvolvimento dos organismos vivos, pois estão envolvidos em inúmeras vias metabólicas onde muitas de suas funções ainda não são bem compreendidas (VIEIRA, 2008; SUTTLE, 2010). Podem ser classificados em macro ou microminerais, dependendo da quantidade encontrada no organismo animal (GEORGIEVSKII et al., 1981). 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 animal (VIEIRA, 2003).

O manganês (Mn) é um dos microminerais essenciais. O Mn é um metal de transição no grupo 7 da tabela periódica, com número atômico 25, e massa atômica de 55. Ele desempenha diversas funções no organismo dos animais, como componente e/ou ativador de enzimas essenciais para o metabolismo, atua na reprodução e funcionamento do sistema nervoso central (BERTECHINI, 2012), sendo também essencial para o desenvolvimento embrionário, crescimento normal dos ossos e metabolismo de carboidratos e lipídios (UNDERWOOD, 1977).

Os principais sais inorgânicos utilizados na avicultura para suplementar manganês são os sulfatos ($MnSO_4$), que normalmente são utilizados para desenvolver as exigências nutricionais para aves e suínos (NRC, 1994). A solubilidade da fonte utilizada, bem como, o status nutricional dos animais, são fatores muito importantes na determinação da exigência de minerais (VIERA, 2008). As diferentes fontes deste mineral podem alterar a disponibilidade e, mesmo que os animais apresentem eficiente eliminação, sugerem indícios de subfornecimento ou sobrefornecimento, sendo de grande valia estudos relacionados com a fonte e exigências de Mn em matrizes.

Os microminerais possuem baixa biodisponibilidade, o que segundo Mabe et al., (2003) pode estar relacionado com a formação de complexos com outras substâncias no trato digestivo reduzindo sua solubilidade. Por conta disso, a suplementação de microminerais em rações para aves é frequentemente feita em quantidades superiores às exigidas, na tentativa de assegurar o bom desempenho das aves (GOMES et al., 2009). As recomendações de Mn para aves reprodutoras podem ser encontradas nos manuais de linhagem, como por exemplo, no manual de linhagem Cobb 500 (2013), a suplementação recomendada para Mn é de 120 ppm na fase produtiva. A tabela brasileira para aves e suínos apresenta recomendação de 70 ppm para fontes inorgânicas (ROSTAGNO et al., 2017), enquanto no *National Research Council* (NRC, 1994) não dispõe de recomendação de Mn para matrizes de frangos de corte.

Tendo em vista a grande importância do Mn para o organismo das aves, há a necessidade de conhecimento dos reais requerimentos exigidos para matrizes reprodutoras. A exigência de um nutriente pode ser definida pela quantidade a ser fornecida na dieta para atender as necessidades de um animal em condições de um ambiente compatível com a boa saúde, e essas necessidades podem ser interpretadas como sendo as quantidades de um nutriente para atender um determinado nível de produção (SAKOMURA &

ROSTAGNO, 2007).

A manutenção de um status adequado dos microminerais é essencial para se atingir uma máxima produção. No entanto, conforme Vieira (2008), a determinação das exigências de microminerais tem sido uma preocupação secundária em nutrição de aves em comparação com outros nutrientes. Assim, tem-se observado dificuldade em encontrar bibliografia científica sobre a exigência de microminerais, especialmente para reprodutoras. Contudo, trabalhos recentes foram desenvolvidos com apresentação de exigências de ferro, cobre e zinco para matrizes pesadas (TASCHETTO et al., 2017; BERWANGER et al., 2018; MAYER et al., 2019). Desse modo, evidencia-se a necessidade de um estudo para determinação da exigência do micromineral manganês para matrizes de frangos de corte, buscando uma nutrição melhor e mais precisa.

REVISÃO BIBLIOGRÁFICA

O Manganês é o quinto metal mais abundante da terra, e em 1930 foi mostrada sua importância na fertilidade e crescimento de animais em laboratório. As pesquisas através de sua suplementação iniciaram por conta da prevenção de duas enfermidades em aves, a perose e a condrodistrofia nutricional de pintos (SUTTLE, 2010).

Funções do Manganês

O Manganês é importante para diversas funções no organismo, como formação e desenvolvimento da matriz orgânica óssea, ativador de várias enzimas (fosfatase, desoxiribonuclease, enolase, glicosiltransferases) e essencial na reprodução e funcionamento normal do sistema nervoso central (BERTECHINI, 2012). Além de ativador de enzimas, o Mn atua como componente de enzimas essenciais no metabolismo dos animais. Um papel bioquímico importante do Mn é no metabolismo de carboidratos e lipídios, fazendo parte da metaloenzima piruvato carboxilase (SCRUTTON et al., 1966, 1972). Interferências na conversão de piruvato em oxalacetato tem sido reportadas em camundongos e reduzindo a deposição de gordura em suínos, ambos com deficiência de Mn (BALY et al., 1985; SUTTLE, 2010).

Outra enzima que possui em sua composição o Mn é a Superóxido Dismutase (MnSOD), isolada a partir de uma mitocôndria localizada em um fígado de frango (SUTTLE, 2010). A MnSOD mitocondrial está presente em grande quantidade em mitocôndrias localizadas no coração, fígado e rins e complementa a enzima CuZnSOD presente no citosol celular. Juntas, elas protegem as células dos peróxidos, principalmente do radical superóxido (O_2-) (SURAI, 2016).

Na ativação de enzimas, o Mn é necessário para a síntese dos mucopolissacarídeos na cartilagem através da ativação da glicosiltransferase (XIAO et al., 2014). Esta enzima é necessária para a síntese de sulfato de condroiditina, presentes nas moléculas de proteoglicano (KEEN et al., 2013). Com a deficiência de Mn, ocorre uma redução de produção de glicosaminoglicanos e oligossacarídeos, prejudicando o crescimento tibial em pintos (LIU et al., 1994). Em poedeiras e matrizes, ovos são produzidos com qualidade da casca ruim, devido a síntese de mucopolissacarídeos prejudicada (HILL & MATHERS, 1968; ZHANG et al., 2017), com consequente redução no teor de hexosamina da matriz da casca dos ovos (LONGSTAFF & HILL, 1972). Xiao et al., (2014) observaram um aumento na deposição de glicosaminoglicanos na membrana da casca de ovos de poedeiras suplementadas com Mn quando comparadas as aves não suplementadas.

Absorção e Metabolismo do Manganês

Minerais como Ca, Fe, Mn, Cu e Zn são absorvidos de acordo com a necessidade do organismo animal. É bastante questionável o quanto o Mn presente nos alimentos é disponível para as aves, mas sabe-se que sua absorção no trato grastrointestinal é pobre (LESSON & SUMMERS, 2001). Apesar dos inúmeros trabalhos publicados a respeito das funções fisiológicas e nutricionais do Mn em aves (ATKINSON et al., 1967; OFFIONG & ABEB, 1980;

OLGUN, 2017), existem poucos trabalhos a respeito de seu mecanismo de absorção.

O Mn pode ser transportado do lúmen intestinal para dentro dos enterócitos pela proteína transportadora de metal divalente-1 (*Divalent Metal Transporter-1*, DMT1), localizado nos enterócitos, responsável por transportar cátions divalentes para dentro dos enterócitos, incluindo o Mn^{2+} (CHUA & MORGAN, 1997). Comparado com o jejuno e íleo, os níveis de expressão gênica de DMT1 são maiores no duodeno, indicando que esse transportador pode estar envolvido na regulação da absorção do Mn no intestino delgado proximal (BAI et al., 2012; LIAO et al., 2019). Por outro lado, alguns trabalhos em laboratório usando sacos intestinais invertidos e alças ligadas reportaram que o íleo foi o principal sítio de absorção do Mn em frangos de corte, maior que o duodeno e jejuno, supondo que o Mn pode estar envolvido em outra forma de absorção na porção distal do intestino delgado (JI et al., 2006a,b).

Após absorvido, o Mn^{2+} é oxidado a Mn^{3+} e se liga a um transportador específico que pode ser a transferrina. Os tecidos ricos em mitocôndrias são os que possuem maior concentração do metal (OBERLEAS, et al., 1999). Sua excreção é quase que em sua totalidade por via intestinal, possuindo também outras rotas. O fígado absorve rapidamente o Mn presente na circulação e o incorpora na bile (SUTTLE, 2010). A quantidade de Mn aumenta consideravelmente no fluido biliar com grandes quantidades administradas aos animais. Quando a via hepática biliar está bloqueada, o Mn pode ser excretado também pelos fluidos pancreáticos (OBERLEAS et al., 1999).

Distribuição do Manganês no Tecido Animal

Os ossos são as maiores fontes de Mn nos tecidos, seguido pelo fígado. As glândulas, pineal e pilulitária também possuem quantidade relativamente alta de Mn no organismo dos animais (LESSON & SUMMERS, 2001). Em pintos as maiores concentrações são encontradas no fígado, seguido pelo rim e ossos. As aves podem armazenar o excesso de manganês da dieta, porém, altos acréscimos devem ser realizados para causar aumentos consideráveis de Mn nos ossos (SUTTLE, 2010).

Os microminerais também estão presentes nas estruturas do ovo, e estão em menor concentração no albúmen quando comparado com a gema (VIEIRA, 2007). Yair & Uni (2011) estudaram a porcentagem de uso de vários minerais pelo embrião até o dia da eclosão e observaram que a gema contribuiu para o uso de minerais e que o maior uso de minerais se deu aos 17 dias de incubação, além disso, observou os minerais da casca, dos quais o manganês da casca foi o mineral com a 5ª maior porcentagem relativa de uso (86,7%), atrás do cobre, ferro, zinco e fósforo (95,5, 94,9, 94,2, 93,3%).

O Mn está presente também nas cascas dos ovos, onde há relatos que sua suplementação em galinhas poedeiras é capaz de aumentar a resistência da casca e espessura, devido ao melhor desenvolvimento das camadas da casca dos ovos ao longo de sua formação e ainda incrementando a formação da membrana das cascas dos ovos (XIAO et al., 2014; ZHANG et al., 2017).

Interações do Manganês com Outros Elementos

Em monogástricos, existem interações antagonistas entre o manganês e diversos elementos, o que pode torná-lo indisponível para absorção e ser perdido no lúmen intestinal. No momento da absorção os minerais podem sofrer interferência mútua, o que pode reduzir as taxas de absorção (VIEIRA, 2008). Minerais como Ca e P estão relacionados com a redução de absorção de Mn, principalmente quando adicionados em excesso (WEDEKIND & BAKER, 1990).

Fatores antinutricionas presentes na dieta podem interagir com o Mn, afetando sua biodisponibilidade. Um destes fatores é o fitato, que limita a absorção de Mn pela formação de quelatos altamente insolúveis, diminuindo a sua disponibilidade para absorção no trato gastrointestinal (HUMER et al., 2015; SANTOS et al., 2015).

