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EXIGÊNCIA DE COBRE EM DIETAS COM OU SEM FITASE PARA FRANGOS DE CORTE

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EXIGÊNCIA DE COBRE COM OU SEM FITASE EM DIETAS PARA FRANGOS DE CORTE[1]

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Orientador: Sergio Luiz Vieira

Resumo – Objetivou-se avaliar os efeitos da suplementação de níveis crescentes de cobre (Cu) na forma de sulfato de Cu em rações com e sem suplementação de fitase para frangos de corte de 8 a 28 d sobre o desempenho zootécnico, variáveis sanguíneas e problemas locomotores. O experimento consistiu em um arranjo fatorial 2 x 5 com 10 tratamentos, 8 repetições de 7 pintos machos Cobb 500 cada, distribuidos em delineamento inteiramente casualizado. A ração basal (8 ppm Cu analisado) foi suplementada com 0; 3; 6; 9 e 12 ppm de Cu, com ou sem suplementação de fitase (2,500 FYT/Kg). Ganho de peso, conversão alimentar e consumo de ração foram avaliado aos 8, 14, 21 e 28 d. Aos 28 dias, realizou-se avaliação de varus, valgus e rotação de tíbia e coletou-se sangue para avaliar hematócrito (Ht) e hemoglobina (Hb). Aos 28 d, todos os animais foram abatidos para coleta de rim, fígado, tíbia, músculo *pectoralis major*, tendão gastrocnêmio e conteúdo ileal. Amostras de ração e digesta ileal foram analisadas para determinação da energia digestível ileal (EDI). Dados foram submetidos ao proc MIXED do SAS e médias, quando significativas, foram comparadas pelo teste Tukey ($P < 0,05$). Tratamentos foram distribuídos em arranjo fatorial 2 x 5. As respostas dos frangos de corte foram ajustadas aos modelos polinomial quadrático (QP) e linear (L). Nenhum efeito dos níveis crescente de Cu foi observado sobre o desempenho quando as rações foram formuladas com fitase ($P > 0,05$). No entanto, níveis crescentes de Cu sem suplementação de fitase afetaram ($P < 0,05$) a conversão alimentar de 8 a 14 dias e de 8 a 28 dias, sendo as menores conversões obtidas com 14,50 e 11,81 ppm, respectivamente. A suplementação com fitase aumentou o ganho de peso e consumo de ração e diminuiu a conversão alimentar durante todo o período experimental ($P < 0,05$) quando comparada com as dietas sem fitase, exceto para conversão alimentar de 15 a 21 d. Não houve diferença entre os tratamentos para Ht e Hb ($P > 0,05$) e para problemas locomotores ($P > 0,05$). Além disso, não observou-se diferença histológica no fígado entre os diferentes tratamentos ($P> 0,05$). Os tratamentos com inclusão de fitase apresentaram maior EDI ($P <0,05$) que os tratamentos não suplementados. Os dados do presente trabalho sugerem que a quantidade de Cu suplementada hoje em rações para frangos de corte pode estar acima da necessidade real para atingir o desempenho zootécnico máximo. Porém, a suplementação com fitase é essencial para atingir a taxa máxima de desempenho.

Palavras chave: desempenho**,** cobre, micromineral, exigência.

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[1]Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil, Março, 2021.

CUPPER REQUIREMENT WITH OR WITHOUT PHYTASE IN BROILER DIETS[1]

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Abstract – The objective of the present study was to evaluate the effects of supplementation of increasing levels of Cu with and without phytase supplementation in broiler diets on growth performance, blood parameters, and locomotor disorders. The experiment consisted of a 2 x 5 factorial arrangement with 10 treatments, 8 replications of 7 male Cobb 500 chicks, distributed in a completely randomized design. The basal diet (8 ppm Cu analyzed) was supplemented with 0; 3; 6; 9 and 12 ppm Cu, with or without phytase supplementation (2,500 FYT / Kg). Weight gain, feed conversion and feed intake were evaluated at 8, 14, 21 and 28 d. At 28 days, varus, valgus and tibia rotation were evaluated and blood was collected for hematocrit (Ht) and hemoglobin (Hb) evaluation. At 28 d, all animals were slaughtered to collect kidney, liver, tibia, *pectoralis major* muscle, gastrocnemius tendon and ileal content. Diet samples and ileal digesta were analyzed to determine the ileal digestible energy (IDE). Data were analysed using the SAS MIXED proc and averages, when relevant, were compared using the Tukey test (P <0.05). Treatments were distributed in a 2 x 5 factorial arrangement. The broiler responses were adjusted to the quadratic (QP) and linear (L) polynomial models. No effect of increasing Cu levels was observed on performance when the diets were formulated with phytase (P> 0.05). However, increasing levels of Cu without phytase supplementation affected (P <0.05) feed conversion from 8 to 14 days and from 8 to 28 days, with the lowest conversions obtained at 14.50 and 11.81 ppm, respectively. Phytase supplementation increased weight gain and feed intake and decreased feed conversion throughout the experimental period (P <0.05), when compared to diets without phytase, except for feed conversion from 15 to 21 d. There was no difference between treatments for Ht and Hb (P> 0.05) and for locomotor problems (P> 0.05). In addition, there was no histological difference in the liver between the different treatments (P> 0.05). Treatments with phytase inclusion showed a higher IDE (P <0.05) than non-supplemented treatments. data from the present study suggest that the amount of Cu supplemented today in broiler rations may be above the real need to achieve maximum zootechnical performance. However, supplementation with phytase is essential to achieve the maximum growth rate.

Keywords: growth performance, cupper, micromineral, requirement.

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SUMÁRIO

LISTA DE TABELAS

CAPÍTULO II

APÊNDICE

RELAÇÃO DE ABREVIATURAS

BWG	Body weight gain
Cp	Ceruloplasmina
FCR	Feed convertion ratio
FI	Feed intake
Ht	Hematócrito
Hb	Hemoglobina
MDA	Malondialdeído

CAPÍTULO I

INTRODUÇÃO

A suplementação de microminerais é essencial para a produção de frangos de corte, a fim de permitir o adequado crescimento dos animais sem sinais de deficiência. A suplementação de Cu é necessária para frangos de corte, uma vez que a quantidade deste micromineral nos ingredientes usuais da ração, majoritariamente milho e farelo de soja, pode não ser suficiente para atender à exigência necessária para otimizar o crescimento, especialmente devido à variabilidade que existe nos alimentos (NRC, 1994).

O cobre (Cu) é um micromineral essencial localizado no subgrupo B do grupo 1 da tabela periódica, apresenta número atômico 29 e peso molecular de 63,54. Apresenta duas formas: cuproso ou cúprico (Cu^{+} e Cu^{+2}). A fonte tradicional de Cu suplementado na dieta de frangos de corte é o sulfato pentahidratado ($CuSO_4 5H_2O$) devido ao baixo custo e à grande disponibilidade comercial (Pang & Applegate, 2006). A suplementação atual de Cu em dietas para frangos de corte tem sugestões variáveis: 5 a 8 ppm (NRC, 1994), 3 a 10 ppm (FEDNA, 2008), 15 ppm (COBB, 2008) e 25 ppm (EFSA, 2012).

O Cu tem várias funções no metabolismo, a maioria delas relacionadas ao funcionamento de enzimas (Abdelrahman, et al., 2010; Richards et al., 2010). Destacam-se as funções deste micromineral como cofator enzimático da superoxidase dismutase, da citocromo oxidase, da lisil oxidase, bem como da ceruloplasmina (Lim & Paik, 2006). Seu papel na atividade enzimática deve-se principalmente por estar relacionado à oxirredução e por ser um intermediário na transferência de elétrons (Silva et al., 2013).

Entre suas várias funções, o Cu participa da formação óssea, do transporte de Fe e, por consequência, da formação da hemoglobina e de outras proteínas do sangue, como a eritrocupreína, ou CuZnSOD, encontrada nos eritrócitos, a qual está envolvida no metabolismo do oxigênio (Leeson, 2009). Assim, a deficiência de Cu resulta em anemia induzida por baixa concentração de hemoglobina no sangue (Baumgartner et al., 1978). Como cofator da lisil oxidase, o Cu favorece a formação de colágeno e de elastina, e sua deficiência leva à formação inadequada de cartilagem em vários tecidos (Medeiros, 2016), podendo ocasionar problemas locomotores como as deformidades em valgus e varus (Leeson, 2009). Nesse sentido, Muszy and Tomaszewska (2017) observaram efeito positivo da suplementação de Cu (4 mg kg) no volume e na espessura trabecular da tíbia de frangos de corte quando comparados àqueles

alimentados com dieta não suplementada.

O ácido fítico pode ser considerado um fator antinutricional, pois tem sido relatado que quelata minerais essenciais, como: Cu, Zn, Mn, Fe e Ca; formando um composto estável denominado fitato (Cheryan & Rackis, 1980). O fitato não está disponível para digestão / absorção pelo trato gastrointestinal das aves (Humer et al., 2015). Nesse contexto, Aoyagi and Baker (1993) afirmam que baixos níveis de Cu presentes no milho e no farelo de soja estão disponíveis para serem absorvidos pelos enterócitos devido a maior ligação do Cu com o ácido fítico.

A biodisponibilidade do Cu no milho e no farelo de soja é em torno de 80% (AW-YONG et al., 1982) e 40%, respectivamente (Aoyagi & Baker, 1993b). A ordem decrescente de estabilidade do complexo fitato de metal é: Zn + ²> Cu + ²> Co + ²> Mn + ²> Ca + ². Porém, segundo (Wise and Gilburt, 1982), o Cu é o cátion mais facilmente dessorvido pela fração solúvel do conteúdo intestinal, na seguinte ordem: Cu + ²> Zn + ²> Ni + ² > Co + ²> Mn + ²> Fe + ²> Ca + ². Em concordância, Vohra et al. (1965) e Obeleas (1973) afirmaram que a ordem decrescente de complexação do metal é Cu + 2 >> Cd + 2> Zn + 2> Pb + 2 em pH 7,4.

