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PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

REQUERIMENTO DE MANGANÊS PARA FRANGOS DE CORTE ALIMENTADOS COM SUPLEMENTAÇÃO DE FITASE

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

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REQUERIMENTO DE MANGANÊS PARA FRANGOS DE CORTE ALIMENTADOS COM SUPLEMENTAÇÃO DE FITASE

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Resumo - A suplementação de manganês (Mn) em rações para frangos de corte é comum e visa reduzir os riscos de sua deficiência. Por outro lado, os efeitos da fitase suplementar têm sido relatados como capazes de aumentar a disponibilidade de microminerais em rações à base de milho e soja. O presente estudo teve como objetivo avaliar as exigências de Mn em frangos de corte alimentados com dietas suplementadas com fitase. Um total de 640 pintinhos machos Cobb × Cobb 500 de um dia de idade foram distribuídos em um arranjo fatorial 2 × 5, com rações contendo ou não fitase e cinco níveis crescentes de Mn na dieta. Foram utilizadas 80 gaiolas de bateria, com oito repetições de oito aves cada. Pintinhos de um dia de idade foram alimentados com uma ração deficiente em Mn, sem fitase (14,58 ± 0,74 mg/kg de Mn analisado), desde a entrada até os 7 dias de idade. Em seguida, foram aplicados tratamentos experimentais por 28 dias (milho-soja, carbonato de cálcio e ácido fosfórico, resultando em um teor de Mn de 19,64 ± 1,5 mg/kg). A fitase foi suplementada a 4.000 FYT/kg (analisada em 4.680 ± 420 FYT/kg), e o Mn foi fornecido como sulfato ($MnSO_4 \cdot H_2O$) em concentrações crescentes para atingir 19,64 ± 1,5, 44,08 ± 0,67, 73,80 ± 1,26, 105,08 ± 2,76 e 129,97 ± 1,59 mg/kg de Mn. Não foram observadas interações entre fitase e Mn para nenhuma variável ($P > 0,05$). No entanto, rações contendo fitase impactaram positivamente o desempenho das aves ($P < 0,05$). O aumento de Mn na dieta não apresentou efeitos sobre o desempenho de crescimento ($P > 0,05$). A retenção de Mn foi linear com o aumento da sua ingestão dietética, inclusive na tíbia e no fígado ($P < 0,05$). Não foram observados efeitos da fitase ou do Mn sobre os parâmetros locomotores (valgo, varo, rotação da tíbia, escore de marcha) ou sobre a morfometria da tíbia aos 28 dias. Os resultados sugerem que a suplementação com Mn, como atualmente proposta, não oferece benefícios e é economicamente excessiva. No presente estudo, melhorias consistentes no desempenho com a fitase ocorreram em paralelo com a utilização de energia e a retenção de Mn.

Palavras-chave: manganês, frangos de corte, fitase, desempenho.

MANGANESE REQUIREMENT FOR BROILER CHICKENS FED DIETS SUPPLEMENTED WITH PHYTASE

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Abstract – Manganese (Mn) supplementation of broiler feeds is routinely done in commercial feeds intending to reduce the risks of its dietary deficiency aiming optimum live performance. On the other hand, phytase added to the feeds has been reported to increase trace mineral availability in corn-soy feeds. The present study aimed to evaluate Mn requirements of broilers fed diets with supplemental phytase. A total of 640 one-day-old male Cobb × Cobb 500 chicks were assigned in a 2 × 5 factorial arrangement (feeds with or without phytase and five graded increases of dietary Mn. A total of 80 battery cages with eight replicates of eight birds each was used. One d old chicks were fed a Mn-deficient feed without phytase (14.58 ± 0.74 mg/kg analyzed Mn) from placement to 7 d of age. Experimental treatments were applied thereafter to 28 d (corn-soybean meal in a total Mn content of 19.64 ± 1.5 mg/kg). Phytase was supplemented at 4,000 FYT/kg (analyzed 4,680 ± 420 FYT/kg) with Mn provided as sulfate ($MnSO_4 \cdot H_2O$) at increasing contents to reach 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76, and 129.97 ± 1.59 mg/kg Mn. No interactions between phytase and Mn were observed for any variable ($P > 0.05$); however, birds fed phytase had improved performance ($P < 0.05$). The increased dietary Mn did not show effects on growth performance as well as effects of phytase and Mn on locomotor parameters (valgus, varus, tibia rotation, and gait score) and tibia morphometry at 28 d. Total Mn retention as well as tibiae and liver content increased linearly as it was added to the feeds ($P < 0.05$). Results indicate that current commercially available suggestions for Mn supplementation do not provide benefits and are excessive. Improvements in growth performance, as well as in energy utilization and Mn retention when birds are fed phytase, are consistent with previous research done with other trace minerals.

Key words: broiler; manganese; phytase.

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CAPÍTULO I

INTRODUÇÃO

A avicultura exerce papel de grande relevância na economia brasileira, consolidando-se como uma das principais atividades do setor agropecuário nacional. Além de sua expressiva contribuição na geração de empregos e na movimentação econômica, destaca-se como um dos pilares da segurança alimentar do país (IBGE, 2024). O Brasil apresenta elevada produção de carne de frango, estimada em 14,97 milhões de toneladas, e de ovos, com aproximadamente 57,6 bilhões de unidades, posicionando-se entre os principais produtores mundiais (ABPA, 2025).

O consumo de produtos provenientes da avicultura tem apresentado crescimento contínuo desde 2014. Nesse período, o consumo per capita de carne de frango passou de 42,8 kg para 45,5 kg em 2024, representando um aumento aproximado de 6,3% ao longo de dez anos. De forma ainda mais expressiva, o consumo de ovos evoluiu de 182 para 269 unidades por habitante entre 2014 e 2024, resultando em um incremento de aproximadamente 48%.

Apesar da elevada eficiência produtiva do setor, os custos de produção permanecem elevados, sendo a alimentação o componente de maior impacto econômico, correspondendo a cerca de 65% do custo total de produção (ABPA, 2025). Diante desse cenário, estratégias que visem à redução dos custos dietéticos, associadas à otimização do aproveitamento nutricional, tornam-se fundamentais. Nesse contexto, o uso de enzimas exógenas destaca-se como ferramenta relevante para maximizar o desempenho produtivo, melhorar a utilização dos nutrientes e auxiliar na definição de níveis nutricionais mais eficientes.

O estabelecimento de níveis ideais de nutrientes é essencial para evitar a excreção excessiva, contribuindo não apenas para a redução de custos, mas também para maior eficiência no processamento das rações, diminuição do tempo e do espaço demandados na fábrica e melhor aproveitamento metabólico pelos animais. Assim, a constante atualização e avaliação desses níveis nutricionais tornam-se indispensáveis para promover ganhos produtivos sustentáveis e econômicos no sistema avícola.

REVISÃO BIBLIOGRÁFICA

Uso de microminerais na avicultura

A avicultura moderna é caracterizada por elevados índices de produtividade, o que exige dietas nutricionalmente precisas para atender às demandas fisiológicas das aves. Entre os nutrientes essenciais, os microminerais desempenham papel fundamental no metabolismo, crescimento, imunidade, formação óssea e sistemas antioxidantes, mesmo sendo requeridos em pequenas quantidades. Na nutrição de aves, destacam-se minerais como manganês (Mn), zinco (Zn), ferro (Fe), cobre (Cu), selênio (Se) e iodo (I).

Usualmente utilizado o premix vitamínico-mineral é uma mistura concentrada de vitaminas e minerais adicionada às rações em pequenas quantidades, com o objetivo de assegurar que as exigências nutricionais das aves sejam atendidas com precisão. O uso do premix permite uniformidade na distribuição dos micronutrientes, reduzindo o risco de deficiências ou excessos nutricionais e na otimização do desempenho zootécnico e na manutenção da saúde intestinal, óssea e imunológica das aves, sendo um componente indispensável na formulação de rações comerciais (Rostagno, 2024).

No caso dos microminerais, o premix pode conter fontes inorgânicas (como sulfatos e óxidos) ou orgânicas (minerais quelatados ou complexados a aminoácidos). Fontes orgânicas têm sido amplamente estudadas por apresentarem maior biodisponibilidade, menor interação com antagonistas dietéticos e menor excreção mineral, contribuindo para melhor eficiência nutricional e menor impacto ambiental. Já os minerais inorgânicos, apesar de sua baixa absorção em comparação com o orgânico, têm menor custo.

