UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

FITASE E FONTES MINERAIS PARA FRANGOS DE CORTE

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

Porto Alegre (RS), Brasil
Março de 2018

CIP - Catalogação na Publicação

Serafini, Natália
Fitase e fontes minerais para frangos de corte / Natália Serafini. -- 2018.
66 f.
Orientador: Liris Kindlein.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, BR-RS, 2018.

1. frango de corte. 2. enzimas exógenas. 3. fitase. 4. minerais. 5. nutrição animal. I. Kindlein, Liris, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

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DISSERTAÇÃO

Submetida como parte dos requisitos

para obtenção do Grau de

MESTRE EM ZOOTECNIA

Programa de Pós-Graduação em Zootecnia

Faculdade de Agronomia

Universidade Federal do Rio Grande do Sul

Porto Alegre (RS), Brasil

Aprovada em: 23.03.2018
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AGRADECIMENTOS

Agradeço à Deus por guiar meus passos e permitir que cada dificuldade seja uma oportunidade de crescimento.

Agradeço à minha filha Helena, que com sua chegada ressignificou toda a minha vida. Ao meu marido Gabriel, por me apoiar, incentivar e sempre acreditar em mim. A eles, que são a motivação diária para tudo que faço, minha mais sincera gratidão.

Aos meus pais, agradeço pela vida e por tudo o que me ensinaram ao longo dela. Obrigada pela educação e minha formação como pessoa.

À toda a minha família e à família Frainer Correa, pela compreensão nos momentos de ausência, pelo apoio e por torcerem por mim.

Ao professor Sergio Luiz Vieira, por todas as oportunidades proporcionadas e orientação, desde a época da graduação.

À professora Liris Kindlein, pela orientação e receptividade de sempre.

Aos colegas do Aviário de Ensino e Pesquisa, pela amizade, momentos de descontração e todo o auxílio nos experimentos. Cada um ocupa espaço especial no coração e nas lembranças. Agradeço também ao senhor Jairo Padilha pelo auxílio nas atividades do aviário.

Aos professores e funcionários do Programa de Pós-Graduação em Zootecnia - UFRGS.

Ao CNPq pela bolsa de Mestrado.

Por fim, agradeço a todas as pessoas que de forma direta ou indireta contribuíram para a realização deste trabalho.

FITASE E FONTES MINERAIS PARA FRANGOS DE CORTE[1]

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RESUMO – Um estudo foi realizado para avaliar os efeitos da suplementação de uma fitase e duas fontes de Zinco (Zn), Cobre (Cu) e Manganês (Mn) sobre o desempenho produtivo e a digestibilidade de nutrientes em frangos de corte. Um total de 528 pintos da linhagem Cobb 500, machos com um dia (d) de idade foram distribuídos em 4 tratamentos com 12 repetições de 11 aves cada. Um arranjo fatorial 2 x 2 foi utilizado, sendo duas suplementações de fitase (com ou sem) e duas fontes minerais (inorgânica ou orgânica). A suplementação de fitase foi de 500 unidades de fitase (FTU)/kg, enquanto Zn-Cu-Mn foram suplementados em concentrações de 32-30-32 ou 100-120-100 ppm para as formas orgânica e inorgânica, respectivamente. Foi utilizado um programa alimentar de duas fases: inicial (1 a 12 d) e crescimento (12 a 25 d). As dietas foram formuladas de forma a atender as exigências nutricionais dos animais de acordo com a idade, exceto para Fósforo (P) disponível (Pd) e Cálcio (Ca), que tiveram níveis reduzidos (0,32% e 0,77 % na dieta inicial e 0,23% e 0,71 % na dieta crescimento para Pd e Ca, respectivamente). Os níveis de metionina nas dietas foram reduzidos conforme a adição de minerais orgânicos, que tinham como agente quelante metionina hidróxi-análoga (HMTBA). As tíbias das aves foram coletadas aos 12 e aos 25 dias de idade para determinação do teor de cinzas, Ca e P. Aos 25 dias, também, foi coletado conteúdo ileal para determinação da digestibilidade ileal aparente da matéria seca (MS), Ca e P. A suplementação de fitase aumentou o ganho de peso (GP) e a conversão alimentar (CA) dos frangos dos 12 aos 25 dias e também no período acumulado (1 a 25 d). Foi observada interação entre fontes minerais e as fitases para digestibilidade de MS e P ($P<0,05$). A digestibilidade ileal da matéria seca foi maior nos frangos alimentados com dietas suplementadas com fitase, e também naqueles que receberam fontes inorgânicas de Zn-Cu-Mn. Os frangos que receberam dietas com fitase tiveram melhores coeficientes de digestibilidade de Ca e P ($P<0,05$). A fonte orgânica de microminerais resultou em maior o conteúdo de tíbia em percentual aos 12 dias. A suplementação de 500 FTU/kg de fitase nas dietas à base de milho e soja também levou a um aumento no conteúdo de cinzas das tíbias aos 12 e 25 dias, mas não houveram diferenças entre o conteúdo de Ca e P entre os animais alimentados com e sem fitase. Conclui-se que a suplementação de fitase melhora o desempenho produtivo, digestibilidade ileal de Ca e P e a mineralização óssea, e que concentrações mais baixas de minerais, através do uso de fontes orgânicas, podem ser utilizadas sem prejuízos ao desempenho animal.

Palavras chave: mineralização óssea, frango de corte, digestibilidade, desempenho, fitase.

[1]Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (66 p.) Março, 2018.

PHYTASE AND MINERAL SOURCES TO BROILER CHICKENS[2]

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ABSTRACT – A study was conducted to evaluate the effects of dietary supplementation of phytase and mineral sources of zinc (Zn), copper (Cu) and manganese (Mn) on growth performance and nutrient digestibility of broiler chickens. A total of 528 Cobb x Cobb 500 male chicks were distributed into 4 treatments with 12 replicates of 11 birds each. A 2 x 2 factorial arrangement was used with two enzyme supplementation (with or without) and two mineral sources (inorganic or organic). Phytase supplementation were 500 phytase units (FTU)/kg whereas Zn-Cu-Mn were supplemented in a concentration of 32-30-32 or 100-120-100 ppm in organic and inorganic forms, respectively. A two-phase feeding program was used, from 1 to 12 (starter) and from 12 to 25 d (grower). Diets were formulated to meet bird's nutritional requirements according to age, except for Available Phosphorus (Av.P) and Calcium (Ca), that were formulated at 0,32% and 0,77% in starter and 0,23% and 0,71% in grower, to Av P and Ca, respectively. Methionine levels were reduced according to organic minerals supplementation, that had hydroxy-analogue methionine (HMTBA) as the chelating agent. Tibiae were collected at 12 and 25 d to measure ash, Ca and P content. Also, at 25 d, ileal contents were collected to determine apparent ileal digestibility of dry matter (DM), Ca and P. Body weight gain (BWG) and feed conversion ratio (FCR) was higher with phytase supplementation from 12 to 25 d and 1 to 25 d. Dry matter (DM) digestibility was higher in animals fed diets with phytase and also in those receiving inorganic minerals. Ca and P digestibility were improved by phytase. Interactions between mineral sources and enzyme were observed to DM and P digestibility. Treatment consisting of inorganic minerals and phytase was associated with higher values of P and DM digestibility. Organic mineral source improved ash content in percentage at 12 d. Supplementing phytase to the diets led to an increase in the percentage of ash content at 12 and 25 d, but there were no statistical differences in Ca and P content between animals receiving diets with or without the enzyme. In conclusion, phytase has benefitial impacts on performance, digestibility and bone mineralisation, and lower concentrations of minerals, with organic source, can be supplied without losses to animal performance.