O ácido fítico (1,2,3,4,5,6 mio-inositol hexakis di-hidrogenofosfato) é a principal forma de armazenamento de fósforo em material vegetal e é amplamente distribuído em cereais, leguminosas e em sementes oleaginosas em concentrações em torno de 7 a 8 g/kg em milho a 50 a 60 g/kg em subprodutos de cereais (Eeckhout & De Paepe, 1994). O envolvimento do ácido fítico na nutrição está associado principalmente à forte carga eletronegativa dos vários grupos fosfato no anel inositol. É a eletronegatividade do ácido fítico sob condições intestinais que reduz a solubilidade e a digestibilidade de P, Ca e vários cátions, como Fe, Zn, Cu, além de proteínas, ocasionando cascatas fisiológicas deletérias (Cosgrove, 1966). É esperado que o Cu proveniente de alimentos comumente usados para frangos de corte seja altamente atraído pela eletronegatividade do ácido fítico e, portanto, ligado a ele.

A fitase é uma hidrolase que tem sido incluída em dietas para frangos de corte, não apenas para reduzir a suplementação de fósforo nas dietas, mas também para liberar outros minerais que podem estar quelados ao fitato (Slominski, 2011). Milho e farelo de soja, que correspondem a aproximadamente 90% das dietas dos frangos, podem apresentar quantidades importantes de fitato em sua composição (milho: 0,19% e farelo de soja: 0,34%) (Rostagno et al., 2011). A suplementação com fitase pode liberar do fitato uma quantidade

significativa de fósforo e de microminerais, permitindo maior biodisponibilidade de nutrientes aos animais (Leytem et al., 2007).

No contexto atual, no qual o desempenho dos frangos de corte modernos tem melhorado consideravelmente ao longo do tempo (Havenstein et al., 2001) e a fitase tem sido tradicionalmente adicionada às dietas dos frangos, os nutrientes precisam ser revistos. As sugestões do NRC (1994) e FEDNA (2008) para a suplementação de Cu podem estar desatualizadas, portanto, podem estar sendo utilizadas doses maiores de Cu para tentar atingir o máximo potencial genético de frangos de corte (Pesti & Bakalli, 1996; Arias and Koutsos, 2004), resultando perdas econômicas e danos ambientais. Portanto, um estudo utilizando doses menores de Cu do que as utilizadas atualmente com a suplementação de fitase, deve ser testado. Diante desse cenário, o presente estudo foi desenvolvido com o objetivo de avaliar os efeitos da suplementação de níveis crescentes de em rações Cu com e sem a suplementação de fitase para frangos de corte sobre o desempenho zootécnico, problemas locomotores e variáveis sanguíneas.

REVISÃO BIBLIOGRÁFICA

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Exigência de cobre

A exigência atual de microminerais na dieta de frangos de corte provavelmente está desatualizada devido às melhorias no desempenho zootécnico e no melhoramento genético nas últimas décadas. Segundo dados do *National Chicken Council* dos EUA, de 1925 a 2017, a idade média de abate dos animais diminuiu 65 dias (de 112 para 47), o percentual de mortalidade caiu 13,5% (de 18% para 4,5%) , o peso ao abate dos animais aumentou 151% (de 1,13kg para 2,84kg) e a conversão alimentar diminuiu 157% (de 4,7 para 1,83). Essas melhorias foram permitidas devido ao grande desenvolvimento de áreas como: genética, nutrição e ambiência; sugerindo que a exigência de microminerais pode ter sido alterada.

Há uma lacuna nos estudos de microminerais para frangos de corte, incluindo o Cu, o que pode estar sendo suplementado de forma inadequada nas dietas. A fim de evitar uma suplementação maior do que a necessária, a legislação em vigor da UE recomenda uma suplementação total máxima de 25 ppm de Cu na dieta de aves, como uma preocupação com a poluição ambiental (EFSA, 2016). No entanto, essa suplementação pode ser ainda maior do que a necessidade atual. A suplementação de Cu em excesso, resulta em um acréscimo no custo da ração e em aumento da concentração de Cu nas excretas, prejudicando o meio ambiente (Schmidt et al., 2005).

A suplementação de fitase tem sido amplamente utilizada na dieta de frangos de corte com o objetivo de reduzir os custos com alimentação e os impactos ambientais. Essa enzima hidroliza o fitato, fator antinutricional presente nos ingredientes vegetais, liberando o fósforo presente nessa estrutura. Nesse sentido, diversos estudos relataram os efeitos da fitase no aumento da biodisponibilidade do fósforo (Denbow et al., 1995; Soares, 1995; Li et al., 2016; Kim et al., 2018). Entretanto, faltam dados na literatura sobre os possíveis efeitos que a fitase apresenta em relação à liberação de microminerais.

Variáveis sanguíneas

Análises hematológicas podem ser ferramentas clínicas muito eficientes no diagnóstico de distúrbios metabólicos. Nesse contexto, há muitas informações sobre as condições de vida das aves que podem ser avaliadas por

variáveis sanguíneas. O Cu está envolvido em várias funções do metabolismo das aves, tendo papéis importantes como cofator de várias enzimas, tais como: ceruloplasmina (Cp), ferroxidase, superoxidase dismutase, citocromo oxidase e lisyl oxidase (Lim and Paik, 2006). Seu papel na atividade enzimática deve-se principalmente por estar relacionado à oxidorredução e por ser um intermediário na transferência de elétrons (Silva et al., 2013). Hematócrito (Ht) e hemoglobina (Hb) têm sido sugeridos como variáveis confiáveis para avaliar o estado nutricional adequado do Cu no organismo animal.

Após ser absorvido pelos enterócitos, o Cu é transferido pelo sistema portal do fígado, onde pode ser incorporado a proteínas, formando a enzima Cp (Suttle, 1998). A Cp contém 0,34% do Cu em sua composição, sendo 95% do Cu presente no plasma transportado por essa proteína (Koh et al., 1996). A Cp está envolvida na proteção de ácidos graxos poli-insaturados das membranas celulares dos radicais ativos de oxigênio, participando do processo antioxidante (Koh et al., 1996). Portanto, a concentração de Cu no fígado, a concentração de Cp no sangue e os produtos do metabolismo oxidante, como as substâncias reativas ao ácido tiobarbitúrico (TBARS), podem estar relacionados à concentração de Cu no sangue.

O Cu é um participante ativo no processo de transporte de oxigênio. Embora o Cu não seja um constituinte da própria hemoglobina, ele está presente em certas proteínas plasmáticas que estão envolvidas no transporte de Fe. O Cu é um componente da ferroxidase, responsável pela conversão do Fe de Fe^{+2} em Fe^{+3} (Leeson, 2009; Grotto, 2010), requisito para sua absorção e transporte através do organismo. Portanto, variáveis sanguíneas como Ht e Hb podem ser usados como critérios para estimar a biodisponibilidade de Cu (Ledoux et al., 1991).

Propriedades antioxidantes

O Cu é um cofator da superóxido dismutase (Cu-Zn-SOD), uma enzima fundamental para a defesa antioxidante das células (Barbosa et al., 2010). Esta enzima está localizada no citosol celular e também no fluido extracelular, sendo especialmente abundante no fígado, rim e hemácias. Cu-Zn-SOD, junto com a catalase e a glutationa peroxidase, são conhecidas como a primeira linha de defesa do organismo para reduzir os radicais livres (Ray and Husain, 2002) e prevenir a peroxidação lipídica (Lanari et al., 2004; Grashorn, 2007).

O principal produto de oxidação dos ácidos graxos poliinsaturados

peróxidos é o malondialdeído (MDA), que é um importante indicador de peroxidação lipídica. O aumento da concentração de espécies reativas de oxigênio (ROS) pode danificar estruturas celulares, como proteínas, lipídios, ácidos nucléicos e membranas celulares (Tan et al., 2010; Kumbhar et al., 2018). O acúmulo de MDA nos tecidos é indicativo de peroxidação lipídica, e sua concentração pode estar ligada ao dano oxidativo das membranas celulares (Karatas et al., 2002). O ensaio de substâncias reativas ao ácido TBARS é usado para estimar a concentração de MDA e, portanto, o nível de peroxidação lipídica. Como o Cu está relacionado à atividade do Cu-Zn-SOD, sua concentração nas dietas pode estar relacionada aos níveis de MDA.

O Cu também é um cofator da enzima lisil oxidase, uma enzima que catalisa a formação de colágeno e de elastina nos tecidos conjuntivos. A deficiência de Cu nas dietas pode diminuir a formação de colágeno devido à diminuição da atividade da enzima lisil oxidase. Nesse contexto, a deficiência de Cu pode falhar na produção de tecidos contendo colágeno e elastina, como vasos sanguíneos, tendões e ossos (Medeiros, 2016).

Adicionalmente, as demandas dos consumidores por altos padrões de qualidade da carne aumentaram nos últimos anos, desafiando a indústria avícola a melhorar sua qualidade, valor nutricional e prazo de validade. A suplementação de Cu pode melhorar essas características, pois está envolvida em alguns processos oxidantes do organismo. Como o Cu é importante para a formação e atividade do Cu-Zn-SOD, é possível que uma suplementação ideal de Cu nas dietas possa aumentar a vida útil dos produtos.

Cu e anormalidades locomotoras

O Cu é cofator da enzima lisil oxidase, responsável por catalisar a formação de colágeno e de elastina. A deficiência de Cu pode diminuir a formação de colágeno devido à diminuição da atividade da enzima lisil oxidase. Nesse contexto, a suplementação de Cu sob a necessidade de frangos de corte pode causar falha na produção de tecidos contendo colágeno e elastina, como tendões e ossos (Medeiros, 2016). Portanto, a deficiência de Cu pode estar relacionada a deformidades como varus, valgus e rotação de tíbia.

O desvio em valgo apresenta uma angulação lateral no segmento distal da articulação, enquanto em varo essa angulação é medial. A tíbia rotacionada é caracterizada por uma rotação externa da articulação tibial metatarso, que resulta no pé apontando lateralmente, de forma que o animal não consegue manter o pé afetado em estação (Julian, 2005).