Os minerais desempenham papéis cruciais em diversas vias metabólicas. Macrominerais, são expressos em percentagem nos alimentos ou tecidos, estão principalmente envolvidos em funções estruturais, enquanto microminerais, tem uma difícil avaliação devido a suas baixas concentrações nos tecidos animais. Os minerais estão frequentemente associados a proteínas, formando metaloenzimas. A absorção de minerais depende do alimento ingerido, onde podem ser encontrados em diversas formas químicas, como moléculas orgânicas ou parte de sais com diferentes solubilidades. O interesse em formas orgânicas inclui diferenças na disponibilidade, mas também está relacionado a possíveis melhorias em suas ações específicas no nível celular (VIEIRA, 2008).

Microminerais inorgânicos geralmente são suplementados nas dietas das aves nas formas de sulfatos e óxidos. No intestino, íons são absorvidos através de difusão passiva ou transporte ativo. Para ingressarem na corrente sanguínea, órgãos e tecidos, esses íons

necessitam se associar a um agente transportador que facilite sua travessia pela parede intestinal. Caso não encontrem esse agente, podem ser eliminados, levando a perdas. Em certas circunstâncias, as perdas podem ocorrer devido a reações com compostos insolúveis ou devido à competição por locais de absorção entre diferentes elementos minerais, resultando em interações antagônicas que prejudicam a absorção (HERRICK, 1993).

Manganês

O manganês (Mn) é um micromineral essencial para frangos de corte, participando de diversas reações metabólicas relacionadas ao crescimento, mineralização óssea, metabolismo de carboidratos e formação de cartilagem. As exigências nutricionais de Mn variam conforme a fase de desenvolvimento, a composição dos ingredientes e a biodisponibilidade da fonte utilizada. Embora o NRC (1994) recomende 60 mg/kg, estudos recentes mostram que dietas modernas à base de milho e farelo de soja possuem níveis basais de Mn reduzidos, o que pode resultar em respostas produtivas e ósseas dependentes da suplementação. Além disso, a presença de fitato nas dietas reduz a disponibilidade de minerais, o que pode aumentar a exigência real de Mn para frangos de corte, especialmente sob condições de rápido crescimento e alta demanda metabólica.

O Mn desempenha uma função crucial em várias operações vitais para os organismos, atuando como um cofator enzimático em processos relacionados à síntese de ATP, ciclo de Krebs, fosforilação oxidativa, além de participar em reações da fosfatase alcalina e piruvato oxidase (GONZÁLEZ; SILVA, 2019). Este micromineral está associado a metaloenzimas ativadas por ele, como glicosiltransferases, arginase, tiaminase, piruvato carboxilase, Mn-superóxido dismutase e dipeptidases intestinais (SUTTLE; UNDERWOOD, 2010). Sua importância estende-se ao desenvolvimento da matriz orgânica óssea e é essencial para a reprodução e o adequado funcionamento do sistema nervoso central (BERTECHINI, 2007).

O manganês desempenha um papel crucial no sistema antioxidante das aves, pois atua como cofator da enzima manganês-superóxido dismutase (Mn-SOD), localizada na matriz mitocondrial. A Mn-SOD é responsável pela detoxificação do radical superóxido ($O_2^{\bullet -}$), convertendo-o em peróxido de hidrogênio, o qual posteriormente é neutralizado por catalase ou glutationa peroxidase. Dessa forma, o manganês contribui diretamente para a proteção das células contra o estresse oxidativo e a disfunção mitocondrial. Deficiências de Mn podem reduzir a atividade da Mn-SOD, resultando em maior peroxidação lipídica, danos celulares e diminuição do desempenho produtivo. Por outro lado, níveis adequados ou elevados de Mn

tendem a aumentar a atividade antioxidante sistêmica, especialmente em situações de desafio metabólico ou inflamatório. (Li, 2006)

Disponibilidade

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

Sulfato, oxido, níveis usados microminerais inorgânicos geralmente são suplementados nas dietas das aves nas formas de sulfatos e óxidos. No intestino, íons são absorvidos através de difusão passiva ou transporte ativo. Para ingressarem na corrente sanguínea, órgãos e tecidos, esses íons necessitam se associar a um agente transportador que facilite sua travessia pela parede intestinal. Caso não encontrem esse agente, podem ser eliminados, levando a perdas. Em certas circunstâncias, as perdas podem ocorrer devido a reações com compostos insolúveis ou devido à competição por locais de absorção entre diferentes elementos minerais, resultando em interações antagônicas que prejudicam a absorção (HERRICK, 1993).

O manganês está presente em ingredientes de origem vegetal, como milho, soja, arroz, trigo e demais, em dietas comerciais de frangos de corte os níveis de manganês ficam em torno de 30 a 40 mg/kg. Para os manuais de nutrição com estes valores precisaria haver uma suplementação para atingir os níveis que eles recomendam para uma ótima performance animal.

Deficiências

O Mn é fundamental para a formação adequada de cartilagem, ossos e matrizes extracelulares, participando como cofator de glicosiltransferases envolvidas na síntese de proteoglicanos. A carência de Mn está associada a deformidades ósseas, como encurvamento de ossos longos, condrodistrofia, fragilidade óssea, perose e problemas locomotores. Em frangos de corte, que apresentam crescimento rápido e alta deposição muscular, a integridade locomotora é particularmente sensível ao equilíbrio mineral. Estudos mostram que níveis insuficientes de Mn podem prejudicar a ossificação, alterar a mineralização da tíbia e aumentar a incidência de lesões articulares. De acordo com Leeson e Summers (2001), a maior parte do Mn no corpo animal concentra-se nos ossos, seguido pelo fígado. Em aves jovens, as maiores concentrações de Mn são encontradas no fígado, rim e ossos (SUTTLE; UNDERWOOD, 2010). Em ovos, estudos de Yair e Uni (2011) indicam que o Mn é o quinto micromineral com

maior concentração na casca de ovos. A deficiência de Mn pode resultar em malformações esqueléticas, crescimento retardado, ataxia em recém-nascidos, infertilidade e anormalidades no metabolismo lipídico e de carboidratos (OBERLEAS; HARLAND; BOBILYA, 1999).

Toxicidade

Embora níveis muito elevados de Mn sejam raramente tóxicos em aves, suplementações abaixo do necessário comprometem a qualidade óssea, o equilíbrio locomotor e o bem-estar das aves, alguns autores com Miles (2003) e Sunder (2006) sugerem valores de toxicidade de 1500 e 1600 mg/kg respectivamente. A toxicidade ocorrerá apenas em situações de aporte excessivo de manganês via premix, uma vez que a composição nutricional dos ingredientes, por si só, não promove níveis excessivos desse mineral.

Fitase

É uma enzima amplamente utilizada na nutrição de aves por sua capacidade de hidrolisar o ácido fítico, aumentando a disponibilidade de fósforo, cálcio, aminoácidos e diversos microminerais, incluindo manganês. Em dietas à base de milho e farelo de soja, uma fração significativa do Mn encontra-se complexada com fitato, o que limita sua absorção intestinal. A suplementação de fitase pode liberar este Mn ligado, aumentando sua biodisponibilidade e reduzindo a necessidade de suplementação mineral adicional. Além disso, a ação da fitase tende a melhorar o desempenho zootécnico, digestibilidade dos nutrientes e saúde óssea. A depender da dose e da eficácia da enzima, a fitase pode até substituir parcialmente o Mn suplementar, contribuindo para formulações mais econômicas e ambientalmente sustentáveis. As fitases (mio-inositol hexafosfato fosfohidrolase) são hidrolases capazes de catalisar a hidrólise gradual de mio-inositol hexafosfato (ácido fítico; IP6). As fitases relevantes para a alimentação animal podem ser divididas em 2 subclasses, 3-fitases ou 6-fitases, dependentes de qual fosfato inicia a catálise no núcleo mio inositol (ADEOLA & COWIESON, 2011).