O Mn está também relacionado ao metabolismo de Fe. Altos níveis de Mn interferem na absorção de Fe, já que o Mn compete pelo mesmo sítio de absorção dos minerais divalentes (CHUA & MORGAN, 1997). Baker e Halpin (1991) demonstraram que o excesso de Mn apresentou efeito na utilização de Fe em pintos, enquanto o excesso de Fe não antagonizou a utilização de Mn.

Fontes de Manganês

As concentrações de manganês nas plantas geralmente são influenciadas pelas concentrações e propriedades do solo (SUTTLE, 2010) Dentre estas propriedades, o pH é o principal fator para absorção, onde solos ácidos possuem maior disponibilidade de Mn, bem como solos com alta quantidade de matéria orgânica e pH básico (KABATA-PENDIAS, 2001).

As matérias primas utilizadas para fabricação de rações apresentam quantidade variadas de manganês. O milho, por exemplo, contém aproximadamente 5 a 15 ppm de Mn, enquanto que o farelo de soja geralmente possui entre 36 a 48 ppm de Mn (SUTTLE, 2010; SPEARS, 2011; FAVERO et al., 2013). Outros ingredientes tais como o farelo de arroz e de trigo são boas fontes desse micromineral, contendo cerca de 190 e 110 ppm de Mn, respectivamente (SUTTLE, 2010; ROSTAGNO et al., 2017).

A principal fonte desse mineral de forma inorgânica é o sulfato de manganês monohidratado de Mn (30%). Existem outras fontes inorgânicas deste mineral, como por exemplo, o óxido de manganês e o carbonato de manganês com 46 e 56% de Mn, respectivamente, porém, estes com menor biodisponibilidade. Estas fontes geralmente são incluídas na dieta via premix mineral (ROSTAGNO et al., 2017). Ainda, há a disponibilidade de microminerais complexados no mercado, que correspondem a sais minerais ligados a aminoácidos, proteínas ou carboidratos (SAKOMURA et al., 2014). Sua utilização tem sido crescente em dietas para matrizes de corte (FAVERO et al., 2013; EBBING et al., 2019).

Deficiência de Manganês

A importância da suplementação de manganês foi mostrada primeiramente na prevenção de “perose” ou “tendão deslocado” em pintos (WILGUS et al., 1936). A perose é caracterizada pelo alargamento e má formação da articulação tibiometatársica, com torção e flexão da tíbia,

espessamento e encurtamento dos ossos longos seguido do escorregamento do tendão gastrocnêmio de seus côndilos (BERTECHINI, 2012).

Posteriormente, Gallup & Norris (1939) demonstraram que reprodutoras alimentadas com dietas deficientes em Mn resultaram em baixa eclodibilidade e produção de ovos. Resultados semelhantes foram encontrados por Atkinson et al. (1967) em perus, onde houve diminuição da produção de ovos com aumento da mortalidade embrionária durante a incubação, resultando em baixa eclodibilidade. No embrião, são observadas alterações como asas e pernas encurtadas, um “bico de papagaio” que resulta em encurtamento da mandíbula. A mortalidade é alta e o defeito subsequente é a condrodistrofia (LIU et al, 1994).

A deficiência de Mn também afeta a qualidade da casca dos ovos. Em poedeiras, a diminuição da espessura da casca foi relacionada com a falta de Mn na dieta (LEACH & GROSS, 1983; SAZZAD et al., 1994). Parâmetros como resistência da casca a quebra, percentagem e espessura da casca aumentaram com a suplementação de Mn nas dietas de poedeiras comerciais (XIAO et al., 2014; ZHANG et al., 2017).

Exigências de Manganês para Reprodutoras

As investigações sobre as exigências de minerais devem levar em conta as suas funções fisiológicas dentro dos sistemas biológicos, onde são esperados para trabalhar. Essas funções podem ser geralmente divididas em construção e manutenção de tecidos duros e moles, bem como na regulação de processos biológicos (SUTTLE, 2010).

Favero et al. (2013) explica que as exigências de microminerais e a disponibilidade de minerais não são bem definidos para aves reprodutoras pesadas, no que se refere a avaliações do embrião, característica importante para a produção de pintinhos de um dia.

As exigências são identificadas principalmente por curvas de dose-resposta. Entretanto, devido aos inúmeros fatores que impactam no resultado de experimentos (critérios utilizados para avaliar os resultados, a composição da dieta e das linhagens dos animais), pode-se encontrar alguns resultados de exigência variáveis, mesmo para uma dada espécie e linhagem (SAKOMURA & ROSTAGNO, 2007).

As indicações de suplementação de Mn para reprodutoras de frangos de corte são encontradas nos manuais das linhagens Cobb 500 (2018) e Aviagen (2017); ambos possuem recomendação de suplementação de 120 mg/kg Mn para matrizes em fase de produção. As tabelas brasileiras para aves e suínos recomendam uma suplementação de manganês inorgânico de 70 mg/kg Mn para aves reprodutoras. Por outro lado, o NRC (1994) não fornece recomendações específicas de manganês para matrizes de frangos de corte, apenas para poedeiras (25 mg/kg Mn). Ressaltando que os valores de recomendação de suplementação e valores de exigência são diferentes, visto que a suplementação não considera os níveis já presentes nos ingredientes da dieta.

HIPÓTESES E OBJETIVOS

Hipóteses

A utilização de manganês nas dietas de matrizes de frango de corte favorece o desempenho e as condições fisiológicas do animal.

A quantidade de manganês utilizada nas dietas não tem interferência nos parâmetros produtivos das aves.

Objetivo Geral

Determinar a influência dos níveis de manganês e períodos (idade das aves) e de suas interações sobre características produtivas das matrizes de frango de corte da linhagem comercial Cobb 500 *Slow Feather* utilizando o Sulfato de Mn.

Objetivos Específicos

Avaliar níveis crescentes de Mn sobre o desempenho produtivo, qualidade da casca dos ovos e produção da progênie de matrizes de corte.

Determinar a exigência de manganês para matrizes de frango de corte em período produtivo.

CAPÍTULO II[1]

[1]Artigo elaborado conforme as normas do periódico *Poultry Science.*

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METABOLISM AND NUTRITION

MANGANESE AND BROILER BREEDER HENS

Manganese requirements of broiler breeder hens

T. L. Noetzold,* S.L. Vieira*,[1], A. Favero,* R. M. Horn* C. M. Silva and G. B. Martins*

[*]Department of Animal Sciences, Federal University of Rio Grande do Sul, Av. Bento Gonçalves, 7712, 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 Limited studies have dealt with micromineral nutrition of broiler breeders. The present research has been conducted with the objective of determine the Mn requirement of broiler breeder hens. One hundred and twenty Cobb 500 hens, 22 wks of age, were allocated individually into electrostatically painted cages having stainless nipple drinkers and plastic feeders. After being fed a Mn deficient diet for 5 wks, hens were randomly split in treatments having 6 graded increments of 30 ppm Mn starting with the lowest Mn content feed (22.2 ppm Mn). The supplemental incremental Mn source was lab grade Mn sulfate ($MnSO_4$ H_2O). Analyses were conducted in all mixing batches (n=4) and had: 22.2 $\pm$ 3.21; 48.5 $\pm$ 3.44; 77.9 $\pm$ 5.49; 103.1 $\pm$ 1.82; 140.0 $\pm$ 7.88 and 168.2 $\pm$ 3.57 ppm. Feeds were formulated with corn, soybean meal, oat hulls and all other mineral supplements were lab grade. No phytase was added to any feeds. Requirements of Mn were estimated using quadratic polynomial (QP) and broken line quadratic (BLQ) models using repeated measures. Experimental feeds were fed during 4 periods of 28 d. There were no interactions between dietary Mn and period for any evaluated response ($P > 0.05$). Requirements of Mn for hen day egg production and settable egg production were 115.8, and 56.6 ppm and 122.1, and 63.6 ppm ($P < 0.05$), respectively, using QP, and BLQ models whereas total eggs and total settable eggs per hen had Mn requirements estimated as 115.7, and 56.6 and 121.8, and 61.7 ppm ($P < 0.05$), respectively for QP, and BLQ models. Eggs having cracked shells as well as defective eggs had 129.5, 66.4 ppm and 118.4 ppm Mn ($P < 0.05$) using QP, and BLQ models and QP model, respectively. Number of cracked, defective and contaminated eggs decreased, whereas hatchability, hatchability of fertile eggs, eggshell percentage and eggshell palisade layer increased when hens were fed diets having from 48.5 to 168.2 ppm Mn ($P < 0.05$). Maximum responses for egg weight and eggshell percentage were 117.7 and 63.6 ppm as well as 131.6, and 71.0 ppm ($P < 0.05$), respectively using QP and BLQ models. Breaking strength and egg specific gravity had Mn requirements estimated at 140.2 and 112.7 ppm as well as 131.3,

68.5 ppm ($P < 0.05$), whereas eggshell palisade layer and eggshell thickness were maximized with 128.8 and 68.8 ppm, and 140.2, 134.2 ppm, respectively for QP and BLQ models ($P < 0.05$). Maximum yolk Mn content values were obtained using 118.0, and 118.4 ppm Mn by QP and BLQ models, respectively. The average of all Mn requirements estimated for QP, and BLQ models are 128.4, and 92.3 ppm Mn (18.7 and 13.5 mg/hen/d), respectively, which are contents much lower than what has been currently recommended in commercial production.

Key words: broiler breeder, micromineral, manganese.

INTRODUCTION

Manganese (Mn) is the fifth most abundant mineral on earth (Suttle, 2010) and it was firstly reported to prevent perosis in broilers by Wilgus et al. (1936). Mn deficiency in poultry is associated with structural and physiological disorders, which include cartilage and skeletal malformation as well as a reduction in the antioxidant defense system (Luo et al., 1992; Tuormaa, 1996).

Since Mn was determined as an essential trace mineral for poultry, a series of involvements of Mn have been demonstrated, mainly as a constituent of metalloenzymes. For instance, Mn superoxide dismutase (MnSOD) is involved in the control of oxidative stress in the mitochondria by converting superoxide to peroxide, which is then reduced to water afterwards (Bottje, 2018). Pyruvate carboxylase (PC) and arginase are also metalloenzymes, respectively involved in the metabolism of pyruvate into oxaloacetate (Reed and Scrutton, 1974; Baly et al., 1985; Moomaw et al., 2009; Suttle, 2010) as well as in the conversion of arginine into urea and ornithine (Wu and Morris, 1998; Fernandes and Murakami, 2010).

Because Mn has an active role in activating a glycosyltransferase, which is involved on formation of proteoglycans, therefore it is essential for the formation of eggshell membranes (Leach and Gross, 1983; Tuormaa, 1996; Xiao et al., 2014). Proteoglycans are major constituents of bone and eggshell extracellular matrixes that are comprised of a protein core with a large number of glycosaminoglycan side chains (Arias et al., 1993; Keen et al., 2013). Mn deficient chicks have been observed to have skeletal and cartilage malformations with substantial reductions in the total proteoglycan bone content (Liu et al., 1994).