Key words: bone mineralization, broiler, digestibility, performance, phytase.

[2]Master of Science dissertation in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (66 p.) March, 2018.

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RELAÇÃO DE ABREVIATURAS

Av P	Available phosphorus
BWG	Body weight gain
Ca	Cálcio
CA	Conversão alimentar
Cu	Cobre
DM	Dry matter
FCR	Feed conversion rate
FI	Feed intake
Mn	Manganês
MS	Matéria seca
P	Fósforo
P_d	Fósforo disponível
Ppm	Partes por milhão
Zn	Zinco

CAPÍTULO I

INTRODUÇÃO

A indústria avícola brasileira utiliza como base da formulação das rações o milho e o farelo de soja, que são as principais fontes de energia e proteína, respectivamente. Estes ingredientes, que têm alta digestibilidade para as aves, também possuem quantidades variáveis de ácido fítico, que está associado à menor digestão e aproveitamento do fósforo presente nas dietas, assim como outros fatores antinutricionais (Bach Knudsen, 1997; Choct, 1997; Meng et al., 2005). Um maior aproveitamento dos nutrientes pode ser obtido através da suplementação de enzimas exógenas. A fitase, enzima que degrada o ácido fítico, tem sido amplamente utilizada na formulação de rações para frangos de corte devido à grande ênfase em pesquisas, aliada à possibilidade de melhorar a disponibilidade de nutrientes e reduzir custos de produção (Jozefiak et al., 2010; Cowieson et al., 2011).

Em dietas comerciais, a maioria dos microminerais é comumente suplementada na forma inorgânica, como sulfatos, óxidos e carbonatos, para fornecer níveis que permitam que as aves atinjam todo o seu potencial de crescimento (Bao et al., 2007). Incertezas relacionadas à absorção e utilização de microminerais inorgânicos e o baixo custo desta fonte mineral levou ao uso excessivo desses minerais nas dietas (Saripinar-Aksu et al, 2012). Entretanto, o uso de grandes quantidades pode reduzir a absorção e biodisponibilidade de outros nutrientes presentes nas dietas (Underwood & Suttle, 1999), devido à interação entre as moléculas quando no processo digestivo. O uso contínuo destes sais inorgânicos como aditivos alimentares tem sido implicado em poluição ambiental devido à sua acumulação nas excretas das aves (Bao & Choct, 2009). Devido as crescentes preocupações sobre o potencial poluidor das moléculas minerais, a utilização de fontes organicamente complexadas ou quelatadas em concentrações mais baixas tem sido sugerida para a produção animal, baseado na hipótese de que minerais orgânicos têm maior biodisponibilidade do que os sais inorgânicos análogos (Saripinar-Aksu et al., 2012).

Assim, este estudo teve como objetivo avaliar os efeitos da suplementação de fitase e de fontes inorgânicas e orgânicas de cobre (Cu), zinco (Zn) e manganês (Mn) sobre o desempenho zootécnico, aproveitamento de nutrientes e mineralização óssea de frangos de corte.

REVISÃO BIBLIOGRÁFICA

Enzimas exógenas

Enzimas são proteínas que possuem estrutura tridimensional e atuam acelerando processos químicos, agindo como catalisadores biológicos no metabolismo animal (Slominski, 2011). Lehninger (2000) as define como moléculas proteicas complexas que catalisam reações químicas; de alta especificidade para as reações que catalisam e para os substratos que estão envolvidos na reação; que exigem que sua estrutura permaneça inalterada para garantir sua atividade; que podem ser inativadas e desnaturadas por pH extremo e calor, e que também possam ser degradadas por outras enzimas. Para que uma reação enzimática aconteça no trato gastrointestinal (TGI), condições ambientais adequadas devem existir, e estas condições são diferentes e pouco previsíveis daquelas existentes em ambientes *in vitro*, onde geralmente as enzimas são avaliadas. Como resultado, pode haver uma maior dificuldade no entendimento da atuação das enzimas em condições ambientais diferenciadas, como é o caso do processamento das dietas e do processo de digestão no TGI (Stefanello, 2016).

Fitases, carboidrases e proteases são enzimas naturalmente secretadas por uma gama de microorganismos para satisfazer suas exigências metabólicas, e estas atividades são rastreadas para características úteis apropriadas para aplicação nas rações. A maioria das enzimas alimentares comercialmente disponíveis atualmente são obtidas através de sistemas de fermentação otimizada contando com o uso de bactérias ou fungos geneticamente modificados (Adeola & Coiweson, 2011). Enzimas exógenas têm sido utilizadas comercialmente nas rações para aves com a finalidade de melhorar o aproveitamento de nutrientes e reduzir o efeito de anti-nutrientes (Stefanello, 2016). Esta suplementação apresenta como principais objetivos 1) complementar a ação de enzimas endógenas e, também, 2) fornecer enzimas que não são sintetizadas no trato gastrointestinal, como é o caso da fitase (Cowieson & Adeola, 2005), que atualmente é o principal aditivo enzimático utilizado no sistema de produção comercial de frangos de corte.

Fitase e fósforo fítico

Ainda que sejam considerados de alta digestibilidade para as aves, o milho e o farelo de soja apresentam em suas composições substâncias que não são eficientemente acessadas pelas enzimas digestivas desta espécie, o que requer a utilização de enzimas exógenas específicas para melhorar o aproveitamento dos nutrientes (Cowieson & Adeola, 2005; Sorbara, 2009).