Fitase e Cobre

O fósforo é armazenado principalmente na forma de fitato em cereais e sementes oleaginosas, como milho e soja, que são os principais ingredientes usados em rações comerciais para aves. Esta molécula não pode ser eficientemente hidrolisada por enzimas endógenas, uma vez que os não ruminantes não produzem a enzima necessária para quebrar suas ligações. Aproximadamente dois terços do fósforo contido nos grãos de cereais e de seus subprodutos ficam indisponíveis para a absorção animal, pois estão presentes na molécula de fitato (Rostagno et al., 2011).

O ácido fítico pode ser considerado um fator antinutricional, pois tem sido relatado que quelata minerais essenciais, como: Cu, Zn, Mn, Fe e Ca; formando um composto estável denominado fitato (Cheryan and Rackis, 1980). O fitato não está disponível para digestão / absorção pelo trato gastrointestinal das aves (Humer et al., 2015). Nesse contexto, Aoyagi and Baker (1993) afirmam que baixos níveis de Cu presentes no milho e no farelo de soja estão disponíveis para serem absorvidos pelos enterócitos devido a maior ligação do Cu com o ácido fítico.

Além disso, o fitato é capaz de alterar o *turnover* das células intestinais, podendo causar irritação da mucosa, aumentando a produção de mucina e, consequentemente, prejudicando a absorção de nutrientes (Cowieson et al., 2011). Os nutrientes indisponíveis ligados ao fitato são eliminados nas excretas dos frangos, causando sérios problemas ao meio ambiente como eutrofização das águas e nitrificação dos solos, reduzindo a quantidade de oxigênio nas águas de rios e de lagos e contaminando o solo, respectivamente.

Para minimizar esses efeitos deletérios, a fitase tem sido amplamente utilizada pela indústria de frangos de corte. Além do fósforo, a enzima pode liberar outros minerais que são quelados na molécula do fitato, aumentando sua biodisponibilidade no organismo (Sebastian et al., 1998). Portanto, a suplementação de fitase pode não apenas aumentar a disponibilidade de fósforo, mas também ter um papel importante a desempenhar na promoção da disponibilidade de Cu, uma vez que o cátion apresenta grande afinidade com a molécula do ácido fítico. Com a suplementação de fitase em dietas para frangos de corte, pode ser possível reduzir a suplementação de Cu, o que pode levar a benefícios econômicos e ambientais.

Diante desse cenário, o presente estudo tem como objetivo avaliar os efeitos de níveis de suplementação de Cu em dietas para frangos de corte de 8

a 28 d sobre o desempenho zootécnico, variáveis sanguíneas e problemas locomotores. Adicionalmente, o experimento tem por objetivo avaliar os efeitos da suplementação de fitase em dietas com níveis crescentes de Cu sobre biodisponibilidade de Cu e energia digestível ileal. A pesquisa torna-se relevante devido ao grande avanço de áreas como: genética, nutrição e sanidade que permitiram melhora no desempenho dos animais. Portanto, as atuais recomendações da suplementação de Cu podem estar superestimadas, tornando-se relevante testar níveis mais baixos de Cu com a suplementação de fitase.

HIPÓTESES E OBJETIVOS

Hipóteses

Níveis crescente de Cu suplementado em rações milho e soja para frangos de corte afetam desempenho, variáveis sanguíneas 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 Cu pode aumentar a biodisponibilidade de Cu em frangos de corte.

Objetivos

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

CAPÍTULO II[1]

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Cupper requirement in diets with or without phytase for broiler

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ABSTRACT The objective of the present study was to evaluate the effects of supplementation of increasing levels of Cu with and without phytase supplementation in broiler diets on growth performance, blood parameters, and locomotor disorders. The experiment consisted of a 2 x 5 factorial arrangement with 10 treatments, 8 replications of 7 male Cobb 500 chicks, distributed in a completely randomized design. The basal diet (8 ppm Cu analyzed) was supplemented with 0; 3; 6; 9 and 12 ppm Cu, with or without phytase supplementation (2,500 FYT / Kg). Weight gain, feed conversion and feed intake were evaluated at 8, 14, 21 and 28 d. At 28 days, varus, valgus and tibia rotation were evaluated and blood was collected for hematocrit (Ht) and hemoglobin (Hb) evaluation. At 28 d, all animals were slaughtered to collect kidney, liver, tibia, *pectoralis major* muscle, gastrocnemius tendon and ileal content. Diet samples and ileal digesta were analyzed to determine the ileal digestible energy (IDE). Data were analysed using the SAS MIXED proc and averages, when relevant, were compared using the Tukey test ($P < 0.05$). Treatments were distributed in a 2 x 5 factorial arrangement. The broiler responses were adjusted to the quadratic (QP) and linear (L) polynomial models. No effect of increasing Cu levels was observed on performance when the diets were formulated with phytase ($P > 0.05$). However, increasing levels of Cu without phytase supplementation affected ($P < 0.05$) feed conversion from 8 to 14 days and from 8 to 28 days, with the lowest conversions obtained at 14.50 and 11.81 ppm, respectively. Phytase supplementation increased weight gain and feed intake and decreased feed conversion throughout the experimental period ($P < 0.05$), when compared to diets without phytase, except for feed conversion from 15 to 21 d. There was no difference between treatments for Ht and Hb ($P > 0.05$) and for locomotor problems ($P > 0.05$). In addition, there was no histological difference in the liver between the different treatments ($P > 0.05$). Treatments with phytase inclusion showed a higher IDE ($P < 0.05$) than non-supplemented treatments. data from the present study suggest that the amount of Cu supplemented today in broiler rations may be above the real need to achieve maximum zootechnical performance. However, supplementation with phytase is essential to achieve the maximum growth rate.

supplementation.*Keywords:* growth performance, cupper, micro-mineral, requirement.

61

62 **INTRODUCTION**

63

64 Trace mineral supplementation is essential in broiler production allowing adequate
65 expression growth without deficiency signals. Dietary Cu supplementation is required for
66 broilers since the amount of this micromineral in usual feed ingredients may be not enough to
67 meet the dietary contents that optimize growth, especially due to variability in feedstuffs (NRC,
68 1994). The traditional source of Cu supplemented in diets for broilers is sulfate pentahydrate
69 ($CuSO_4 5H_2O$) due to low cost and commercial availability (Pang and Applegate, 2006). Current
70 Cu supplementation of broiler diets has variable suggestions: 8 ppm (NRC, 1994), 8 to 10 ppm
71 (FEDNA, 2008) and 15 ppm (Cobb, 2018).

72 Among its various functions, Cu participates in bone formation, in Fe transport, and,
73 consequently, in hemoglobin formation. Thus, Cu deficiency results in low blood hemoglobin
74 inducing anemia (Baumgartner et al., 1978) as it is a component of ferroxidase, responsible for
75 the conversion of Fe from Fe^{+2} into Fe^{+3} (Leeson, 2009; Grotto, 2010), a requirement for its
76 absorption and transport through the organism. Therefore, blood parameters such as Ht and Hb
77 can be used as criteria to estimate the bioavailability of Cu (Ledoux et al., 1991). As a cofactor
78 of lysyl oxidase, it supports the formation of collagen and elastin, and its deficiency leads to
79 inadequate cartilage formation in various tissues (Medeiros, 2016). This has been shown to
80 induce valgus and varus deformities as well as other locomotor disorders (Leeson, 2009). When
81 in excess, it can be noticed possible signals of Cu excess in the liver as: hepatocellular
82 degeneration, necrosis or Kupffer cells (Bozynski et al., 2009).

83 Bioavailability values for Cu in corn and soybean meal (SBM) are around 80% (Aw-
84 Yong et al., 1982) and 40%, respectively (Aoyagi and Baker, 1993b). Presenting high electro
85 positivity (Vohra et al., 1965; Obeleas, 1973; Wise and Gilburt, 1982), Cu has high stability

with phytic acid, forming a phytate complex.

Phytic acid (1,2,3,4,5,6 myo-inositol hexakis dihydrogen phosphate) is the principal storage form of phosphorus in plant material and is found widely distributed in cereals, grain legumes, and oilseed meals at concentrations from around 7–8 g/kg in corn to 50–60 g/kg in cereal by-products (Eeckhout and De Paepe, 1994). The involvement of phytic acid in nutrition is associated mainly with the strong electronegative charge of the various phosphate groups on the inositol ring. It is the electronegativity of phytic acid under intestinal conditions that reduce the solubility and digestibility of several cations and proteins (Cosgrove, 1966). Cu in commonly used feedstuffs for broilers is expected to highly affect by phytic acid electronegativity and therefore, mainly bound to it.

Phytase is a hydrolase that has been included in feed diets, not only to reduce phosphorus supplementation in diets, but also to release other minerals from phytate that can be chelated to it (Slominski, 2011). Corn and soy-bean meal (SBM), which correspond to approximately 90% of chicken diets, can present important amounts of phytate in their composition (corn: 0.19% and SBM: 0.34%) (Rostagno et al., 2011). Phytase supplementation may be able to release from phytate a significant amount of phosphorus and microminerals, allowing higher bioavailability of nutrients (Leytem et al., 2007).

In the current context, in which modern broiler chicken performance has been considerably improved over time (Havenstein et al., 2001) and phytase has traditionally been added to broiler diets, nutrients need reviewing. The suggestions of NRC (1994) and FEDNA (2008) for Cu supplementation may be outdated, therefore, it is being used higher doses of Cu to try to reach the maximum genetic potential of broilers (Pesti and Bakalli, 1996; Arias and Koutsos, 2004), resulting in economic losses and environmental damage. Therefore, a study using lower doses of Cu than the currently used and with the supplementation of phytase should be tested. The present study had the objective to evaluate the effects of ncreasing levels of

supplemented Cu in feed formulated with and without phytase on growth performance, leg problems, and blood parameters of broilers until 28 d.

MATERIALS AND METHODS

The Ethics and Research Committee of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil, approved all procedures used in the present study (Project number UFRGS 276).