In plant feedstuffs, Mn varies upon soil composition (Gupta et al., 2008). Therefore, Mn contents in broiler breeder feeds is expected to vary with its dietary source. Mn contents in wheat bran, which is largely used in broiler breeder feeds, varies from 88 to 163.9 mg/kg (Suttle, 2010; Rostagno et al., 2017) whereas in corn, it been reported to vary from 5 to 15

mg/kg and in SBM from 36 to 48 mg/kg (Halpin and Baker, 1886; NRC, 1994; Suttle, 2010; Spears and Engle, 2011). Macro mineral supplements, such as phosphates and limestone, can have high content of Mn, also at variable concentrations (from 174 to 726 and 15 to 250 ppm Mn, respectively) (Reid and Weber, 1976; Lima et al., 1999; Wilkinson et al., 2013).

Availability of Mn in plant feedstuffs is dependent of its total chelation with phytic acid, similarly to what happens with other positively charged minerals. Differently from Fe, Cu and Zn, overall contents of Mn in routinely used broiler breeder feeds are low (an exception is wheat bran, which has a high proportion of phytate and, then, potentially reduces Mn availability for poultry (Mohanna and Nys, 1999; Attia et al., 2010). High dietary Ca and P has also been reported to reduce Mn absorption, probably because of the increased insolubility of phytate when reacted with these minerals (Wedekind and Baker, 1990; Spears and Engle, 2011).

Reports on Mn supplementation have been published recently with broiler chickens (Lu et al., 2006; Li et al., 2011; Lu et al., 2016; Pacheco et al., 2017) and laying hens (Olgun and Cufadar, 2010; Xiao et al., 2014; Zhang et al., 2017). However, limited research has been conducted with broiler breeder hens. Present recommendations for Mn as a dietary supplement for broiler breeder hens are mostly based on suggestions, which range from 70 to 90 mg Mn/kg of feed (FEDNA, 2008; Rostagno et al., 2011; Rostagno et al., 2017) to 120 mg Mn/kg (Aviagen, 2017; Cobb-Vantress, 2018). These suggestions, however, lack representative *in vivo* research of support.

Mn is usually supplemented in broiler breeder feeds as part of the micro mineral premix, frequently as a sulfate salt, but sometimes incorporated in a diversity of organic minerals. Oversupplying minerals to commercial livestock has become an environmental issue, which has been shown to contaminate groundwater. Recent regulations have stablished 150 ppm as maximum Mn content in poultry feeds in the European Union (EFSA, 2016). In

the Eastern Shore, USA, regulations have been put in place to reduce that threat (Subramanian and Gupta, 2006).

The present study was conducted using Mn sulfate monohydrate ($MnSO_4\ H_2O$) as the supplemental source in a Mn deficient feed. Evaluated responses were related to egg production, but also extended to other parameters that can affect overall hen nutrition as well as hatching chick production. The main goal of the present study is to provide Mn requirements, such that daily Mn supply to broiler breeder hens do not limit competitive production, but also are not excessive, such that an increase in the cost of production parallels unnecessary Mn excretion, turning it into environmental pollutant.

MATERIAL AND METHODS

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.

Birds and Management

One hundred and twenty Cobb 500 broiler breeder hens and 30 Cobb broiler breeder males were obtained from a commercial breeder farm (Grupo Vibra Agroindustrial S. A, Montenegro, RS, Brazil) at 22 wk of age. Hens were individually weighed at arrival such that their coefficient of variation could be assessed. They were then individually placed in cages (0.33 m length x 0.46 m width x 0.40 m height) respecting a representative distribution of body weights throughout similar ranges in the different treatments. In parallel, 30 Cobb breeder males were placed in 3 collective floor pens (2.0 x 1.5 m), 10 in each, for semen collection. The utilized experimental cages are electrostatically painted and have one stainless steel nipple drinker and a plastic trough feeder. Temperature control, lighting, and feeding

programs followed Cobb-Vantress (2016) recommendations. Semen collection, and hen insemination were done as described by Taschetto et al. (2017).

Experimental Feeds

Breeder hens were given a common pre-experimental adaptation feed followed by a Mn depletion one (Table 1). The adaptation feed followed recommendations from Cobb-Vantress (2018) and was provided from the arrival of hens at the farm until 30 wks of age whereas the Mn deficient depletion feed (16.4 ppm formulated and 22.2 $\pm$ 3.21 ppm analyzed Mn) was fed from 31 to 35 wks. Starting at 36 wks, the hens were individually weighed and assigned to the experimental treatments following a complete randomized block experimental design. Breeder males were fed diets providing nutrients and energy as recommended by Cobb (2018) throughout the end of the study.

The experimental feeds were graded supplemented, at the expense of an inert (kaolin), with laboratory grade Mn sulfate monohydrate ($MnSO_4H_2O$) (Sigma Aldrich, St. Louis, USA) into the deficient feed providing increments of 0, 30, 60, 90, 120, and 150 ppm Mn. Analyses of Mn were performed in every batch of feed mixed (n=4) using the atomic absorption spectrophotometric method of AOAC (AOAC, 2016; No.968.08). Average analyzed contents of Mn in feeds supplemented with $MnSO_4\ H_2O$ throughout the treatments were: 22.2 $\pm$ 3.21; 48.5 $\pm$ 3.44; 77.9 $\pm$ 5.49; 103.1 $\pm$ 1.82; 140.0 $\pm$ 7.88 and 168.2 $\pm$ 3.57 mg Mn/kg, respectively. The experiment was divided in 4 periods of 28 d, in 6 x 4 factorial arrangement of 6 dietary Mn contents and 4 periods. Each one of the 6 dietary treatments was replicated 20 times with one individually caged hen being the replicated experimental unit.

All ingredients and feeds were analyzed for Mn prior to feed formulation using the method described above. Breeder hens were fed daily restricted amounts as recommended by Cobb-Vantress (2016). Consumption of Mn in mg/hen/d per hen was calculated using daily

feed intakes present in the feeds (Table 2). Water Mn content was analyzed using atomic absorption (ZEEnit 650 P, Analytik Jena, Jena, Germany). Averaged duplicate analysis of Mn in water was <0.006 ppm, which was not considered a significant dietary source of this mineral.

Hen Performance

Eggs were daily collected 4 times per d and classified as hatchable or not (broken, defective or without shells). Body-checked (wrinkled equator), elongated, and rounded eggs were classified as defective eggs. The percentage of eggs in each period was calculated on the basis of the number of live hens. All hatchable eggs laid in the last wk of each 28 d period were weighted and grouped in 4 replicates (eggs of each five replications were added together) per treatment and set into a single-stage incubator at 37.5 ºC and 65% relative humidity until 18 d. Eggs were then transferred to a hatcher set to 36.6 ºC and 80% RH. Incubator and hatcher were equipment commercially produced by Avicomave (Rua Edjan Barçalobre, 161 – Distrito Industrial, Iracemápolis, SP, Brazil). Overall hatchability and hatchability of fertile eggs were calculated as the percentage of hatching chicks to the total and fertile eggs set, respectively. All unhatched eggs were broken, and evaluations were conducted to estimate the moment and cause of embryonic death as described by Favero et al (2013). Contaminated eggs were those visually rotten (having uncommon green or black contents, emitting putrid odor or exploding at opening).

Hen Blood Measurements

Hematocrit (Ht) and hemoglobin (Hb) as well as alkaline phosphatase (ALP) concentrations were obtained from blood samples pooled from 3 hens randomly selected from each treatment per period. Blood obtained was partially transferred to 0.5 ml test tubes

containing EDTA for Ht and Hb analyses. Ht was determined using micro capillaries containing blood centrifuged for 5 min from 15,650 to 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 (1970), using a digital bench colorimeter (Model Labquest, Vernier Software & Technology, Beaverton, United States). Collection of blood was done such that none of the hens was bled more than once.

Hatching Chicks

Hatching chicks were weighed and had their length measured (distance from the tip of the beak to the end of the middle toe) as described by Molenaar et al. (2008). Hb and Ht concentrations were determined with 15 chicks hatched per treatment per period. Chick blood samples were obtained from the jugular vein after euthanasia by cervical dislocation. Six hatching chicks per treatment each period were randomly selected for tibia collection as follow: right tibias were stripped of any adhering tissue leaving intact cartilage bone caps, which were further submitted to fat extraction using diethyl ether overnight. Dried tibias were weighed, and their lengths were determined using a digital micrometer (Mitutoyo CSX-B, Japan). Bones were later left for 10 h at 600°C in a muffle furnace (Sanchis & Cia Ltda, Porto Alegre, RS, Brazil) to determine percent ash [(dry ash weight/dry tibia weight) × 100].

Egg Analysis

Eggs were collected in the last 4 d of each period with a total of 45 eggs per treatment. Eggs (n=25) were used to measure egg weight, specific gravity, yolk, albumen and eggshell percentage of the egg. 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

223 Gutteridge, 1949). Eggshell weight was obtained after washing and drying shells at 105ºC
224 overnight, whereas eggshell thickness was measured using a micrometer (Model IP65,
225 Mitutoyo Corp., Kawasaky, Japan) in the basal, equatorial and apical regions with these
226 values being averaged for statistical analysis. The other 20 eggs were used to determine
227 eggshell breaking strength, using a texture analyzer (Model TA.XT. plus, Texture
228 Technologies Corp., Hamilton, United States) with a 75-mm (P/75) breaking probe (Molino,
229 et al., 2015). Additionally, 3 eggs with the same average weight ± 10% SD per treatment
230 obtained in the last 3 d of each period (39, 43, 47 and 51 wks) were used in the analysis of
231 eggshell ultrastructure using scanning electron microscopy (King and Robinson, 1972). In the
232 preparation for this analysis, eggshell samples were taken from three 0.5 cm^2 samples in the
233 apical, equatorial, and basal areas. Eggshell samples were mounted transversely and
234 horizontally on aluminum stubs using carbon tape, to measure the thickness of eggshell layers
235 and the number of mammillary buttons/mm^2, respectively. These were metallized with gold
236 at 35 nm for 3 minutes (BAL-TEC SCD050 Sputter Coater, Capovani Brothers Inc., Scotia,
237 New York, United States). Mammillary and palisade layer magnification was done according
238 to Dennis et al. (1996). Microscopy images were analyzed in the Image-Pro Plus software
239 (Media Cybernetics, Rockville, United States). Average of eggshell layer thickness (µm)
240 were estimated from three different locations per image.
241
242 ***Statistical Analysis***
243 Data were submitted to the normalcy of variance test and Levene's test for
244 homogeneity of variance (Levene, 1960; Shapiro and Wilk, 1965). Data were transformed
245 using the arcsine square root percentage (z = asin (sqrt (y $^+$ 0.5))) whenever not normally
246 distributed (Ahrens et al., 1990). Data were submitted to ANOVA using the MIXED
247 procedure of SAS (2013) using each one of the 28 d periods as repeated measures. Total egg

production and settable egg production per hen at 51 wks were analyzed using the general linear models (PROC GLM). The Tukey-Kramer test was used for means comparison with differences being considered significant at $P < 0.05$ (Tukey, 1991). The covariance structures of PROC MIXED were chosen based on the Akaike criteria (Littell et al., 1998). Non-parametric data, such as hatching chick leg length and navel button scores, were analyzed by the GLIMMIX procedure, and the means were also compared by the Tukey-Kramer test ($P < 0.05$). Estimates of maximum responses to total dietary Mn were done using quadratic polynomial (QP), and broken line quadratic (BLQ) models (Robbins et al., 1979; Pesti et al., 2009). The QP model ($Y = a + b \times Mn + c \times (Mn)^2$) had Y as the dependent variable and as a function of dietary level of Mn; *a* as the intercept; *b* as the linear coefficient and *c* as the quadratic coefficient with the maximum response for Mn defined as $Mn = - b \div (2 \times c)$. The BLQ model ($Y = a + b \times (c - Mn)^2$) had ($c - Mn$) = 0 for Mn > *c* had Y as the dependent variable as a function of the dietary level of Mn, *a* the value of the dependent variable at the plateau and *b* as the slope of the line.