O ácido fítico (hexafosfato de mio-inositol) é a maior forma de estocagem de fósforo (P) na maioria das plantas, sendo abundante nas sementes (Zeng et al., 2011), e requer a ação da enzima fitase para a hidrólise e posterior liberação de P para o metabolismo animal. Estima-se que 40 a 60% do conteúdo de P de dietas típicas para frangos de corte nas diferentes fases de crescimento, à base de milho e farelo de soja, esteja na forma de ácido fítico (NRC, 1994). Contudo, as fitases não são secretadas por monogástricos, o que torna necessária a suplementação de P a partir de fontes inorgânicas nas dietas, para atender às exigências nutricionais das aves. Este, além de ser um recurso mineral caro e não renovável, aumenta o impacto ambiental da atividade avícola, já que o P não disponível é excretado em grandes quantidades pelos animais e, quando aplicado ao solo, contribui, através de escoamento ou lixiviação, para a eutrofização e poluição de águas (Summers, 1997). Além de limitar a disponibilidade do P, o ácido fítico traz outros efeitos deletérios para os animais monogástricos. Em pH baixo, o ácido fítico forma ligações eletrostáticas com alguns aminoácidos, minerais, amido e proteínas, resultando em complexos insolúveis. Conforme o pH aumenta, o complexo se torna mais solúvel e, nesta forma, se complexa com proteínas devido a presença de cátions divalentes, principalmente cálcio, magnésio ou zinco, que agem como uma ponte entre os grupos carboxila com carga negativa das proteínas e o fitato (Ravindran et al., 1999). Estas proteínas complexadas com o fitato estão menos sujeitas à ação de enzimas proteolíticas endógenas, não sendo eficientemente digeridas (Ravindran et al., 1995). Portanto, além de melhorar a liberação de P para o metabolismo, espera-se que a fitase melhore a liberação de aminoácidos e proteínas dietéticas, além da energia a partir do amido.

A utilização de fitases exógenas na dieta de frangos de corte foi a alternativa encontrada para aumentar a disponibilidade do P das dietas, reduzindo a sua adição por fonte inorgânica. Contudo, caso esta adição não seja diminuída ao se utilizar fitase, a excreção de P disponível aumentará (Angel et al., 2005). Por isso, frente à possibilidade do uso de aditivos enzimáticos, é necessário que se realize uma análise criteriosa da composição nutricional da dieta na qual será aplicada. A adição da fitase em rações para frangos de corte está amplamente consolidada na indústria devido à grande ênfase em pesquisas envolvendo sua utilização e efeitos, comprovando sua eficácia na disponibilização de nutrientes, sendo o primeiro estudo conduzido sobre a eficácia de uma fitase microbiana sobre a hidrólise do P datando de 1971, por Nelson e colaboradores. Além de atuar diretamente na disponibilização de P, o que também aumenta a deposição óssea deste mineral, a fitase também tem sido associada a ganhos indiretos, como uma melhor digestibilidade de outros nutrientes da dieta. O aumento da digestibilidade de aminoácidos e da energia metabolizável aparente (EMA) da ração pode ser o resultado da liberação das moléculas decorrente da hidrólise realizada pela fitase e também pela redução das perdas endógenas (Cowieson et al., 2006; Selle & Ravindran, 2007).

As fitases relevantes para a alimentação animal são divididas em 2 subclasses (3- ou 6-fitases), dependentes de por qual fosfato se inicia a catálise no núcleo mio-inositol. Quando adicionadas às dietas, são capazes de hidrolisar a ligação de éster entre o carbono 3 (no caso das 3-fitases) ou o carbono 6 (no caso de 6-fitases) e o grupo fosfato associado, liberando o fosfato do fitato para o animal. Após a hidrólise no site de eleição, as fitases então se movem sequencialmente em torno do anel de inositol, liberando grupos fosfato adicionais (Adeola & Coiweson, 2011).

Não há denominação comum para as unidades de fitases nos produtos comerciais, podendo ser denominadas FYT, PU, U e FTU. Uma unidade de fitase é definida como a quantidade de enzima que libera 1 micromol (µmol) de fósforo inorgânico por minuto, a partir de 5,1 µmol de fitato de sódio em pH 5,5 e temperatura de 37ºC (Engelen et al.,1994).

Microminerais na alimentação animal

Os minerais são nutrientes presentes em todos os tecidos e fluidos corporais dos seres vivos e envolvidos em uma série de processos essenciais à vida. Entre outras funções, compõem as membranas celulares e regulam a síntese hormonal. Além disso, são componentes essenciais de sistemas enzimáticos, portanto, deficiências destes compostos têm efeitos profundos no metabolismo e estrutura de tecidos (Soetan et al., 2010).

Os minerais são classificados em macrominerais e microminerais ou elementos traços. O que difere macro e microminerais são as quantidades em que são necessários para as funções que desempenham no organismo, sendo acima e abaixo de 100 mg/dL, respectivamente (Murray et al., 2000). Dos 109 elementos conhecidos, 26 são considerados essenciais para os animais Destes, 11 são macroelementos e 15 são microelementos (Vieira, 2008).

Minerais Inorgânicos

Na produção de frangos de corte, a principal forma de suplementação de microminerais é através de premixes que utilizam fontes inorgânicas como sulfatos, óxidos e carbonatos para promover níveis dietéticos adequados para os animais. Incertezas relacionadas à absorção e utilização de moléculas inorgânicas, aliado ao baixo custo desta fonte levou ao uso excessivo desses minerais nas dietas (Saripinar-Aksu et al, 2012). Isto pode, entretanto, reduzir a absorção e biodisponibilidade de outros nutrientes presentes nas dietas (Underwood & Suttle, 1999), devido à interação entre as moléculas no processo digestivo. Além disso, o uso contínuo destes sais inorgânicos em altas concentrações tem sido implicado em poluição ambiental devido à sua acumulação nas excretas das aves (Bao e Choct, 2009). Devido às crescentes preocupações sobre o potencial poluidor das moléculas minerais inorgânicas, a utilização de fontes organicamente complexadas ou quelatadas tem sido sugerida para a produção animal, que são utilizadas em concentrações mais baixas, baseado na hipótese de que esta fonte tem maior biodisponibilidade (Saripinar-Aksu et al., 2012), além de maior solubilidade e estabilidade no trato gastrointestinal (Vieira, 2008).

O denominador comum nas interações de antagonismos entre os minerais inorgânicos é a dissociação dos sais inorgânicos no pH relativamente baixo do trato gastrointestinal superior. Quando os minerais atingem o pH mais alto dos segmentos intestinais, se ligam a outras moléculas, sejam elas minerais ou frações da digesta, como fitato e fibras, que o tornam insolúvel (Dibner et al., 2007). Estas formas insolúveis, como mencionado, não são aproveitadas e são excretadas pelo animal.