Bird Husbandry and Dietary Treatments

A total of 560 one-day-old male Cobb × Cobb 500 slow feathering chicks were allocated into 80 metallic cages using a completely randomized design with 10 treatments and 8 replications. On day 7, birds were randomly allocated by weight into groups of 7 birds to the 10 dietary treatments and housed in the battery cages. From 8 to 28 d, birds were fed the experimental diets.

Treatment and replication numbers were visible at each pen or cage. The temperature at placement was 32 ºC, which was adjusted weekly to maintain bird comfort throughout the study. Birds had *ad libitum* access to water and mash feed. Lighting was provided 24 h continuously throughout the study. All pens were daily checked for sick and dead birds. The pen number and body weight of each dead bird were registered as soon as it was observed.

The dietary treatments consisted of increasing levels of Cu supplementation: 0, 3, 6, 9, and 12 ppm from $CuSO_5H_20$ with or without phytase (2,500 FYT/Kg) (Ronozyme HiPhos with 10,000 FYT/g, Novozymes A/S, Bagavaerd, Denmark) supplementation (Table 1). All diets had 1% Celite as indigestible marker (Celite, Celite Corp., Lompoc, CA).

Corn-SBM diets were formulated based on local broiler industry levels (Table 2). A 2-phase feeding program (pre-starter: 1 to 7 d and starter: 8 to 28 d) was used. Feed samples were

collected from each batch, separated by treatment, and analyzed for crude protein, gross energy (GE), Ca, P, and Cu. All feeds from starter treatments had a minimum and maximum geometric diameter of 1.052 and 1.117 μm, respectively, with a standard geometric deviation 1.79.

Growth Performance and Ileal Digestible Energy

Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) corrected for the weight of dead birds were evaluated at 8, 14, 21, and 28 days of age.

Ileal contents were collected from all birds per pen at 28 d after euthanasia by electrical stunning using 45 V for 3 s. Contents were collected from the terminal 2/3' of ileum, which was defined as the region between Meckel's diverticulum to 2 cm cranial to the ileocaecal junction. The content was 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. Calculations of ileal digestible energy (IDE) was done afterwards.

Apparent ileal digestibility were calculated using the following equations (Kong and Adeola, 2014):

$$\text{Digestibility (\%)} = [1 - (M_i/M_o) \times (E_o/E_i)] \times 100,$$

where M_i represents the concentration of acid insoluble ash in the diet in grams per kilogram of DM; M_o represents the concentration of acid insoluble ash in the ileal digesta in grams per kilogram of DM output; E_i represents the concentration of DM, CP, GE, Cu, Ca and P in the diet in milligrams per kilogram of DM; and E_o represents the concentration of DM, CP, GE, Cu, Ca and P in the ileal digesta in milligrams per kilogram of DM.

Feed samples and ileal content were analyzed to determine dry matter (DM), gross energy (GE), Cu, Ca, and P.

Organs and blood sampling

Blood samples were taken minutes before euthanasia at 28 d from 3 broilers randomly selected from each treatment. Blood obtained was partially transferred to 0.5mL test tubes containing EDTA for hematocrit (Ht) and hemoglobin (Hb) analyzes. Determination of Ht was done using micro capillaries containing blood centrifuged for 5 min at 15,650 to 18,510 × g. Concentration of Hb was determined using the cyanmethemoglobin method as described by Crosby et al. (1954). Blood (3 mL) also were centrifuged to obtain serum, which was transferred to Eppendorf tubes. A portion of the serum was used for Cu concentration analysis (Meret and Henkin, 1971).

Breast muscle, liver, kidney, tibia, and gastrocnemius tendon were collected from all birds after euthanasia at 28 days. All samples were frozen by pen in plastic bags at -20 °C until analysis. After thawing, the samples were ashed and Cu concentration was determined as described by AOAC (1990) using a PerkinElmer 5000 Atomic Absorption Spectrophotometer. Bone ash was expressed by cage as percent of the dry defatted bone weight and as the absolute weight of the tibia in grams.

Liver samples were fixed in 10% formaldehyde and processed routinely for histology. Processing includes cutting the sample into small fragments, dehydrating in rising concentrations of alcohol, bleaching in xylol and soaking in paraffin. Subsequently, the samples were sectioned (3-5 µm), stained with hematoxin and eosin (HE) and with Rubiic Acid and evaluated through optical microscopy. The histological lesions considered were necrosis, degeneration of hepatocytes and intracytoplasmic accumulation of copper pigment (positive in rubyic acid staining) in hepatocytes and kupffer cells. When present, the changes were graded according to distribution (focal, extensive, multifocal or diffuse) and intensity (discrete, moderate or accentuated).

185 ***Leg abnormalities***

186 At 28 d, *valgus*, *varus*, and rotated tibia deviations in the tibiometatarsal joint were
187 evaluated in all birds. Broilers were maintained in the normal anatomic position and their leg
188 status were evaluated by 3 different researchers. The results considered the average of the three
189 evaluators. Broilers were classified by the presence or the absence of each deformity. *Valgus*
190 was classified as a deviation of the tibiometatarsal joint presenting an outward angulation of
191 the distal segment of tibiometatarsal joint. The *varus* deviation was considered the joints that
192 presented a medial deviation of the distal tibiometatarsal joint (Julian, 1984). Rotated tibia
193 above 90º was considered abnormal and characterized as a torsional rotation of the shaft of the
194 tibiotarsus of 1 or both legs, causing the metatarsus to point laterally and the broiler to assume
195 a spraddle leg posture (Thorp, 1994).

196

197 ***Statistical analysis***

198 Data were tested for homoscedasticity and normality of residuals distribution. Data that
199 were not normal were square root transformed. Data were analyzed using the GLM procedure
200 of SAS Institute (SAS, 2009). Significance was accepted at $P < 0.05$. Data were submitted to a
201 one-way ANOVA and mean differences were separated using Tukey's HSD test.

202 Estimation of maximum responses to total dietary Cu was done using linear (L) and
203 quadratic polynomial (QP) regression models. The L model ($Y = \beta1 + \beta2 \times X$) has Y as the
204 dependent variable, X as the dietary level of Se, β1 as the intercept, and β2 as the linear
205 coefficient. The QP model ($Y = \beta1 + \beta2 \times Se + \beta3 \times (Cu)^2$) has Y as the dependent variable as
206 a function of dietary level of Cu; β1 as the intercept; β2 as the linear coefficient and β3 as the
207 quadratic coefficient. The maximum response for Cu was defined as $Se = -\beta2 \div (2 \times \beta3)$.

208

209

RESULTS

Analyzed Cu in the experimental feeds was similar to the expected values from feed formulation from treatment 1 to 10: 7.8, 11.1, 14.6, 16.5, 19.0, 8.3, 11.4, 13.8, 16.6 and 19.9 ppm, respectively. Analyzed phytase content was also similar to the expected values from feed supplementation, being 0 FYT/kg: 2752, 3200, 2831, 2446 and 2947 from treatment 6 to 10, respectively (Table 3).

No effects of increased Cu levels were observed on growth performance (Table 4) and cumulative growth performance (Table 5) of broilers from 8 d or at 28 d ($P > 0.05$). However, 2,500 FYT/kg phytase supplementation improved BWG, FI, and FCR during all the experimental periods ($P < 0.05$), except for FCR from 15 to 21 d.

There were no differences among treatments for Ht and Hb (Table 7), varus, valgus and tibia rotation (Table 8) at 28 d ($P > 0.0.5$). Through histological evaluation, there were no significant changes in the samples evaluated. However, treatments with phytase inclusion presented higher IDE ($P < 0.05$) when compared to broilers that had no phytase on their fed (Table 9).

Estimations of Cu requirements were determined using L and QP regression models shown in Table 6. The L and QP fitting were not significant at any period for treatments with phytase inclusion. On the other hand, dietary increases of Cu affected ($P < 0.05$) FCR from 8 – 14 d, and 8 – 28 d when no phytase was supplemented, being the maximum responses 14.5 and 11.8 ppm, respectively. As responses did not fit adequately for blood parameters and locomotor problems, requirement estimation is not presented.

DISCUSSION

The basal diets used in this experiment were formulated with corn and SBM containing the comparable nutrient content and energy levels than commercial diets in Brazil, except for

Cu. These diets were supplemented with increasing levels of Cu from 3 to 12 ppm and the control diet had no Cu supplementation. The basal diet was analyzed for Cu content and it was found 8 ppm of Cu.

Cupper has been added to poultry diets. Since Cu concentration in feedstuffs is variable depending on soil concentration, it is a common practice to supplement the mineral to reduce the risk of deficiency and, therefore, avoid losses in performance. Traditionally, Cu supplementation is done using sulfate form (CuSO4). Nonetheless, there is evidence of an excess of Cu in poultry manure, resulting in soil phytotoxicity (Mohanna and Nys, 1998), inferring that feed formulation needs to be revised. In the present experiment, broilers fed increased CuSO4 levels diets did not present a difference for growth performance and cumulative growth performance from 8 to 28 d ($P > 0.05$). It may be suggested that the amount of Cu present in the diet may be sufficient to achieve ideal growth performance. Therefore, traditional suggestions of Cu supplementation present in the current literature of 8 ppm (NRC, 1994), 8 - 10 ppm (FEDNA, 2008), 15 ppm (Cobb, 2018) may be excessive.

However, results observed in this experiment indicated that phytase supplementations in diets could improve cumulative growth performance from 8 to 28 d. This finding is in agreement with the results of previous studies which reported that phytase supplementation increased cumulative BWG ($P = 0.001$) and decreased cumulative FCR ($P = 0.005$) in broilers (Kriseldi et al., 2021). Song et al. (2021) also stated that a diet with phytase supplementation of 500, 1,000, 2,000, 4,000, 8,000 U of phytase/kg could improve BWG ($p < .05$) FCR on day 42 when compared with a group that did not receive phytase supplementation.