RESULTS

Analyses of Mn in the dietary treatments were conducted on samples from one pool of each 4 mixed batches throughout the study and averaged 22.2 ± 3.21; 48.5 ± 3.44; 77.9 ± 5.49; 103.1 ± 1.82; 140.0 ± 7.88 and 168.2 ± 3.57 ppm (Table 2). Analyses of variance and regressions were conducted with the analyzed data. Analyses performed to check for further deviations between formulated and analyzed feeds showed no important differences that could affect the treatments with different levels of Mn.

There were no interactions between dietary Mn and period for any response. Therefore, data is presented as main factors throughout this report. Defective eggs, shell-less eggs, breeder ALP (Table 3), hatchability of fertile eggs, embryo mortality, contaminated eggs

(Table 4), eggshell percentage, yolk Mn content, specific gravity, membrane thickness (Table 5) and leg scores were not affected by period (Table 6) ($P > 0.05$); however, hen day and settable egg production, hatching chick Hb (Table 3), fertility, hatchability of total eggs (Table 4) as well as albumen as a proportion of the whole egg decreased as hens aged (Table 5) ($P < 0.05$). On the other hand, egg yolk percentage (Table 5), egg weight, hatching chick weight, hatching chick length, tibia length and hatching chick tibia ash increased as hens aged (Table 6) ($P < 0.05$). Number of mammillary buttons (Table 5) and hatching chick navel button scores (Table 6) were higher in the period of 36 to 39 weeks when compared to all other periods, whereas hen Ht and Hb were lower in the same period compared to all others ($P < 0.05$). Palisade layer and mammillary layer peaked highest in the period of 44 to 47 wk (Table 5) decreasing afterwards ($P < 0.05$). Eggshell breaking strength as well as eggshell thickness increased in the second period (40 to 43 wk) decreasing afterwards (44 to 51 wk) (Table 5).

Supplementing Mn at any level did not affect shell-less eggs, hen Hb and ALP, chick Hb and ALP (Table 3), egg fertility, embryo mortality (Table 4), yolk and albumen percentage, number of mammillary buttons (Table 5), egg and chick weights, hatching chick length and scores, navel button score as well as tibia ash (Table 6) ($P > 0.05$). On the other hand, dietary increases of Mn affected ($P < 0.05$) the total and settable hen egg production, and total number of eggs and settable eggs per hen, cracked eggs, defective eggs, hen and chick Ht (Table 3). Contaminated eggs decreased, whereas total hatchability, hatchability of fertile eggs (Table 4), shell percentage, palisade and mammillary layer (Table 5) and tibia length increased as dietary Mn was higher (Table 6) ($P < 0.05$).

Estimation of Mn requirements determined using BLQ and QP regression models are shown in Tables 7 and 8. In a few cases responses did not fit adequately, therefore, in those cases requirement estimation is not presented. Maximum responses for hen day egg

298 production and total egg production were the same (115.8, and 56.6 ppm Mn from QP and
299 BLQ models, respectively) whereas requirements for percent settable eggs were 122.1 and
300 63.6 ppm Mn whereas total eggs produced were maximized at 121.8 and 61.7 ppm Mn, QP
301 and BLQ respectively. Cracked eggs were minimized at 129.5, and 66.4 ppm Mn using a QP
302 and BLQ regressions respectively, whereas maximum response was obtained at 118.4 by QP
303 on defective eggs. The highest egg hatchability and the lowest contaminated eggs were
304 optimized at 125.7 ppm and 127.9 ppm, 69.5 ppm and 71.9 ppm respectively, using the QP,
305 and BLQ models. The maximum values for hatchability of fertile eggs were obtained at
306 124.5, and 65.8 ppm Mn by QP and BLQ models, respectively. Breeder hen Ht and ALP
307 requirements using QP and BLQ models were estimated at 142.7 and 148.0, and 126.5 and
308 145.2 ppm Mn, whereas 135.5 and 122.4 ppm Mn were highest for chick Ht, respectively.
309 Mn requirement for highest egg weight was 117.7, and 63.6 ppm Mn by QP and BLQ
310 models respectively, while, hatching chick weight using the same models were 120.4 and
311 85.6 ppm Mn. Requirements of dietary Mn to maximize eggshell and albumen as egg
312 percentages were 131.6 and 127.5 ppm Mn using the QP model; 71.0 and 113.0 ppm using
313 the BLQ model, respectively.
314 Egg yolk Mn content was positively related to dietary Mn increased, with maximum
315 responses obtained at 118.0 and 118.4 ppm Mn with QP and BLQ models, respectively,
316 whereas yolk percentage was highest when dietary Mn was fed at 124.4 using the QP model.
317 The highest hatching chick Ht and breeder Ht values were obtained when hens were fed
318 dietary Mn at 103.1 ppm, whereas specific gravity increased up to 140.0 Mn ppm ($P < 0.05$).
319 Eggshell thickness, membrane thickness and hatching chick length were highest ($P < 0.05$)
320 when hen dietary Mn was at 103.1 ppm or above, while eggshell breaking strength was
321 highest at the hen dietary level above 77.9 ppm Mn ($P < 0.05$). The QP model estimated
322 140.2 ppm as Mn requirement, and the BLQ regression estimated 128.0 and 134.2 ppm Mn

requirement for eggshell membrane layer and thickness, respectively, whereas Mn requirements for palisade layer was 128.8, and 68.8 ppm Mn with QP and BLQ models. Requirements of Mn that maximized breaking strength using QP and BLQ models were 140.3 and 112.7 ppm Mn, respectively, whereas, maximum specific gravity was obtained at 131.3, and 68.5 ppm Mn with QP and BLQ models, respectively. Ma Additionally, close Mn requirements of 142.2 and 138.1 ppm Mn were estimated for navel button score using QP and BLQ regressions, and 140.8 ppm Mn by QP model for hatching chick tibia length.

DISCUSSION

In the present study, broiler breeder hens fed Mn deficient diets demonstrated signs of deficiency throughout most of the evaluated responses. General improvements were observed as dietary Mn increased in the feeds. Since Mn plays several roles in animal metabolism, observed changes in the studied responses varied depending on the amounts of Mn demanded to successfully support each of its biological involvement.

Feeding depleted feeds on the studied micromineral is of utmost importance when conducting requirement studies such that adequate supplemental recommendations are presented. For instance, supplementing Mn to laying hens, broiler breeders, and broilers without previous body depletion did not allow full recovery responses (Sazzad et al., 1994; Mabe et al., 2003; Xiao et al., 2014; Zhu et al., 2015; Zhang et al., 2017).

Nutrient requirement studies are generally presented by modeling data with QP and BLQ and less frequently as exponential asymptotic or others. Data in the present study were modelled using QP and BLQ with the objective of allowing the reader to compare the majority of published results with the ones in this text, at least for these two models. Main differences, however, could be found in the estimation of Mn requirements when using these models. For instance, almost no difference was found for egg yolk, which was maximized by

118.0 and 118.4 ppm Mn using QP and BLQ, whereas estimation to optimize cracked eggs was more than doubled (129.5 ppm Mn) with QP compared to BLQ (66.4 ppm Mn). Shape and sensitivity of curvature derivates from each model cause variations in points that optimize responses. It is well known that QP tends to overestimate requirements (Runho et al., 2001) when compared to BLQ, which tends to underestimate them (Baker et al., 2002). Therefore, it is important to previously note the potential differences due to statistical models utilized when comparisons between different publications are made (Robbins et al., 1979).

In the present study, egg production responded with a significant increase as Mn was gradually added in the feeds. Several mechanisms that control egg production can be related to Mn. For example, decreases in egg production were observed when PC activity was low, which is associated with reduction in the use of glucose (Baly et al., 1985). Reduction in circulating estradiol, progesterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are also suggestive of Mn deficiency since it can affect the function of the hypothalamic-pituitary-gonadal axis (Cao and Chen, 1987; Feng and Feng, 1998). Interestingly, loss of PC activity induced by Mn deficiency in chickens can be partially alleviated by Mg (Scrutton et al., 1972; Reed and Scrutton, 1974).

Essential metal microminerals are transferred from hens to embryos in order to allow successful development through stored yolk phosvitin (Richards, 1997). Mabe et al (2003) have found increases in Mn content of egg yolks in laying hens when 60.0 ppm Mn was added to a corn-soybean meal diet (24.7 ppm Mn) using Mn oxide as the supplemental source. Li et al. (2018) have reported a similar response using an organic source, where 60 ppm supplementation in a corn-soybean-wheat diet increased Mn yolk content. These results were lower than what have been found in the present study (average 118.2 ppm Mn). The higher original Mn content in their diets as well as differences in bioavailability of Mn could explain the differences.

In the present study, egg hatchability increased as the addition of Mn was increased in the deficient diet. Decreases in embryo mortality, cracked eggs as well as in contaminated eggs were also reduced as Mn was gradually added to the feeds. Other authors also observed decreased hatchability in turkeys, and Guinea fowls when Mn were supplemented at low levels (27 ppm Mn in a sorghum-corn-soybean basal diet, and 54 ppm Mn supplemented in a corn-sorghum-groundnut basal diet, respectively) (Atkinson et al., 1967; Offiong and Abed, 1980). Furthermore, Zhu et al., (2015) supplementing 120 ppm Mn on a deficient diet (14.3 ppm Mn in a corn-soybean meal diet) have observed improvements on hatchability of eggs from broiler breeder hens (88.8 to 95.1%). The number of settable eggs and percent hatchability are expected to be affected by eggshell integrity since well-formed membranes and eggshells protect against egg contamination (Swiatkiewicz and Koreleski, 2008).

