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EFEITOS DA SUPLEMENTAÇÃO DE FITASE EM DIETAS À BASE DE MILHO E FARELO DE SOJA PARA SUÍNOS EM CRESCIMENTO E FRANGOS DE CORTE

PORTO ALEGRE

2020

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EFEITOS DA SUPLEMENTAÇÃO DE FITASE EM DIETAS À BASE DE MILHO E FARELO DE SOJA PARA SUÍNOS EM CRESCIMENTO E FRANGOS DE CORTE

Tese apresentada como requisito para obtenção do Grau de Doutor em Zootecnia, na Faculdade de Agronomia, da Universidade Federal do Rio Grande do Sul

Orientador: Drª. Ines Andretta

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Agradeço a todos os pesquisadores cujos trabalhos foram utilizados para esta tese. Todos os animais utilizados nos experimentos geraram resultados que foram aproveitados para a construção da base de dados. Cada meta-análise pode ser elaborada graças a toda a ciência produzida até hoje e a todos os animais que já foram e serão utilizados em experimentos.

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À UFRGS e à Faculdade de Agronomia por fornecer a estrutura e os recursos para realizarmos os nossos trabalhos.

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EFEITOS DA SUPLEMENTAÇÃO DE FITASE EM DIETAS À BASE DE MILHO E FARELO DE SOJA PARA SUÍNOS EM CRESCIMENTO E FRANGOS DE CORTE[1]

Autora: Carolina Schell Franceschina

Orientador: Drª Ines Andretta

Resumo: Duas meta-análises foram elaboradas para avaliar a eficiência da suplementação de fitase em dietas a base de milho e farelo de soja sobre a digestibilidade e o desempenho de suínos em crescimento e frangos de corte, e identificar os fatores que, potencialmente, modulam o efeito da enzima. As duas bases de dados foram construídas a partir de informações de estudos realizados entre 2007 e 2017/2019 e que avaliavam experimentos com suínos (22 artigos; 2161 animais em 28 experimentos), e frangos de corte (102 artigos; 69472 animais em 104 experimentos), respectivamente. O critério principal para a seleção dos artigos foi: (a) avaliação da suplementação de fitase; (b) dietas formuladas a base de milho e farelo de soja; (c) suínos desde as fases de crescimento até a terminação/frangos de corte em todas as fases produtivas; e (d) respostas de desempenho e/ou digestibilidade. A meta-análise indicou que a alimentação de suínos com dietas suplementadas com fitase não influenciou o consumo de ração e a conversão alimentar, embora o ganho de peso tenha sido maior nos tratamentos com suplementação. Os coeficientes de digestibilidade total da proteína bruta e do cálcio não foram influenciados pela fitase. Porém, a fitase melhorou a digestibilidade do fósforo. As equações de regressão foram positivas para todas as respostas, mas significativas somente para as digestibilidades, com valor R^2 acima de 95%. A decomposição da variância indicou que o estudo e a marca da enzima foram os fatores que afetaram o efeito da fitase sobre o ganho de peso e a digestibilidade do fósforo. A alimentação de frangos de corte com dietas suplementadas com fitase influenciou tanto o desempenho quanto a digestibilidade, melhorando todos os resultados. A digestibilidade ileal do fósforo foi a resposta mais afetada, como esperado. O conteúdo de cinzas na tíbia também apresentou aumento significativo, embora a quantidade de cálcio e fósforo não tenha sido afetada, assim como o rendimento de carcaça. O efeito da idade foi significativo para o desempenho e para a digestibilidade. Já o estudo foi o fator mais impactante para todas as respostas avaliadas. Finalmente, a relação Ca:P parece ser mais importante do que o conteúdo de cálcio ou de fósforo para explicar o efeito da fitase sobre o ganho de peso e a digestibilidade ileal do cálcio. É possível concluir que a fitase parece não tem efeito sobre o desempenho de suínos em crescimento e terminação alimentados com dietas baseadas em milho e farelo de soja. Entretanto, este trabalho apresenta resultados importantes sobre a digestibilidade do fósforo, principalmente com relação às fitases bacterianas. A suplementação de fitase em dietas para frangos de corte melhorou o desempenho, a digestibilidade ileal e o conteúdo de cinzas ósseas das aves. Alguns fatores, como a idade e a composição da dieta, afetaram essas respostas de formas diferentes e, até mesmo, contrárias.

Palavras-chave: cálcio; enzima; nutrição; fósforo; monogástricos

Tese de doutorado em produção animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (84 p.). Março, 2020.

ASSESSING THE EFFECT OF PHYTASE SUPPLEMENTATION TO CORN- AND SOYBEAN MEAL-BASED DIETS FOR GROWING PIGS AND BROILERS

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Abstract: Two meta-analysis were performed to assess the efficiency of phytase supplementation to corn- and soybean meal-based diets on nutrient digestibility and performance of growing pigs and broilers and to identify the factors that potentially modulated the enzyme effects. The two database was constructed using information from previous studies carried out between 2007 and 2017/2019 that evaluated trials with growing pigs (22 papers; 2161animals in 28 experiments), and broilers (102 papers; 69472animals in 104 experiments), respectively. The main criteria for the paper selection were: (a) experimental evaluation of phytase supplementation; (b) feed formulas based on corn and soybean meal; (c) pigs from growing to finishing rearing phases/broilers in all production phases; and (d) performance and/or digestibility responses. The meta-analysis indicated that feeding growing pigs with diets supplemented with phytase did not influence feed intake and feed conversion responses although weight gain tended to be higher in supplemented treatments. The apparent total tract digestibility coefficients of crude protein and calcium were not influenced by the phytase supplementation. However, phytase improved phosphorus digestibility. The regression equations were positive for all responses, but significant only for apparent digestibility variables, with R^2 values above 95%. The variance decomposition indicated that the study code and the enzyme brand were the factors that affected the phytase effect on weight gain and phosphorus digestibility. Feeding broilers with diets supplemented with phytase influenced both performance and digestibility, with improvements in all result. Phosphorus ileal digestibility was the most affected response, as expected. Bone ash content also showed a significant increase, although the bone amounts of calcium and phosphorus were not affected, as well as the carcass yield. The effect of age was significant for performance and AID responses. The study effect was the most important factor for all responses, and the Ca:P ratio appears to be more important than the calcium or the phosphorus content of the diet for explaining the effect of phytase on weight gain and calcium ileal digestibility. It was possible to conclude that phytase has no effect on performance of growing pigs fed diets based on corn-soybean meal. However, it presents important results on phosphorus digestibility, especially bacterial phytases. Supplementing phytase to broiler diets improves performance, ileal digestibility and bone ash content of birds. Some factors, such as age and diet composition, affect these responses in different and even opposite ways.

Keywords: calcium; enzyme; nutrition; phosphorus; monogastric

Doctoral thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (84 p.). March, 2020.

SUMÁRIO

RELAÇÃO DE TABELAS

CAPÍTULO II

CAPÍTULO III

COMPLEMENTARY MATERIAL

RELAÇÃO DE FIGURAS

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

RELAÇÃO DE ABREVIATURAS

ABPA – Associação Brasileira de Proteína Animal

AID – Apparent ileal digestibility

ATTD – Apparent total tract digestibility

Ca – Cálcio/calcium

FTU – Unidade de fitase/phytase unit

Kg – Quilograma/kilogram

N – Nitrogen

P – Fósforo/phosphorus

CAPÍTULO I

1. Introdução

Até 2050, espera-se que o aumento da população mundial duplique a demanda por alimento (Tilman et al., 2002), de modo que a produção animal adquire importância ainda maior pela geração de proteína de excelente qualidade. No Brasil, o consumo das carnes de frango e de suíno também está aumentando expressivamente (ABPA, 2019), e o setor de nutrição animal passou a consumir mais recursos econômicos e ambientais. A criação de aves e suínos se caracteriza pela conversão eficiente de proteína vegetal em proteína animal (Broch et al., 2018), mas muitos ingredientes de origem vegetal apresentam fatores antinutricionais, sendo o fitato um dos mais importantes.

O ácido fítico, também conhecido como fitato (quando está na forma de sal, ligado a outros componentes), é um componente essencial das sementes, responsável por manter as reservas de fósforo para o adequado crescimento das plantas (Broch et al., 2018). Nos ingredientes de origem vegetal, 80% do fósforo está ligado ao fitato, formando o fósforo fítico. Outros minerais e aminoácidos também podem estar ligados ao fitato, tornando-se indisponíveis para animais monogástricos (Nelson, 1976). O uso de aditivos na dieta, como a fitase exógena, pode aumentar o aproveitamento de nutrientes (Viana et al., 2009) e melhorar o desempenho de aves (Amerath et al., 2014; Hughes et al., 2009) e suínos (Zeng et al., 2014). Atualmente, a fitase é a enzima exógena mais difundida no mercado de enzimas, o que se deve à ampla aceitação desta tecnologia em substituição ao fosfato bicálcico (Adeola & Cowieson, 2011), que é uma fonte não-renovável de fósforo inorgânico.

Por outro lado, a efetividade da adição de fitase às dietas não é observada em todos os estudos desenvolvidos nesta área (Adeola & Cowieson, 2011). Frente à variabilidade entre resultados de diferentes experimentos, é importante explorar os dados obtidos para cada resposta para identificar os fatores que modulam os efeitos da suplementação nos diversos cenários estudados. Para isso, a elaboração de bancos de dados com informações de trabalhos prévios e seu estudo através de ferramentas como meta-análise pode ser uma alternativa para aumentar o conhecimento nesta área de pesquisa.

A meta-análise é uma ferramenta que compara e combina os efeitos de tratamentos oriundos de diferentes experimentos conduzidos em condições variadas, o que é interessante principalmente quando o tamanho da amostra ou o número de repetições é baixo em alguns estudos. Este estudo é uma alternativa sustentável com relação à experimentação animal, já que trabalhos envolvendo animais são caros, com dificuldades práticas, riscos e implicações éticas. Ainda, é necessário avaliar a produção de aves e suínos conforme a realidade em que estamos inseridos, com a escolha de ingredientes relevantes no Brasil - opção que a meta-análise permite destacar. Assim, este trabalho foi desenvolvido para avaliar os efeitos da inclusão de fitase em dietas para frangos de corte e suínos sobre o aproveitamento de nutrientes e o desempenho zootécnico.

2. Revisão bibliográfica

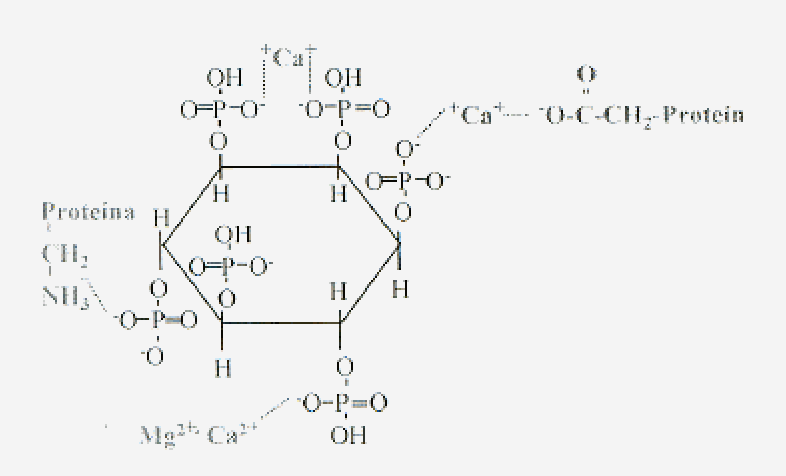
Segundo o relatório da ABPA (2019), a produção de carne de frango no Brasil foi de 12,86 milhões de toneladas em 2018 – aumento de 25% com relação ao ano de 2007 – e o consumo foi de 41,99 kg por habitante neste mesmo ano. Em 2018, o Brasil perdeu a posição de maior produtor de carne de frango para os Estados Unidos. A produção brasileira de carne suína foi de 3.974 mil toneladas em 2018, um aumento de 32% com relação a 2007, e o consumo foi de 15,9 kg por habitante neste mesmo ano. O Brasil é o quarto maior produtor de carne suína no mundo, atrás da China, União Europeia e Estados Unidos. Para que aves e suínos possam expressar ao máximo o seu potencial de desempenho, é necessário que os sistemas de produção estejam amparados pelo que há de mais avançado em termos de conhecimento científico, especialmente em áreas-chave, como a nutrição.