It could be observed quadratic responses for FCR for broilers fed no phytase from 8 to 14 d and from 8 to 28 d, and the requirement estimates obtained in the present study for broiler with no phytase supplementation were 14.50 and 11.81 ppm, respectively, considerably lower than the NRC (1994) recommendations for broilers. However, broiler with phytase

supplementation did not present quadratic responses. This may suggest that the conventional amount of Cu supplementation is above the real need for broilers. In agreement, Banks et al., (2004) observed that BWG was not different among broiler groups fed with 0 or 250 ppm of different sources of Cu (Cu sulfate, Cu citrate, Cu lysinate or CuCl) from 9 to 22 d.

Just the presence of a nutrient in a diet is not an indication of its bioavailability. The ability of Cu to form stable metal-phytate complexes within the intestinal tract seems to reduce its availability (Maddaiah et al., 1964; Vohra et al., 1965) as it was seen by Davis et al. (1962) when fed chicks with an isolated soya-bean protein, due to the high phytate content of SBM. In this context, Davies and Nightingale (1975) reported that rats fed phytate (10g/Kg) in a basal diet containing 5 ppm of Zn, 25 ppm of Cu and 50 ppm of Fe presented reduced average daily accumulation of Zn, Cu, Fe and Mn, increased growth rate and food intake when compared with the control groups. Besides, when results were expressed as retention relative to intake of rats had a different intake of these trace metals, phytate fed rats also presented significantly reduced retention of all trace metals, suggesting that dietary phytate reduces the availability of these microminerals.

In the last years has become common the addition of exogenous phytase in poultry diets to increase phosphorus, protein, amino acid, and mineral digestibility and, consequently, to reduce their excretion in manure (Woyengo and Nyachoti, 2010). The benefits from phytase supplementation in broilers` diet on performance are well documented in the literature (Pirgozliev et al., 2008; Muszy and Tomaszewska, 2017; Song et al., 2021). However, the efficacy of the enzyme in microminerals liberations is not deeply known. Results observed in this experiment indicated that 2,500 FYT phytase supplementations in diets could improve cumulative growth performance from 8 to 28 d and may reduce the requirement of Cu.

In agreement, Pirgozliev et al. (2008) observed that broilers fed corn and SBM supplemented with 0, 250, 500, or 2500 phytase (FYT/k)g increased in a linear manner feed

intake and weight gain in response to phytase dose. Besides, 2,500 FYT supplementation presented 6,6% higher and 2,4% lower BWG and FCR, respectively. In this context, since the conditions in the gut are not ideal for phytase activity and considering also the limited time spend on it, it is expected that the enzyme will not be able to completely dephosphorylate dietary phytates. Therefore, it is likely that adding higher doses than the conventional amount of phytase can increase the efficiency of phytate breakage. Regarding the action of phytase on microminerals, Bikker et al. (2012), in an experiment with swine, added 1,500 FYT/kg in a diet containing 160 ppm of Cu (CuSO4) in addition to Cu from feed ingredients (7 ppm) and it was observed an increase in Cu digestibility by 16% units when compared to a no phytase supplemented diet.

Treatments with phytase inclusion presented higher IDE ($P < 0.05$) when compared to broilers that had no phytase supplementation on their feed. Similar results were found by Leyva-Jimenez et al. (2019) and Mohiti-Asli et al. (2020), where energy digestibility was improved ($P < 0.05$) with phytase inclusion in broiler`s diet. Thus, in the present experiment, the significant improvements in growth performance of broiler supplemented phytase can be explained by the increased IDE.

Hematological analyzes can be very efficient clinical tools in the diagnosis of metabolic disorders. Although Cu is not a constituent of hemoglobin itself, Cu is an active participant in the oxygen transport process as it is present in certain plasma proteins that are involved in the transport of Fe. In an experiment testing different levels of Cu and Fe, in a no Fe supplemented group, the hemoglobin was markedly lower than in those supplemented with 14.2 ppm of Cu, 164.6 ppm of Fe or no Cu supplementation. While in an experiment where both Cu and Fe were deficient, there was a small increase with the addition of Cu, showing that hemoglobin decreased to a greater extent with a deficiency of Fe, however, small amounts of Cu appear to be sufficient to achieve adequate blood parameters (Davis et al., 1962). Since there was no

difference among treatments for blood parameters, it can be suggested that the amount of Cu in the basal diet is sufficient to meets broiler requirements. Confirming the quote of Hill and Matrone (1961) that Cu deficiency alone has little effect on hemoglobin concentration and hematocrit values.

There were no effects of Cu supplementation with or without phytase in the frequency of varus or valgus deviations and tibia rotation. In corroboration, day-old chicks were fed skim-milk-based diets containing 0, 2, 4, 8, or 16 ppm of copper (CuSO4) for 21 days, and it could be observed that the requirement for optimal growth was 6-8 ppm copper, while the requirement for normal cross-link formation and mineralization in bone was less than 2 ppm copper, concluding that the amount of Cu needed to maintain the mechanical integrity of bone was lower than for growth performance (Opsahl et al., 1982). Therefore, adding copper to achieve maximum performance allows the proper development of the locomotor members.

Accumulation of copper in the liver can be extremely toxic and increases oxidant stress (Boal and Rosenzweig, 2009). In the present study, liver observed through histological evaluation, did not present changes among treatments with increasing level of Cu with or without phytase, indicating that there was no excess of the micromineral stored in this organ for any treatment.

CONCLUSIONS

In conclusion, data from the present study suggests that the amount of copper supplemented today in broiler diets may be above the real need to achieve maximum growth performance. Besides, phytase supplementation is essential to achieve maximum growth rate and increases ileal digestibility.

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Table 1.

Outline of experimental treatments.

Treatment	Suplementation, ppm	Cu, ppm[1]	Phytase Supplementation
T1	0.0	7.01	No Phytase
T2	3.0	10.01	
T3	6.0	13.01	
T4	9.0	16.01	
T5	12.0	19.01	
T6	0.0	7.01	2500 FYT/kg
T7	3.0	10.01	
T8	6.0	13.01	
T9	9.0	16.01	
T10	12.0	19.01	

[1] Considering the amount of Cu analyzed in corn and in SBM.

Table 2. Ingredient and nutrient composition of the basal diets.

	Pre-Starter (1 to 7 d)	Starter (8 to 28 d)									
		Cu supplementation, ppm without phytase					Cu supplementation, ppm with phytase 2,500 FYT/Kg				
Item		0	3	6	9	12	0	3	6	9	12
Ingredient, %											
Corn	48.47	46.99									
Soybean meal	45.47	41.69									
Soybean oil	2.80	5.41									
Calcium carbonate	1.55	2.23									
Celite	-	1.00									
Phosphoric acid	0.37	1.36									
Salt	0.53	0.51									
DL-Methionine 99%	0.38	0.35									
L-Lysine HCl 76%	0.10	0.10									
L-Threonine 98.5%	0.08	0.07									
Choline chloride	0.02	0.04									
Monensina	0.02	0.02									
Vitamin mix[1]	0.10	0.10									
Mineral mix[2]	0.10	0.10									
Vitamin C	-	0.02									
Total	100.00	100.00									
Calculated nutrient composition, % unless noted											
AME_n, kcal/kg	2,960	3,050									
CP	24.97	23.07									
Ca	1.05	0.95									
Non-phytate P	0.52	0.47									
Na	0.23	0.22									
K	0.97	0.90									
Cl	0.39	0.38									
Choline, mg/kg	1,60	1,60									
Dig. Lys [3]	1.32	1.22									
Dig. TSAA	0.99	0.91									
Dig. Thr	0.86	0.79									
Dig. Trp	0.28	0.26									
Dig. Arg	1.57	1.44									
Dig. Val	1.02	0.94									
Dig. Ile	0.94	0.86									
Cu[4], ppm	8.63	7.8 ± 0.2	11.1 ± 0.4	14.6 ± 0.1	16.5 ± 0.2	19.0 ± 0.1	8.3 ± 0.5	11.4 ± 0.6	13.8 ± 0.3	16.6 ± 0.2	19.9 ± 0.4

[1]Premix mineral by kg of premix: Zn, 110 mg; Fe, 50 mg; Se, 0.3 mg and I, 2 mg.

[2]Premix Vitaminic by kg of premix: vitamin A, 12000 IU; vitamin D_3, 3000 IU; vitamin E, 100 IU; vitamin C, 50 mg; vitamin K_3, 6 mg; vitamin B12, 35 µg; tiamin, 3 mg; riboflavin, 15 mg; vitamin B6, 6 mg; niacin, 40 mg; pantothenic acid, 25 mg; folic acid, 4 mg; biotin, 0.3 mg.

[3] Amino acid: lysine (digestible) ratio = Met + Cys 0.75; Thr 0.65; Val 0.77; Ile 0.67; Arg 1.17, and Trp 0.17.

[4] Analysed Cu.

Table 3. Declared and analyzed phytase in the experimental diets.

Supplemented Cu, ppm	Phytase (U/kg) [1]	Phytase (U/kg) [2]
0 ppm Cu no phytase	0	0
3 ppm Cu no phytase	0	0
6 ppm Cu no phytase	0	0
9 ppm Cu no phytase	0	0
12 ppm Cu no phytase	0	0
0 ppm Cu with phytase	2500	2752 ± 171
3 ppm Cu with phytase	2500	3200 ± 464
6 ppm Cu with phytase	2500	2831 ± 391
9 ppm Cu with phytase	2500	2446 ± 282
12 ppm Cu with phytase	2500	2947 ± 149

[1]From laboratory phytase supplementation.
[2]Analyzed phytase from the batch.

Table 4. Growth performance of broilers fed diets supplemented with increasing levels of cupper with or without phytase from 8 to 28 d, g.