In commercial settings, egg contamination is a major source of embryo mortality (Khabisi et al., 2012). In the present study, contaminated eggs were minimized at 127.9 and 71.9 ppm Mn using the QP and BLQ models, respectively. Cracked eggs were minimized at 129.8, and 66.5 ppm Mn using the QP and BLQ models, respectively. Venglovska et al. (2014) demonstrated that the percentage of cracked eggs was decreased when 120 ppm dietary Mn supplementation was used in a wheat-corn-soybean basal diet whereas eggshell quality increased. The QP and BLQ adjustments occurred with maximum hen responses for eggshell breaking strength at 140.3, and 112.7 ppm and of eggshell thickness at 140.2, and 134.2 ppm dietary Mn, respectively for QP and BLQ. These results are in accordance with other published studies, where 100, and 120 ppm Mn supplementation had a positive effect on eggshell breaking strength as well as eggshell thickness in laying hens using corn-soybean meal diets (Xiao et al., 2014; Zhang et al., 2017), and increased eggshell breaking strength without affecting the thickness in broiler breeders fed with 120 ppm Mn based on a corn-soybean diet (Xie et al., 2014).

The general amelioration in eggshells as Mn was increased in the diets (eggshell breaking strength, eggshell thickness, specific gravity and eggshell percentage) occurred in parallel with increases in the palisade and mammillary layer as well as in the eggshell membrane, even though mammillary buttons were not changed (Figure 1). Xiao et al., (2014) and Zhang et al., (2017) have shown that corn-soybean basal diets supplemented with 100 and 120 ppm Mn, respectively, increased eggshell breaking strength and thickness in laying hens. Additionally, Stefanello et al., (2014) have observed improvements on eggshell percentage, thickness, and strength with 125 ppm dietary Mn added to the corn-soybean meal basal diet in laying hens. Stefanello et al., (2014) have linked these results to interferences on eggshell membrane, palisade and mammillary layer. Glycosyltransferase is an enzyme involved in the formation of proteoglycans, components of the organic matrix (Xiao et al., 2014). Nys et al. (2004) reported that the protein matrix affects the size and orientation of the calcite crystals during eggshell build up and increments in membrane glycosaminoglycans support the mammillary buttons to grow oriented outward forming well structured columnar units of palisade layer (Xiao et al., 2014). Mn supplementation have been associated with improvements of glycosaminoglycan contents in membrane, which might be also responsible for increments on eggshell morphology (Ha et al., 2007).

Proteoglycans have also been associated with normal bone growth and development in chicks since the bone formation is linked to extracellular matrix formation (Velleman, 2000). It has been reported that Mn deficient chicks have less proteoglycan in the cartilage of the tibial growth plate, which may result in chondrodystrophy and abnormal bone growth (Leach et al., 1969; Liu et al., 1994). In the current study, Mn supplementation improved hatching chick tibia length with a maximum response at 140.8 ppm when the QP model was used (Table 6). Several studies have demonstrated a reduction in ash content and length of legs and wing bones in chicks fed Mn deficient diets (Caskey et al., 1939; Caskey et al., 1944; Watson

et al., 1971). Increases in leg abnormalities and weakness have been reported in chicks fed Mn deficient diets (Leach and Muenster, 1962; Watson et al., 1970; Watson et al., 1971; Stock and Latshaw, 1981). Hatching chick navel button score was decreased but it was minimized at 142.2 and 138.1 ppm Mn (QP and BLQ models, respectively). Hatching chicks with unhealed navels are more likely to die during the production periods due to yolk sac infections or even gain less body weight than chicks with healthy navels (Fasenko and O'Dea, 2008). In the present study, eggs from hens fed a Mn deficient diet led to a higher navel score in hatching chicks (Table 06).

The data from this study indicate Mn requirements ranging from 56.6 to 148.0 ppm dietary Mn (8.3 to 22.6 mg/hen/d), depending on production objectives. Average requirements for egg production and hatchability were 93.5 ppm (13.6 mg/hen/d), and 97.6 ppm (14.2 mg/hen/d), respectively, whereas averaged values for egg quality responses were 117.5 ppm (17.1 mg/hen/d). The average of all requirement estimates using both models (QP and BLQ) was 111.5 ppm total dietary Mn (16.3 mg/hen/d), while averaged values for QP, and BLQ models are 128.4, and 92.4 ppm Mn (18.7 and 13.5 mg/hen/d), respectively.

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655 **Table 1.** Experimental diets provided to breeder hens during adaptation as well as in the course of the
656 Mn requirement experiment[1].

Ingredient, % as-is	Basal diet (22 to 30 wks)	Mn deficient diet (31 to 51 wks)
	Adaptation phase	Pre-experimental and experimental phases[2]
Corn	54.50	61.70
Soybean meal	19.82	23.44
Calcium carbonate	-	7.53
Oat hulls	-	3.87
Wheat meal	14.21	-
Limestone	8.15	-
Dicalcium phosphate	0.73	-
Soybean oil	1.44	1.00
Phosphoric acid, 85% P	-	1.36
Potassium carbonate	-	0.03
Sodium bicarbonate	0.26	0.18
Sodium chloride	0.28	0.39
Choline chloride	0.13	0.11
DL-methionine, 99%	0.18	0.18
L-threonine 98.5%	0.04	0.02
L-tryptophan, 98%	0.01	0.01
Vitamin and mineral mix[3]	0.25	0.17
Antioxidant	0.01	0.01
Total	100.00	100.00
Formulated composition, % or as shown		
AME_n, kcal/kg		2,760
CP		15.40
Ca		2.90
Available P		0.43
Na		0.20
Choline, mg/kg		1,500
Mn, ppm	175.1	16.4 (22.2)

657 [1] Calcium carbonate, phosphoric acid, potassium carbonate, sodium bicarbonate and sodium chloride were laboratory
658 grade and had only trace amounts of Mn (10.0; 0.7; 0.0; 3.9 ppm, respectively).
659 [2] Experimental treatments resulted from feed additions with $MnSO_4H_2O$.
660 [3] Mineral and vitamin premix supplied the following per kg of feed: Cu, 15 mg; Zn, 110 mg, Fe, 50 mg; Se, 0.3 mg; I, 2
661 mg; 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
662 µg; thiamine, 3.5 mg; riboflavin, 16 mg; vitamin B6, 6 mg; niacin, 40 mg; pantothenic acid, 25 mg; folic acid, 4 mg;
663 biotin, 0.3 mg.

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668 **Table 2.** Supplemented, calculated and analyzed Mn concentrations in the experimental diets, feed
669 intake and Mn intake per hen day in each period.[1]

Supplemented Mn, ppm[1]	Total dietary Mn, ppm		Period, wk				Average 36 to 51
	Calculated	Analyzed[2]	36 to 39	40 to 43	44 to 47	48 to 51	
			Mn intake, mg/hen/d				
0	16.4	22.2 ± 3.21	3.9	3.0	2.9	3.1	3.2
30	46.4	48.5 ± 3.44	6.9	6.9	6.7	7.8	7.1
60	76.4	77.9 ± 5.49	10.8	11.3	10.8	12.5	11.4
90	106.4	103.1 ± 1.82	14.7	15.1	15.3	15.0	15.0
120	136.4	140.0 ± 7.88	20.9	19.6	19.3	21.8	20.4
150	166.4	168.2 ± 3.57	24.7	24.2	25.2	24.0	24.5
Mn intake, mg/hen/d			13.7	13.3	13.4	14.0	13.6
Feed intake, g/hen/d			145.8	144.2	145.6	147.7	145.8

670 [1] From laboratory grade Mn sulfate monohydrate.
671 [2]Analyzed Mn from one pooled sample from each feed mix batch (n=4).

672 **Table 3.** Broiler breeder hen performance as affected by increased dietary Mn[1].

	Eggs[2]							Breeders			Hatching chicks		
Mn, ppm (mg/day)	Hen day production	Settable	Cracked	Defective	Shell-less	Total[3]	Settable[4]	Ht[5], %	Hb[6], g/dL	ALP[7], U/L	Ht, %	Hb, g/dL	ALP, U/L
	%												
22.2 (3.2)	58.5^{b}	45.9^{b}	10.4^{a}	1.64^{a}	0.33	65^{b}	51^{b}	28.4^{b}	9.4	237	30.8^{c}	9.8	3,126
48.5 (7.1)	64.0^{ab}	57.6^{a}	4.9^{b}	0.50^{b}	0.32	72^{ab}	65^{a}	29.1^{ab}	9.8	262	31.2^{bc}	10.0	3,402
77.9 (11.4)	64.1^{ab}	58.4^{a}	4.5^{b}	0.55^{b}	0.18	72^{ab}	66^{a}	28.7^{b}	9.7	313	31.8^{bc}	10.3	3,391
103.1 (15.0)	64.9^{a}	60.0^{a}	4.0^{b}	0.45^{b}	0.10	73^{a}	67^{a}	30.9^{a}	10.2	322	33.8^{a}	10.8	3,208
140.0 (20.4)	64.2^{ab}	59.9^{a}	3.5^{b}	0.59^{b}	0.09	72^{ab}	67^{a}	30.2^{ab}	9.9	385	31.8^{bc}	10.0	3,380
168.2 (24.5)	64.1^{ab}	59.6^{a}	3.2^{b}	0.46^{b}	0.05	72^{ab}	67^{a}	30.0^{ab}	9.9	301	32.9^{ab}	10.8	3,438
Period, wk													
36 to 39	71.3^{a}	64.3^{a}	5.4^{a}	0.82	0.33	-	-	27.0^{c}	9.0^{c}	266	32.2^{ab}	10.89^{a}	2,358^{c}
40 to 43	66.0^{b}	59.1^{b}	5.7^{a}	0.67	0.15	-	-	29.6^{b}	9.7^{b}	315	32.8^{a}	10.25^{ab}	2,947^{b}
44 to 47	60.4^{c}	53.7^{c}	5.3^{a}	0.71	0.12	-	-	31.6^{a}	10.9^{a}	326	32.9^{a}	10.37^{ab}	4,013^{a}
48 to 51	54.8^{d}	50.0^{d}	3.8^{b}	0.58	0.10	-	-	29.9^{ab}	9.6^{bc}	306	30.2^{b}	9.67^{b}	3,961^{a}
SEM	0.55	0.65	0.29	0.081	0.042	0.74	1.05	0.32	0.13	12.5	0.35	0.11	96.0
Probability <													
Mn	0.0289	0.0001	0.0001	0.0010	0.3024	0.0429	0.0001	0.0486	0.4032	0.0714	0.0021	0.0523	0.2526
Period	0.0001	0.0001	0.0012	0.7437	0.3076	-	-	0.0001	0.0001	0.2576	0.0139	0.0011	0.0001
Mn *vs*. period	0.8895	0.7715	0.1793	0.7287	0.7196	-	-	0.2882	0.2984	0.3836	0.0977	0.6206	0.3292

673 $^{a>b}$ Means with different letters in the same column indicate significant differences ($P \leq 0.05$).
674 [1] Probabilities of hen day production, settable, cracked, defective, and shell-less eggs are presented after arcsine transformation.
675 [2] As a percentage of total live hens at the time of measurement.
676 [3] Total eggs at the end of the experiment.
677 [4] Total settable egg at the end of the experiment.
678 [5] Hematocrit.
679 [6] Hemoglobin.
680 [7] Serum alkaline phosphatase.
681

682

683 **Table 4.** Broiler breeder hen incubation performance as affected by increased dietary Mn[1].