A alta taxa de crescimento e de ganho de peso das aves e suínos modernos e, portanto, de produção de carne, originou uma das maiores cadeias produtivas do agronegócio no mundo. Essa cadeia é caracterizada pela conversão de plantas em proteína animal (Broch et al., 2018). O uso desses ingredientes vegetais na dieta dos animais monogástricos geralmente está associado à adição de enzimas exógenas devido à presença de fatores antinutricionais, como o fitato e os polissacarídeos não-amídicos. O melhor aproveitamento de nutrientes e da energia por meio desses aditivos pode representar uma economia significativa ao final da formulação das rações (Viana et al., 2009), além de proporcionar a melhora do desempenho zootécnico e ambiental desses animais.

O fitato

O fitato, também chamado de fitina ou ácido fítico, é um componente essencial das sementes, responsável por manter as reservas de fósforo para o adequado crescimento das plantas (Broch et al., 2018), sendo um dos principais fatores antinutricionais para animais monogástricos. Nos ingredientes de origem vegetal, cerca de 80% do fósforo está ligado a esse componente do alimento, formando o fósforo fítico. Outros minerais e aminoácidos também podem estar complexados com fitato (Figura 1), o que é um problema para esses animais, que não conseguem hidrolisá-lo por não produzirem a enzima (Nelson, 1976).

Figura 1 - Molécula de fitato: interação com proteínas e minerais.



Fonte: Dominguez et al. (2002).

Embora a afinidade do cálcio pelo fitato seja menor com relação a outros minerais da dieta, o seu impacto é alto devido à ampla suplementação de cálcio nas rações (Hamdi et al., 2018). Além disso, a ligação do fitato com o cálcio tem uma grande importância devido à formação de um complexo indigestível pela fitase e, portanto, é indispensável que o fitato seja hidrolisado, de modo a disponibilizar tanto o cálcio quanto o fósforo fítico (Selle et al., 2009). Se não fosse o fitato, a quantidade de fósforo total presente nos ingredientes de origem vegetal seria suficiente para o crescimento e o desenvolvimento ósseo animal (Hamdi et al., 2018).

Em pH ácido, a ligação do complexo fitato-cálcio é solúvel, e no lúmen intestinal, onde o pH é mais básico, a ligação torna-se insolúvel e ocorre a precipitação do complexo (Selle et al., 2009). Por outro lado, a ligação do fitato com as proteínas ocorre a nivel estomacal (pH ácido), quando há ligação eletrostática com os resíduos básicos de alguns aminoácidos. Em pH acima de 7, as ligações se desfazem e só voltam a ocorrer na presença de cátions divalentes (Ca^{2+}, Mg^{2+} e Zn^{2+}), que formam uma ponte entre os grupos carboxila das proteínas e o fitato (Finely & Hopkins, 1985).

Enquanto os produtores e os nutricionistas procuram meios de melhorar a eficiência alimentar com um baixo custo, a inclusão de nutrientes em excesso ou em formas que apresentam baixa digestibilidade nas dietas leva à maior excreção de componentes poluidores no ambiente, além de ser desvantajoso economicamente. O fósforo é um dos maiores poluentes gerados nos sistemas de produção animal, o que é agravado pela presença de fatores antinutricionais como o fitato. Estima-se que mais de 14 milhões de toneladas de fósforo fítico sejam produzidos por ano na agricultura (Lott et al., 2000). Além disso, as fontes minerais de fósforo são limitadas, tornando as fontes não-renováveis de fósforo cada vez mais vulneráveis à escassez (Humer et al., 2014). A limitação das fontes naturais de fósforo representa um custo significativo para a nutrição animal. Na produção de suínos, o fósforo é o terceiro nutriente mais caro na formulação das dietas, ficando atrás apenas da proteína e da energia (Létourneau-Montminy et al., 2011). Dessa forma, a suplementação das dietas de aves e suínos com fitase exógena é uma alternativa para melhorar o desempenho zootécnico e reduzir a excreção de fósforo ou outros nutrientes no ambiente.

Enzimas exógenas na nutrição de aves e suínos

Hastings (1946) testou uma enzima de origem fúngica pela primeira vez na nutrição de aves e percebeu o efeito positivo no desempenho somente quando a suplementação foi feita em dietas com alto teor de fibra. Alguns anos depois, Jensen et al. (1957) verificaram que frangos de corte não possuíam determinadas enzimas capazes de hidrolisar alguns tipos de carboidratos da dieta. Nelson et al. (1968), ao adicionarem fitase na dieta de frangos de corte, observaram o aproveitamento do fósforo fítico.

Muitas pesquisas foram realizadas desde então. De modo geral, o aumento das informações disponíveis na área é positivo, pois garante que praticas mais modernas e eficazes estejam disponíveis para serem aplicadas na produção animal. Por outro lado, o aumento no volume de dados tornou difícil a tarefa de sintetizar toda a informação disponível nesta área de conhecimento utilizando apenas metodologias tradicionais de revisão de literatura.

Atualmente, o mercado de enzimas para alimentação animal divide-se entre fitases (cerca de 60% da produção de enzimas) e não fitases (carboidrases, proteases, amilases) (Lei et al., 2013). O rápido crescimento desse mercado, principalmente nos últimos dez anos, deve-se à ampla aceitação da tecnologia das fitases em substituição ao fosfato bicálcico e à adoção das carboidrases em dietas à base de milho (Adeola & Cowieson, 2011). Atualmente, as fitases são muito usadas na alimentação animal porque, além de terem se tornado mais baratas, podem reduzir o uso de fósforo mineral (ingrediente muito caro) (Hamdi et al., 2018).

Diversos fatores podem interferir na resposta observada nos animais alimentados com dietas suplementadas com enzimas exógenas. A eficiência do aditivo pode variar com características da enzima (como sua fonte, sendo provenientes de vegetais ou de microorganismos), do animal (como a idade, a espécie ou a categoria) e da dieta (como a composição de ingrediente, a disponibilidade de substrato e, no caso da fitase, o nível de cálcio utilizado para a formulação da dieta) (Hamdi et al., 2018). O cálcio, ao se ligar ao fitato e formar um complexo que pode precipitar no lúmen intestinal, dificulta a ação da fitase, embora os resultados ainda sejam inconsistentes (Li et al., 2018). Portanto, a utilização de fontes de cálcio de alta solubilidade (como o calcário calcítico) podem afetar a atividade da enzima. Algumas referências sugerem que um aumento na digestibilidade do cálcio de 0,2% deve ser considerado na formulação de dietas suplementadas com fitase (Bertechini, 2012). Singh (2008) também afirma que a eficácia da fitase é menor em dietas ricas em cálcio ou com alta relação entre o cálcio e o fósforo, pois há maiores chances da formação de complexos insolúveis com o cálcio.

A fitase

A fitase é uma enzima que hidrolisa o fosfato a partir do fitato, produzindo ortofosfato, inositol fosfato ou inositol. A primeira fitase usada como aditivo na alimentação animal foi desenvolvida ainda na década de 80, através da tecnologia do DNA recombinante (Lei et al., 2013). Em 1991, foi lançada a primeira fitase no mercado, de origem fúngica (*Aspergillus niger*). A partir de então, o desenvolvimento do mercado das fitases foi lento, e foram necessários 15 anos para que uma nova fitase fosse desenvolvida, de origem bacteriana. A segunda fitase apresentava o DNA da *Escherichia coli* e parecia ser mais efetiva do que a fitase fúngica (Rodriguez et al., 1999). Com o marco da produção da fitase bacteriana, surgiu a segunda geração de fitases que é, em muitos aspectos, superior à primeira geração. Em 2010, o mercado de fitases compreendia 60% do total do mercado de enzimas (Lei et al., 2013).

Existem quatro fontes de fitase para aves e suínos: fitase intestinal (fitase endógena, produzida pelos microorganismos presentes no trato gastrointestinal dos animais, mas em quantidade insuficiente para hidrolisar o fitato da dieta), fitase de origem vegetal (necessária para que as plantas liberem o fósforo fítico para o seu próprio desenvolvimento), fitase bacteriana e fitase fúngica (Lei et al., 2013). As fitases de maior relevância para a alimentação são divididas em dois subgrupos (3-fitase e 6-fitase), dependendo do grupo fosfato no qual elas iniciam o catabolismo do fitato (Adeola & Cowieson, 2011). O fitato apresenta estrutura cíclica (mio-inositol) e seis grupos fosfato ligados aos átomos de carbono.

O fitato se torna mais solúvel conforme os grupamentos fosfatos são hidrolisados, de modo que a função principal das fitases exógenas é impedir que o mio-inositol com mais de dois grupos fosfato chegue ao duodeno (Adeola & Cowieson, 2011). As fitases também podem ser classificadas como ácidas ou básicas, dependendo da faixa de pH em que elas atuam (3 a 6 e 5,5 a 8, respectivamente; Bertechini, 2012). As fitases de origem fúngica apresentam maior termoestabilidade, mas menor intervalo de pH ótimo em comparação com as fitases bacterianas, o que pode levar a diferentes respostas de digestibilidade e de desempenho (Yin et al., 2007).

A habilidade catalítica da fitase nos diferentes compartimentos do trato digestório depende do pH dessas regiões. Geralmente a atividade da enzima é mais alta em meio ácido (Hamdi et al., 2018), como aquele presente no estômago dos suínos e no papo, na moela e no proventrículo das aves.Uma menor hidrólise do fitato na moela pode aumentar, por exemplo, o impacto do cálcio sobre o fitato no intestino, que é onde ocorre a precipitação desse mineral com o fitato não digerido e dificulta a ação da fitase. Aves mais jovens, que estão com o trato gastrointestinal em desenvolvimento, possuem uma taxa de passagem do alimento maior, com menos tempo de retenção na moela, prejudicando o contato da enzima com o seu substrato (Li et al., 2018).

Fitase e aproveitamento de nutrientes em frangos de corte e suínos Ensaios de digestibilidade são importantes para avaliar a qualidade de uma dieta. Estes estudos permitem estimar o aproveitamento dos nutrientes e da energia de determinados ingredientes e da ração. O grau de digestão dos nutrientes no sistema digestório é baseado no tipo de alimento que é oferecido aos animais - dietas de alta digestibilidade levam a uma maior retenção de nutrientes e, portanto, a um melhor desempenho (Alabi et al., 2019)

Através da liberação dos nutrientes ligados ao fitato, a fitase é capaz de aumentar a sua disponibilidade e, assim, melhorar a digestibilidade das dietas. Porém, o efeito da fitase varia conforme o nutriente estudado. Adeola e Cowieson (2011) avaliaram trabalhos de aves e suínos ao longo de 20 anos, e o efeito da fitase na digestibilidade de aminoácidos foi nula ou pouco expressiva em alguns estudos, mas com melhores resultados para aves em comparação com suínos. Selle e Ravindran (2007) observaram diferenças na digestibilidade ileal de aminoácidos dependendo do tipo de marcador utilizado em suínos (com os piores resultados para o Cr_2O_3). Além disso, uma descrição mais detalhada sobre as interações fitato-proteína é necessária, já que o fitato desencadeia a formação de agregados insolúveis de proteína refratários à ação da pepsina em pH 2 a 3, de modo que início da digestão proteica é prejudicado (Selle et al., 2012).