Minerais Orgânicos

Os minerais quelatados são definidos por Leeson & Summers (2001) como sendo uma mistura de elementos minerais que são ligados a algum tipo de carreador, que pode ser um aminoácido ou polissacarídeo, e que possui a capacidade de se ligar ao metal por ligações covalentes, através de grupamentos amino ou oxigênio, formando assim uma estrutura cíclica. A vantagem dos microminerais orgânicos é que a ligação à molécula orgânica promove estabilidade do complexo no TGI superior. Os minerais orgânicos resistem à dissociação no papo, proventrículo e moela, permitindo que o complexo chegue intacto ao epitélio absortivo do intestino delgado (Leeson & Summers, 2001). Quando um aminoácido quelatado é formado, a molécula assume características de um di ou tripeptídeo, preservando sua estrutura molecular. Desta forma, o metal transita pelo intestino como uma destas moléculas, sem ser alterada pelo processo digestivo, e seu baixo peso molecular permite que seja absorvido intacto nas células da mucosa sem hidrólise luminal (Ashmead, 1993).

Assim, fontes orgânicas ou quelatos de minerais têm sido avaliadas devido a sua perspectiva de serem mais biodisponíveis do que na forma inorgânica (Kidd, 2003; Dibner et al., 2007).

Cobre, Zinco e Manganês

Estes três microminerais são catalistas ou constituintes de vários sistemas enzimáticos, e são parte de diversas proteínas e moléculas orgânicas envolvidas no metabolismo intermediário, cascatas de secreção hormonal e sistemas de defesa imunológica (Dieck et al., 2003). Estas moléculas influenciam o crescimento, desenvolvimento ósseo, empenamento, estrutura e função enzimática e apetite em frangos de corte (Nollet et al., 2007). Em suma, os microminerais são essenciais para a manutenção da sanidade, influenciando o crescimento corporal, produção e reprodução animal (Santos et al., 2015).

Vieira (2008) destaca que a matriz óssea é composta principalmente por colágeno, e a correta mineralização do esqueleto depende de seu crescimento e qualidade. Cu, Zn e Mn, assim como vitaminas, estão diretamente relacionadas com a formação da matriz óssea.

Os tecidos ósseo e muscular contêm a maior parte do zinco no organismo e possuem a capacidade de reter e acumular o excedente, liberando para o metabolismo quando em escassez na dieta (Emmert & Baker, 1995). O zinco é importante para o desenvolvimento de tecido ósseo e da pele (Peric et al., 2007) e está envolvido na síntese de colágeno e queratina (Pardo & Selman, 2005; Richards et al., 2010). Este metal promove síntese de colágeno e turnover da cartilagem e desenvolvimento ósseo (Caterson et al., 2000; Krane & Inada, 2008).

O cobre age como co-fator da lisil-oxidase, a enzima que controla o cross-linking do colágeno e da elastina (Kagan & Wande, 2003). A ingestão de quantidades adequadas de cobre é crucial para obter massa óssea em ossos longos (Heaney, 1988). Além disso, o cobre tem função na utilização de ferro no estágio inicial da hematopoiese, compondo o plasma sanguíneo como uma proteína carreadora de chamada eritrocuprina (Hays & Swenson, 1985).

O manganês é um cofator das enzimas hidrolases, descarboxilases e transferases (Murray et al., 2000). Está envolvido na formação dos glicosaminoglicanos contendo sulfatos de condroitina (Beattie & Avenell, 1992) e também na síntese dos proteoglicanos presentes na placa de crescimento ósseo das espécies avícolas (Liu et al., 1994), sendo essencial para o desenvolvimento de tendões e ossos. Acredita-se que a suplementação de cobre, zinco e manganês a partir de fontes orgânicas melhorem a mineralização óssea, bem como sua integridade estrutural, em comparação a fontes inorgânicas (Sirri et al., 2016).

HIPÓTESES E OBJETIVOS

Hipóteses

A utilização de fitase exógena em dietas para frangos de corte melhora o desempenho produtivo e a digestibilidade de nutrientes da ração quando comparada a dietas sem inclusão desta enzima.

A inclusão de fitase exógena nas rações aumenta a deposição de cálcio e fósforo na tíbia de frangos de corte em comparação a animais alimentados sem a inclusão enzimática.

O desempenho zootécnico e a digestibilidade de nutrientes de frangos de corte são melhorados pela utilização de fontes orgânicas dos minerais zinco, cobre e manganês, em comparação à utilização de fontes inorgânicas destes minerais nas dietas.

A inclusão de fontes orgânicas de zinco, cobre e manganês nas rações aumenta a deposição de cálcio e fósforo na tíbia de frangos de corte em comparação a animais alimentados com suplementos a partir de fontes inorgânicas.

Objetivos

Avaliar o efeito da inclusão de fitase exógena em rações milho-farelo de soja sobre o desempenho produtivo e digestibilidade de nutrientes em frangos de corte de 1 a 25 dias, comparando os efeitos em dois grupos: animais que receberam uma dieta com a inclusão enzimática e animais que receberam uma dieta sem.

Avaliar o efeito da inclusão de fitase exógena em dietas para frangos de corte sobre a mineralização óssea. Os efeitos foram avaliados e comparados tomando como base a observação dos animais que receberam *versus* os que não receberam a enzima, aos 12 e aos 25 dias de idade.

Avaliar os efeitos da inclusão de fontes minerais de zinco, cobre e manganês sobre o desempenho zootécnico e digestibilidade de nutrientes em frangos de corte de 1 a 25 dias. Os resultados foram avaliados e comparados entre os animais alimentados com fonte orgânica e os alimentados com fonte inorgânica daqueles minerais.

Avaliar comparativamente os efeitos da inclusão de fontes orgânicas e inorgânicas de zinco, cobre e manganês na deta para frangos de corte sobre a mineralização óssea. A avaliação das fontes minerais foi realizada aos 12 e aos 25 dias de idade dos animais, comparando os que receberam fontes inorgânicas e os que receberam fontes orgânicas dos minerais.

CAPÍTULO II[1]

[1] Artigo elaborado conforme as normas da revista *Animal Feed Science and Technology* (apêndice).