		Hatchability, %		Embryo mortality[2], %				
Mn, ppm (mg/day)	Fertility[5], %	Total eggs[3]	Fertile eggs[4]	Early dead	Middle dead	Late dead	Pips	Contaminated eggs[6], %
22.2 (3.2)	87.3	62.0[b]	70.8[b]	5.50	1.91	6.73	6.14	7.75[a]
48.5 (7.1)	87.6	75.2[a]	85.9[a]	3.11	1.19	3.35	2.93	3.18[ab]
77.9 (11.4)	87.8	75.8[a]	86.3[a]	3.10	0.85	3.14	3.34	2.92[ab]
103.1 (15.0)	88.5	79.6[a]	89.9[a]	2.09	0.62	3.07	2.78	1.43[b]
140.0 (20.4)	89.5	79.8[a]	89.2[a]	2.13	0.61	3.14	2.55	1.58[b]
168.2 (24.5)	88.2	78.6[a]	89.2[a]	2.32	0.61	3.16	2.85	1.63[b]
Periods, wk								
36 to 39	92.4[a]	78.0[a]	84.3	4.23	1.67	3.46	3.67	2.43
40 to 43	88.1[ab]	76.4[ab]	87.0	2.15	0.51	3.85	3.13	3.03
44 to 47	87.9[b]	74.8[ab]	84.6	2.72	0.98	3.80	4.06	2.92
48 to 51	84.2[b]	71.5[b]	84.8	3.06	0.70	3.91	2.87	3.95
SEM	0.66	1.15	1.11	0.450	0.263	0.484	0.469	0.407
Probability <								
Mn	0.9320	0.0018	0.0001	0.1841	0.8143	0.3488	0.4942	0.0039
Period	0.0001	0.0366	0.8002	0.4902	0.4277	0.9937	0.6999	0.4830
Mn *vs*. period	0.1807	0.1719	0.8593	0.9882	0.6833	0.7009	0.9985	0.8444

684 [a>b] Means with different letters in the same column indicate significant differences ($P \leq 0.05$).
685 [1] All probabilities presented after arcsine transformation.
686 [2] All data were calculated as a percentage of fertile eggs; early, middle and late dead were estimated, respectively at 1st, 2nd and 3rd week of incubation.
687 [3] Hatchability as a proportion of total eggs, % = (number of chicks hatched/number of eggs set) × 100.
688 [4] Hatchability as a proportion total of fertile eggs, % = (number of chicks hatched/number of fertile eggs set) × 100.
689 [5] Fertility, % = (number of fertile eggs/numbers of total egg set) × 100.
690 [6] Contaminated eggs calculated as a percentage of total eggs.
691

692 **Table 5.** Broiler breeder hen egg characteristics as affected by increased dietary Mn.

	Yolk	Albumen	Shell			Eggshell					
				Yolk Mn, ppm	Specific gravity, kg/cm^3	Palisade layer	Mammillary layer	Membrane	Thickness	Breaking strength, kg/cm^2	Number of mammillary buttons/mm^2
Mn, ppm (mg/day)		%[1]						μm			
22.2 (3.2)	28.7	63.2	8.12^{b}	1.81^{b}	1.080^{c}	196.3^{b}	88.4^{b}	63.6^{d}	359^{c}	3.53^{b}	213
48.5 (7.1)	29.3	62.1	8.54^{a}	2.00^{ab}	1.084^{b}	222.5^{a}	90.0^{ab}	70.3^{c}	377^{b}	3.84^{ab}	206
77.9 (11.4)	29.3	62.2	8.54^{a}	2.09^{ab}	1.084^{b}	224.0^{a}	91.7^{ab}	70.9^{bc}	377^{b}	3.96^{a}	195
103.1 (15.0)	29.6	61.8	8.56^{a}	2.37^{a}	1.085^{ab}	226.5^{a}	92.8^{ab}	74.7^{a}	391^{a}	4.00^{a}	194
140.0 (20.4)	29.5	61.7	8.77^{a}	2.29^{a}	1.086^{a}	235.1^{a}	99.9^{a}	74.3^{ab}	391^{a}	4.06^{a}	180
168.2 (24.5)	29.5	61.8	8.65^{a}	2.12^{ab}	1.085^{ab}	228.6^{a}	95.0^{ab}	75.4 ^{a}	390^{a}	4.07^{a}	194
Period, wk											
36 to 39	28.1^{c}	63.3^{a}	8.50	2.13	1.085	215.0^{b}	86.5^{b}	70.8	366^{c}	3.82^{ab}	217^{a}
40 to 43	29.2^{b}	62.2^{b}	8.56	2.17	1.084	220.3^{b}	90.6^{ab}	71.2	404^{a}	4.05^{a}	183^{b}
44 to 47	29.8^{a}	61.6^{c}	8.60	2.09	1.084	229.5^{a}	101.7^{a}	71.9	378^{b}	4.00^{ab}	183^{b}
48 to 51	30.1^{a}	61.4^{c}	8.47	2.06	1.084	223.8^{ab}	93.0^{ab}	72.2	374^{b}	3.76^{b}	205^{ab}
SEM	0.09	0.09	0.028	0.037	0.001	2.1	1.5	0.53	1.2	0.034	4.1
*P*robability <											
Mn	0.4979	0.0636	0.0003	0.0122	0.0001	0.0001	0.0432	0.0001	0.0001	0.0023	0.5311
Period	0.0001	0.0001	0.1789	0.5632	0.1632	0.0380	0.0449	0.6983	0.0001	0.0111	0.0059
Mn *vs.* period	0.3157	0.2733	0.6117	0.4424	0.9005	0.9450	0.9335	0.0646	0.2298	0.9106	0.9886

693 [a>b] Means with different letters in the same column indicate significant differences ($P \leq 0.05$).
694 1 Probabilities of yolk, albumen and eggshell percentage are presented as arcsine transformation.
695
696

Table 6. Hatching chick characteristics as affected by increased dietary Mn.

		Hatching chicks[1]					
	Egg weight	Chick weight	Chick length[2], cm	Leg score[3]	Navel button score[4]	Tibia length, mm	Tibia ash, %
Mn, ppm (mg/day)	g						
22.2 (3.2)	72.4	51.7	18.5	1.17	1.58	29.2^{b}	24.9
48.5 (7.1)	70.7	50.7	18.6	1.14	1.43	29.5^{ab}	25.4
77.9 (11.4)	70.5	50.6	18.6	1.07	1.43	29.8^{ab}	25.6
103.1 (15.0)	70.6	50.4	18.6	1.05	1.28	30.6^{a}	26.0
140.0 (20.4)	70.2	50.3	18.6	1.05	1.30	30.3^{a}	26.3
168.2 (24.5)	70.7	50.4	18.6	1.06	1.31	30.3^{a}	26.1
Period, wk							
36 to 39	69.6^{c}	48.7^{c}	18.4^{c}	1.18	1.61^{a}	28.8^{c}	24.1^{b}
40 to 43	70.7^{b}	50.3^{b}	18.5^{bc}	1.04	1.14^{b}	29.5^{bc}	26.3^{a}
44 to 47	71.2^{b}	51.1^{b}	18.6^{ab}	1.05	1.31^{b}	30.2^{b}	26.1^{a}
48 to 51	72.3^{a}	52.6^{a}	18.8^{a}	1.02	1.36^{ab}	31.3^{a}	26.3^{a}
SEM	0.11	0.12	0.020	0.009	0.018	0.13	0.22
Probability <							
Mn	0.6832	0.8745	0.9540	0.8219	0.0549	0.0032	0.5748
Period	0.0001	0.0001	0.0001	0.3538	0.0001	0.0001	0.0004
Level *vs.* period	0.9853	0.1020	0.9053	0.9999	0.9719	0.1180	0.6161

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

[1]Leg and navel scores were analyzed using proc Glimmix (variables are non-parametric data).

[2]From the tip of the beak to the end of the middle toe (third toe).

[3]Leg scores: 1 – normal legs and toes; 2 – Sings of inflammation or redness in the legs.

[4]Navel scores: 1 – completely closed and clean; 2 – not completely closed and not discolored; 3 – not completely closed and discolored.

Table 7. Regression equations of egg production and incubation of breeders fed with Mn supplementation**.**

	Model	Regression equations[1]	R^2	*Probability* <	Requirement
Total egg production[2], %	QP	$y=56.37698+0.15410x-0.00066530x^2$	0.03	0.0016	115.8
	BLQ	$y=64.2859-0.00487(56.6483-x)^2$	0.03	0.0004	56.6
Settable egg production[3], %	QP	$y=41.23796+0.33202x-0.00136x^2$	0.11	0.0001	122.1
	BLQ	$y=59.4306-0.00788(63.6067-x)^2$	0.12	0.0001	63.6
Cracked eggs, %	QP	$y=12.31387-0.14512x+0.00056028x^2$	0.13	0.0001	129.5
	BLQ	$y=3.8040+0.00336(66.4374-x)^2$	0.15	0.0001	66.4
Defective eggs, %	QP	$y=1.95438-0.02744x+0.00011585x^2$	0.04	0.0001	118.4
Hatchability, %	QP	$y=55.87404+0.39736x-0.00158x^2$	0.27	0.0001	125.7
	BLQ	$y=78.4475-0.00734(69.4930-x)^2$	0.29	0.0001	69.5
Hatchability of fertile eggs, %	QP	$y=64.53912+0.42841x-0.00172x^2$	0.33	0.0001	124.5
	BLQ	$y=88.6570-0.00942(65.7774-x)^2$	0.37	0.0001	65.8
Contaminated eggs, %	QP	$y=9.88679-0.13776x+0.00053837x^2$	0.28	0.0001	127.9
	BLQ	$y=1.8898+0.00237(71.9020-x)^2$	0.29	0.0001	71.9
Total egg production[4]	QP	$y=63.09205+0.17237x-0.00074517x^2$	0.08	0.0124	115.8
	BLQ	$y=71.9315-0.00544(56.5923-x)^2$	0.09	0.0056	56.6
Settable egg production[5]	QP	$y=46.53535+0.37016x-0.00152x^2$	0.21	0.0001	121.8
	BLQ	$y=66.7397-0.00973(61.7100-x)^2$	0.25	0.0001	61.7
Hen Ht, %	QP	$y=27.48538+0.03812x-0.00013359x^2$	0.07	0.0900	142.7
	BLQ	$y=30.1971-0.00012(148.0-x)^2$	0.07	0.0917	148.0
Chick Ht, %	QP	$y=29.61752+0.04676x-0.00017260x^2$	0.07	0.0856	135.5
	BLQ	$y=32.6856-0.00022(122.4-x)^2$	0.07	0.0778	122.4
Hen ALP[6]	QP	$y=168.97433+2.74723x-0.01086x^2$	0.15	0.0035	126.5
	BLQ	$y=340.1-0.00717(145.2-x)^2$	0.13	0.0053	145.2

[1]Regression equations obtained using the increasing analyzed Mn in the diets (22.4; 48.5; 77.9; 103.1, 140.0, and 168.2 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 eggs produced by live hens at the end of the experiment.
[6]Alkaline Phosphatase.