No geral, a suplementação dietética de fitase exógena varia entre 250 e 1000 FTU/kg, mas acredita-se que as superdoses de fitase podem maximizar a hidrólise do fitato e a utilização do fósforo da dieta (Walk et al., 2014). Até recentemente, esperava-se que uma dose padrão de fitase (500 FTU/kg) era capaz de alcançar a hidrólise máxima de 70% de todo o fitato da dieta, mas hoje, com o desenvolvimento de novas fitases adicionadas em altas doses (três a quatro vezes a dose padrão), o objetivo é a quebra de 90% do fitato dietético (Hamdi et al., 2018). Estudos recentes demonstraram a eficácia da fitase na digestibilidade proteica em frangos de corte. Amerah et al. (2014) e Truong et al. (2015) observaram o aumento na digestibilidade de aminoácidos com a suplementação de fitase a dietas de milho e farelo de soja para frangos de corte.

A função principal da fitase é a liberação do fósforo fítico (Lei et al., 2013), mas outros minerais também são liberados, como o cálcio, o zinco, o manganês e o ferro (Bertechini, 2012). Em frangos de corte, a adição de fitase em dietas à base de milho e farelo de soja aumentou a digestibilidade do fósforo total (Rutherfurd et al., 2004). Para suínos, a suplementação da dieta com fitase melhorou a digestibilidade do fósforo (Adeola et al., 2004; Jendza et al., 2006; Kerr et al., 2010), o que nem sempre foi acompanhado pela melhora do desempenho (Dersjant-Li et al., 2017). Em comparação ao interesse dos efeitos da fitase sobre o fósforo, menos ênfase é dada aos efeitos sobre o cálcio. Isso ocorre porque o cálcio é um ingrediente relativamente barato e a maioria dos trabalhos não estuda a sua interação com o fósforo e com a fitase (a atenção maior é relacionada à interação do cálcio com o fitato), com resultados ainda inconclusivos (Li et al., 2018).

Fitase e desempenho de frangos de corte e suínos

Uma das consequências da maior disponibilidade e assimilação de nutrientes pelos animais recebendo suplementação de enzimas exógenas é a melhora do desempenho. Em dietas com ingredientes alternativos/co-produtos ou com muitos fatores antinutricionais, o objetivo da suplementação enzimática é a valorização nutricional das dietas, tornando-as mais eficientes para a produção animal (Lei et al., 2013). Além da liberação de nutrientes e minerais, a redução da estrutura do fitato também pode gerar a provisão de inositol, que é uma fonte energética e promove o crescimento (Lee & Bedford, 2016).

Em um estudo com frangos de corte, a adição de uma superdose (5000 FTU/kg) de fitase à dieta aumentou do ganho de peso e melhorou a eficiência alimentar (Ptak et al., 2015) devido à maior disponibilidade e assimilação de nutrientes. Os autores também mencionaram a alteração da microflora intestinal em função da suplementação enzimática: a liberação de cálcio e fósforo alterou a microflora intestinal, aumentando a fermentação e maximizando o processo digestivo. Quando ocorre a redução nutricional das dietas, a piora no desempenho é compensada pela adição de fitase. Por outro lado, sugere-se que a idade pode afetar a eficiência da enzima, com os maiores benefícios observados em aves jovens (Tiwari et al., 2010). Dessa forma, é interessante avaliar as necessidades fisiológicas e nutricionais de acordo com a categoria do animal, alterando a dose de suplementação conforme a ave envelhece.

Alguns trabalhos mostram benefícios para o desempenho e a mineralização óssea de suínos com o uso de fitase (Santos et al., 2014), e a superdose (20000 FTU/kg) pode maximizar esse efeito (Zeng et al., 2014). Por outro lado, em uma série de experimentos com leitões realizados por Olukosi et al. (2007), os dados são contraditórios, apresentando resultados positivos ou a falta de efeito da enzima. No primeiro experimento, a redução nutricional das dietas provocou uma queda no desempenho de leitões recém-desmamados, o que foi compensado com a adição de fitase. Em um segundo experimento, não houve quaisquer efeitos. Outros trabalhos mostram que o uso do aditivo não afetou o desempenho de suínos em crescimento (Samson et al., 2017) e terminação (Dersjant-Li et al., 2017)

No geral, os bons resultados de desempenho de frangos de corte pelo uso de fitase se devem à liberação de nutrientes e minerais, principalmente o fósforo, e à redução dos fatores antinutricionais do fitato (Dessimoni et al., 2018; Hamdi et al., 2018; Li et al., 2018), mas outros fatores relacionados à ave (genética, sexo, idade) e à enzima (origem microbiana, modo de produção) também afetam esses resultados. Diferente de frangos de corte, os resultados de desempenho de suínos são contraditórios. Há menos experimentos de desempenho com suínos, pois eles costumam ser mais complexos e vulneráveis e erros metodológicos, pois possuem uma carga de trabalho maior, além de demandarem maiores recursos econômicos (Cowieson et al., 2017). Também é importante considerar as diferenças fisiológicas e do trato digestório entre as espécies.

A meta-análise na produção animal

A meta-análise é uma ferramenta que permite integrar os resultados de diferentes estudos e extrair informações adicionais desses dados (Lovatto et al., 2007). O termo também se refere a um conjunto de procedimentos para estimar a magnitude e a direção dos efeitos (Sauvant et al., 2008). Dessa forma, a meta-análise compara e combina os efeitos de tratamentos oriundos de diferentes experimentos conduzidos em condições variadas, o que é interessante principalmente quando o tamanho da amostra e/ou o número de repetições são limitantes. As meta-análises que utilizam modelos com efeitos aleatórios também permitem estimar a heterogeneidade desses efeitos e avaliar os fatores que podem explicar essa heterogeneidade (Bougouin et al., 2014). Isso é importante, já que existem efeitos entre os estudo que não podem ser controlados pelo pesquisador, dentre eles o ambiente em que o estudo foi construído, o clima, e o acúmulo de erros. Já os efeitos fixos são aqueles que podem ser controlados pelo condutor do trabalho, como os tratamentos.

Atualmente, devido à pressão que existe sobre a experimentação animal, a meta-análise é uma alternativa que permite unir diversos resultados sem que seja necessário conduzir um novo trabalho utilizando mais animais, de modo que esta metodologia está cada vez mais difundida nas áreas de ciências agrárias. A meta-análise surge como uma alternativa sustentável à experimentação animal, que são estudos caros, com dificuldades práticas, riscos e implicações éticas (Oster et al., 2018). Assim, com um amplo número de trabalhos existentes sobre determinados assuntos, esse método estatístico é escolhido para ilustrar a complexidade dos processos (Létourneau-Montminy et al., 2012). Além disso, é importante explorar e examinar o padrão de certas respostas, obtidos através de trabalhos prévios, comparando-o com publicações mais recentes (Cowieson et al., 2017). Com essa visão geral de como os dados se comportam ao longo do tempo, é possível entender como certos fatores (evolução das genéticas, das gerações das enzimas e da qualidade dos insumos, por exemplo) atuam sobre os resultados e como eles podem ser melhorados.

O uso de fitase em dietas para aves e suínos é bastante estudado. A quantidade de artigos científicos na plataforma *Web of Science* com as palavras “*pigs/broilers*” e “*phytase*” no título é de 202 e 306, respectivamente. Frente a tantos resultados diferentes e da amplitude de condições experimentais dos projetos prévios, é necessária uma padronização dos dados obtidos para as principais respostas produtivas. Para isso, a elaboração de um banco de dados com informações de diferentes trabalhos pode ser uma alternativa para aumentar o entendimento dos resultados já obtidos.

3. Hipóteses e objetivos

Hipótese

A meta-análise permite quantificar os diversos fatores que podem modular a ação e os efeitos da fitase sobre as respostas avaliadas, ao considerar a variabilidade entre os estudos.

Objetivo

Estudar diferentes fatores que podem modular a eficiência da fitase sobre as respostas avaliadas, ao considerar e reduzir a grande variabilidade existente entre os diferentes estudos, gerando resultados homogêneos.

CAPÍTULO II

Assessing the effect of phytase supplementation to corn- and soybean meal-based diets on digestibility and performance of growing pigs

Este capítulo é apresentado conforme as normas de publicação da revista Animal Trabalho submetido em janeiro de 2020.

Assessing the effect of phytase supplementation to corn- and soybean meal-based diets on digestibility and performance of growing pigs

Short title: Meta-analysis of phytase effects for growing pigs

Abstract
A meta-analysis was performed to assess the efficiency of phytase supplementation to corn- and soybean meal-based diets on nutrient digestibility and performance of growing pigs and to identify the factors that potentially modulated the phytase effects. The database was constructed using information from previous studies carried out between 2007 and 2017 that evaluated the phytase supplementation in diets for growing pigs (22 papers; 2161animals in 28 experiments). The main criteria for the paper selection were: (a) experimental evaluation of phytase supplementation; (b) feed formulas based on corn and soybean meal; (c) pigs from growing to finishing rearing phases; and (d) performance and/or digestibility responses. The meta-analysis followed three sequential analyses: graphical, correlation, and variance-covariance. Equations were used to predict the effect control treatment on the phytase supplementation. Variance decomposition was done using the sum of square from the analysis of variance. The meta-analysis indicated that feeding growing pigs with diets supplemented with phytase did not influence feed intake and feed conversion responses (P>0.05) although weight gain tended to be higher (+6%; P=0.067) in supplemented treatments. The digestibility coefficients of crude protein and calcium were not influenced by the phytase supplementation (P>0.05). However, phytase improved apparent digestibility of phosphorus (+42%; P< 0.05) compared to non-supplemented pigs. The regression equations were positive for performance and digestibility, but significant

(*P*<0.001) only for apparent digestibility variables, with R^2 values above 95%. The variance decomposition indicated that the study code and the enzyme brand were the factors that affected the phytase effect on weight gain and phosphorus digestibility. It was possible to conclude that phytase appears to have no or little effect on performance of growing pigs fed diets based on corn-soybean meal. However, it presents important results on phosphorus digestibility, especially bacterial phytases.

Keywords: calcium, enzyme, nutrition, phosphorus, swine

Implications

Meta-analysis can be used as a tool to predict the impact of phytase on growing pig performance and nutrient digestibility without the need for a new animal experiment. Phosphorus is one of the most expensive ingredients of the diets, and, in the medium term, their natural inorganic reserves will be depleted due to intense rock extraction. The results of this study may help other researchers in the development of mathematical models, the industry in the identification of increasingly efficient phytases, as well as assist the nutritionists in the formulation of economically and environmentally sustainable diets.

Introduction

Increasing world population is expected to double demand for food until 2050 (Tilman *et al.*, 2002). In this context, animal production becomes even more important due to the generation of a protein of excellent quality. Thus, the consumption of animal foods has grown significantly in recent years (ABPA,

2018) and animal nutrition improved the consumption of economic and environmental resources (Oster *et al.*, 2018).