A fitase é comumente adicionada às dietas de aves para reduzir os efeitos antinutricionais do fitato, liberando quantidades significativas de P do ácido fítico, assim como a liberação de outros minerais e demais nutrientes, consequentemente, melhorando o desempenho dos frangos de corte, contribuindo para menor excreção de P no ambiente (COWIESON et al., 2013). O fitato está presente nos alimentos de origem vegetal, como por exemplo, milho e farelo soja contém 0,19% e 0,34%, respectivamente (ROSTAGNO et al., 2017). Esta molécula não pode ser eficientemente hidrolisada por enzimas endógenas em aves e aproximadamente dois terços do P acabam ficando indisponíveis para a absorção animal, podendo dessa forma alterar o turnover das células intestinais e pode causar irritação da mucosa,

aumentando a produção de mucinas e, consequentemente, a perda de nitrogênio endógeno (COWIESON et al., 2009). Existe uma variação no que rege a liberação de P ligado ao fitato em rações a base de milho e farelo de soja, podendo variar de 50 a 100%, isso depende principalmente da dose suplementada (SIMONS et al., 1990; Waldroup et al., 2000; SOMMERFELD et al., 2019).

O uso de fitase tem demonstrado efeito positivo na deposição de Ca e P na tíbia de frangos de corte, principalmente quando se tem redução nos níveis de fósforo disponível (VIEIRA et al., 2015; BROCH et al., 2020). Recentes investigações demonstraram que a fitase pode melhorar a digestibilidade de microminerais como zinco e cobre, uma vez que esses e outros microminerais podem estar complexados com o fitato (SOSTER et al., 2023). A digestibilidade de aminoácidos e energia podem ser melhorados com o uso de fitase em função da liberação das moléculas ligadas ao inositol hexafosfato, assim como também pela redução das perdas endógenas (COWIESON et al., 2006).

HIPÓTESES E OBJETIVOS

HIPÓTESES

Níveis crescente de Mn suplementado em rações milho e soja para frangos de corte afetam desempenho e problemas locomotores em frangos de corte.

A suplementação de fitase pode aumentar a energia digestível ileal em frangos de corte.

A suplementação de fitase em dieta com níveis crescentes de Mn pode aumentar a biodisponibilidade de Mn em frangos de corte.

OBJETIVO GERAL

Avaliar os efeitos de níveis de suplementação de Mn em dietas para frangos de corte de 8 a 28 dias sobre o desempenho zootécnico e problemas locomotores. Adicionalmente, o experimento tem por objetivo avaliar os efeitos da suplementação de fitase em dietas com níveis crescentes de Mn sobre biodisponibilidade de Mn e energia digestível ileal.

CAPÍTULO II

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RUNNING TITLE: BROILER AND MANGANESE

Reevaluating manganese requirements of broiler chickens under phytase supplementation

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ABSTRACT

Manganese (Mn) is routinely supplemented in broiler diets to prevent deficiency and sustain optimal perfor mance, while phytase is known to enhance trace mineral availability in corn–SBM feeds. This study evaluated graded Mn supplementation in diets with or without phytase. A total of 640 one-day-old male Cobb × Cobb 500 chicks were assigned to a 2 × 5 factorial design (with or without phytase × five Mn levels). Birds were housed in 80 battery cages, with 8 replicates of 8 birds each. From placement to 7 days, all chicks received a Mn-deficient diet without phytase (14.58 ± 0.74 mg/kg analyzed Mn). From 7 to 28 days, corn–SBM diets (19.64 ± 1.5 mg/kg analyzed Mn) were fed with phytase at 4,000 FYT/kg (analyzed 4,680 ± 420 FYT/kg) and Mn, supplied as $MnSO_4 \cdot H_2O$, to achieve 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76, and 129.97 ± 1.59 mg/kg analyzed Mn. No interactions between phytase and Mn were detected ($P > 0.05$). Phytase significantly improved growth performance, dry matter and Mn retention ($P < 0.001$), and AMEn ($P < 0.05$). Increasing dietary Mn had no effect on growth performance, locomotor parameters, or tibia morphometry at 28 days ($P > 0.05$), whereas Mn excretion and retention increased linearly with higher dietary inclusion ($P < 0.001$). Tissue Mn concentra tions in tibia and liver, as well as heart Mn superoxide dismutase (Mn-SOD) activity, responded quadratically, with estimated maxima at 124.2, 180.6, and 105.8 mg/kg Mn, respectively ($P < 0.05$). These results indicate that current Mn supplementation practices in commercial broiler feeds may exceed requirements when growth performance is the primary response criterion. Improvements associated with phytase are consistent with pre vious findings for other trace minerals. However, Mn-SOD responses suggest that dietary Mn levels required to support antioxidant defense and mitigate oxidative stress may be higher than those needed to optimize con ventional performance metrics.

Key words: broiler; manganese; phytase.

INTRODUCTION

Manganese (Mn) is dietary essential and has important functions in animal metabolism. McHargue (1926) firstly demonstrated that Mn was essential for rats, whereas other authors have shown Mn deficiency in chicks (Wilgus et al., 1936) and pigs (Miller et al., 1940; Greenberg et al., 1943). The main functions of Mn are related to its role as cofactor for several enzymes, including mitochondrial superoxide dismutase (MnSOD), pyruvate carboxylase, and arginase, which are involved in antioxidant defense, energy metabolism, and proteoglycan synthesis (Suttle, 2010; Tufarelli et al., 2017). Chicks store high amounts of Mn in liver, kidneys and bones (Carvalho et al., 2021).

Bone formation is usually referred as a main target of Mn because of its function in the synthesis of proteoglycans and collagen, which are structural for bone and cartilage matrices (Luo et al., 1992; Tuormaa, 1996). Manganese deficiency in broilers is, therefore, primarily associated with skeletal abnormalities due to compromised activity of Mn-dependent enzymes, resulting in lower incorporation of sugars into proteoglycan molecules and deficient cartilage and bone formation (Luo et al., 1992; Lu et al., 2006). This condition has been called perosis and is characterized by joint deformities and tendon displacements (Wilgus et al., 1936). Poor productive performance and impaired antioxidant systems have also been reported during Mn deficiencies (Lu et al., 2006; Carvalho et al., 2021).

Excessive Mn intake causes toxicity, reduced growth, and increase its fecal excretion, which eventually leads to an unnecessary increase in environmental Mn load (Oberleas, 1999; Tufarelli, 2017). Toxicity of Mn, however, occurs with abnormally high Mn feed contents at 1,500 mg/kg feed or higher (Miles et al., 2003; Sunder et al., 2006).

Present commercial recommendations for trace mineral supplementation in broilers vary widely. For instance, the NRC (1994), Rostagno et al. (2024), Cobb (2024), and Aviagen (2022) suggest 60, 70, 100, and 120 mg Mn/kg feed, respectively. Variable requirements are expected in nutritional studies due to experimental factors such as the evaluation criteria used,

feed composition, and differences due to broiler genetics (Sakomura and Rostagno, 2007). However, Mn requirement studies that evaluate metabolic responses as well as growth in the presence of phytase are scarce with broiler chickens. Among the different sources of Mn available for poultry nutrition, Mn sulfate ($MnSO_4 \cdot H_2O$) is widely used in commercial feeds due to its low cost and reasonable bioavailability.

Supplementation with phytase is widely adopted in animal feeds, mainly focusing into the increase in the availability of P and Ca bound to phytate. Recent studies, however, have shown that phytase can also increase the utilization of trace minerals such as Fe (Feijo et al., 2023), Zn (Maria et al., 2024) and Cu (Soster et al., 2023). The wide disparity among current Mn supplementation recommendations, as well as the disregard of the positive effects of phytase on trace mineral availability, underscores the need for a reassessment of trace mineral requirements in phytase supplemented feeds. This is reinforced by the need to reduce production costs and mitigate unnecessary buildup of waste in the environment.

The objective of the present study was to evaluate the Mn requirements of broilers using corn-soy feeds and graded increases of Mn sulfate. Phytase was added in excess to the feeds such that phytate could be extensively and quickly degraded allowing for a maximum trace mineral release. The evaluations were conducted based on productive performance and other responses that are particularly sensitive to Mn.