Effects of micro mineral sources and phytase on nutrient digestibility and mineral retention in broilers

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ABSTRACT

A study was conducted to evaluate the effects of phytase and two sources of zinc (Zn), copper (Cu) and manganese (Mn) on growth performance, bone mineralisation and nutrient digestibility of broiler chickens. A total of 528 Cobb × Cobb 500 one-day(d)-old male chicks were distributed into 48 battery cages, in 4 treatments with 12 replicates of 11 birds each. A 2 x 2 factorial arrangement of 2 phytase supplementation [without or with 500 phytase units (FTU)/kg] and two sources of Zn-Cu-Mn (inorganic or organic). The inorganic form was included as sulphate at 100-120-100 ppm while the organic mineral had Zn-Cu-Mn chelated with DL-2-hydroxy-(4-methylthio) butanoic acid (HMTBA) and was added at 32-30-32 ppm. A two-phase feeding program was used, from 1 to 12 and 12 to 25 d. Performance was evaluated from 1 to 25 d. Tibiae were collected at 12 and 25 d to measure ash, Ca and P content. At 25 d, ileal content was collected to determine ileal digestibility of Ca, P and dry matter. Broilers fed diets supplements with phytase had higher BWG and lower FCR ($P<0.05$) compared to birds not supplemented from 12 to 25 and from 1 to 25 d. At 25 d, bone ash and digestibility of Ca and P also were improved ($P<0.05$) by phytase supplementation. Inorganic mineral source resulted in lower FCR and FI from 1 to 12 d ($P<0.05$). An interaction between mineral sources and phytase was observed on dry matter and P digestibility where the diet with inorganic source formulated with 500 FTU/kg was associated to higher P digestibility ($P<0.05$). In conclusion, phytase has benefitial impacts on performance, digestibility and bone mineralisation parameters. Zinc, copper and manganese in lower concentrations and organic source can be supplied without losses to animal performance.

Keywords: bone mineralization, broiler, digestibility, performance, phytase.

Abbreviations: Av. P., available phosphorus; BWG, body weight gain; Ca, calcium; Cu, cupper; CP, crude protein; d, days; dig., digestible; DM, dry matter; FCR, feed conversion ratio corrected for dead birds; FI, feed intake; FTU, phytase units; HMTBA, DL-2-hydroxy-(4-methylthio) butanoic acid; IDM, ileal digestibility of dry matter; Mn, manganese; P, phosphorus; SBM, soybean meal; Zn, zinc.

1. Introduction

With concerns about human, animal and environmental health and respecting international restrictions imposed by some countries, increasingly amounts of vegetable ingredients has been utilized by nutritionists when formulating diets to broilers. Since the BSE (Bovine spongiform encephalopathy) outbreaks around the world on the final of the 90's, concerns upon animal and human health have been raised (Vieira and Lima, 2005). As a preventive measure designed to stop the transmission of this disease and to minimize the potential risks to humans, the use of animal by-products in the manufacture of animal feeds given to all farmed animals destined to the production of human food, which included poultry, was prohibited in the European Union (CEC, 2000). Authorities from other countries, such as Saudi Arabia, a major broiler chicken importer, also adopted this policy. Currently, there is a trend in the global market of all-vegetable-fed animals, including poultry, leaving no choice for the industry but to utilise ingredients that met those requirements. Furthermore, concerns about the contribution to environmental pollution that poultry industry represents is a well known issue, since poultry wastes are rich mainly in P. In feeds of plant origin, the majority of phosphorus (P) is bound as phytate, the salt of phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate or $InsP_6$). $InsP_6$-P has to be cleaved in the gastrointestinal tract by phytases and other phosphatases prior to absorption, but insufficient secretion of endogenous enzymes in nonruminants limits phytate hydrolysis (Ingelmann et al., 2018). This inefficient phytic-P absorption causes inorganic P supplementation needed. Currently, exogenous phytase is the main enzyme additive utilized by nutritionists in poultry nutrition world wide. Microbial phytases, when added to the diet, are able to hydrolyze the ester bond between carbon and the associated phosphate group, liberating the phosphate from phytate and some nutrients as amino acids, starch and minerals (Adeola and Coiweson, 2011). Phytase supplementation has enabled a more efficient utilisation of phytate-P and reduction of P pollution (Santos et al., 2015).

Trace minerals such as zinc (Zn), copper (Cu) and manganese (Mn) are essential for maintaining health and immunity as well as being involved in animal growth, production and reproduction (Santos et al., 2015). In commercial poultry diets, the majority of micro minerals is usually supplemented with inorganic forms. However, uncertainties related to the absorption and utilisation of inorganic trace minerals and their low cost lead to the excessive use of trace mineral in these diets (Saripinar-Aksu et al, 2012), and consequently, reduced nutrient absorption and bioavailability (Underwood and Suttle, 1999), thus producing wastes with higher mineral content. Due to the increasing concerns about potential mineral pollution, the use of organic trace minerals, which are chelated with amino acids, has been suggested for livestock diets, based on the hypothesis that such mineral complexes have a higher bioavailability than inorganic salts analogues (Saripinar-Aksu et al., 2012). Exogenous sources of phytase and trace elements are regularly supplemented to monogastric diets to meet animal's nutritional requirements. However, the possibility for negative interaction between individual components within the premix is high and is often overlooked (Santos et al., 2015). Phytic acid is considered to be an antinutritional factor for humans and animals as it may chelate nutritionally important cations, which include Cu^{2+}, Zn^{2+}, Mn^{2+} and Ca^{2+}, among others (Persson et al., 1998; Tran et al., 2011).

The objective of this study was to evaluate the effects and interactions of an exogenous phytase and inorganic or organic sources of micro minerals on growth performance, nutrient utilisation and bone mineralisation in broilers fed maize-soybean meal (SBM) based diets from 1 to 25 days (d) of age.

2. Materials and methods

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

2.1. Birds and housing

A total of 528 slow-feathering, Cobb × Cobb 500 one-d-old male chicks was obtained from a commercial hatchery (BRF, Arroio do Meio, RS, Brazil). Chicks were vaccinated for Marek's disease at the hatchery and averaging 39 g ± 0.5, were allocated into 48 metallic battery cages (0.90 m × 0.40 m) located in a temperature controlled room. Temperature at placement was 32°C, which was adjusted to maintain bird comfort throughout the study. Birds had ad libitum access to water and feeds. Lighting was continuous throughout the study.

2.2. Experimental diets

Broilers were fed maize-soybean meal based diets in a two-phase feeding program composed by starter, from 1 to 12 d and grower, from 12 to 25 d (Tables 1 and 2). Experimental diets were formulated using nutrients and energy as usual in Brazilian integrations, except for P and calcium (Ca) levels. Diets were low in available phosphorus (Av. P) and Ca (3.2 g/kg and 7.7 g/kg in starter; 2.3 g/kg and 7.1 g/kg in grower for Av. P and Ca, respectively). The grower diet was formulated with 10 g/kg of

Celite (Celite Corp., Lompoc, CA) obtained from a local distributor as the indigestible marker.

Birds were distributed according to body weight at placement in a completely randomized design with 4 treatments, 12 replications and 11 birds per cage. A 2 x 2 factorial arrangement was used with two enzyme supplementation (with or without phytase) and two mineral sources (inorganic or organic). The dietary 4 treatments consisted of: inorganic mineral source without phytase supplementation; inorganic mineral source with phytase; organic mineral source without phytase; and organic mineral source with phytase. Enzyme supplementation were 500 phytase units (FTU)/kg to both treatments receiving supplementation, whereas Zn-Cu-Mn were supplemented in a concentration of 32-30-32 or 100-120-100 ppm in organic and inorganic forms, respectively.