Many ingredients of plant origin have antinutritional factors and phytate is one of the most important. In these ingredients, approximately 80% of the phosphorus is bound to phytate, but other minerals and amino acids can also bind phytate, becoming unavailable to monogastric animals (Nelson, 1976). Phytase is currently the most widespread exogenous enzyme on the market, which is due to the acceptance of this technology in place of dicalcium phosphate (Lei *et al.*, 2013), a non-renewable source of inorganic phosphorus (Scholz *et al.*, 2013).

On the other hand, the effectiveness of phytase addition to diets is not observed in all studies (Adeola and Cowieson, 2011). Given the great variability between results of different experiments, it is necessary to standardize the data obtained for each response and to identify the factors that act by modulating the effects of supplementation in the studied scenarios (Selle *et al.*, 2012). For this, the elaboration of databases with information from previous research projects and the study of these databases with tools such as meta-analysis may be an alternative to increase the available knowledge in this research area.

Meta-analysis is a tool that combines the effects of treatments from different experiments conducted under varying conditions (Sauvant *et al.*, 2008). This is especially interesting when the sample size or number of repetitions is low in some studies. This type of study is a sustainable alternative to animal trials, as animal studies are expensive, with practical difficulties, risks and ethical implications (Oster *et al.*, 2018). Thus, the aim of the present study was to assess the efficiency of phytase supplementation to corn- and soybean meal-based diets on nutrient digestibility and performance of growing pigs. In addition, the study attempted to identify the factors that potentially modulated the phytase effects.

Material and Methods

Digital databases (Google Scholar, Web of Science, and Scopus) were searched to identify studies published in scientific journals that reported the performance and apparent digestibility results of pigs fed diets supplemented with phytase. The keyword ‘phytase’ combined with ‘pigs’ was used in the search. The main criteria for the paper selection were: (a) experimental evaluation of phytase supplementation; (b) feed formulas based on corn and soybean meal (other main ingredients were not tolerated); (c) pigs from growing (25 kg of body weight) to finishing rearing phases; and (d) performance and/or digestibility responses. The literature search was performed in August, 2018. After the paper selection, the information related to the proposed theoretical model and other additional variables were copied from both material and methods and results sections in the original publications, and transferred to an electronic spreadsheet. Results collected in animals subjected to any health challenge were not included in the database because it could change the effect of the enzyme.

The methodology applied to database construction and coding followed the proposals described in literature (Lovatto *et al.*, 2007; Sauvant *et al.*, 2008). Codes were used with qualitative grouping criteria in the analytical models. In this item, the main codes were applied for supplementation (control or phytase supplemented diet) and dietary phosphorus content (adequate levels according to the growth phase or lower levels than Rostagno *et al.* (2017) recommendations, which represents the reality of Brazilian production). Other codes were used to consider the variability among all compiled experiments (e.g.: effect of study or trial).

Performance and digestibility results were evaluated as raw data (as presented in the original papers) or as relativized information (indicated in the current study as ‘phytase effect’). For the second approach, the responses of phytase supplemented treatments were relativized to the respective control treatment and expressed as percentage of variation between the results. This procedure was adopted because it considerably reduced the effect of variations among experiments in the database. Other calculations were performed in the database. When the content of phytate phosphorus was not provided in the publication, it was calculated from Brazilian Tables for Pigs and Poultry (Rostagno *et al.*, 2017). Nutrient intake was obtained multiplying the nutritional composition of the diets by the feed intake. Additionally, feed conversion ratio was calculated from feed intake and weight gain results.

Statistical analyses were performed using the software Minitab (Minitab for Windows, v. 18, Pine Hall Rd, Pennsylvania, USA). Meta-analysis was performed following three sequential analyses: graphical (to control database quality and to observe biological coherence of data), correlation (to identify related factors among all variables), and variance-covariance (to compare the treatments and to obtain the prediction equations).

Equations were used to predict the effect of control treatment on the phytase supplementation. In this procedure, phytase effect (% of change over control) was the dependent variable and the response of the control treatment was considered as the independent variable. The code of study effect was considered in all analytical models as a random effect.

General Linear Model procedure was also used to perform a subsequent variance decomposition, used to observe the intensity of model's variables on the variables under analysis. Factors that showed high correlation with the phytase effect on the weight gain and on the apparent total tract digestibility of phosphorus were considered in the models. Due to the collinearity among some factors, four models were developed for each variable. So, one model considered the ingredient inclusions in the feed formulas (i.e. inclusion of phosphate, corn, limestone, and soybean meal), while other model included the factors related to the nutritional composition of diets (i.e. level of phytate phosphorus, total phosphorus, and total calcium). Variance decomposition was done using the sum of square from the analysis of variance. In the procedure, the total sum of square was considered as the total variance and the sum of square of each variable was considered as a fraction of it.

Results

The database was composed of 22 scientific papers (Table 1) published from January 2007 to August 2017, which used 2161 pigs in 28 experiments. Initial and final mean body weights were 44.08 and 83.68 kg, respectively. Barrows and females were used mixed in 15% of the studies, while 63% of the trials used only barrows, and 22% of the trials used only females. All trials were developed using commercially available enzymes. Phytase of bacterial origin was tested in 50% of the studies, while 36% used fungal phytase. Phytate phosphorus content varied between 0.18 and 0.25% in the experimental feeds.

The relation between dietary microbial phytase and total phosphorus is shown in Figure 1. Each cross represents a study of the database. Only one study with two experiments was excluded from the graph because it did not present the total phosphorus content of the diets. Only 39% of the studies tested a single concentration of phytase, while 43% of the studies tested more than one level of available phosphorus. Most studies (69%) used doses of supplemented phytase up to 1000 FTU/kg. Available phosphorus content of diets varied between 0.20 and 0.70%. Feeds with reduced levels of phosphorus (nutritional reduction - lower than the requirement for the phase) were used in 36% of the studies.

Performance variations between phytase supplemented treatments and their respective control treatments are presented in Figure 2. Numerical improvements in weight gain responses were observed in 96% of the comparisons between supplemented and control treatments. In addition, 51% of all treatments containing phytase reported improved feed intake compared to control treatments. However, the weight gain improvement was significant (considering the statistical analysis provided in the original publication) in 27% of the comparisons, while only one study reported a significant variation for feed intake. The heterogeneity observed in the results of previous studies is probably due to the large diversity of experimental conditions, such as phytase source, enzyme supplementation in the feeds, and animal's age. Meta-analysis is a useful tool to deal with this experimental variability as it allows establishing systematic responses adjusted to the diversity of available publications. Meta-analysis also increases the sample number, thereby highlighting possible effects that would not be observed in conventional studies due to the limited number of observations.

The meta-analysis indicated that feeding growing pigs with diets supplemented with phytase did not influence feed intake and feed conversion responses (P>0.05), although weight gain tended to be higher (+6%; P=0.067) in supplemented treatments (Table 2). The digestibility coefficients of crude protein and calcium were not influenced by the phytase supplementation (P>0.05). However, phytase improved apparent digestibility of phosphorus (+42%; P<0.05) compared to non-supplemented pigs, as expected.

The general regression equations for the effect of phytase supplementation on performance and apparent digestibility based on the control results are presented in Table 3. The control effect was positive for both responses, but significant (P<0.001) only for apparent digestibility variables, with R^2 values above 95%. This indicates that the digestibility of the control diet itself explains the significant effect of phytase on the digestibility of the treatments. Some models were developed to help understanding the factors that affect the most on the phytase effect on weight gain (Models I and II; Table 4). The inclusion of phosphate, corn, limestone, and soybean meal were the most important factors (P<0.01) in Model I. Together, these factors accounted for 66% of the variation. Phytase brand was also an important factor (P<0.06) explaining the effect of supplementation in both models (9% in Model I and 13% in Model II). However, the phytase dose and body weight of the animals did not influence the effect of phytase on weight gain. In the same way, the dietary level of phytate phosphorus, total phosphorus, and total calcium were neither significant in Model II.

The phytase dose was one of the main factors (*P*<0.01) in the models developed to explain the phytase effect on apparent total tract digestibility of phosphorus (Models III and IV; Table 5). Phytase brand was also an important factor (*P*<0.01) in Model III, but it was not included in the Model IV. The inclusion of phosphate, corn, limestone, and soybean meal were not significant and accounted together for 7% of the variation of Model III. Lastly, the dietary level of phytate phosphorus, total phosphorus, and total calcium were not significant in Model IV, and neither was the body weight in any developed model.

Discussion

Phytate is the most important antinutritional factor of plant-based feed in monogastric nutrition due to its production losses and its negative environmental effects related to the elimination of phytic phosphorus. When phytate binds to dietary phosphorus and calcium, some of these nutrients become unavailable to the animal and are excreted to the environment (Lei *et al.*, 2013). The adverse environmental consequences of high phosphorus excreted and the need of inorganic phosphate supplementation to meet the phosphorus requirements of these species motivated the development of phytases, phosphohydrolytic enzymes that remove phosphate from phytate and make this mineral available for absorption (Singh and Satyanarayana, 2015). Pigs and poultry account by 70% of the global meat production, reinforcing the need for sustainable management of the world's limited sources of phosphorus (Oster *et al.*, 2018).

According to the data presented in this study, phosphorus digestibility was the most affected variable by the use of phytase on corn- and soybean meal-based diets for growing pigs. The fact that the basal ingredients of all diets were always the same suggests that the determining factor for the increase in phosphorus digestibility was the effect of phytase on phosphorus release. High amounts of dietary calcium may impair phosphorus digestibility due to the binding of these minerals during digestion and the formation of salts (Heaney and Nordin, 2002). However, the models do not always present significant interactions between calcium levels and microbial phytase (Létourneau-Montminy *et al.*, 2012; Zouaoui *et al.*, 2018). The content of dietary non-phytic phosphorus also directly affects the use of phosphorus by the animal (Létourneau-Montminy *et al.*, 2012). However, these effects were not observed in this study. The factors that contributed to improve phosphorus digestibility were the enzyme dose and brand.

There is controversy on the effect of phytase on the digestibility of protein, calcium, and other dietary minerals (Selle *et al.*, 2012). In this study, protein and calcium digestibility were not directly affected by enzyme inclusion, but there was an effect of control diets on phytase effect. The limitation of ingredients included in this database was crucial to verify this effect. This indicates that there may be a positive action of phytase when added to balanced diets without nutritional reduction, but when these diets already have a high digestibility the enzyme effectiveness is reduced.

It is well accepted that phytate stimulates mucin secretion into the gut and alters amino acid flow which impairs its digestibility (Selle *et al.*, 2012), but this effect is lower in pigs than in poultry (Woyengo *et al.*, 2009).A meta-analysis study showed that dietary supplementation with phytase increased amino acid digestibility, mainly threonine, and this response was not affected by diet composition (Zouaoui *et al.*, 2018). According to the authors, the increase in digestibility may be a result of reduced endogenous amino acid losses. The hydrolysis of phytate would decrease the production of ileal secretions such as mucin, which is rich in threonine. On the other hand, a recent experiment with pigs showed that although supplementing diets with at least 3000 FTU/kg of phytase increased degradation of dietary phytate, it did not change intestinal mucin synthesis, gastric protein hydrolysis, and protein digestibility (Mesina *et al.*, 2019).

Performance responses did not differ significantly between treatments. The number of studies that included all the criteria evaluated in this meta-analysis may have been a limiting factor. Unlike poultry, experiments with pigs are more complex, laborious, and expensive (Cowieson *et al.*, 2017) and most of the articles included in this database evaluated only digestibility. In a literature review of trials testing phytase, 70% of studies using broiler chickens studied performance, and only 14% of experiments with pigs evaluated this response (Singh and Satyanarayana, 2015). It should be noted that phytase was developed to reduce phosphorus excretion (Selle and Ravindran, 2008), not for weight gain, although performance is not impaired with enzyme supplementation. The lower R^2 values of the models also indicated a low influence of phytase on the variables.