MATERIAL AND METHODS

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

Bird Husbandry and Dietary Treatments

A total of 640 1-d-old male Cobb × Cobb 500 chicks were randomly placed into 80 wire cages (0.9 × 0.4 m2). Each cage was equipped with one trough feeder and one 3 nipple drinkers

and *ad libitum* access to water and mash feeds. Temperature at placement was 32 °C, which was daily adjusted to maintain bird comfort throughout the study. Lighting was provided 24 h continuously throughout the first week followed by a 16:8 light: dark program to the end of the experiment. Chicks were given a common Mn-deficient feed (analyzed total 14.58 ± 0.74 mg/kg of Mn, Table 1) produced with corn, soybean meal (SBM), soybean protein concentrate (60% CP) and laboratory grade calcium carbonate and phosphoric acid from 1 to 7 d. At d 8 chicks were randomly allocated into a 2 X 5 factorial arrangement of feeds with or without phytase or having five graded increases of supplemental Mn. Each treatment was replicated 8 times, in a total of 10 treatments of 8 birds each. Feeds were formulated with energy and nutrients to optimize live performance as usual in commercial integrations (2,975 kcal/kg AME and 23% CP in the first week (depletion) and 3,000 kcal kg/kg AME 22% CP, 1.10% Ca 0.50% Av. P, digestible P 0,41%, in the experimental phase (Table 1). Feed formulations were done such that the total Mn was reduced as possible while maintaining nutrient allowances as in commercial feeds in the first week. In the experimental treatments from 8 to 28 d, formulation was done such that Mn was only originated from corn and SBM.

A commercial phytase preparation (Ronozyme HiPhorius, 40,000 FYT/g, Novozymes A/S, Bagsvaerd, Denmark) was used at 100 g per metric ton feed to deliver 4,000 FYT/kg (4,680 ± 420 analyzed) in half of the feeds. This was added in the feeds at a higher concentration when compared to commercial recommendations of 1,000 FYT to have the phytate in corn and SBM overwhelmingly and quickly degraded. Phytase was added into the feeds without attributing value for P and Ca in 1 kg mixes diluted with ground SBM. Laboratory-grade monohydrate Mn sulfate ($MnSO4{\cdot}H2O$) was added into the experimental feeds to a final supplementation of 0, 30, 60, 90, and 120 mg Mn/kg. As with the depletion feed, both Ca carbonate and phosphoric acid were laboratory grade and guarantee no to have significant Mn contents. Analyzed phytase in the experimental feeds ranged from the lowest to

the highest supplemental Mn as follows: 4,015, 4,290, 4,170, 4,350 and 4,457 FYT/kg whereas analyzed Mn in the feeds with and without phytase were: 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76, 129.97 ± 1.59 mg/kg, respectively. Celite (Celite Corp., Lompoc, CA) was added at 1% in the feeds as an indigestible marker. Feeds had an average geometric diameter of 1.118 μm ± 1.72. Phytase activity in the feeds was expressed in FYT units, defined as the activity that releases 1 μmol of inorganic phosphate from 5.0 mM sodium phytate/min at pH 5.5 and 37 °C (Engelen et al. (1994). Analyses of Mn were done by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

Growth Performance, Total excreta, Ileal Contents

Live performance was evaluated by body weight gain (BWG) and feed intake and calculation of feed conversion ratio (FCR) corrected for the weight of dead birds at 8, 14, 21, and 28 d. Excreta were collected twice daily on wax paper from 24 to 27 d being immediately mixed and pooled by cage and stored at −20 °C until analysis. Ileal contents were collected from all birds at 28 d after euthanasia by electrical stunning using 45 V for 3 s from a section of intestine between Meckel's diverticulum to approximately 2 cm cranial to the ileocecal junction. Contents were flushed with distilled water into plastic containers, pooled by cage, immediately frozen in liquid nitrogen, and stored in a freezer at −20 °C until lyophilized (Christ Alpha 2-4 LD Freeze Dryer, Newtown, UK). Diet and freeze-dried samples of ileal digesta were ground to pass a 0.5-mm screen in a grinder (Tecnal, TE-631/2, São Paulo, Brazil).

Analyses and calculations

Ileal digesta, excreta, and feed samples were analyzed for gross energy (GE) using a calorimeter calibrated with benzoic acid as a standard (IKA Werke, Parr Instruments, Staufen, Germany). Calculations of ileal digestible energy (IDE) and AME were done afterwards. Crude protein (CP, N × 6.25) was determined by combustion (method 968.06; AOAC International,

2006). Acid insoluble ash, ileum samples, and excreta were determined as described by Vogtmann et al. (1975) and Choct and Annison (1992). Apparent ileal digestibility, total tract retention, IDE, and AME were calculated using the following equations (Kong and Adeola, 2014): digestibility (%) = [1 − (Mi /Mo) × (Eo /Ei)] × 100, IDE and AME (kcal/kg) = GEi − [GE × (Mi /Mo)], where Mi represents the concentration of acid insoluble ash in the diet in grams per kilogram of DM; Mo represents the concentration of acid insoluble ash in the excreta and ileal digesta in grams per kilogram of DM output; Ei represents the concentration of DM, PB, GE, Mn in the diet in milligrams per kilogram of DM; and Eo represents the concentration of DM, PB, GE, Mn in the excreta and ileal digesta in milligrams per kilogram of DM. Retention of Mn was determined (Zhang et al., 2018) as follows: Mn retention (mg/bird) = [feed intake (g/bird) × feed Mn content (mg/kg)] − [fecal output (g/bird) × fecal Mn content (mg/kg)]. The diet and fecal Mn contents were based on their analyzed values on DM. All analyses of Mn in the present research were conducted using Inductively Coupled Plasma Atomic Emission Spectrometry (5800 ICP-OES, Agilent Technologies, Santa Clara, CA, USA) (AOAC Internacional, 2016).

Locomotor Parameters and Tibia Evaluation

At 14 and 28 d, valgus, varus, and rotated tibiae deviations at the tibia metatarsal joints were evaluated in all birds standing in anatomic position and evaluated by a 3 individual panel, classifying them by the presence or the absence of each deviation. Tibiae-metatarsal joints with valgus deviations were those presenting an outward angulation, whereas the varus deviations were inward. Rotated tibiae above 90° were considered abnormal and characterized as a torsional rotation of the shaft of the tibiotarsus of 1 or both legs, causing the metatarsus to point laterally and a spraddle leg conformation. Scores taken were averaged for the sake of statistical analysis as described by Santiago et al. (2020).

Gait score assessments were performed on d 14 and 28 of the experiment, following the methodology described by Garner et al. (2002), which classifies broiler locomotion on a scale from 0 (N walking) to 5 (unable to walk). Three trained evaluators, blinded to treatment groups, independently scored the locomotion of chickens through a linear path of one meter.

The left tibiae of all birds from each cage were collected and the surrounding sampled muscle tissue removed and frozen until analysis. Tibia breaking strength was evaluated using a texture analyzer (TA.XTPlus, Texture Technologies, Hamilton, MA, United States), according to the method proposed by Shim et al (2012). Tibia morphometry was performed with a micrometer (Model IP65, Mitutoyo Corp., Kawasaki, Japan) measuring length, diaphysis, caudal zone, and cranial region, and these values were calculated for statistical analysis (Maria, 2024). The tibiae were then defatted and ashed for Mn content analysis.

Tissue sampling and analysis

Livers from all birds were collected at 28 d and immediately weighed being stored in plastic bags per battery cage at -20 °C. Liver samples were lyophilized (Christ Alpha 2-4 LD Freeze Dryer, Newtown, UK), subsequently ashed, and analyzed for Mn content. A separate livers sample was defatted using ethyl ether to determine the fat content of the sample.

Heart samples were homogenized by sonication in PBS buffer solution (pH 7.4), and then the supernatant was collected after centrifugation at 4,000 rpm for 3 min at 4 °C. Activity of MnSOD was measured in the supernatant using a Chicken Superoxide Dismutase 2, Mitochondrial (SOD2) ELISA Kit (Ref. MBS742418, My Bio Source, San Diego, CA, USA). Immediately, the protein concentration in the supernatant was determined by the Bradford method using BSA as a standard. MnSOD activity was expressed in ng/mg of protein.