One FTU is defined as the activity that releases 1 μmol of inorganic phosphate from 5.0 mM sodium phytate at pH 5.5 and 37°C (Engelen et al., 1994). The phytase used in the current study was a 6-phytase produced by a strain of *Pseudomonas fluorescens* expressing a pool of bacterial genes. A commercial product with this enzyme is available under the name of Cibenza®Phytaverse®, by Novus International.

The inorganic minerals were included as zinc, copper and manganese sulphates ($ZnSO_47H_2O$, $CuSO_45H_2O$ and $MnSO_4H_2O$, respectively). The organic minerals consisted of a combination of the metal and the chelant agent, methionine hydroxy-analogue [DL-2-hydroxy-(4-methylthio) butanoic acid (HMTBA)]. Each metal is combined with the DL-Met analogue individually, consisting of three products used in this study. These products are commercially available under the trade mark of Mintrex®: MintrexZn®, MintrexMn® and MintrexCu®, by Novus International.

2.3. Experimental procedures and chemical analysis

Birds and feeds were weighed to evaluate growth performance. Bird weight and average by cage were recorded at 12 and 25 d. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) corrected for the weight of dead birds were calculated from 1 to 12 d, 12 to 25 d and 1 to 25 d.

At 12 d, 4 birds per cage were randomly taken from each pen and euthanized by cervical dislocation following electrical stunning at 45 V for 3 s to collect tibia from the right leg. Bones were collected and pooled per cage to measure ash, Ca and P contents. Bone ash were expressed by cage as percent of the dry defatted bone weight and as the absolute weight of the tibia in grams. Ash and total P and Ca concentration were determined according to AOAC International (2000) methods for ash (930.15), Ca (968.08), and P (946.06).

At 25 d, all remaining birds were euthanized to allow ileal digesta collection. Ileal samples were collected by cutting the ileum from the Meckel’s diverticulum to approximately 2 cm cranial to the ileum-ceca 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. Tibia from the right leg from all birds were also collected following the same methods used in the 12 d slaughtering.

Dry matter (DM), acid insoluble ash (AIA), Ca and P were determined in ileum contents; DM, ash, Ca and P in tibia samples and DM, AIA, Ca and P in diet samples. Cartilaginous caps were manually removed from the tibia. Bone samples were dried at 105ºC for 12 h, defatted for 16 h in a Soxhlet apparatus using petroleum ether, dried again at 110ºC for 12 h, weighed and then ashed in a muffle furnace at 600ºC for 18 h. Diet and freeze-dried samples of ileal contents were ground to pass through a 0.5-mm screen in a grinder (Tecnal, TE-631/2, São Paulo, Brazil). Dry matter analysis of samples was performed after oven drying the samples at 105°C for 16 h (method 934.01; AOAC International, 2006). Acid insoluble ash concentration in the diets and ileum samples was determined using the method described by Vogtmann et al. (1975) and Choct and Annison (1992).

2.4. Calculations

Apparent ileal digestibility was calculated using the following equation (Kong and Adeola, 2014):

Digestibility (%) = $[1 - (Mi/Mo) \times (Eo/Ei)] \times 100$, 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, Ca, or P, in the diet in milligrams per kilogram of DM; and E_o represents the concentration of DM, Ca, or P in the ileal digesta in milligrams per kilogram of DM.

2.5. Statistical analysis

The experimental design was a completely randomized using a factorial arrangement of 2 micro mineral sources (inorganic or organic) × 2 phytase supplementation (0 or 500 FTU/kg). Data were submitted to a 2-way ANOVA using the GLM procedure of SAS Institute (SAS, 2009). Significance was accepted at $P \leq 0.05$ and mean differences were separated using Tukey's HSD test (Tukey, 1991).

3. Results and discussion

No differences (P>0.05) were observed on growth performance of broilers fed phytase and micromineral sources from 1 to 12 d. The effects of dietary treatments on broiler performance from 1 to 25 d are presented in Table 3. There was no interaction between phytase and mineral sources on animal performance. Mortality was not affected by mineral source nor phytase in any of the evaluated periods. Phytase supplementation increased (P < 0.05) BWG and FCR of broilers from 12 to 25 d and in the cumulative period. The BWG and FCR were not affected by mineral sources (P > 0.05). The use of phytase in low-Av. P diets has been demonstrated by several studies to have further benefits in poultry diets outside digestibility effects, as an improvement on performance parameters (Żyła et al., 2000; Dilger et al., 2004; Onyango et al., 2005; Cowieson et al., 2006; Olukosi et al., 2007). This was observed in this study, with higher BWG in broilers fed diets with 500 FTU/kg from 12 to 25 d and in the cumulative period, in comparison with birds receiving diets without enzyme supplementation. This may be due to extra-phosphoric effects of phytase, that allows better overall utilisation of all dietary nutrients (Shirley and Edwards, 2003). A combination of precise phosphorus nutrition and addition of proper levels of microbial phytase is expected to optimize broiler performance while reducing the reliance on inorganic phosphorus sources through improving utilisation of phytate-bound P from the diet (Nelson, 1967).

Organic mineral source was found to increase ash content, in percentage. Ca and P content of tibia were not affected by any of the dietary treatments. Phytase supplementation led to an increase in ash content of tibia, in miligrams and percentage, in the two evaluated periods (Table 4). No interaction was observed between mineral sources and phytase supplementation to bone parameters. Organic mineral source was found to increase ash content, in percentage. Ca and P content of tibia were not statistically affected by any of the dietary treatments. This was possibly due to the low levels of those minerals in the experimental diets, which allows for sensitivity to phytase well stablished improvements regarding BWG and digestibility parameters, while small differeces were not statistically significant. Phytase supplementation led to an increase in ash content of tibia, in miligrams and percentage, in the two evaluated periods.

Apparent ileal digestibility results are presented in Table 5. Inorganic Zn-Cu-Mn when included at concentrations of 100-120-100ppm in the diets for broilers increased DM digestibility, whereas Ca and P digestibility did not differ from the organic source. Phytase inclusion at 500 FTU/kg resulted in the highest Ca and P digestibility ($P<0.05$). In the present study, broilers fed diets supplemented with phytase had improved P digestibility, in agreement with several studies upon the effects of this enzyme on digestibility and retention of P in chickens (Żyła et al., 2000; Dilger et al., 2004; Juanpere et al., 2005; Onyango et al., 2005). The presence of phytate limits the efficiency of digestion by decreasing the access of digestive enzymes to substrates, after forming indigestible complexes especially with Ca and Zn (Singh and Krikorian, 1982; Matyka et al., 1990). Therefore, with the use of exogenous phytase in broiler diets is expected to improve digestibility not only of P itself, as seen in this study, but of nutrients in general. This was assessed in this study, with an improvement in Ca digestibility by phytase supplementation. The usual standard supplementation of this enzyme additive at an industrial and global scale demonstrates the solid knowledge of the performance and economic benefits obtaining by poultry industry regarding its use.