Item	8 to 14 d			15 to 21 d			22 to 28 d		
Treatment	BWG	FCR	FI	BWG	FCR	FI	BWG	FCR	FI
Phytase, FYT/kg									
0	268	1.288	344	507	1.226	622	775	1.247	967
2500 FYT/Kg	309	1.222	377	525	1.221	641	834	1.221	1018
Suplemental cupper, ppm									
0	282	1.287[a]	362	512	1.226	628	795	1.247	990
3	290	1.248[b]	361	519	1.226	636	809	1.234	997
6	287	1.250[b]	358	527	1.222	644	814	1.231	1002
9	292	1.242[b]	361	517	1.216	628	808	1.225	989
12	290	1.248[b]	361	506	1.228	621	796	1.235	982
Mean	288	1.255	361	516	1.224	632	615	1.376	845
SEM	2.700	0.006	2.380	3.241	0.003	3.976	3.644	0.005	4.072
P-values									
Phytase	0.001	0.001	0.001	0.007	0.419	0.020	0.001	0.001	0.001
Cupper	0.191	0.044	0.929	0.313	0.762	0.392	0.376	0.132	0.635
Phytase x Cupper	0.418	0.600	0.079	0.923	0.133	0.601	0.757	0.123	0.189

[a-b]Means within the same column with different superscripts differ by Tukey test ($P \leq 0.05$).

[1]From laboratory grade Cu sulfate.

Table 5. Cumulative growth performance of broilers fed diets supplemented with increasing levels of cupper with or without phytase from 8 to 28 d, g.

Item	8 to 21 d			8 to 28 d		
Treat	BWG	FCR	FI	BWG	FCR	FI
Phytase						
No phytase	775	1.247	967	1384	1.305	1.807
2500 FYT/Kg	834	1.221	1018	1453	1.286	1.868
Cupper						
0 ppm	795	1.247	990	1405	1.302	1828
3 ppm	809	1.234	997	1434	1.293	1854
6 ppm	814	1.231	1002	1433	1.292	1851
9 ppm	808	1.225	989	1419	1.290	1829
12 ppm	796	1.235	982	1404	1.290	1825
Mean	804	1.234	992	1419	1.295	1837
SEM	4.924	0.003	5.218	6.825	0.003	7.487
P-value						
Phytase	0.001	0.001	0.001	0.001	0.090	0.001
Cupper supl.	0.370	0.132	0.6331	0.261	0.448	0.478
Phytase x Cupper	0.753	0.123	0.1914	0.592	0.061	0.344

[1]From laboratory grade Cu sulfate.

Table 6. Regression equations of FCR of broilers fed diets supplemented with no phytase with increasing levels of cupper.

Day	Regression equations[1]	Model	r^2	P	Requeriment
15-21	$Y = 1.5281\ X^2 – 0.0359 * X + 0.0012$	QP	0.2820	0.0022	14.50
8 - 28	$Y = 1.3740\ X^2 – 0.0144 * X + 0.0006$	QP	0.1524	0.0469	11.81

[1]Regression equations obtained using the increasing analyzed Cu in the diets (8.05; 11.25; 14.2; 16.55 and 19.45 ppm).

Table 7. Hematocrit (Ht) and hemoglobin (Hb) of broilers fed diets supplemented with increasing levels of cupper with or without phytase from 8 to 28 d, g.

Treat	Ht	Hb
Phytase		
No phytase	27.1	8.22
2500 FYT/Kg	27.5	8.25
Cupper		
0 ppm	26.4	8.38
3 ppm	27.1	8.09
6 ppm	27.5	8.26
9 ppm	27.3	8.24
12 ppm	28.0	8.20
Mean	27.3	8.23
SEM	0.197	0.068
P-value		
Phytase	0.442	0.806
Cupper supl.	0.129	0.772
Phytase x Cupper	0.091	0.456

[a-b]Means within the same column with different superscripts differ by Tukey test ($P \leq 0.05$).
[1]From laboratory grade Cu sulfate.

Table 8. Valgus, varus and tibia rotation of broilers fed diets supplemented with increasing levels of cupper with or without phytase from 8 to 28 d, g.

Treat	Normal	Valgus	Varus	Tibia Rotation
Phytase				
No phytase	45.5	48.9	1.71	3.69
2500 FYT/Kg	43.5	51.3	2.05	3.16
Cupper				
0 ppm	46.6	48.8	1.84	2.77
3 ppm	42.8	54.5	1.24	1.19
6 ppm	40.3	48.9	2.39	8.49
9 ppm	46.9	49.4	1.79	1.94
12 ppm	46.1	49.1	2.14	2.73
Mean	44.5	50.1	1.88	3.42
SEM	1.416	1.392	0.456	0.862
P-value				
Phytase	0.473	0.395	0.712	0.755
Cupper supl.	0.503	0.644	0.949	0.067
Phytase x Cupper	0.191	0.117	0.085	0.934

[a-b]Means within the same column with different superscripts differ by Tukey test ($P \leq 0.05$).
[1]From laboratory grade Cu sulfate.

Table 9. IDE of broilers at 28 d fed diets supplemented with increasing levels of cupper with or without phytase.

Treat	IDE[2], kcal/kg
Phytase	
No phytase	2675
2500 FYT/Kg	2881
Cupper	
0 ppm	2636
3 ppm	2741
6 ppm	2721
9 ppm	2926
12 ppm	2866
Mean	2778
SEM	23.993
P-value	
Phytase	0.037
Cupper supl.	0.281
Phytase x Cupper	0.985

[1]From laboratory grade Cu sulfate.

CAPÍTULO III

CONSIDERAÇÕES FINAIS

Atender à necessidade real de Cu para frangos de corte tornaria possível reduzir os custos da alimentação e os impactos ambientais. O presente trabalho sugere que a quantidade de Cu suplementada hoje nas dietas para frangos de corte está acima da real necessidade para atingir o máximo desempenho zootécnico. No entanto, a suplementação com fitase mostrou-se essencial para atingir a taxa máxima de crescimento, independentemente da quantidade de Cu suplementada. Ainda, níveis crescentes de cu suplementado em rações milho e soja para frangos de corte não afetam variáveis sanguíneas e problemas locomotores em frangos de corte.

Os resultados apresentados neste estudo permite maior conhecimento sobre a suplementação de Cu em dietas de frangos de corte de 8 a 28 d, possibilitando a utilização de dados referentes a histologia, variáveis sanguíneas e problemas locomotores para a realização de estudos futuros.

Devido à pandemia, dados referentes à concentração de Cu no conteúdo ileal, no rim, no fígado, na tíbia, no peito e no tendão gastrocnêmio ainda não puderam ser analisados. Tais dados também serão incluídos no artigo.

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APÊNDICES

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

POULTRY SCIENCE INSTRUCTIONS TO AUTHORS [1]

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Full-Length Articles. The majority of papers pub- lished in *Poultry Science* are full-length articles. The jour- nal emphasizes the importance of good scientific writing and clarity in presentation of the concepts, apparatus, and sufficient backgroundinformation that would be required for thorough understanding by scientists in other disciplines. One of the hallmarks for experimental evidence is repeatability. The results of experiments published in *Poultry Science* must be replicated, either by replicating treatments within experiments or by repeating experiments. Care should be taken to ensure that experiments are adequately replicated.

Research Notes. Research Notes are short notes giv- ing the results of complete experiments but are less com- prehensive than full-length articles. Preliminary or progress reports will not be accepted. The running head shall be -RESEARCH NOTE.‖ Research Notes will be pub- lished as a subsection of the scientific section in which they were reviewed. Research Notes are limited to five printed pages including tables and figures. Manuscripts should be prepared according to the guidelines for full- length articles.

Symposium Papers. The symposium organizer or chair must present the proposal and tentative budget to the Board of Directors at the summer meeting one full year before the symposium is to be scheduled. The sym- posium chair must then develop detailed symposium plans, including a formal outline of the talks approved and full budgetary expectations, which must be brought to the Board of Directors at the January meeting prior to the meeting at which the symposium is scheduled. The symposium chair must decide whether or not the symposium is to be published and willinform the ed- itor-in-chief of this decision at the January meeting. If the decision is not to publish the symposium, the indi- vidual authors retain the right to submit their papers for consideration for the journal as ordinary manuscripts. If publication is decided upon, all manuscript style and form guidelines of the journal shall be followed. Manuscripts must be prepared electronically, including figures and tables, and then uploaded onto the *Poultry Science* Manuscript Central site within 2 weeks after the annual meeting. The symposium chair will review the papers and, if necessary, return them to the authors for revision. The symposium chair then forwards the re- vised manuscript to theeditor-in-chief for final review. Final revisions by the author and recommendations for acceptance or rejection by the chair must be completed by December 31 of the year in which the symposium was presented. Manuscripts not meeting this deadline will not be included in the published symposium pro- ceedings. Symposium papers must be prepared in accordance with the guidelines for full-length articles and are subject to review. Offprints and costs of pages are the responsibility of the author.

Invited Papers. Invited papers, such as the World's Poultry Science Association lecture, should be submitted online; the editorial office will then make these papers available to the editor-in-chief. These 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 but not offprint charges.

Review Papers. Review papers are accepted only if they provide new knowledge or a high-caliber synthesis of important knowledge. Reviews are not exempt from pages charges. All *Poultry Science* guidelines for style and form apply.

Invited Reviews. Invited Reviews will be approxi- mately 10 published pages and in review format. The editor-in-chief will send invitations to the authors and then review these contributions when they are submitted. Nominations or suggestions for potential timely reviews are welcomed and should be sent directly to the editor- in-chief.

Contemporary Issues. Contemporary Issues in *Poul- try Science* will address critical issues facing poultry sci- entists and the poultry industry. As such, submissions to this section should be of interest to any poultry scien- tist, to the industry, to instructors and faculty teaching contemporary issues classes, and to undergraduate and graduate students. The section will consist of short pa- pers (approximately 2 published pages) written in essay format and will include an abstract, appropriate subhead- ings, and references.

Rapid Communications. We aim for receipt-to-deci- sion times of a month or less, and accepted papers will have priority for publication in the next available issue of *Poultry Science.* These papers will present informative and significant new findings, such as tissue-specific gene expression profile data with full-length cDNA and genom- ic gene structure characterization. These papers will be short (2 to 4 published pages), adhere to journal format, and include references and an abstract. Rapid Communi- cations should **not** be preliminary reports or incomplete studies. Authors will select Rapid Communications as the paper type when submitting the paper.