Statistical Analysis

Data were tested for homoscedasticity and normality of the variance prior to statistical analyses (Shapiro and Wilk, 1965). Data without normal distribution was analyzed after arc-sin transformation, but the actual means are presented in the tables. A two-way ANOVA was used to test for differences in the effects of the independent variables (phytase and dietary Mn) on the dependent variables measured. Analyses were done using the GLM procedure of SAS (SAS, 2012) with significance accepted as $P \leq 0.05$. Mean separation was done using Tukey multiple-range test when the model effect was significant (Tukey, 1991). Estimations of maximum responses to total dietary Mn were done using quadratic polynomial (QP) regression models. The L model ($Y = \beta1 + \beta2 \times X$) has Y as the dependent variable, X as the dietary level of Mn, β1 as the intercept, and β2 as the linear coefficient. The QP model ($Y = \beta1 + \beta2 \times Mn + \beta3 \times (Mn)^2$) has Y as the dependent variable as a function of dietary level of Mn; β1 as the intercept; β2 as the linear coefficient and β3 as the quadratic coefficient. The maximum response for Mn was defined as $Mn = -\beta2 \div (2 \times \beta3)$.

RESULTS AND DISCUSSION

The formulated feeds were accepted as adequate for the experimental purposes since analyzed phytase and Mn in feeds were very close to the expected from formulations. Phytase concentrations had an average of 4,680 ± 420 FYT/kg whereas Mn content analyzed increased as formulated from 19.64 ± 1.5; 44.08 ± 0.67; 73.80 ± 1.26; 105.08 ± 2.76 and 129.97 ± 1.59 mg/kg.

There were no interactions between phytase and Mn for any of the responses evaluated throughout the present study; therefore, results and discussion in the present text are done based on individual effects. Lack of interaction effects of phytase on the requirements of trace minerals has been previously demonstrated when requirement studies were previously done using similar methodologies with Cu (Soster et al, 2023), Fe (Feijo et al., 2023) and Zn (Maria et al., 2024).

In the present study, phytase supplementation led to a significant improvement in BWG as well as in FCR between 8 and 28 d of age ($P < 0.05$) (Table 2). These positive effects reinforce the importance of phytase in the degradation of phytate, a compound that forms insoluble complexes with Ca, P and microminerals, limiting their bioavailability. However, in the present study these improvements are not expected to be delivered by P and Ca since phytase was added on top of the feeds. Instead, the likely have corresponded to the increases in the observed energy utilization. Similar responses have been observed earlier when phytase was tested in conjunct with trace minerals (Soster et al., 2023, Feijó et al., 2023, and Maria et al., 2024).

Chicks fed increased dietary Mn in the present study did not show significant differences in live performance; however, increased liver and tibiae Mn content was found as dietary Mn was gradually added to the feeds ($P < 0.05$). Quadratic adjustments were detected for tibia and liver Mn content at 28 d ($Y = 2.5712 + 0.04488x - 0.000180708x^2$; $R^2 = 0.1615$; $P < 0.0004$, and $Y = 5.1833 + 0.02013x - 0.000055722x^2$; $R^2 = 0.773$; $P < 0.0001$) (Tables 5

and 7). Maximum responses, respectively for tibiae and liver contents, occurred at 124.18 mg (Table 5) and 180.60 mg Mn/kg of diet (Table 7). Previous research has not shown live performance benefits when Mn was supplemented in commercial type corn–soybean feeds for broilers indicating that they provide sufficient Mn to sustain maximum growth (Wedekind et al., 1992; Lu et al., 2006). The increased Mn content found in bones and livers occurred without parallel improvements in growth responses, therefore, they do not seem to indicate an increase in Mn metabolic demand. This rather seems to be an increase in Mn deposition as intake increases, as it occurs with other trace minerals (Bao et al., 2007). In parallel, liver Mn content, is also expected to be higher under increased dietary intake since the liver is a means for Mn biliary excretion (Bao and Choct, 2009). Therefore, both tibiae and liver are expected to have high Mn content as dietary Mn increases. Phytase, however, did not impact hepatic Mn concentration.

Incidence of leg deformities (Table 3), and other parameters, such as gait scores (Table 4), bone strength, and tibia morphometry (Table 5) did not differ among treatments in the present study. This is an indication that Mn, although essential for enzymes related to bone matrix formation, was not needed in addition to the contents already present in corn and SBM. Similarly, Li et al. (2004) reported that Mn supplementation, at relatively higher levels above requirements, did not affect leg deformities.

Total DM retention and AME_n were higher in birds fed supplemental phytase, confirming the positive effects of the enzyme on nutrient and energy utilization efficiency. Increasing dietary Mn levels did not affect DM or CP digestibility; however, it was increasingly excreted as its intake was augmented (mg/kg Mn): intake = 0.0226 + 0.1279x, $R^2 = 0.9552$, $P < 0.001$; excretion = 0.8223 + 0.0313x, $R^2 = 0.666$, $P < 0.001$; retention = -0.8004 + 0.0966x, $R^2 = 0.9177$, $P < 0.001$. Without phytase, Mn excretion and retention were described by Y = 0.9868 + 0.0283x, $R^2 = 0.627$; $P < 0.0001$ and Y = −0.7103 + 0.0976x, $R^2 = 0.901$; $P < 0.0001$,

271 respectively, whereas with phytase the equations were $Y = 0.7234 + 0.0329x$, $R^2 = 0.680$; $P <$
272 0.0001 and $Y = -1.0888 + 0.0999x$, $R^2 = 0.913$; $P < 0.0001$, respectively (Table 6). The
273 proportion of Mn retained, however, decreased as Mn intake increased, which indicates a
274 reduction in the absorption efficiency as Mn becomes dietary excessive. This pattern has been
275 shown in previous studies indicating that Mn absorption in broilers is tightly controlled, with
276 excess being rapidly excreted through bile and then excreta (Tufarelli, 2005; Li et al., 2008).
277 Phytase added to the feeds led to increased Mn retention in the present study. This was
278 likely to be due to its ability to hydrolyze Mn bound to phytate complexes, releasing it for the
279 absorption. Similar results were reported by Li et al. (2016) and Akter et al. (2017), who
280 observed greater Mn availability and tissue deposition in broilers fed diets supplemented with
281 phytase.
282 The concentration of Mn in the organism has been directly related to the activity of
283 MnSOD, a mitochondrial isoform of SOD that uses Mn as a catalytic cofactor (Surai, 2015).
284 Its activity, therefore, has been utilized as a dependent variable in the present study. It is a
285 specific and sensitive biomarker for circulating Mn without being directly influenced by other
286 minerals within the same metabolic pathway and particularly pronounced in tissues with a high
287 mitochondrial density, such as the heart (Li et al., 2008). Heart MnSOD activity evaluated in
288 the present study showed a significant response to dietary Mn. A linear increase in MnSOD
289 activity occurred as a function of dietary Mn ($MnSOD = 16.6054 + 0.08341x$; $R^2 = 0.108$; $P =$
290 0.0125) (Table 7). Overall, the results show that cardiac MnSOD is sensitive to dietary Mn
291 levels and that its activity has not reached a plateau response. This behavior is consistent with
292 the physiological mechanism of the enzyme, which depends directly on the incorporation of
293 Mn as cofactor. Therefore, in contrast to the lack of response for growth and locomotor
294 responses, increased dietary Mn seem to be needed to support heart's mitochondrial antioxidant

protection. The commercial significance of this impact, however, could not be detected in the present study.

Results of the present study indicate that Mn supplementation of corn-SBMs does not improve productive performance or skeletal health in broiler chickens. Considering the low retention efficiency at high intake levels, supplementation strategies should aim to balance the real need of chickens with excesses that will not pay back the investment in supplementation but also will eventually build up in the environment. However, investigations to support a more definitive recommendation for increased dietary Mn requirements need to take in consideration the MnSOD responses obtained with heart tissue and further evaluations of Mn-dependent enzymatic systems across multiple organs.

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DISCLOSURES

All authors declare that they have no conflict of interest or personal relationships that could have appeared to influence the work reported in this article.

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Table 1. Ingredient and nutrient composition of feeds having supplemental phytase and graded increases of Mn.