The variance decomposition indicated that the study code and the enzyme brand were the factors that affected the phytase effect on weight gain and phosphorus digestibility. The enzyme brand is probably related to the microbial origin of phytase (bacterial or fungal), since bacterial phytases are more resistant to larger pH ranges and therefore have action over a larger portion of the gastrointestinal tract (Jain *et al.*, 2016). The "study factor" may consider animal characteristics, such as genetics or sex.

Some factors related to diets affected only weight gain, such as the addition of corn, soybean meal, limestone and phosphate, but were not enough to cause statistical difference, corroborating with other results already discussed in this paper. Phytase dose only affected phosphorus digestibility. In another study, the authors state that doses of the enzyme between 600 and 800 FTU/kg are already sufficient to mitigate the effects of phosphorus deficiency on broilers fed diets based on corn and soybean meal (Létourneau-Montminy *et al*., 2010). In the present study, most of the trials presented phytase doses lower than 1000 FTU/kg. Despite the different species, this could explain the effect of phytase dose on phosphorus digestibility without affecting weight gain. Generally, the recommended dose from phytase manufacturers is 500 FTU/kg for broilers and pigs. Since weight gain did not differ statistically between diets, this result is relevant to explain how phytase improved phosphorus digestibility. The study effect is expected because each one represents a random result from a population, and the study effect represents the sum of the effects of various factors (Sauvant *et al.*, 2008).

The effect of the enzyme brand is probably a consequence of the microbial origin of phytase (fungal or bacterial).The first globally commercialized phytase, from *Aspergillus niger*, was launched on market in 1991. More than a decade later, the first bacterial phytase, from *Escherichia coli*, was developed and many studies were published (Lei *et al.*, 2013). In the last decade new generations of the enzyme have been developed and the advance of animal genetic improvement has ameliorated the responses obtained (Singh and Satyanarayana, 2015). Studies have shown the superior effects of bacterial phytase in releasing phosphate from phytate (Rodriguez *et al.*, 1999; Augspurger *et al.*, 2003; Kerr *et al.*, 2010). Bacterial phytases are more resistant to heat processing, pepsin action, and more acidic pH than fungal phytase, which is more resistant to pancreatic enzymes, and these characteristics improves its action in the stomach – the site with the highest phytase activity (Rodriguez *et al.*, 1999; Jain *et al.*, 2016). Therefore, the temporal limitation that considers the last decade of phytase evolution plus the limitation of ingredients included in the diets, which keeps the substrate base homogeneous, were important measures to reduce the variation of the collected results for this meta-analysis. Despite the various factors considered in an attempt to justify the causes of phosphorus digestibility and weight gain variation, approximately 40% of the effects that contributed to these results could not be explained and may be particular characteristics of animals (genetic, sex) or enzymes.

This work has shown that meta-analysis can be used as a tool to predict the impact of phytase on growing pig production, but further studies on the performance of these animals are needed. The scarcity of works (due to the limitations imposed) was an important factor that hindered the performance of some statistical analysis. On the other hand, the large amount of work on the effect of phytase on phosphorus digestibility allowed the choice of some criteria for the formation and homogenization of the database, such as temporal and ingredient limitation.

Conclusions

In conclusion, phytase appears to have no or little effect on performance of growing pigs fed diets based on corn-soybean meal. However, it represents important results on phosphorus digestibility, since this response is directly affected by characteristics of phytase (microbial origin and dose) and, possibly, of the animal (genetics, sex).

Declaration of interest

We declare no conflict of interest, including financial, personal, or other relationships with other people or organizations that could inappropriately influence this work**.**

Ethics statement

This study was performed with data from published papers; consequently, there was no use of animals. In this situation, it is not necessary for the study to be approved by the Animal Ethics Committee of the Universidade Federal do Rio Grande do Sul.

Software and data repository resources

None of the data were deposited in an official repository.

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Table 1 *Description of the pigs database*

Reference			Body weight, kg		Microbial phytase origin
Code	First author	Year	Min.	Max.	
1 [b]	McCormick	2017	-	-	*Escherichia coli*
2 [b]	Velayudhan	2015	25.00	-	*Buttiauxella* spp.
3 [b]	Favero	2014	39.30	40.40	*E. coli*
4 [a]	Santos	2014	22.90	55.95	*E. coli*
5 [a]	Almeida	2013	-	-	*Citrobacter braakii*
6 [b]	Almeida	2012	-	-	*E. coli*
7 [b]	Jolliff	2012	41.00	-	*Peniophora lycii* *Aspergillus niger* *E. coli*
8 [b]	Kiefer	2012	44.90	-	*A. niger*
9 [b]	Salyer	2012	46.60	117.95	*E. coli*
10 [b]	Zeng	2011	-	-	*Trichoderma reesei*
11 [b]	Kerr	2010	83.20	111.00	*E. coli* *P. lycii* *A. niger, E. coli, P. lycii*
12 [a]	Rodrigues	2010	25.00[a] 49.00[b]	49.81 -	*E. coli* + *C. braakii*
13 [b]	Trujillo	2010	-	-	*A. niger*
14 [a]	Corassa	2009	93.71	119.75	*P. lycii*
15 [a]	Hill	2009	45.30	-	*A.niger*
16 [b]	Jendza	2009	51.30[a] 20.00[b]	- -	*E. coli*
17 [a]	Sands	2009	18.20	31.75	*A.niger*
18 [b]	Tolón	2009	31.20	-	
19 [a]	Veum	2008	51.51	122.45	*A.niger*
20 [a]	Augspurger	2007	80.00	120.20	*E. coli*
21 [a]	Beaulieu	2007	40.26	68.11	*E. coli*
22 [b]	Sands	2007	28.00	-	*A.niger*

[a]= Study of performance
[b]= Study of digestibility

Table 2 *Performance, inherent apparent total tract digestibility (ATTD) and the effect of exogenous phytase in growing pigs*

	Control performance		Phytase effect		
	Mean	S.E.	Effect, %	S.E.	*P-value*
Feed intake, g/day	2249.00	143.00	+2.03	0.53	0.500
Weight gain, g/day	878.00	30.20	+6.29	0.65	0.067
Feed conversion, g/g	2.80	0.11	-4.65	0.71	0.336
ATTD crude protein, %	84.37	1.55	+1.21	0.59	0.798
ATTD calcium, %	66.04	3.11	+12.88	2.02	0.576
ATTD phosphorus, %	29.25	2.47	+42.13	2.58	0.027

S. E., standard error.
[a]Responses of phytase supplemented treatments were relativized to the respective control treatment and expressed as percentage of variation between the results.

Table 3 *Prediction of the effect of phytase supplementation on pig performance and apparent total tract digestibility (ATTD) in function of control results*

Variable	Regression equation (model)	*P-value*		R^2
		Intercept	Variable	
Feed intake, g/day	y = -2.81 + 0.0020x	0.630	0.467	0.38
Weight gain, g/day	y = -7.20 + 0.0138x	0.465	0.213	0.63
Feed conversion, g/g	y = -23.00 + 7.0200x	0.123	0.190	0.31
ATTD crude protein, %	y = 83.71 + 0.8816x	<0.001	<0.001	0.99
ATTD calcium, %	y = -71.48 + 1.1361x	<0.001	<0.001	0.99
ATTD phosphorus, %	y = -37.24 + 1.4126x	<0.001	<0.001	0.95

y, phytase effect; x, control result.

Table 4 *Factors that explained the phytase effect on the weight gain of pigs*

Factors	Contribution to the total variance, %	*P-value*
Model I		
Phosphate inclusion in feed formula, %	18.55	0.001
Corn inclusion in feed formula, %	16.02	0.001
Limestone inclusion in feed formula, %	15.59	0.002
Soybean meal inclusion in feed formula, %	15.56	0.002
Study effect	11.14	0.018
Phytase brand	9.18	0.010
Phytase dose, FTU/kg	1.03	0.327
Body weight, kg	0.00	0.721
Error	12.93	
Model II		
Study effect	36.17	0.028
Phytase brand	13.35	0.050
Dietary phytate phosphorus, %	2.86	0.341
Dietary total phosphorus, %	3.73	0.279
Phytase dose, FTU/kg	1.49	0.488
Dietary total calcium, %	1.31	0.516
Body weight, kg	0.00	0.777
Error	41.10	

Table 5 *Factors that explained the phytase effect on the apparent total tract digestibility of phosphorus in pigs*

Factors	Contribution to total variance, %	*P-value*
Model III		
Phytase dose, FTU/kg	41.65	<0.001
Study effect	16.41	0.018
Brand	16.41	0.001
Phosphate inclusion in feed formula, %	4.52	0.095
Body weight, kg	4.36	0.100
Limestone inclusion in feed formula, %	1.48	0.331
Soybean meal inclusion in feed formula, %	0.45	0.589
Corn inclusion in feed formula, %	0.34	0.640
Error	14.38	
Model IV		
Study effect	45.15	0.019
Phytase dose, FTU/kg	12.26	0.006
Dietary total phosphorus, %	1.40	0.323
Body weight, kg	0.54	0.537
Dietary phytate phosphorus, %	0.39	0.599
Dietary total calcium, %	0.04	0.867
Error	40.21	

Figure captions

Figure 1 Meta-design: within-study relation between dietary microbial phytase and total phosphorus concentration. Each study is indicated by a cross, the horizontal line indicates the available phosphorus content of diets, and the vertical line indicates the range of microbial phytase supplementation.