Book Reviews. *Poultry Science* publishes reviews of books considered to be of interest to the readers. The editor-in-chief ordinarily solicits reviews. Unsolicited reviews must be sent directly to the editor-in-chief for ap- proval. Book reviews shall be prepared in accordance to the style and form requirements of the journal, and they are subject to editorial revision. No page charges will be assessed.

Letters to the Editor. The purpose of letters will be to discuss, critique, or expand on scientific points made in articles recently published in *Poultry Science*. Intro- duction of unpublished data will not be allowed, nor will material based on conjecture or speculation. Letters must be received within 6 months of an article's publica- tion. Letters will be limited to 400 words and 5 references (approximately 3 double-spaced, typed pages including references). Letters shall have a title. Author name(s) andaffiliation(s) shall be placed between the end of the text and list of references. Letters will be sent electroni- cally directly to the editor-in-chief for consideration. The author(s) of the original paper(s) will be provided a copy of the letter and offered the opportunity to submit for consideration a reply within 30 days. Replies will have the same page restrictions and format as letters, and the titles shall end with -—Reply.‖ Letters and replies will be published together. Acceptability of letters will be decided by the editor- in-chief. Letters and replies shall follow appropriate *Poultry Science* format and may be edited by the editor-in-chief and a technical editor. If multiple let- ters on the same topicare received, a representative letter concerning a specific article will be published. All letters may not be published. Letters and replies will be pub- lished as space permits.

SUBMISSION OF ELECTRONIC MANUSCRIPTS

Authors should submit their papers electronically (http://mc.manuscriptcentral.com/ps). Detailed instruc- tions for submitting electronically are provided online at that site. Authors who are unable to submit electronically should contact the editorial office (nes.diaz@oup.com) for assistance.

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Authors shall complete the Manuscript Submission and Copyright Transfer form for each new manuscript submission; faxed copies are acceptable. The form is publishedin *Poultry Science* as space permits and is avail- able online (http://ps.oxfordjournals.org). The copyright agreement is included in the Manuscript Submission and Copyright Transfer Form and must be completed by all authors before publication can proceed. The correspond- ing author is responsible for obtaining the signatures of coauthors. Persons unable to sign copyright agreements, such as federal employees, must indicate the reason for exemption on the form.

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If an author desires to reprint a figure published else- where, copyright permission to use the figure must be ob- tained by the author and forwarded to the PSA editorial office.

REVIEW OF MANUSCRIPTS

After a manuscript is submitted electronically, the edi- torial office checks the manuscript. If a manuscript does not conform to the format for *Poultry Science*, it willbe returned to the author (rejected) without review. Manu- scripts that pass initial screening will be forwarded to the appropriate section editor, who pre-reviews the manu- script and may suggest rejection at this early stage for fatal design flaw, inappropriate replications, lack of nov- elty, deviation from the Instructions for Authors, or other major concerns.

The section editor assigns two reviewers, at least one of whom is an associate editor. Each reviewer has 3 weeks to review the manuscript, after which his or her comments are forwarded to the section editor. The sec- tion editor may recommend rejection or acceptance at this point, after which the manuscript and reviewer com- ments are made available to the editor-in-chief for a final decision. More commonly, the manuscript will be sent back to the corresponding author for revision according to the guidelines of the reviewers. Authors have 6 weeks to complete the revision, which shallbe returned to the section editor. Failure to return the manuscript within 6 weeks will cause the paper to be purged from the files. Purged manuscripts may be reconsidered, but they will have to be processed as new manuscripts. Section editors handle all initial

correspondence with authors during the review process. The editor-in-chief will notify the author of the final decision to accept or reject. Rejected manu- scripts can be resubmitted only with an invitation from the section editor or editor-in-chief. Revised versions of previously rejected manuscripts are treated as new sub- missions. Therefore, authors must complete a new Manu- script Submission and CopyrightTransfer Form.

PRODUCTION OF PROOFS

Accepted manuscripts are forwarded by the editor-in- chief to the editorial office for technical editing and type- setting. At this point the technical editor may contact the authors for missing information or figure revisions. The manuscript is then typeset, figures reproduced, and au- thor proofs prepared.

Proofs

Author proofs of all manuscripts will be provided to the corresponding author. Author proofs should be read care- fully and checked against the typed manuscript, because the responsibility for proofreading is with the author(s). Corrections may be returned by fax (217-378-4083), mail, or e-mail. For faxed or mailed corrections, changes to the proof should be made neatly and clearly in the margins of the proof. If extensive editing is required, corrections should be provided on a separate sheet of paper with a symbol indicating location on the proof. Changes sent by e-mail to the technical editor must indicate page, column, and line numbers for each correction to be made on the proof. Corrections can also be marked using the note and highlight tools to indicate necessary changes. Author al- terations to copy exceeding 10% of the cost of composi-tion will be charged to the author.

Editor queries should be answered on the galley proofs; failure to do so may delay publication. Proof corrections should be made and returned to the technical editor within 48 hours of receipt. The publication charge form should be returned with proof corrections so as not to delay publication of the article.

Publication Charges and Offprints

Poultry Science has two options available for the pub- lication of articles: conventional page charges and Open Access (**OA**).

OA. For authors who wish to publish their papers OA (available to everyone when the issue is posted online), au- thors will pay the OA fee when proofs are returned to the editorial office. Charges for OA are $1,500 if at least one au- thor is a current professional member of PSA; the charge is $2,000 when no author is a professional member of PSA.

Conventional Page Charges. The current charge for publication is $100 per printed page (or fraction thereof) in the journal if at least one author is a professional mem-
ber of PSA. If no author is a member of PSA, the publication charge is $170 per journal page.

Offprints. Offprints may be ordered at an additional charge. When the galley proof is sent, the author is askedto complete an offprint order requesting the number of offprints desired and the name of the institution, agency, or individual responsible for publication charges.

Color Charges. The cost to publish in color in the print journal is $600 per color image; a surcharge for off- prints will also be assessed. At the time of submission on ScholarOne Manuscripts, authors will be asked to ap- prove color charges for figures that they wish to have published in color in the print journal. Color versions of figures will be included in the online PDF and full-text article at no charge.

MANUSCRIPT PREPARATION: STYLE AND FORM

General

Papers must be written in English. The text and all sup- porting materials must use American spelling and usage as given in *The American Heritage Dictionary, Webster's Third New International Dictionary,* or the *Oxford Ameri- can English Dictionary.* Authors should follow the style and form recommended in *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers.* 2006. 7th ed. Style Manual Committee, Council of Science Editors, Reston, VA.

Authors should prepare their manuscripts with Microboldface and italic. Text that follows a first subheading should be in a new paragraph.

Second Subheadings. Second subheadings begin the first line of a paragraph. They are indented, boldface, italic, and followed by a period. The first letter of each important word should be capitalized. The text follows immediately after the final period of the subheading.

Title Page

The title page shall begin with a running head (short title) of not more than 45 characters. The running head is centered, is in all capital letters, and shall appear on the top of the title page. No abbreviations should be used.

The title of the paper must be in boldface; the first letter of the article title and proper names are capitalized, and the remainder of the title is lowercase. The title must not have abbreviations.

Under the title, names of authors should be typed (first name or initial, middle initial, last name). Affili- ations will be footnoted using the following symbols:

*, †, ‡, §, #, ‖, and be placed below the author names. Do not give authors' titles, positions, or degrees. Num- bered footnotes may be used to provide supplementary information, such as present address, acknowledgment of grants, and experimentstation or journal series num- ber. The corresponding author should be indicated with 1 soft Word and upload them using the fewest files pos a numbered footnote (e.g., Corresponding author: mysible to facilitate the review and editing process.

Authors whose primary language is not English are strongly encouraged to use an English-language service to facilitate the preparation of their manuscript. A partial list of services can be found in the *Poultry Science* Manuscript checklist.

Preparing the Manuscript File

Manuscripts should be typed double-spaced, with lines and pages numbered consecutively, using Times New Roman font at 12 points. All special characters (e.g., Greek, math, symbols) should be inserted using the sym- bols palette available in this font. Complex math should be entered using MathType from Design Science (http:// www.dessci.com). Tables and figures should be placed in separate sections at the end

of the manuscript (not placed within the text). Failure to follow these instructions may result in an immediate rejection of the manuscript.

Headings

Major Headings. Major headings are centered (ex- cept ABSTRACT), all capitals, boldface, and consist of ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), ACKNOWLEDGMENTS (optional), AP- PENDIX (optional), and REFERENCES.

First Subheadings. First subheadings are placed on a separate line, begin at the left margin, the first letter of all important words is capitalized, and the headings are name@university.edu). Note that there is no period after the corresponding author's e-mail address.

The title page shall include the name and full address of the corresponding author. Telephone and FAX numbers and e-mail address must also be provided. The title page must indicate the appropriate scientific section for the paper (i.e., Education and Production; Environment, Well-Being, and Behavior; Genetics; Immunology, Health, and Disease; Metabolism and Nutrition; Molecular, Cellular, and Developmental Biology; Physiology, Endocrinology, and Reproduction; or Processing, Products, and Food Safety).

Authors may create a full title page as a one-page document, in a file separate from the rest of the paper. This file can be uploaded and marked ‑not for review.‖ Authors who choose to upload manuscripts with a full title page at the beginning will have their papers forwarded to reviewers as is.

Abbreviations

Author-derived abbreviations should be defined at first use in the abstract and again in the body of the manuscript. The abbreviation will be shown in bold type at first use in the body of the manuscript. Refer to the Miscellaneous Usage Notes for more information on abbreviations.

Abstract

The Abstract disseminates scientific information through abstracting journals and through conveniencefor the readers. The Abstract, consisting of not more than 325 words, appears at the beginning of the manuscript with the word ABSTRACT without a following period. It must summarize the major objectives, methods, results, conclusions, and practical applications of the research. The Abstract must consist of complete sentences and use of abbreviations should be limited. References to other work and footnotes are not permitted. The Abstract and Key Words must be on a separate sheet of paper.