Ingredient, kg/ton	Mn Deficient (1 to 7 d)	Experimental (8 to 28 d)
Corn 7.8%	65.29	51.97
Soybean meal 45%	5.38	37.68
Soybean protein concentrate 66%	23.20	-
Soybean oil	0.5	4.15
Ca carbonate[1]	2.70	2.15
Phosphoric acid 85%	1.50	1.34
Common salt	0.40	0.54
DL-Methionine, 99%	0.28	0.34
L-Lysine, 62,4%	0.15	0.24
L-Threonine, 98.5%	0.07	0.11
Choline chloride	0.28	0.23
Vitamin and mineral mix[2]	0.25	0.25
Celite[3]	-	1.00
Total	100.00	100.00
Calculated nutrient composition, % unless noted		
AME_n, kcal/kg	2,975	3,000
CP	23.0 (23.3 ± 0.78)	21.7 (22.02 ± 0.43)
Ca	1.10 (1.09 ± 0.02)	0.90 (1.02 ± 0.03)
Available P	0.50	0.45
Digestible P	0.44	0.41
Total P	0.63 (0.64 ± 0.03)	0.67 (0.68 ± 0.02)
Total phytate[4]	0.23	0.22
Na	0.18	0.23
Choline, mg/kg	2,500	2,500
Dig. Lys	1.24	1.22
Dig. TSAA	0.93	0.92
Dig. Thr	0.84	0.81
Dig. Trp	0.25	0.24
Dig. Arg	1.43	1.34
Dig. Val	0.94	0.91
Mn, mg/kg[5]	14.46 (14.58 ± 0.74)	17.48 (19.64 ± 1.5)
Phytase, FYT/kg[6]	-	4,000 (4,680 ± 420)

[1]Calcium carbonate and phosphoric acid were laboratory-grade and guaranteed to not present significant amounts of other minerals.

[2] Composition per kilogram of feed: vitamin A, 12,000 IU; vitamin D 3 , 3,000 IU; vitamin C, 50 mg; vitamin E, 100 IU; vitamin K 3 , 6mg; vitamin B12, 35 µg; thiamin, 3 mg; riboflavin, 15 mg; vitamin B6, 6 mg; niacin, 40 mg; pantothenic acid, 25 mg; folic acid, 4mg; biotin, 0.3 mg; Zn, 100 mg, Cu, 15 mg, Fe, 40 mg, Se, 0.45 mg and I, 2 mg.

3Indigestible marker (Celite, Celite Corp., Lompoc, CA).

[4]Phytate values analyzed with NIRS, considering the levels of 0.18% for corn, 0.38% for soybean meal and 0.45% for soybean protein concentrate, adjusted proportionally to the composition of the diets.

[5] Formulated Mn were 17.48, 47.48, 77.48, 107.48 and 137.48 mg/kg; analyzed Mn in the diets with and without phytase were 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76, 129.97 ± 1.59 mg/kg.

[6] Ronozyme HiPhorius 40,000 FYT/g, Novozymes A/S, Bagsvaerd, Denmark.

422 Table 2. Growth performance of broilers fed diets with supplemental phytase and graded increases of Mn from 8 to 28d.[1,2]

Item	BWG, g				FCR				FI, g			
	8 – 14	15 – 21	22 – 28	8 - 28	8 – 14	15 – 21	22 – 28	8 - 28	8 – 14	15 – 21	22 – 28	8 - 28
Phytase, FYT/kg[3]												
None	304[a]	551[a]	693[a]	1,548[a]	1.286[a]	1.300[a]	1.390[a]	1.335[a]	391	717	970	2,083
4,000	311[b]	566[b]	713[b]	1,600[b]	1.265[b]	1.283[b]	1.354[b]	1.316[b]	393	727	962	2,097
Mn, mg/kg[4]												
17.5	312	548	701	1,561	1.263	1.290	1.373	1.326	393	712	964	2,081
47.5	304	554	697	1,557	1.292	1.292	1.392	1.329	392	718	965	2,105
77.5	305	566	701	1,579	1.287	1.302	1.379	1.338	392	737	969	2,112
107.5	308	557	701	1,566	1.263	1.289	1.348	1.312	389	715	964	2,065
137.5	310	569	714	1,594	1.270	1.283	1.376	1.323	394	727	978	2,086
SEM	1.508	2.666	4.088	5.840	0.005	0.003	0.006	0.003	1.670	3.271	7.560	9.691
Probability												
Phytase	0.024	0.004	0.013	<0.0001	0.045	0.016	0.006	0.003	0.588	0.080	0.425	0.434
Mn	0.332	0.061	0.685	0.093	0.242	0.565	0.243	0.155	0.879	0.058	0.671	0.286
Phytase*Mn	0.066	0.203	0.659	0.960	0.735	0.055	0.078	0.236	0.302	0.105	0.143	0.085

423 [a,b] Means with different letters in the same column indicate significant differences ($P \leq 0.05$).
424 [1]BWG, body weight gain; FCR, feed conversion ratio corrected for the weight of dead birds; FI, feed intake.
425 [2]Chick body weight at 1 and 8 d respectively: 51.0 g ± 0.99; 207.6 g ± 4.67.
426 [3]Ronozyme HiPhorius 40,000 FYT/g, Novozymes A/S, Bagsvaerd, Denmark.
427 [4]Formulated Mn: 17.48, 47.48, 77.48, 107.48 and 137.48 mg/kg; analyzed Mn in feeds with and without phytase: 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76,
428 129.97 ± 1.59 mg/kg.

429 Table 3. Leg deviations of broilers fed diets with phytase and graded increases of Mn from 8 to 28 d[1,2].

Item	Leg deviations 14 d, %				Leg deviations 28 d, %			
	Unaffected	Valgus	Varus	Rotated tibia	Unaffected	Valgus	Varus	Rotated tibia
Phytase, FYT/kg[3]								
None	89.8	5.4	4.6	0.2	82.6	10.6	7.1	0.4
4,000	86.8	5.4	7.0	0.6	81.7	12.1	7.1	1.0
Mn, mg/kg[4]								
17.5	85.4	7.8	6.8	0	81.2	8.3	8.9	1.6
47.5	87.5	5.7	6.8	0	79.5	13.5	11.5	0.5
77.5	92.7	3.1	2.6	1.6	80.7	12.0	7.3	0
107.5	89.0	4.7	5.7	0.5	89.0	7.8	3.1	0
137.5	86.9	5.7	7.2	0	79.5	15.1	4.7	1.6
SEM	10.40	1.62	1.25	0.13	10.97	1.08	1.23	0.42
Probability								
Phytase	0.218	0.985	0.157	0.529	0.781	0.445	0.986	0.296
Mn	0.356	0.543	0.448	0.498	0.071	0.070	0.075	0.238
Phytase*Mn	0.792	0.534	0.354	0.340	0.083	0.134	0.238	0.265

430 [1] Unaffected had no visible deviation in leg conformation, valgus had an outward angulation, varus had an inward angulation, and rotated tibia had abnormal internal or external
431 rotation of the tibia relative to the longitudinal axis of the leg.
432 [2] Data were arcsine transformed prior to analysis, but the values in the table are the real means.
433 [3] Ronozyme HiPhorius 40,000 FYT/g, Novozymes A/S, Bagsvaerd, Denmark.
434 [4] Formulated Mn: 17.48, 47.48, 77.48, 107.48 and 137.48 mg/kg; analyzed Mn in feeds with and without phytase: 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76, 129.97
435 ± 1.59 mg/kg.

436 Table 4. Gait scores of broilers fed diets with phytase and graded increases of Mn from 8 to 28d[1,2].

Item	Gait score 14 d, %						Gait score 28 d, %					
	0	1	2	3	4	5	0	1	2	3	4	5
Phytase, FTY/kg[3]												
None	85.0	13.3	1.7	1.7	-	-	61.9	30.0	7.1	1.1	-	-
4,000	82.7	15.0	2.1	2.3	-	-	57.1	32.1	9.4	1,5	-	-
Mn, mg/kg[4]												
17.5	80.7	18.2	1.0	1.0	-	-	60.9	30.2	7.8	1.1	-	-
47.5	84.9	13.0	2.1	2.1	-	-	57.8	28.6	11.5	2.1	-	-
77.5	85.9	11.5	2.1	2.6	-	-	58.8	32.3	8.3	0.6	-	-
107.5	86.4	10.9	2.6	2.6	-	-	60.9	33.8	5.2	0.5	-	-
137.5	81.2	17.2	1.6	1.6	-	-	58.8	30.2	8.3	2.7	-	-
SEM	1.198	1.079	0.980	0.587	-	-	1.552	1.079	0.980	0.587	-	-
Probability												
Phytase	0.371	0.460	0.633	0.525	-	-	0.171	0.464	0.232	0.232	-	-
Mn	0.477	0.144	0.827	0.819	-	-	0.971	0.798	0.368	0.368	-	-
Phytase*Mn	0.742	0.853	0.827	0.721	-	-	0.774	0.420	0.785	0.785	-	-

437 [1] Locomotion scores ranged from 0 to 5 indicating: 0-skillful and agile movement, 1- a slight and vague abnormality, 2- a defined and identifiable abnormality, 3-an obvious
438 gait issue affecting mobility, 4- a severe abnormality with limited movement, and 5- birds unable to move (Garner et al., 2002).
439 [2] Data were arcsine transformed prior to analysis, but the values in the table are the real means.
440 [3] Ronozyme HiPhorius 40,000 FYT/g, Novozymes A/S, Bagsvaerd, Denmark.
441 [4] Formulated Mn: 17.48, 47.48, 77.48, 107.48 and 137.48 mg/kg; analyzed Mn in feeds with and without phytase: 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76,
442 129.97 ± 1.59 mg/kg.