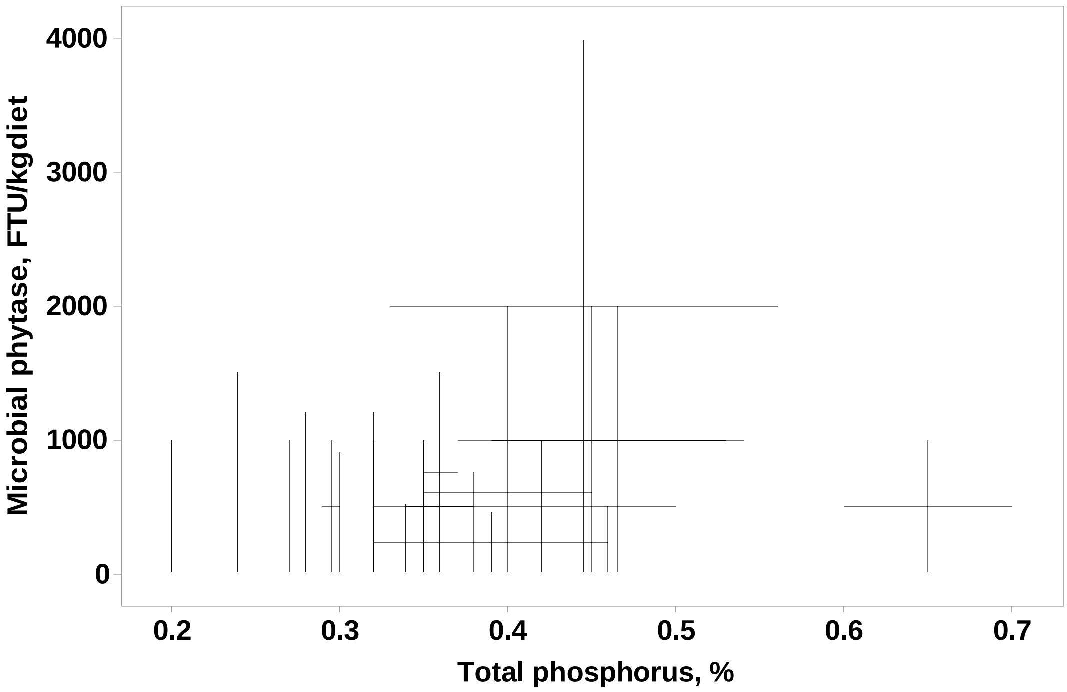
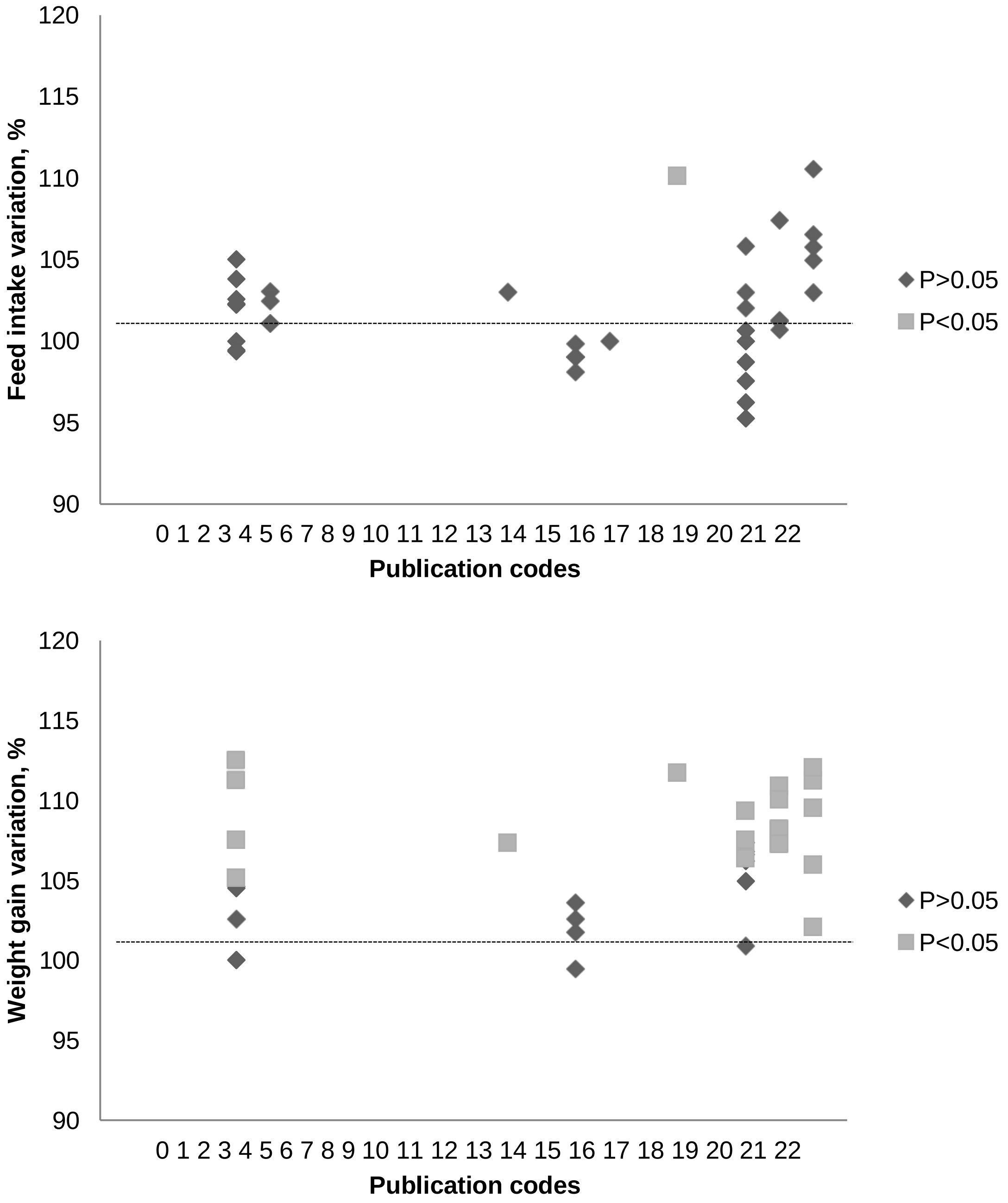


Figure 2 Performance responses of pigs fed phytase supplemented treatments relativized to the respective control treatment.



[a]Codes are the same indicated in Table 1. Probabilities were indicated in the original publication.

CAPÍTULO III

Phytase supplementation to corn- and soybean meal-based diets affects digestibility and performance of broilers

Este capítulo é apresentado conforme as normas de publicação da revista Animal. Submissão ainda não realizada.

Phytase supplementation to corn- and soybean meal-based diets affects digestibility and performance of broilers

Short title: Meta-analysis of phytase effects for broilers

Abstract

A meta-analysis was performed to assess the efficiency of phytase supplementation to corn- and soybean meal-based diets on performance, apparent ileal digestibility (AID), bone characteristics and carcass yield of broilers and to identify the factors that potentially modulated the phytase effects. The database was constructed using information from previous studies carried out between 2007 and 2019 that evaluated the phytase supplementation in diets for broiler (102 papers; 69472 animals in 104 experiments).The main criteria for the paper selection were: (a) experimental evaluation of phytase supplementation; (b) feed formulas based on corn and soybean meal; (c) broilers in all production phases; and (d) performance and/or digestibility responses. The meta-analysis followed three sequential analyses: graphical, correlation, and variance-covariance. Equations were used to predict the effect control treatment on the phytase supplementation. Variance decomposition was done using the sum of square from the analysis of variance. The meta-analysis indicated that feeding broilers with diets supplemented with phytase influenced both performance and digestibility, with improvements in all results ($P < 0.05$). Feed intake (+5.22%), weight gain (+7.09%), and feed conversion ratio (-2.75%) were positively influenced by phytase supplementation. Phosphorus ileal digestibility was the most affected response (+27.42%). Bone ash content (left tibia) also showed a significant increase ($P<0.01$), although the bone amounts of calcium and phosphorus were not affected, as well as the carcass yield (*P*>0.05). The age of the birds was used as a covariate in the regression models due to its wide variation between studies. As expected, the effect of age was significant for performance and AID responses. The study effect was the most important factor for all responses, followed by the phytase dose (weight gain and AID of phosphorus) or age (AID of calcium). Lastly, the Ca:P ratio appears to be more important than the calcium or the phosphorus content of the diet for explaining the effect of phytase on weight gain and AID of calcium. Supplementing phytase to broiler diets improves performance, ileal digestibility and bone ash content of birds. Some factors, such as age and diet composition, affect these responses in different and even opposite ways, while the dose of the enzyme shows effect only on the phosphorus AID.

Keywords: calcium, data modeling, enzyme, phosphorus, phytate

Implications

Meta-analysis is an alternative tool to predict the impact of phytase on broilers production without the need for a new experiment. This approach also allows the identification of the factors that influence the phytase effect, which is very important and difficult in conventional trials. The knowledge of these factors is important to maximize the production and to reduce possible losses caused by environmental, genetic and nutritional variations. These results can help in planning the broiler production according to the available resources and encourage future studies on the environmental impact of phytase supplementation for broilers.

Introduction

The use of ingredients of plant origin in the diet of monogastric animals is often associated with the addition of exogenous enzymes due to the presence of anti-nutritional factors, such as phytate. About 80% of the phosphorus in these ingredients is bounded to phytate, forming the phytate-phosphorus. Other minerals and amino acids can also be bounded to phytate and are excreted in the environment (Nelson, 1976). The addition of phytase to diets for broilers can increase the use of nutrients (Li *et al.*, 2018) and improve the performance of birds (Hughes *et al.*, 2009; Lee and Bedford, 2016), but the results still vary widely between studies.

Phytase represents 60% of the enzyme market for animal nutrition (Lei *et al.*, 2013). The rapid growth of this market is due to the wide acceptance of phytase technology to replace the use of dicalcium phosphate (Adeola & Cowieson, 2011). Currently, phytases are cheaper and the price of mineral phosphorus is very high, so the cost-benefit is higher with enzyme supplementation (Hamdi *et al.*, 2018). In addition, dicalcium phosphate is a finite source of inorganic phosphorus (Lei *et al.*, 2013). Several factors can interfere with the performance and digestibility responses observed in animals fed diets supplemented with phytase, and it is important that these factors are determined and quantified.

Meta-analysis is a sustainable alternative to animal experimentation (Oster *et al.*, 2018). Thus, with a large number of existing works on certain subjects and the grouping of data from several previous studies, this statistical method is chosen to illustrate the complexity of the processes (Sauvant *et al.*, 2008; Létourneau-Montminy *et al.*, 2012). This work was developed to evaluate the effects of the inclusion of phytase in diets for broilers on the use of nutrients and productive performance through a meta-analysis, in addition to determining and quantifying the factors that influence the effect of this enzyme.

Material and Methods

Digital databases (Google Scholar, Web of Science, and Scopus) were searched to identify studies published in scientific journals that reported the performance, apparent ileal digestibility, bone characteristic (ash, calcium, and phosphorus content), and carcass yield results of broilers fed diets supplemented with phytase. The keyword ‘phytase’ combined with ‘broilers’ was used in the search. The main criteria for the paper selection were: (a) experimental evaluation of phytase supplementation; (b) feed formulas based on corn and soybean meal (other main ingredients were not tolerated); (c) broilers in all production phases; and (d) performance and/or digestibility responses. The results of total and ileal digestibility were collected, but only the data of ileal digestibility were used in this article. After the paper selection, the information related to the proposed theoretical model and other additional variables were copied from both material and methods and results sections in the original publications, and transferred to an electronic spreadsheet. Results collected in animals subjected to any health challenge were not included in the database because it could change the effect of the enzyme.

The methodology applied to database construction and coding followed the proposals described in literature (Lovatto *et al.*, 2007; Sauvant *et al.*, 2008). Codes were used with qualitative grouping criteria in the analytical models. In this item, the main codes were applied for supplementation (control or phytase supplemented diet) and dietary phosphorus content (adequate levels according to the growth phase or lower levels, following Rostagno *et al.* (2017), which represents the reality of Brazilian production recommendations). Other codes were used to consider the variability among all compiled experiments (e.g.: effect of study or trial).

Performance and digestibility results were evaluated as raw data (as presented in the original papers) or as relativized information (indicated in the current study as ‘phytase effect’). For the second approach, the responses of phytase supplemented treatments were relativized to the respective control treatment and expressed as percentage of variation between the results. This procedure was adopted because it considerably reduced the effect of variations among experiments in the database. Other calculations were performed in the database. Nutrient intake was obtained multiplying the nutritional composition of the diets by the feed intake. Additionally, feed conversion ratio was calculated from feed intake and weight gain results.

Statistical analyses were performed using the software Minitab (Minitab for Windows, v. 18, Pine Hall Rd, Pennsylvania, USA). Meta-analysis was performed following three sequential analyses: graphical (to control database quality and to observe biological coherence of data), correlation (to identify related factors among all variables), and variance-covariance (to compare the treatments and to obtain the prediction equations).

Equations were used to predict the effect of control treatment on the phytase supplementation. In this procedure, phytase effect (% of change over control) was the dependent variable and the response of the control treatment was considered as the independent variable. The code of study effect was considered in all analytical models as a random effect.

General Linear Model procedure was also used to perform a subsequent variance decomposition, used to observe the intensity of model's variables on the responses under analysis. Factors that showed high correlation with the phytase effect on the weight gain and on the apparent ileal digestibility of phosphorus and calcium were considered in the models. Due to the collinearity among some factors, the final model was developed considering only the most correlated variables of nutritional composition of diets (i.e. the level of total phosphorus was contrasted with the level of available phosphorus and only one was chosen for the final model because these variables were highly correlated). Variance decomposition was done using the sum of square from the analysis of variance. In the procedure, the total sum of square was considered as the total variance and the sum of square of each factor was considered as a fraction of it.