Interaction between mineral source and phytase was observed to DM and P digestibility where broilers fed diets with inorganic mineral source and 500 FTU/kg had higher P and DM digestibility. Abdallah et al. (2009) reported that chicks fed diets containing 100% organic minerals (Zn, Cu, Mn, and Fe) had higher BW and better feed conversion in comparison with those fed diets with inorganic minerals. Similar results were obtained by El-Husseiny et al. (2012). This was not seen in this study, in agreement with Nollet et al. (2007) and Peric et al. (2007), who observed no significant differences between birds fed inorganic and organic minerals as for productive performance. Although improvements were not observed, it demonstrates that it is possible to supply organically complexed minerals in lower concentrations that the usually performed with inorganic forms, without losses in animal performance. On the other hand, inorganic mineral source was found to improve DM digestibility, contrary to the hypothesis regarding the bioavailability characteristics of organic minerals. Therefore, it has yet to be conclusively proven that mineral chelates are better absorbed in the monogastric enterocyte (Nollet et al., 2007).

4. Conclusion

In conclusion, phytase has beneficial impacts on performance, digestibility and bone mineralisation parameters of broilers. Phytase supplementation at 500 FTU/kg in maize-SBM diets with reduced available P resulted in improved BWG and FCR in broilers from 1 to 25 d of age. Also, the usual inorganic source of trace minerals in animal feeds can be replaced by organic source without any losses to animal performance.

Acknowledgements

The authors acknowledge the partial funding from Conselho Nacional de Pesquisa (CNPq – Brasilia, DF, Brazil) and Novus International for their financial support to this project.

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Table 1
Ingredient and nutrient composition of starter diets (1 to 12 d)

Item	0 FTU/kg		500 FTU/kg	
	Zn-Mn-Cu Sulfate	Zn-Mn-Cu Organic	Zn-Mn-Cu Sulfate	Zn-Mn-Cu Organic
Ingredient, g/kg				
Maize		555.7		
Soybean meal		395.5		
Soybean oil		12.2		
Dicalcium phosphate		10.8		
Limestone		10.9		
Salt		5.3		
Alimet 880 g/kg	4.50	3.93	4.50	3.93
L-Lysine 780 g/kg		1.60		
L-Threonine 985 g/kg		0.40		
Choline		0.30		
Vitamin mix[1]		1.00		
Coban 260		0.25		
Cu sulfate 250 g/kg	0.48	0.00	0.48	0.00
K iodate 590 g/kg		0.0016		
Fe sulfate 200 g/kg		0.20		
Mn sulfate 310 g/kg	0.322	0.00	0.322	0.00
Zn sulfate 220 g/kg	0.454	0.00	0.454	0.00
Mintrex Zn	0.00	0.20	0.00	0.20
Mintrex Cu	0.00	0.20	0.00	0.20
Mintrex Mn	0.00	0.25	0.00	0.25
Kaolin/phytase	0.05	0.05	0.00	0.00
Calculated nutrient composition, g/kg or as noted				
AME_n, MJ/kg		12.08		
CP		224.4		
Ca		7.7		
Av P		3.2		
Total P		5.6		
Sodium		2.3		
Chloride		4.1		
Choline, mg/kg		1,500		
Dig. Lys		12.5		
Dig. TSAA		10.0		
Dig. Thr		8.1		
Dig. Trp		2.6		
Dig. Arg		14.4		
Dig. Val		9.6		
Dig. Ile		8.9		

[1]Composition per kg of feed: vitamin A, 8,000 UI; vitamin D3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.

Table 2
Ingredient and nutrient composition of grower diets (12 to 25 d).

Item	0 FTU/kg		500 FTU/kg	
	Zn-Mn-Cu Sulfate	Zn-Mn-Cu Organic	Zn-Mn-Cu Sulfate	Zn-Mn-Cu Organic
Ingredient, g/kg				
Maize			568.6	
Soybean meal			366.0	
Soybean oil			22.2	
Dicalcium phosphate			6.20	
Limestone			12.6	
Salt			5.80	
Alimet 880 g/kg	4.20	3.63	4.20	3.63
L-Lysine 780 g/kg			1.70	
L-Threonine985g/kg			0.40	
Choline			0.60	
Vitamin mix[1]			1.00	
Coban 260			0.25	
Cu sulfate 250 g/kg	0.48	0.00	0.48	0.00
K iodate 590 g/kg			0.0016	
Fe sulfate 200 g/kg			0.20	
Mn sulfate 310 g/kg	0.322	0.00	0.322	0.00
Zn sulfate 220 g/kg	0.454	0.00	0.454	0.00
Mintrex Zn	0.00	0.20	0.00	0.20
Mintrex Cu	0.00	0.20	0.00	0.20
Mintrex Mn	0.00	0.25	0.00	0.25
Kaolin/phytase	0.05	0.05	0.00	0.00
Celite[2]			10.0	
Calculated nutrient composition, g/kg or as noted				
AME_n, MJ/kg			12.35	
CP			212.3	
Ca			7.1	
Av P			2.3	
Total P			4.6	
Sodium			2.1	
Chloride			3.8	
Choline, mg/kg			1,600	
Dig. Lys			11.8	
Dig. TSAA			9.4	
Dig. Thr			7.7	
Dig. Trp			2.4	
Dig. Arg			13.5	
Dig. Val			9.1	
Dig. Ile			8.4	

[1]Composition per kg of feed: vitamin A, 8,000 UI; vitamin D3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.
[2] Indigestible marker (Celite, Celite Corp., Lompoc, CA).

Table 3
Growth performance of broilers fed diets formulated with organic or inorganic minerals and formulated or not with phytase, from 1 to 25 d, g.

Item	1 to 12 d			12 to 25 d			1 to 25 d		
	BWG	FCR	FI	BWG	FCR	FI	BWG	FCR	FI
Mineral source									
Inorganic	220	1.278[b]	280.5[b]	971	1.402	1,358	1,203	1.370	1,628
Organic	220	1.324[a]	290.4[a]	984	1.409	1,384	1,191	1.383	1,667
Phytase, FTU/kg									
0	216	1.321	284.7	929	1.435	1,332	1,144	1.405	1.607
500	222	1.290	285.8	1,002	1.390	1,391	1,224	1.364	1.667
SEM	1.703	0.010	2.101	7.811	0.007	8.860	8.802	0.006	9.650
P-value									
Minerals	0.5845	0.0130	0.0548	0.1797	0.6946	0.0561	0.2964	0.1308	0.0156
Phytase	0.0820	0.1236	0.7967	0.0001	0.0022	0.0012	0.0001	0.0011	0.0022
Minerals x Phytase	0.1833	0.4987	0.3710	0.1541	0.6859	0.1858	0.3416	0.9876	0.2764

Table 4
Tibia mineral content of broilers on DM basis.