443 Table 5. Proximal analyses, Mn content and tibia morphometry of broiler chickens at 28 d of age.

Item	Weight g	DM %	Ash %	Mn, mg/kg[2]	Breaking strength kgf	Length	Proximal Epiphysis	Diaphysis	Distal Epiphysis
						cm			
Phytase, FYT/kg[1]									
None	8.8	45.1	21.7	4.8	50.8	7.74	2.04	0.83	1.67
4,000	8.8	45.0	22.0	4.5	50.5	7.67	2.09	0.85	1.67
Mn, mg/kg[3]									
17.5	8.6	44.8	21.6	3.3[b]	50.5	7.68	2.12	0.86	1.68
47.5	8.7	45.4	22.0	4.3[ab]	51.5	7.74	2.05	0.82	1.64
77.5	8.8	45.1	21.8	4.9[ab]	52.8	7.69	1.97	0.86	1.69
107.5	8.9	45.1	22.0	5.1[a]	48	7.75	2.11	0.84	1.68
137.5	8.9	45.0	21.8	5.5[a]	50.2	7.67	2.08	0.84	1.66
SEM	0.71	0.13	0.97	0.28	0.77	0.259	0.253	0.061	0.090
Probability									
Phytase	0.522	0.737	0.150	0.452	0.886	0.183	0.310	0.141	0.854
Mn	0.610	0.599	0.655	0.010	0.401	0.827	0.366	0.328	0.306
Phytase*Mn	0.162	0.489	0.168	0.669	0.935	0.278	0.371	0.462	0.313

444 [1] Ronozyme HiPhorius 40,000 FYT/g, Novozymes A/S, Bagsvaerd, Denmark.
445 [2]Tibia Mn content at 28d: $Y = 2.5712 + 0.04488x - 0.000180708x^2$, $R^2 = 0.1615$, $P = 0.0004$; X = dietary Mn content.
446 [3] Formulated Mn: 17.48, 47.48, 77.48, 107.48 and 137.48 mg/kg; analyzed Mn in feeds with and without phytase: 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76,
447 129.97 ± 1.59 mg/kg.

448 Table 6. Apparent ileal digestibility and total tract retention of broilers as affected by increased dietary Mn with or without phytase, DM basis[1,2].

Item	Apparent ileal digestibility					Total tract retention		Intake	Excretion	Retention	
	DM, %	IDEC, %	IDE, kcal/kg	CP, %	Mn, %	DM, %	AME, kcal/kg	Mn, mg/bird		Mn, mg/bird	Mn, %
Phytase, FYT/kg[3]											
None	75.4	75.7	3,431	83.8	17.9	76.7	3,600	9.7	4.3[a]	5.3[a]	55.5[a]
4,000	75.6	75.9	3,485	84.1	18.2	79.0	3,642	9.5	3.6[b]	6.0[b]	63.7[b]
Mn, mg/kg[4]											
17.5	76.2	76.6	3,450	83.9	16.3	77.9	3,610	2.5[e]	1.0[e]	1.5[e]	60.9
47.5	76.3	75.1	3,461	84.9	17.5	77.3	3,620	5.6[d]	2.2[d]	3.4[d]	60.8
77.5	74.2	74.3	3,383	83.1	17.7	76.0	3,608	9.6[c]	3.8[c]	5.8[c]	60.1
107.5	74.5	76.5	3,475	83.3	19.4	77.6	3,630	13.3[b]	5.5[b]	7.7[b]	58.5
137.5	76.9	76.8	3,534	85.4	17.8	78.1	3,654	16.9[a]	7.1[a]	9.8[a]	57.9
SEM	0.366	0.713	18.580	0.288	0.327	0.530	10.831	0.594	0.348	0.263	0.563
Probability											
Phytase	0.457	0.571	0.628	0.978	0.838	0.034	0.0252	0.6476	<0.0001	0.0014	<0.0001
Mn	0.174	0.785	0.304	0.146	0.958	0.163	0.4738	<0.0001	<0.0001	<0.0001	0.1766
Phytase*Mn	0.433	0.673	0.074	0.350	0.742	0.510	0.4452	0.4200	0.4878	0.1650	0.7835

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

450 [1]IDCE = Ileal digestible energy coefficient; IDE = Ileal digestible energy.

451 [2] Intake, excretion and retention, of Mn (mg/kg), respectively: $Y = 0.0226 + 0.1279x$, $R^2 = 0.9552$, $P < 0.001$; $Y = 0.8223 + 0.0313x$, $R^2 = 0.666$, $P < 0.001$; $Y = -0.8004 +$
452 $0.0966x$, $R^2 = 0.9177$, $P < 0.001$; X = dietary Mn content. Without phytase excretion and retention, respectively: $Y = 0{,}9868 + 0{,}0283x$, $R^2 = 0{,}627$, $P < 0.0001$; $Y = -0{,}7102 +$
453 $0{,}0975x$, $R^2 = 0{,}901$, $P < 0{,}0001$. With phytase excretion and retention, respectively: $Y = 0{,}7234 + 0{,}0329x$, $R^2 = 0{,}680$, $P < 0{,}0001$; $Y = -1{,}0887 + 0{,}0999x$, $R^2 = 0{,}913$, $P <$
454 0,0001.

455 [3] Ronozyme HiPhorius 40,000 FYT/g, Novozymes A/S, Bagsvaerd, Denmark; analyzed phytase in the Mn supplemented diets were (from the lowest to the highest Mn content
456 diets) 4,680 ± 420 FYT/kg.

457 [4] Formulated Mn: 17.48, 47.48, 77.48, 107.48 and 137.48 mg/kg; analyzed Mn in feeds with and without phytase: 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76, 129.97
458 ± 1.59 mg/kg.

459 Table 7. Concentration of Mn in liver and MnSOD in heart tissue at 28 d[1].

Item	Liver			Heart	
	Weight, g	Mn, mg/kg	Fat, %	Weight, g	MnSOD, ng/mg protein
Phytase, FYT/kg[2]					
None	39.2	7.1	9.1	10.4	23.1
4,000	38.8	7.0	8.6	10.3	23.1
Mn, mg/kg[3]					
17.5	40.4	5.6^{c}	9.7	10.7	13.5^{b}
47.5	39.0	6.2^{bc}	8.0	10.2	24.9^{ab}
77.5	39.1	6.9^{b}	9.6	10.4	23.1^{ab}
107.5	38.5	7.9^{a}	8.4	10.3	24.8^{ab}
137.5	38.2	8.7^{a}	8.2	10.2	26.7^{a}
SEM	0.343	0.206	0.405	0.121	0.582
Probability					
Phytase	0.599	0.723	0.357	0.621	0.844
Mn	0.342	<0.0001	0.473	0.570	0.036
Phytase*Mn	0.496	0.947	0.169	0.766	0.711

460 [1]Liver Mn content at 28 d: $Y = 5.18328 + 0.02013x + 0.000055722x^2$, $R^2 = 0.773$, $P < 0.0001$. X = dietary
461 Mn content; MnSOD in heart content at 28 d: $Y = 16.6054 + 0.08341$ x, $R^2 = 0.108$, $P = 0.0125$; X =
462 dietary Mn content.
463 [2]Ronozyme HiPhorius 40,000 FYT/g, Novozymes A/S, Bagsvaerd, Denmark.
464 [3] Formulated Mn: 17.48, 47.48, 77.48, 107.48 and 137.48 mg/kg; analyzed Mn in feeds with and without
465 phytase: 19.64 ± 1.5, 44.08 ± 0.67, 73.80 ± 1.26, 105.08 ± 2.76, 129.97 ± 1.59 mg/kg.