Table 8. Regression equations of egg and chick parameters of breeders fed with Mn supplementation**.**

	Model	Regression equations[1]	R^2	*Probability* <	Requirement
Egg weight	QP	$y=72.99526-0.04801x+0.00020389x^2$	0.02	0.0001	117.7
	BLQ	$y=63.6036+0.00110(63.6036-x)^2$	0.02	0.0001	63.6
Yolk[2], %	QP	$y=28.47918+0.01918x-0.00007709x^2$	0.02	0.0072	124.4
Eggshell[2], %	QP	$y=7.94128+0.01178x-0.00004474x^2$	0.08	0.0001	131.6
	BLQ	$y=8.6426-0.00022(70.9837-x)^2$	0.08	0.0001	71.0
Albumen[2], %	QP	$y=63.57118-0.03072x+0.00012048x^2$	0.04	0.0001	127.5
	BLQ	$y=61.7111+0.000152(113.0-x)^2$	0.04	0.0001	113.0
Yolk Mn, ppm	QP	$y=1.51516+0.01306x-0.00005533x^2$	0.31	0.0001	118.0
	BLQ	$y=2.2399-0.00005(118.4-x)^2$	0.28	0.0001	118.4
Breaking strength, kg/cm^2	QP	$y=3.44749+0.01035x-0.00003689x^2$	0.06	0.0001	140.3
	BLQ	$y=4.0377-0.00006(112.7-x)^2$	0.06	0.0001	112.7
Specific gravity, kg/cm^3	QP	$y=1078.22139+0.11957x-0.00045540x^2$	0.12	0.0001	131.3
	BLQ	$y=1085.3-0.00254(68.4776-x)^2$	0.12	0.0001	68.5
Eggshell membrane layer, μm	QP	$y=59.81601+0.21964x-0.00078347x^2$	0.23	0.0001	140.2
	BLQ	$y=74.7744-0.00095(128.0-x)^2$	0.24	0.0001	128.0
Eggshell Palisade Layer, μm	QP	$y=185.13330+0.74424x-0.00289x^2$	0.41	0.0001	128.8
	BLQ	$y=228.5-0.0149(68.7501-x)^2$	0.43	0.0001	68.8
Eggshell thickness, μm	QP	$y=347.23371+0.63094x-0.00225x^2$	0.15	0.0001	140.2
	BLQ	$y=390.5-0.00243(134.2-x)^2$	0.15	0.0001	134.2
Chick body weight, g	QP	$y=52.25175-0.04050x+0.00016815x^2$	0.01	0.0004	120.4
	BLQ	$y=49.9994+0.000414(85.6426-x)^2$	0.02	0.0002	85.6
Chick navel button score	QP	$y=1.67508-0.00538x+0.00001892x^2$	0.02	0.0001	142.2
	BLQ	$y=1.2988+0.000020(138.1-x)^2$	0.02	0.0001	138.1
Chick tibia length, cm	QP	$y=28.50842+0.02661x-0.00009448x^2$	0.09	0.0013	140.8

[1]Regression equations obtained using the increasing analyzed Mn in the diets (22.4; 48.5; 77.9; 103.1, 140.0, and 168.2 ppm).
[2]Percentage in relation to egg weight.

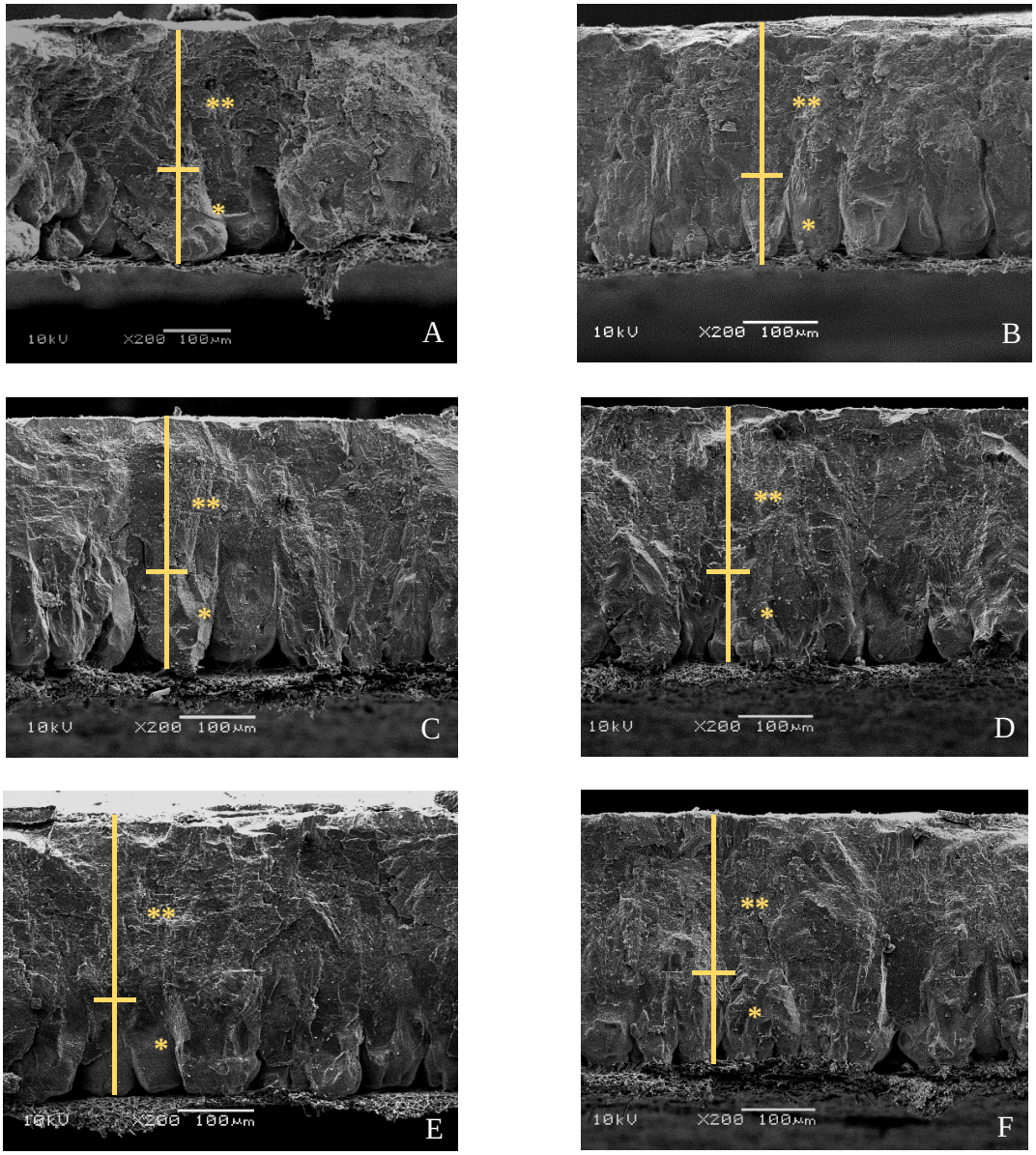

Figure 1. Scanning electron cross-sections and inner surface of the eggshell from broiler breeder hens fed a Mn-deficient diet (22.2 ppm) (A), and diets with 48.5 ppm (B), 77.9 ppm (C), 103.1 ppm (D), 140.0 ppm (E), and 168.2 ppm (F) Mn (200x). * Mammillary layer. **Palisade layer.

CAPÍTULO III

CONSIDERAÇÕES FINAIS

Com base nos resultados obtidos neste estudo podemos concluir que a adição de manganês nas dietas de matrizes de corte se mostrou essencial para os resultados de desempenho e condições fisiológicas das matrizes, bem como para os parâmetros de incubação e qualidade da progênie. O nível de 22,2 ppm de manganês na dieta das reprodutoras foi claramente deficiente, levando a diminuição do desempenho. Ficou evidente a necessidade de Mn para manter a produção e qualidade dos ovos de reprodutoras e consequente produção de pintinhos, visto que esse é seu principal objetivo.

Um ponto a ser observado é o aproveitamento dos microminerais na matriz nutricional dos ingredientes, permitindo assim uma redução da quantidade de manganês no premix mineral. O tipo de dieta utilizada implica diretamente no nível de suplementação a ser oferecido no premix. Em dietas a base de milho e soja, deve-se levar em consideração fatores antinutricionais, como por exemplo a presença ácido fítico, o qual pode diminuir a disponibilidade do manganês. Atualmente, utiliza-se uma margem de segurança na suplementação de manganês nas rações, o que além de aumentar o custo das dietas, também aumenta sua excreção para o ambiente.

As variáveis relacionadas a qualidade da casca se mostraram mais sensíveis aos níveis de manganês na dieta. Diversas alterações como diminuição de produção de ovos incubáveis e eclodibilidade, aumento de ovos quebrados, defeituosos e ovos contaminados foram influenciadas pela qualidade da casca dos ovos, mostrando que o manganês é essencial na formação da casca.

Baseado nos pontos de máxima das equações de regressão, pode-se recomendar o uso entre 56,6 e 148,0 ppm de manganês na dieta de matrizes pesadas, dependendo da variável estudada. Levando em consideração o modelo não linear quadrático polinomial, que tende a explicar melhor os modelos biológicos, a média para exigência de manganês é de 128,4 ppm na dieta. No entanto, dependendo da variável a ser considerada no sistema produtivo, a exigência de manganês pode ser maior ou menor, conforme os valores encontrados neste trabalho. Considerando os dois modelos utilizados, a quantidade de manganês requerida para produção (93,5) e eclodibilidade dos ovos (97,6 ppm) foi menor que a quantidade necessária para a qualidade dos ovos (117,5 ppm) e progênie.