Item	12 d				25 d			
	Ash, %	Ash, g	Ca, %	P, %	Ash, %	Ash, g	Ca, %	P, %
Mineral source								
Inorganic	48.90	0.374	13.23	7.94	50.10	3.40	16.65	7.94
Organic	49.44	0.388	13.26	8.10	50.17	3.34	16.04	7.90
Phytase, FTU/kg								
0	48.09	0.344	12.88	7.87	47.48	2.69	16.66	7.49
500	49.71	0.399	13.42	8.10	51.34	3.69	16.21	8.12
SEM	0.160	0.005	0.157	0.13	0.289	0.069	0.298	0.152
P-value								
Minerals	0.0331	0.2309	0.9358	0.4434	0.5734	0.8413	0.6973	0.5511
Phytase	0.0001	0.0001	0.1072	0.4179	0.0001	0.0001	0.4494	0.0619
Minerals x Phytase	0.5348	0.3530	0.5818	0.4724	0.3047	0.1443	0.1389	0.2002

Table 5
Apparent ileal digestibility of broilers fed diets formulated with organic or inorganic minerals, formulated or not with phytase, at 25 d.

Item		Dry matter, %	Ca, % of DM	P, % of DM
Mineral source				
Inorganic		68.24	59.50	61.6
Organic		66.50	60.42	58.0
Phytase, FTU/kg				
0		66.20	56.84	53.91
500		67.96	61.51	62.62
Mineral source	Phytase, FTU/kg			
Inorganic	0	66.07[b]	55.57	51.75[c]
Inorganic	500	69.33[a]	61.46	66.59[a]
Organic	0	66.33[b]	58.11	56.07[b]
Organic	500	66.58[b]	61.57	55.65[b]
SEM		0.33	0.51	0.80
P-value				
Minerals		0.0482	0.1571	0.0842
Phytase		0.0058	0.0001	0.0001
Minerals x Phytase		0.0174	0.1948	0.0001

[a,b,c] Means not sharing the same letter differ significantly on Tukey Test (P<0.05).

CONSIDERAÇÕES FINAIS

O presente estudo objetivou avaliar a inclusão de fitase em concentrações de 500 FTU/kg de ração, e também comparar fontes orgânicas e inorgânicas de microminerais suplementadas em rações à base de milho e farelo de soja para frangos de corte. Os efeitos foram avaliados sobre o desempenho zootécnico, digestibilidade de nutrientes e mineralização óssea, sobre os seguintes parâmetros: ganho de peso, consumo de ração, conversão alimentar e mortalidade; conteúdo de cálcio, fósforo e cinzas; digestibilidade de cálcio, fósforo e de matéria seca.

Os benefícios da utilização de fitase como aditivo nutricional já são amplamente conhecidos na avicultura. A melhor utilização do conteúdo de fósforo da ração, menor formação de complexos que sequestram nutrientes e minerais importantes para o metabolismo, além da menor excreção ambiental de P e menor custo com suplementação inorgânica de P promovem a fitase como aditivo de eleição na formulação de rações. Os resultados observados neste estudo corroboram todas as hipóteses formuladas para a sua utilização. Contudo, as observações dos resultados sobre os minerais permitem inferir que o emprego de fontes inorgânicas pode ser repensado, já que ao comparar-se ambas as fontes, não houve diferença entre as mesmas. Ou seja, não houve prejuízo ao desempenho, mineralização óssea ou digestibilidade de nutrientes quando da utilização de fontes orgânicas e portanto, de concentrações menores de micro minerais. O que se infere é que existe uma grande tendência de se utilizar moléculas que tenham menor impacto no meio ambiente e que possam ser utilizadas mais racionalmente, além do benefício direto ao desempenho animal. Assim, pesquisas futuras devem ser feitas a fim de se avaliar a digestibilidade dos minerais orgânicos, a fim de promover um melhor entendimento sobre as complexas interações que as moléculas sofrem tanto na dieta quanto no metabolismo animal.

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APÊNDICES

Apêndice 1. Instruções para publicação na revista Animal Feed Science and Technology

POULTRY SCIENCE INSTRUCTIONS TO AUTHORS [1]

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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 shall be 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 Copyright Transfer 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 experiment station 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 methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. 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 same sample 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 prior to 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 any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not “statistically significant” is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by “±” to a number implies that the second value is its standard error (not its standard deviation). Adequate 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 well to 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. More than one article listed in the same sentence of text must be in chronological order first, and alphabetical order for two publications in the same year. Work that has not been accepted for publication shall be listed in the text as: "J. E. Jones (institution, city, and state, personal communication)." The author's own un- published work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and un- published data must not be included in the References section.

References Section. To be listed in the References 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 Methods section 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:

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

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

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

Book:
Metcalfe, J., M. K. Stock, and R. L. Ingermann. 1984. The effects of oxygen on growth and development of the chick embryo. Pages 205-219 in Respiration and Metabolism of Embryonic Vertebrates. R. S. Seymour, ed. Dr. W. Junk, Dordrecht, the Netherlands.
National Research Council. 1994. Nutrient Requirements of
Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
Federal Register:
Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Reg- ist. 69:10137–10151.
Other:
Choct, M., and R. J. Hughes. 1996. Long-chain hydrocarbons as a marker for digestibility studies in poultry. Proc. Aust. Poult. Sci. Symp. 8:186. (Abstr.)
Dyro, F. M. 2005. Arsenic. WebMD. Accessed Feb. 2006. http://
www.emedicine.com/neuro/topic20.htm.
El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, as- signee. US Pat. No. 6,766,767.
Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium, MD. Univ. Maryland, College Park.
Luzuriaga, D. A. 1999. Application of computer vision and elec- tronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gaines- ville.
Peak, S. D., and J. Brake. 2000. The influence of feeding program on broiler breeder male mortality. Poult. Sci. 79(Suppl. 1):2. (Abstr.)

Tables

Tables must be created using the MS Word table 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 the printed 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 facilitate place- 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. All terms 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. German nouns shall begin with capital letters.

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

Number Style. Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits. Measures must be in the metric system; however, US equivalents may be given in parentheses. *Poultry Science* requires that measures of energy be given in calories rather than joules, but the equivalent in joules may be shown in parentheses or in a footnote to tables. Units of measure not preceded by numbers must be 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

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