CAPÍTULO III

CONSIDERAÇÕES FINAIS

A suplementação adequada de manganês é essencial para garantir o desenvolvimento saudável de frangos de corte, especialmente devido ao seu papel fundamental na formação da matriz óssea, na integridade locomotora e na manutenção da capacidade antioxidante celular. Como cofator da Mn-SOD, o manganês contribui diretamente para o controle do estresse oxidativo, o que é particularmente relevante em sistemas de produção intensivos, nos quais as aves são expostas a maior demanda metabólica.

As anormalidades locomotoras observadas na deficiência de manganês reforçam a importância de níveis mínimos adequados do mineral na dieta, uma vez que o comprometimento da mineralização óssea e da síntese de proteoglicanos pode interferir significativamente na saúde, desempenho e bem-estar das aves.

A suplementação de fitase surge como uma ferramenta nutricional valiosa, aumentando a biodisponibilidade de minerais complexados ao fitato, como o manganês, e permitindo que parte da exigência nutricional seja suprida pelo próprio ingrediente vegetal. Contudo, mesmo com o uso de fitase, a suplementação de Mn continua necessária para atingir níveis ótimos de desempenho e de saúde óssea.

Em conjunto, os resultados evidenciam que o manejo nutricional do manganês deve considerar tanto sua função antioxidante quanto sua importância estrutural, além da interação com enzimas como a fitase. A compreensão desses fatores contribui para formulações mais eficientes, seguras e capazes de promover maior desempenho, saúde locomotora e bem-estar em frangos de corte.

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APÊNDICES

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

Journal Poultry Science Guide to Authors[1]

Aims And Scope

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For assistance with Editorial Manager manuscripts, manuscript submission, and manuscript status contact David Busboom at david.busboom@poultryscience.org.

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Article Structure

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

Age, sex, breed, and strain or genetic stock of animals used in the experiments shall be specified. Animal care guidelines should be referenced if appropriate.

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When describing the composition of diets and vitamin premixes, the concentration of vitamins A and E should be expressed as IU/kg on the basis of the following equivalents:

Vitamin A

1 IU = 0.3 μg of all-trans retinol 1 IU = 0.344 μg of retinyl acetate

1 IU = 0.552 μg of retinyl palmitate

1 IU = 0.60 μg of ß-carotene

Vitamin E

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

1 IU = 0.67 mg of d-α-tocopherol

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

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

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

When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately.

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

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

• A parameter [mean (μ), variance (ó2)], 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 x 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).

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• 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 con- sequences 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.

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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.

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.

References

Citations in text

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

References section

To be listed in the References section, papers must be published or accepted for publication. Manuscripts submitted for publication can be cited as "personal communication" or "unpublished data" in the text.

In the References section, references shall first be listed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the authors' names. As with text citations, two or more publications by the same author or set of authors in the same year shall be differentiated by adding lowercase letters after the date. The dates for papers with the same first author that would be abbreviated in the text as et al., even though the second and subsequent authors differ, shall also be differentiated by letters. All authors' names must appear in the Reference section. Journals shall be abbreviated according to the conventional ISO abbreviations given in journals database of the National Library of Medicine. One-word titles must be spelled out. Inclusive page numbers must be provided. Sample references are given below. Consult recent issues of *Poultry Science* for examples not included below.

N.B. - The online version of Poultry Science uses a reference format that differs from that prescribed by the journal. The Guide for Authors is the sole source for the reference format. Any papers that do not follow this format risk rejection.

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 $P \leq 0.01$ 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

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

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

GLM general linear model

h hour

HEPES N-2-hydroxyethyl piperazine-N'-ethane-sulfonic acid

HPLC high-performance (high-pressure) liquid chromatography

i.m. intramuscular

i.p. intraperitoneal

i.v. intravenous

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

ppm parts per million

QTL quantitative trait loci

r correlation coefficient

r2 coefficient of determination, simple

R2 coefficient of determination, multiple

RH relative humidity

RIA radioimmunoassay

RNA ribonucleic acid

rpm revolutions per minute

s second

s.c. subcutaneous

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.

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.

Capitalization

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

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).

Nucleotide sequences

Nucleotide sequence data must relate to poultry or poultry pathogens and must complement biological data published in the same or a companion paper. If sequences are excessively long, it is suggested that the most relevant sections of the data be published in *Poultry Science* and the remaining sequences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submission of sequence data to one of the databases. The following statement should appear as a footnote to the title on the title page of the manuscript. "The nucleotide sequence data reported in this paper have been submitted to Embank Submission (Mail Stop K710, Los Alamos National Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN." Publication of the description of molecular clones is assumed by the editors to place 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 x 27.9 cm in standard (portrait) orientation. Abbreviations should follow *Poultry Science* guidelines.

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.

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 (=, -, +, x, >, or <, etc.) when these signs occur between two items.

• Items in a series should be separated by commas (e.g., a, b, and c).

• Restrict the use of "while" and "since" to meanings related to time.

• Appropriate substitutes include "and," "but," or "whereas" for "while" and "because" or "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.

•Registered (®) and trademark (©) symbols should not be used, unless as part of an article title in the References section. Trademarked product names should be capitalized.

Figures/Illustrations

General points

• Submit each illustration as a separate file.

• Make sure you use uniform lettering and sizing of your original artwork.

• Embed the used fonts if the application provides that option.

• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.

• Number the illustrations according to their sequence in the text.

• Use a logical naming convention for your artwork files.

• Provide captions to illustrations separately.

• Size the illustrations close to the desired dimensions of the published version.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.

Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings, embed all used fonts.

TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.

TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.

TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;

• Supply files that are too low in resolution;

• Submit graphics that are disproportionately large for the content.

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Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Supplementary Data

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

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

Proofs

Corresponding authors will receive an e-mail with a link to their proof. Please annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors.

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Articles In Press

For *Poultry Science*, manuscripts arrive at Elsevier and go through the production process until the final versions are ready to publish. These are then published on the journal's articles in press page. They will remain on the page up until the issue that they are assigned to is published.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication.

Reporting sex- and gender-based analyses

Reporting guidance

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/sponsor requirements and best practices within a field. Authors should address the sex and/or gender dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the Sex and Gender Equity in Research (SAGER) guidelines and the SAGER guidelines checklist. These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

Definitions

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous--thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER

guidelines, the resources on this page offer further insight around sex and gender in research studies.

Apêndice 2: Documento de aprovação CEUA.

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 40248
Título: EXIGENCIA DE MICROMINERAIS PARA FRANGOS DE CORTE

Vigência: 01/07/2021 à 01/07/2023

Pesquisadores:

Equipe UFRGS:

SERGIO LUIZ VIEIRA - coordenador desde 01/07/2021

Comissão De Ética No Uso De Animais aprovou o mesmo em seus aspectos éticos e metodológicos, para a utilização de 2240 pintos machos (Cobb × Cobb 500), de um dia de idade, oriundos da Granja Comercial Vibra Agroindustrial SA, Montenegro/RS. CNPJ: 93586303/0020-81, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Quinta-Feira, 29 de Abril de 2021

ALEXANDRE TAVARES DUARTE DE OLIVEIRA
Coordenador da comissão de ética

VITA

Walter Edmundo Altevogt, filho de Walter Altevogt e Helena Maria Altevogt, nascido em 5 de maio de 1999, em Encantado – RS. Completou o ensino fundamental e o ensino médio no Colégio Evangélico Alberto Torres – Região Alta, Roca Sales - RS, concluindo os estudos em dezembro de 2017. Em 2018, ingressou no curso de Zootecnia na Universidade Federal do Rio Grande do Sul. No último semestre da faculdade foi estagiário na empresa MercoAves LTDA, localizada em Bom Princípio- RS. Formou-se Zootecnista em abril de 2024. No primeiro semestre de 2024 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 de eventos científicos na área de avicultura. Foi submetido à banca de defesa de Dissertação em março de 2026.