Key Words

The Abstract shall be followed by a maximum of five key words or phrases to be used for subject indexing. These should include important words from the title and the running head and should be singular, not plural, terms (e.g., broiler, not broilers). Key words should be formatted as follows: **Key words:** . . .

Introduction

The Introduction, while brief, should provide the read- er with information necessary for understanding research presented in the paper. Previous work on the topic should be summarized, and the objectives of the current research must be clearly stated.

Materials and Methods

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 num- bers 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 refer- ences.

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.

Papers must contain analyzed values for those dietary ingredients that are crucial to the experiment. Papers deal- ing with the effects of feed additives or graded levels of a specific nutrient must give analyzed values for the rel- evant additive or nutrient in the diet(s). If products were used that contain different potentially active compounds, then analyzed values for these coupounds must be given for the diet(s). Exceptions can only be made if appropri- ate methods are not available. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as ap- propriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phos- phorus contents should also be specified.

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 equiv- alents:

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 β-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 ac- ceptable 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. Statisti- cal methodscommonly 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. Refer- ence to a statistical package without reporting the sourc- es 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 anal- ysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar ex-periments 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 chemi- cal analyses of the samesample usually do not consti- tute independent experimental units. Measurements on the same experimental unit over time also are not inde- pendent and must not be considered as independent ex- perimental 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 ex- ample, it is recommended that percentage data between 0 and 20 and between 80 and 100 be subjected to arc sin transformation priorto analysis. Most statistical pro- cedures are based on the assumption that experimental units have been assigned to treatments at random. If ani- mals are stratified by ancestry or weight or if some other initial measurement should be accounted for, the model should include a blocking factor, or the initial measure- ment 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 vari- able, 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 ar- rangement in 5 randomized complete blocks consisting of initial BW.‖ Note that **a factorial arrangement is not a de- sign**; 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 er- ror 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 anystatistical 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 readeras 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 re- porting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of ex- perimental 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 ob- servations 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* sta- tistic. Unbalanced factorial data can present special prob- lems. Accordingly, it is wellto state how the computing was done and how the parameters were estimated. Ap- proximations should be accompanied by cautions con- cerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was de- signed; they should form the basis for comparing treat- ment means. Nonorthogonal contrasts may be evalu- ated by Bonferroni *t* statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally ar- ranged. 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 ar- rangements, 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 impor- tant 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 tradition- ally have been reserved for $P < 0.05$ and $P < 0.01$, re- spectively; 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 deci- mal. 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‖ rela- tionship 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 con- tained 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.

Results and Discussion

Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Re- sults 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 submit- ting. One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experi- ments are adequately replicated. The results of experi- ments must be replicated, either by replicating treatments within experiments or by repeating experiments.

Acknowledgments

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.

Appendix

A technical Appendix, if desired, shall follow the Dis- cussion section or Acknowledgments, if present. The Appendix may contain supplementary material, expla- nations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel com- puter programs or mathematical computations would be appropriate. The Appendix will not be a repository for raw data.

References

Citations in Text. In the body of the manuscript, re- fer 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. Morethan 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 listedin 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 sec- tion, papers must be published or accepted for publica- tion. Manuscripts submitted for publication can be cited as -personal communication‖ or -unpublished data‖ in the text.

Citation of abstracts, conference proceedings, and oth- er works that have not been peer reviewed is strongly discouraged unless essential to the paper. Abstract and proceedings references are not apropriate citations in the Materials and Methodssection of a paper.
In the References section, references shall first be list- ed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the au- thors' names. As with text citations, two or more publi- cations 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 abbrevia-tions given in journals database of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/que- ry.fcgi?db=journals). 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.
Article:
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Tables

Tables must be created using the MS Word table fea- ture 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 theprinted page when planning tables (use of more than 15 columns will create layout prob- lems). 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 body should be done sparingly; such use must be defined in a footnote. Each table must be on a separate page. To facilitateplace- ment 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 $\dagger P \leq 0.10$. Consult a recent issue of *Poultry Science* for examples of tables.

Figures

To facilitate review, figures should be placed at the end of the manuscript (separated by section breaks). Each figure should be placed on a separate page, and identi- fied by the manuscript number and the figure number. A figure with multiple panels or parts should appear on one page (e.g., if Figure 1 has parts a, b, and c, place all of these on the same page). Figure captions should be typed (double spaced) on a separate page.

• ***Figure Size.*** Prepare figures at final size for publi- cation. Figures should be prepared to fit one column (8.9 cm wide), 2 columns (14 cm wide), or full-page width (19 cm wide).

• ***Font Size.*** Ensure that all type within the figure and axis labels are readable at final publication size. A minimum type size of 8 points (after reduction) should be used.
• ***Fonts.*** Use Helvetica or Times New Roman. Sym- bols may be inserted using the Symbol palette in Times New Roman.
• ***Line Weight.*** For line graphs, use a minimum stroke weight of 1 point for all lines. If multiple lines are to be distinguished, use solid, long-dash, short-dash, and dotted lines. Avoid the use of color, gray, or shaded lines, as these will not reproduce well. Lines with different symbols for the data points may also be used to distinguish curves.
• ***Axis Labels.*** Each axis should have a description and a unit. Units may be separated from the de- scriptor by a comma or parentheses, and should be consistent within a manuscript.
• ***Shading and Fill Patterns.*** For bar charts, use dif- ferent fill patterns if needed (e.g., black, white, gray, diagonal stripes). Avoid the use of multiple shades of gray, as they will not be easily distinguishable in print.
• ***Symbols.*** Identify curves and data points using the following symbols only: □, ■, ○, ●, ▲, ▼, n, ,, e, r, +, or ×. Symbols should be defined in a key on the figure if possible.
• ***File Formats.*** Figures can be submitted in Word, PDF, EPS, TIFF, and JPEG. Avoid PowerPoint files and other formats. For the best printed quality, line art should be prepared at 600 ppi. Grayscale and color images and photomicrographs should be at least 300 ppi.
• ***Grayscale Figures.*** If figures are to be reproduced in grayscale (black and white), submit in grayscale. Often color will mask contrast problems that are ap- parent only when the figure is reproduced in gray- scale.
• ***Color Figures.*** If figures are to appear in color in the print journal, files must be submitted in CMYK color (not RGB).
• ***Photomicrographs.*** Photomicrographs must have their unmagnified size designated, either in the cap- tion or with a scale bar on the figure. Reduction for publication can make a magnification power desig- nation (e.g., 100×) inappropriate.
• ***Caption.*** The caption should provide sufficient in- formation that the figure can be understood with excessive reference to the text. All author-derived abbreviations used in the figure should be defined in the caption.
• ***General Tips.*** Avoid the use of three-dimensional bar charts, unless essential to the presentation of the data. Use the simplest shading scheme possible to present the data clearly. Ensure that data, symbols, axis labels, lines, and key are clear and easily read- able at final publication size.
Color Figures. Submitted color images should be at least 300 ppi. The cost to publish each color figure is $600; a surcharge for color reprints ordered will be assessed. Authors must agree in writing to bear the costs of color production after acceptance and prior to publication of the paper.

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 scien- tific 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 ab- breviations 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 edi- tors 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. Allterms are to be spelled out in full with the abbrevia- tion following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbre- viations 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, ab- breviations shall be defined within each of these units of the manuscript.

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 2
R coefficient of determination, multiple

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 ab- breviated 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
RFLP restriction fragment length polymorphism
RH relative humidity
RIA radioimmunoassay
RNA ribonucleic acid

rpm revolutions per minute
s second
SD standard deviation
SDS sodium dodecyl sulfate
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., invitro, in vivo, in situ, a priori). However, genus and spe- cies of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. Ger- man 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 precededby numbers must be writ- ten 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 se- quences be submitted to one of the sequence databases. Acceptance for publication is contingent

on the submis- sion of sequence data to one of the databases. The fol- lowing statement should appear as a footnote to the title on the title page of the manuscript.

-The nucleotide se- quence data reported in this paper have been submitted to GenBank Submission (Mail Stop K710, Los Alamos Na- tional Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN.‖

Publication of the description of molecular clones is as- sumed 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.

Gene and Protein Nomenclature. Authors are re- quired to use only approved gene and protein names and symbols. For poultry, full gene names should not be itali- cized. Gene symbols should be in uppercase letters and should be in italics. A protein symbol should be in the same format as its gee 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 num- bered 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, 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.

Supplemental Information

The following information is available online and up- dated regularly. Please refer to these pages when prepar- ing a manuscript for submission.

Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online: http://www.oxfordjournals.org/our_journals/ps/for_authors/index.html

SI Units. The following site (National Institute of Standards and Technology) provides a comprehensive guide to SI units and usage: http://physics.nist.gov/Pubs/SP811/contents.html

Figure Preparation Guidelines. Current detailed information on figure preparation can be found at http:// www.oxfordjournals.org/for_authors/figures.html

ScholarOne Manuscripts Instructions. Manuscripts are submitted online (http://mc04.manuscriptcentral. com/ps). Full user instructions for using the ScholarOne Manuscripts system are available on the ScholarOne Manuscripts home page.

VITA

Patrícia Soster de Carvalho, filha de Celso Troian de Carvalho e de Denise Beatriz Soster de Carvalho, nascida em 8 de julho de 1991, em Porto Alegre – RS. Completou o ensino fundamental no colégio São João e o ensino médio no Colégio João Paulo, ambos localizados na cidade de Porto Alegre – RS, concluindo os estudos em dezembro de 2008. Em 2012, ingressou no curso de Medicina Veterinária na Universidade Federal do Rio Grande do Sul. No último semestre da faculdade foi Estagiária Nível Superior na empresa BRF S.A., na unidade de Lajeado – RS, em frigorífico, sob a supervisão do Dr. Guayba, e na empresa Hy-Line do Brasil em Nova Granada - SP, em incubatório de matrizes leves, sob supervisão do Dr. Bruno Vieira. Formou-se Médica Veterinária em dezembro de 2018. No primeiro semestre de 2019 ingressou como aluna 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 um evento científico internacional, onde realizou apresentaçãos oral em inglês. Foi submetida à banca de defesa de Dissertação em Março de 2021.