Results

The database was composed of 102 scientific papers (listed in Table 1 – complementary material) published from January 2007 to March 2019, which used 69472 broilers in 114 experiments. Most articles (56%) were published between 2007 and 2012. Mean initial and final ages were 10 and 28 days, respectively. Males and females were used mixed in 8% of the studies, while 69% of the trials used only males, 6% of the trials used only females, and the sex was not mentioned in 17% of the studies. All trials were developed using commercially available enzymes. Phytase of bacterial origin was tested in 49% of the studies, while 43% used fungal phytase. In relation to the nutritional matrix, phytase was added to diets with reduced phosphorus content in 29% of the trials, while 24% of the studies tested the enzyme in diets with reduced calcium content.

The relation between dietary microbial phytase and available phosphorus is shown in Figure 1. Each cross represents a study of the database. Of the 114 trials, only ten studies were excluded from the graph because they did not present the available phosphorus content of the diets. Most studies (58%) used doses of supplemented phytase up to 1000 FTU/kg. Among phytase super-dosing (above 1000 FTU/kg), the highest dose used was 12500 FTU/kg. Available phosphorus content of diets varied between 0.08 and 0.85% (and it is important to note here that part of these studies used reduced doses of phosphorus).

Performance and digestibility variations between phytase supplemented treatments and their respective control treatments are presented in Figures 2 and 3. In most studies there was a numerical improvement in feed intake (67% of the comparisons) and weight gain (70% of the comparisons), which is indicated by the points that are above the dotted line. A single study showed drastic reductions in these responses compared to control treatments (i.e. -68% for feed intake and -42% for weight gain). In this case, the data has been reviewed and maintained, but it is important to note the singular response of this experiment. When compared to performance responses, there are less results available for the apparent ileal digestibility (AID) responses, both for phosphorus and calcium, probably due to the characteristics and costs of the methods. The results on AID of calcium were much more variable between studies than the AID of phosphorus. Even so, most of the comparisons (71%)

were numerically favorable to phytase when assessing AID of calcium, while only one work showed an impaired AID result for phosphorus in supplemented group compared to the control treatment.

The meta-analysis indicated that feeding broilers with diets supplemented with phytase influenced both performance and digestibility, with improvements in all results (Table 1). Feed intake (5.22%), weight gain (7.09%), and feed conversion ratio (-2.75%) were positively influenced (P<0.01) by phytase supplementation. Phosphorus ileal digestibility was the most affected response (+27.42%; *P*<0.01). Bone ash content (left tibia) also showed a significant increase (+6.04%; *P*<0.01), although the bone amounts of calcium (*P*=0.772) and phosphorus (*P*=0.518) were not affected, as well as the carcass yield (*P*=0.562).

The general regression equations for the effect of phytase supplementation based on the control results are presented in Table 2. The equations were generated only for the responses significantly improved by phytase (previous analysis), so the results for bone phosphorus/calcium content and carcass yield were not evaluated in the current approach. The control effect was positive for all responses except for AID of nitrogen, for which it was not significant. This indicates that the digestibility of the control diet itself may explain part of the effect of phytase in most responses. In addition, the age of the birds was also used as a covariate in the regression models due to its wide variation between studies. As expected, the effect of age was significant for performance and AID responses. The high coefficient of determination values indicated that these models explain most of the variation in the effect of phytase in relation to the diets that were not supplemented with the enzyme (control).

Some models were developed to help understanding the factors that affect the most on the phytase effect on weight gain and on AID of calcium and phosphorus (Table 3). The study effect was the most important factor for all responses, followed by the phytase dose (weight gain and AID of phosphorus) or age (AID of calcium). Dietary phosphorus content did not influenced AID of phosphorus, AID of calcium, neither weight gain. Few factors that affected weight gain and AID of phosphorus also affected AID of calcium, and the dietary calcium itself also did not affect these responses. Lastly, the Ca:P ratio appears to be more important than the calcium or the phosphorus content of the diet for explaining the effect of phytase on weight gain and AID of calcium.

Discussion

According to the results, the addition of phytase to corn- soybean meal-based diets improved feed intake, daily weight gain, and feed conversion ratio for broilers, due in part to the greater use of nutrients, which will be further discussed. Initially, phytase was produced to reduce the excretion of phytic phosphorus to the environment and also to minimize the use of inorganic phosphorus in diets (Lei *et al.*, 2013). In addition to hydrolyzing phytic phosphorus, other nutrients are benefited by the use of the enzyme, improving its absorption and the growth of birds, which, consequently, may also consume more feed.

The variance decomposition indicated that the dose of the enzyme and the age of the birds were the factors that contributed the most to the variation in weight gain, in addition to the effect of the study. In addition, the Ca:P ratio of the diets proved to be more important in the models than the calcium or phosphorus content considered as isolated factors. The age had a negative effect on performance responses, indicating that young broilers are most benefited by the effects of phytase, which may have a direct relationship with the rapid bone growth and weight gain of current genetics. The birds' gastrointestinal tract is still developing until 14 days of age (Li *et al.*, 2018). For that reason, the young birds are less able to digest phosphorus and are more susceptible to the dietary calcium concentration and, mainly, to the possible imbalance of the Ca:P ratio (Obst and Diamond, 1992). The lower development of digestive compartments and intestinal villi compromises the production of digestive enzymes, in addition to increasing the rate of passage of feed, impairing its digestion (Li *et al.*, 2018). In this case, the addition of phytase up to three weeks of age can mitigate these negative effects, facilitating the digestion of phosphorus and other dietary components that promote bone and muscle growth in the bird.

The phytase dose also affected the performance responses. Doses of 1000 FTU/kg already improve performance and bone mineralization, but below that, the effects on these responses may not be seen (Hamdi *et al.*, 2018). Depending on the extent of the reduction in phytate structure, in addition to releasing minerals and amino acids, it can generate inositol, a well-known growth promoter (Lee and Bedford, 2016). In fact, the new studies carried out with phytase superdosing aim to improve the performance of birds (Cowieson *et al.*, 2011), since these high doses would cause an almost complete hydrolysis of the phytate in inositol, whose concentration in the intestine would increase (Walk *et al.*, 2014). However, it was not possible to generate a regression equation estimating the recommended dose of phytase due to the lack of studies testing more than 3 doses (intra-study effect). In other words, most of the variation among doses are a inter-study effect in the database.

Phytase supplementation in diets based on corn and soybean meal increased the AID of nitrogen, calcium and phosphorus. The deleterious effects of phytate on dietary protein are already well known. As pH changes along the digestive tract, phytate-protein complexes are formed, which reduce the solubility and utilization of these proteins by animals and are refractory to digestion by pepsin at stomach pH (Selle *et al.*, 2012). Phytase supplementation can reverse this effect and increase the AIDN, but the magnitude of this response varies between different amino acids and the type of dietary ingredient (Ravindran *et al.*, 1999), which was highlighted in the current study by the significant effect of study in most of the analyzed variables.

According to Shirley and Edwards (2003), nitrogen retention reaches a limit with doses close to 750 FTU/kg. This indicates that phytase may have a limited effect on the release of amino acids, or that the formation of phytate-protein complexes occurs on a smaller scale when compared, for example, with the phytate-phosphorus bond. For proper protein digestion, the most important fact is that the breakdown of the complex must occur between the crop and the gizzard, where the catalytic effect of phytase is greater due to acidic pH, and the released protein is mixed and digested by proteolytic enzymes of the proventricle and pancreas in the gizzard (Selle *et al.*, 2012; Hamdi *et al.*, 2018). The model equation for AIDN showed a very low coefficient of determination, indicating that factors other than the digestibility of the control diet itself and age can interfere in this response, such as the ingredients of the diet or characteristics of the enzyme, for example. However, these factors could not be assessed due to limited information in the previous studies.

Data on the effects of phytate and phytase on AIDCa in the literature are controversial. It is known that the binding of phytate with calcium is of great importance due to the formation of an indigestible complex that becomes insoluble and precipitates at a pH above 5 (Selle *et al.*, 2009). Thus, less hydrolysis of phytate in the gizzard may increase the impact of calcium on this phytate in the intestine and impair the action of phytase (Li *et al.*, 2018). Although the affinity of calcium for phytate is lower compared to other minerals, its impact is high due to high calcium supplementation in diets (Hamdi *et al.*, 2018). Considering this information, the high inclusion of limestone in diets can limit the action of phytase (Akter *et al.*, 2016), but these effects are not always observed (Powell *et al.*, 2011). In our study, Ca levels in diets were not significant in the models that explained the effect of phytase on weight gain and AIDP. The inclusion of limestone was not selected by previous procedures as a factor for the same models. However, it should be noted that the studies included in the database worked with acceptable levels of this mineral, avoiding very high concentrations that could cause the negative effect.

According to the results of this study, supplementation of broiler diets with phytase increases AIDCa. The factors that most influenced the effect of the enzyme were, in addition to the variability between studies, the age and the dietary Ca:P ratio. Since the effect of age is positive, according to the model, the older the bird, the greater the efficiency of the enzyme on the use of calcium. Older birds have the most developed gastrointestinal tract and the bolus spends more time being mixed and digested in the gizzard, whose low pH turns the phytate-Ca complex more soluble, facilitating the action of phytase (Li *et al.*, 2018).

The Ca:P ratio of the diet affects AIDCa, but this factor was not significant for AIDP. Calcium supplementation receive less emphasis in the research compared to phosphorus, as this mineral is much cheaper (Li *et al.*, 2018), although the effectiveness of phytase is impaired indirectly by the formation of the phytate-calcium complex. Thus, calcium is often added in excess, modifying its intestinal flow, its digestibility (Akter *et al.*, 2018), and the performance of broilers (Hamdi *et al.*, 2015). On the other hand, some authors have observed that the increase in dietary calcium improves performance when phytase is also supplemented, as the use of phosphorus also increases (Tamim *et al.*, 2004; Driver *et al.*, 2005). Thus, the effects of calcium and phytase on AIDCa are variable among the available studies, as demonstrated in the variance decomposition. Again, we emphasize that the results of this study apply only to the practical levels of Ca:P, since they represent the totality of studies that were used in the database.

As expected, AIDP was the most benefited response to the phytase supplementation. The study and the phytase dose were the factors that directly affected AIDP. Age, on the other hand, had a significant effect on digestibility, showing that younger birds are more benefited by the enzyme's effects on AIDP, as already observed in performance responses. Younger birds have rapid bone growth and are developing the gastrointestinal tract. Therefore, supplementation with phytase will have a greater impact on the use of phosphorus, corroborating the data already presented by Li *et al.* (2018). These results are different from those obtained for AIDCa, in which older birds were most benefited, even though the results in the literature are controversial for this response. The maximum phytase dose tested in the studies that composed the database was 12500 FTU/kg. Total phosphorus retention has already been observed up to doses of 12000 FTU/kg, with the disappearance of up to 95% of phytic phosphorus and the removal of almost all anti-nutritional effects of phytate (Shirley and Edwards, 2003).