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APÊNDICES

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

POULTRY SCIENCE INSTRUCTIONS TO AUTHORS [1]

Scope and General Information

Scope

Poultry Science publishes the results of fundamental and applied research concerning poultry, poultry products, and avian species in general. Submitted manuscripts shall provide new facts or confirmatory data. Papers dealing with experimental design, teaching, extension endeavors, or those of historical or biographical interest may also be appropriate. Opinions or views expressed in papers published by *Poultry Science* are those of the author(s) and do not necessarily represent the opinion of the Poultry Science Association or the editor-in-chief.

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ii.) Review Papers

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v.) Invited Papers

Invited papers are subject to review, and all manuscript style and form guidelines of the journal shall be followed. Invited papers are exempt from page charges.

vi.) Invited Reviews

Invited Reviews will be approximately 10 published pages and in review format. Nominations or suggestions for potential timely reviews are welcomed and should be sent directly to the editor-in-chief.

vii.) Contemporary Issues

Contemporary Issues will address critical issues facing poultry scientists and the poultry industry. As such, submissions to this section should be of interest to any poultry scientist, to the industry, to instructors and faculty teaching contemporary issues classes, and to undergraduate and graduate students. The section will consist of short papers (approximately 2 published pages) written in essay format and will include an abstract, appropriate subheadings, and references.

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1 IU = 0.552 µg of retinyl palmitate
1 IU = 0.60 µg of β-carotene

Vitamin E
1 IU = 1 mg of dl-α-tocopheryl acetate 1 IU = 0.91 mg of dl-α-tocopherol
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Statistical analysis: Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately.
The experimental unit is the smallest unit to which an individual treatment is imposed. For group-fed animals, the group of animals in the pen is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use time-sequence analysis.

Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to

deviations from these assumptions, but occasionally data transformations or other techniques are helpful. For example, it is recommended that percentage data between 0 and 20 and between 80 and 100 be subjected to arc sin transformation prior to analysis. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals are stratified by ancestry or weight or if some other initial measurement should be accounted for, they should include a blocking factor, or the initial measurement should be included as a covariate.

A parameter [mean (μ), variance (σ2)], which defines or describes a population, is estimated by a statistic (x, s2). The term **parameter** is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment.

Standard designs are adequately described by name and size (e.g., “a randomized complete block design with 6 treatments in 5 blocks”). For a factorial set of treatments, an adequate description might be as follows: “Total sulfur amino acids at 0.70 or 0.80% of the diet and Lys at 1.10, 1.20, or 1.30% of the diet were used in a 2 × 3 factorial arrangement in 5 randomized complete blocks consisting of initial BW.” Note that **a factorial arrangement is not a design**; the term “design” refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted).

Standard deviation refers to the variability in a sample or a population. The standard error (calculated from error variance) is the estimated sampling error of a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not “statistically significant” is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by “±” to a number implies that the second value is its standard error (not its standard deviation). Adequate reporting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of experimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of observations or the heterogeneity of the error variance is to be emphasized. Presenting individual standard errors clutters the presentation and can mislead readers.

For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator of each F statistic. Unbalanced factorial data can present special problems. Accordingly, it is well to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases.
Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by Bonferroni t statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged. Fixed-range, pairwise, multiple-comparison tests should be used only to compare means of treatments that are unstructured or not related. Least squares means are the correct means to use for all data, but arithmetic means are identical to least squares means unless the design is

unbalanced or contains missing values or an adjustment is being made for a covariate. In factorial treatment arrangements, means for main effects should be presented when important interactions are not present. However, means for individual treatment combinations also should be provided in table or text so that future researchers may combine data from several experiments to detect important interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

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

Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information contained in the data is sacrificed. Significant digits in data reported should be restricted to 3 beyond the decimal point, unless warranted by the use of specific methods.

iv.) Results and discussion

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

v.) Acknowledgements

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

vi.) Appendix

A technical Appendix, if desired, shall follow the Discussion section or Acknowledgments, if present. The Appendix may contain supplementary material, explanations, and elaborations that are not essential to other major sections but are

helpful to the reader. Novel computer programs or mathematical computations would be appropriate. The Appendix will not be a repository for raw data.

vii.) References

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

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Article:

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

Bagley, L. G., V. L. Christensen, and R. P. Gildersleeve. 1990. Hematological indices of turkey embryos incubated at high altitude as affected by oxygen and shell permeability. Poult. Sci. 69:2035– 2039.

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

Book:

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

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

Federal Register:

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

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

Tables

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

Units of measure for each variable must be indicated. Papers with several tables must use consistent format. All columns must have appropriate headings. Abbreviations not found on the inside front cover of the journal must be defined in each table and must match those used in the text. Footnotes to tables should be marked by superscript numbers. Each footnote should begin a new line. Superscript letters shall be used for the separation of means in the body of the table and explanatory footnotes must be provided [i.e., “Means within a row lacking a common superscript differ ($P < 0.05$).”]; other significant P-values may be specified. Comparison of means within rows and columns should be indicated by different series of superscripts (e.g., a,b, . . . in rows; x–z . . . in columns) The first alphabetical letter in the series (e.g., a or A) shall be used to indicate the largest mean. Lowercase super- scripts indicate $P \leq 0.05$. Uppercase letters indicate $P \leq 1.1$ or less.

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

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

Miscellaneous usage notes

i.) Abbreviations

Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD). A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the scientific field. Abbreviations may be used in heads within the paper, if they have been first defined within the text. The inside back cover of every issue of the journal lists abbreviations that can be used without definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used less than three times must be spelled out in full rather than abbreviated. All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter, rather than the full term.

The abstract, text, each table, and each figure must be understood independently of each other. Therefore, abbreviations shall be defined within each of these units of the manuscript.

Plural abbreviations do not require “s.” Chemical symbols and three-letter abbreviations for amino acids do not need definition. Units of measure, except those in the standard *Poultry Science* abbreviation list, should be abbreviated as listed in the *CRC Handbook for Chemistry and Physics* (CRC Press, 2000 Corporate Blvd., Boca Raton, FL, 33431) and do not need to be defined.
The following abbreviations may be used without definition in *Poultry Science*:
A adenine
ADG average daily gain
ADFI average daily feed intake
AME apparent metabolizable energy
AMEn nitrogen-corrected apparent metabolizable energy ANOVA analysis of variance
B cell bursal-derived, bursal-equivalent derived cell bp base pairs
BSA bovine serum albumin BW body weight
C cytosine
cDNA complementary DNA
cfu colony-forming units CI confidence interval CP crude protein
cpm counts per minute CV coefficient of variation d day
df degrees of freedom DM dry matter
DNA deoxyribonucleic acid
EDTA ethylenediaminetetraacetate
ELISA enzyme-linked immunosorbent antibody assay EST expressed sequence tag
g gram g gravity
G guanine
GAT glutamic acid-alanine-tyrosine G:F gain-to-feed ratio GLM general linear model
h hour
HEPES N-2-hydroxyethyl piperazine-N′-ethane-sulfonic acid HPLC high-performance (high-pressure) liquid chromatography ICU international chick units
Ig immunoglobulin IL interleukin
IU international units kb kilobase pairs kDa kilodalton

L liter*
L:D hours light:hours darkness in a photoperiod (e.g., 23L:1D) m meter
µ micro M molar
MAS marker-assisted selection ME metabolizable energy
MEn nitrogen-corrected metabolizable energy MHC major histocompatibility complex
mRNA messenger ribonucleic acid min minute
mo month
MS mean square
n number of observations
N 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
*Also capitalized with any combination, e.g., mL.

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

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

iv.) Number style**:** Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits. Measures must be in the metric system; however, US equivalents may be given in parentheses. Poultry Science requires that measures of energy be given in calories rather than joules, but the equivalent in joules may be shown in parentheses or in a footnote to tables. Units of measure not preceded by numbers must be written out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract and in the body of the paper at first use. Units of measure for feed conversion or feed efficiency shall be provided (i.e., g:g).

***v.) Nucleotide sequences*:** Nucleotide sequence data must relate to poultry or poultry pathogens and must complement biological data published in the same or a companion paper. If sequences are excessively long, it is suggested that the most relevant sections of the data be published in Poultry Science and the remaining sequences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submission of sequence data to one of the databases. The following statement should appear as a footnote to the title on the title page ofthe manuscript. "The nucleotide sequence data reported in this paper have been submitted to Embank Submission (Mail Stop K710, Los Alamos National Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN." Publication of the description of molecular clones is assumed by the editors to place them in the public sector. Therefore, they shall be made available to other scientists for research purposes.
Nucleotide sequences must be submitted as camera- ready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow Poultry Science guidelines.

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

vii.) General usage:
Note that "and/or" is not permitted; choose the more appropriate meaning or use "x or y or both."
Use the slant line only when it means "per" with numbered units of measure or "divided by" in equations. Use only one slant line in a given expression (e.g., g/d per chick). The slant line may not be used to indicate ratios or mixtures.
Use "to" instead of a hyphen to indicate a range. Insert spaces around all signs (except slant lines) of operation (=, –, +, ×, >, or <, etc.) when these signs occur between two items.
Items in a series should be separated by commas (e.g., a, b, andc).
Restrict the use of "while" and "since" to meanings related totime.
Appropriate substitutes include "and," "but," or "whereas" for "while" and "because" or "although" for "since."
Leading (initial) zeros should be used with numbers less than 1 (e.g., 0.01).
Commas should be used in numbers greater than 999.
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VITA

Thiago Luiz Noetzold, filho de Edson Luiz Noetzold e Elisabete Bauer Noetzold, nascido em 03 de abril de 1995, em Palmitos – SC. Completou o ensino fundamental nos colégios Rodolpho Walter Schreiner e Ida Hilda Casella Vidori, e o ensino médio no colégio Felisberto de Carvalho, todos localizados no município de Palmitos – SC, concluindo os estudos em dezembro de 2012. Em 2013 ingressou no curso de Medicina Veterinária na Faculdade de Itapiranga – FAI. No último semestre da faculdade foi estagiário no Aviário de Ensino e Pesquisa – UFRGS, na cidade de Porto Alegre – RS, na área de produção e nutrição de frangos e matrizes de corte, sob supervisão do professor PhD. Sergio Luiz Vieira. Formou-se em dezembro de 2017. No primeiro semestre de 2018 ingressou como aluno de mestrado com dedicação exclusiva no Programa de Pós-Graduação em Zootecnia da UFRGS, sob orientação do professor PhD. Sergio Luiz Vieira. Além de ter se envolvido em diversos projetos de pesquisa ao longo do seu mestrado, teve a oportunidade de participar em dois eventos científicos internacionais, onde em ambos realizou apresentações orais em inglês sobre trabalhos desenvolvidos no Aviário de Ensino e Pesquisa. Foi submetido à banca de defesa de Dissertação em março de 2020.