Finally, there was a significant difference in the ash content of the tibias between birds that received diets supplemented or not with phytase, but there was no difference in the calcium and phosphorus content of the bones or in the carcass yield. The level of ash in the tibia is a good indicator of the bird's phosphorus status, since phytase supplementation increases the level of bone ash proportionally to the release of phosphorus (Lalpanmawia *et al.*, 2014), corroborating the data of this work, where there was a pronounced increase in AIDP, in contrast to a much less expressive increase in AIDCa. On the other hand, bone is in a constant process of deposition by osteoblasts, and resorption by osteoclasts, so that the levels of calcium and phosphorus in the bones vary in a single day (Vitti and Kebreab, 2010). The analysis of the calcium and phosphorus content of the bones seems to be a less accurate measure than the total ash content to assess the nutritional status of the bird, especially when diets with various levels of these minerals are offered, regardless of the addition of phytase.

A recent study on the inclusion of increasing levels of phytase in diets based on corn and soybean meal for broilers did not show differences in the carcass yield of the animals (Freitas *et al.*, 2019). Similar results were found by Broch *et al.* (2018) and Van Emmenes *et al.* (2018). Our data showed that phytase benefited broiler performance, so possibly an analysis of carcass traits could be performed to check for any possible effects. Although these data were collected in the database, the number of samples (carcass traits responses appeared in less than 10 articles) was too low to perform a representative analysis. The variation between studies is high, but with the meta-analysis technique it is possible to obtain accurate and reliable results, since the study is inserted as a random factor in the models

Conclusions

Supplementing phytase to broiler diets improves performance, ileal digestibility, and bone ash content of birds without affecting carcass yield. Some factors, such as age and diet composition, affect these responses in different and even opposite ways, while the dose of the enzyme shows effect only on the phosphorus ileal digestibility.

Declaration of interest

We declare no conflict of interest, including financial, personal, or other relationships with other people or organizations that could inappropriately influence this work**.**

Ethics statement

This study was performed with data from published papers; consequently, there was no use of animals. In this situation, it is not necessary for the study to be approved by the Animal Ethics Committee of the Universidade Federal do Rio Grande do Sul.

Software and data repository resources

None of the data were deposited in an official repository.

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Table 1 *Performance, apparent ileal digestibility (AID), bone characteristics (left tibia), carcass yield, and the effect of exogenous phytase in broilers*

	Control		Phytase effect[a]		
	Mean	S.E.	Effect, %	S.E.	*P-value*
Performance					
Feed intake, g/day	89.81	2.02	+5.22	0.47	<0.001
Weight gain, g/day	52.84	1.02	+7.09	0.53	<0.001
Feed conversion, g/g	1.67	0.01	-2.75	0.32	<0.001
AID					
Nitrogen, %	79.07	0.67	+2.66	0.41	0.001
Calcium, %	53.16	1.18	+5.35	1.92	0.023
Phosphorus, %	45.30	1.13	+27.02	1.70	<0.001
Bone characteristics					
Ash, %	42.41	0.47	+6.04	0.55	<0.001
Calcium, %	23.97	1.08	+1.62	1.04	0.772
Phosphorus, %	11.80	0.49	+4.69	0.67	0.518
Carcass yield	71.96	0.60	+0.29	0.25	0.562

S. E., standard error.
[a]Responses of phytase supplemented treatments were relativized to the respective control treatment and expressed as percentage of variation between the results.

Table 2 *Prediction of the effect of phytase supplementation on broiler performance, apparent ileal digestibility (AID), bone characteristics (left tibia), and carcass yield in function of control results*

Variable	Regression equation (model)	*P-value*			R^2
		Intercept	Variable	Age	
Performance					
Feed intake, g/day	y = 7.57+ 0.1122x – 0.5830a	<0.001	<0.001	<0.001	0.78
Weight gain, g/day	y = 9.80 + 0.0634x – 0.1674a	<0.001	0.066	0.020	0.84
Feed conversion, g/g	y = -28.92 + 20.7100x – 0.4438a	<0.001	<0.001	<0.001	0.72
AID					
Nitrogen, %	y = 15.00 – 0.1150x – 0.1942a	0.081	0.271	0.039	0.13
Calcium, %	y = -120.90 + 1.9310x + 1.1350a	<0.001	<0.001	0.027	0.76
Phosphorus, %	y = -17.60 + 0.8620x – 0.8080a	0.088	<0.001	0.010	0.76
Bone ash, %	y = -33.92 + 1.0090x – 0.1825a	<0.001	<0.001	0.020	0.88

y, phytase effect; x, control result; a, average age.

Table 3 *Factors that explained the phytase effect on the daily weight gain and apparent ileal digestibility of phosphorus (AIDP) and calcium (AIDCa) in broilers*

Factors	Contribution to the total variance, %	*P-value*
Daily weight gain		
Study effect	77.08	<0.001
Phytase dose, FTU/kg	0.69	<0.001
Average age, d	0.31	0.008
Dietary Cat:Pt	0.28	0.010
Dietary total phosphorus, %	0.05	0.266
Dietary total calcium, %	0.03	0.444
R^2	0.89	
Error	10.59	
AIDP		
Study effect	59.32	<0.001
Phytase dose, FTU/kg	4.62	0.005
Average age, d	1.17	0.137
Dietary total phosphorus, %	0.67	0.260
Dietary total calcium, %	0.28	0.460
Dietary Cat:Pt	0.27	0.464
R^2	0.81	
Error	19.32	
AIDCa		
Study effect	40.23	0.002
Average age, d	7.61	0.003
Dietary Cat:Pt	3.50	0.037
Dietary total phosphorus, %	2.19	0.095
Dietary total calcium, %	1.90	0.120
Phytase dose, FTU/kg	0.00	0.989
R^2	0.71	
Error	29.30	

Figure captions

Figure 1 Meta-design: within-study relation between dietary microbial phytase and available phosphorus concentration. Each study is indicated by a cross. The horizontal line indicates the available phosphorus content of diets, and the vertical line indicates the range of microbial phytase supplementation.

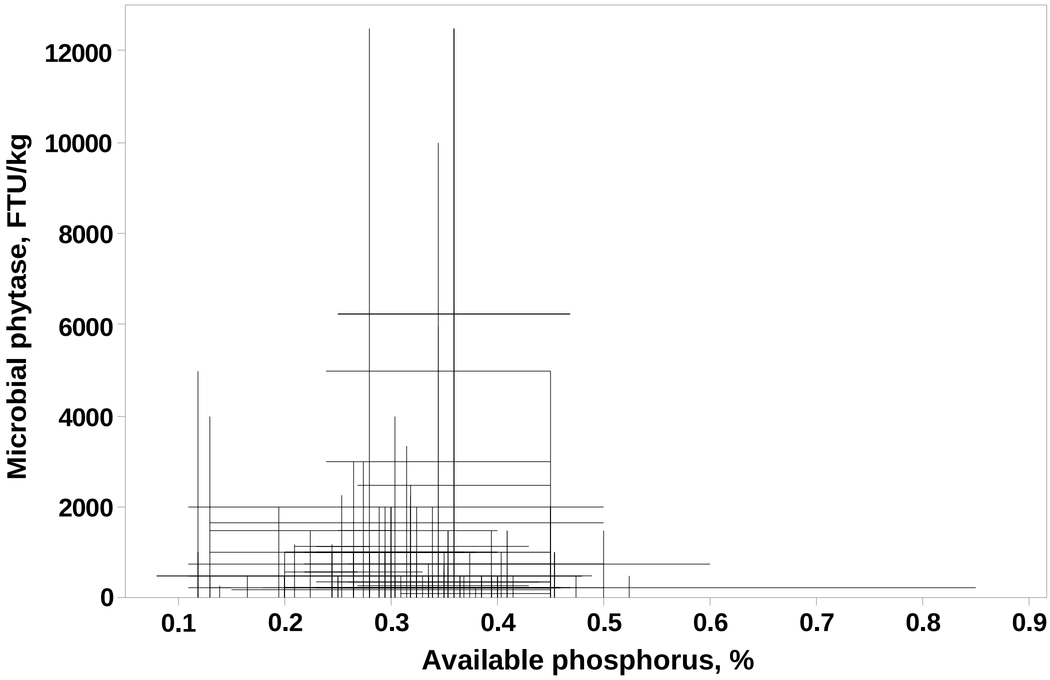
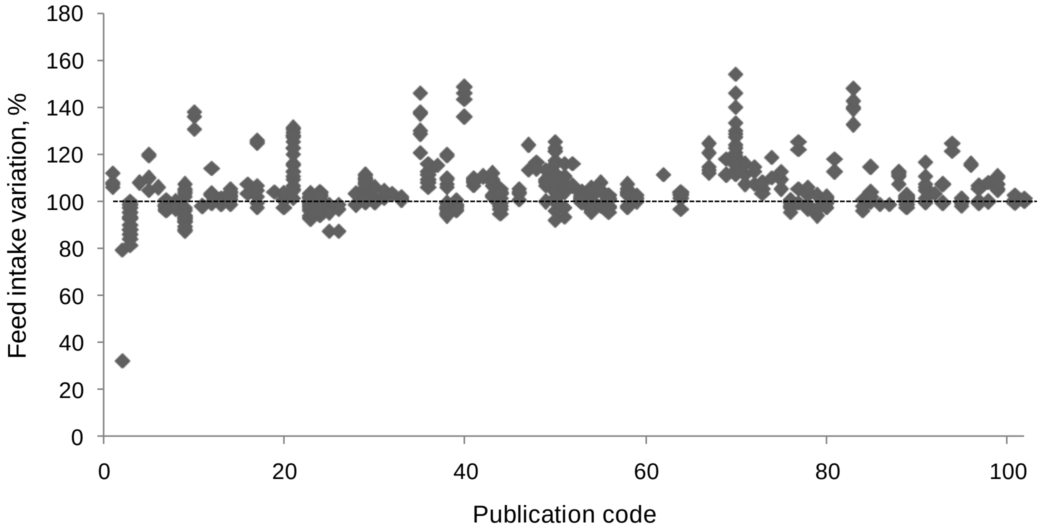


Figure 2 Performance responses of broilers fed phytase supplemented treatments relativized to the respective control treatment.



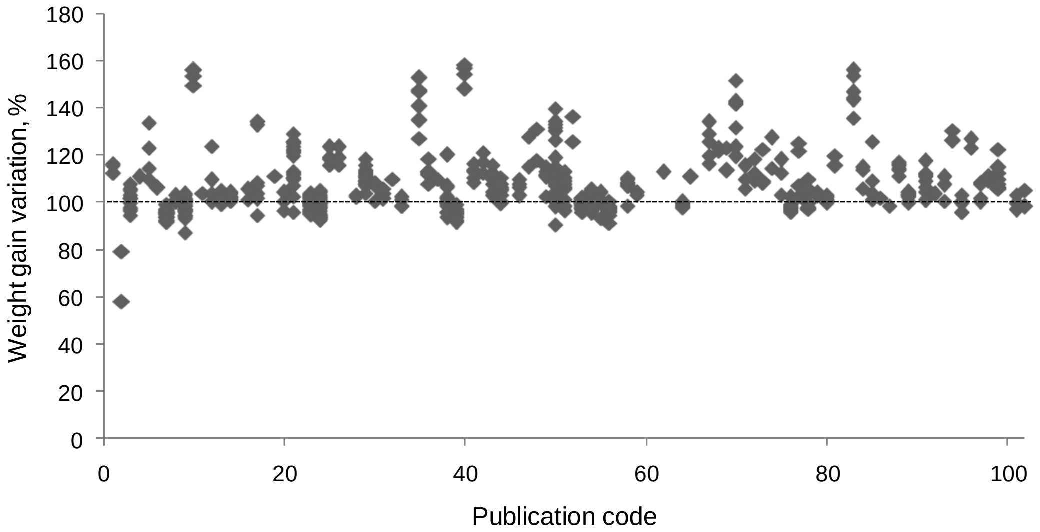
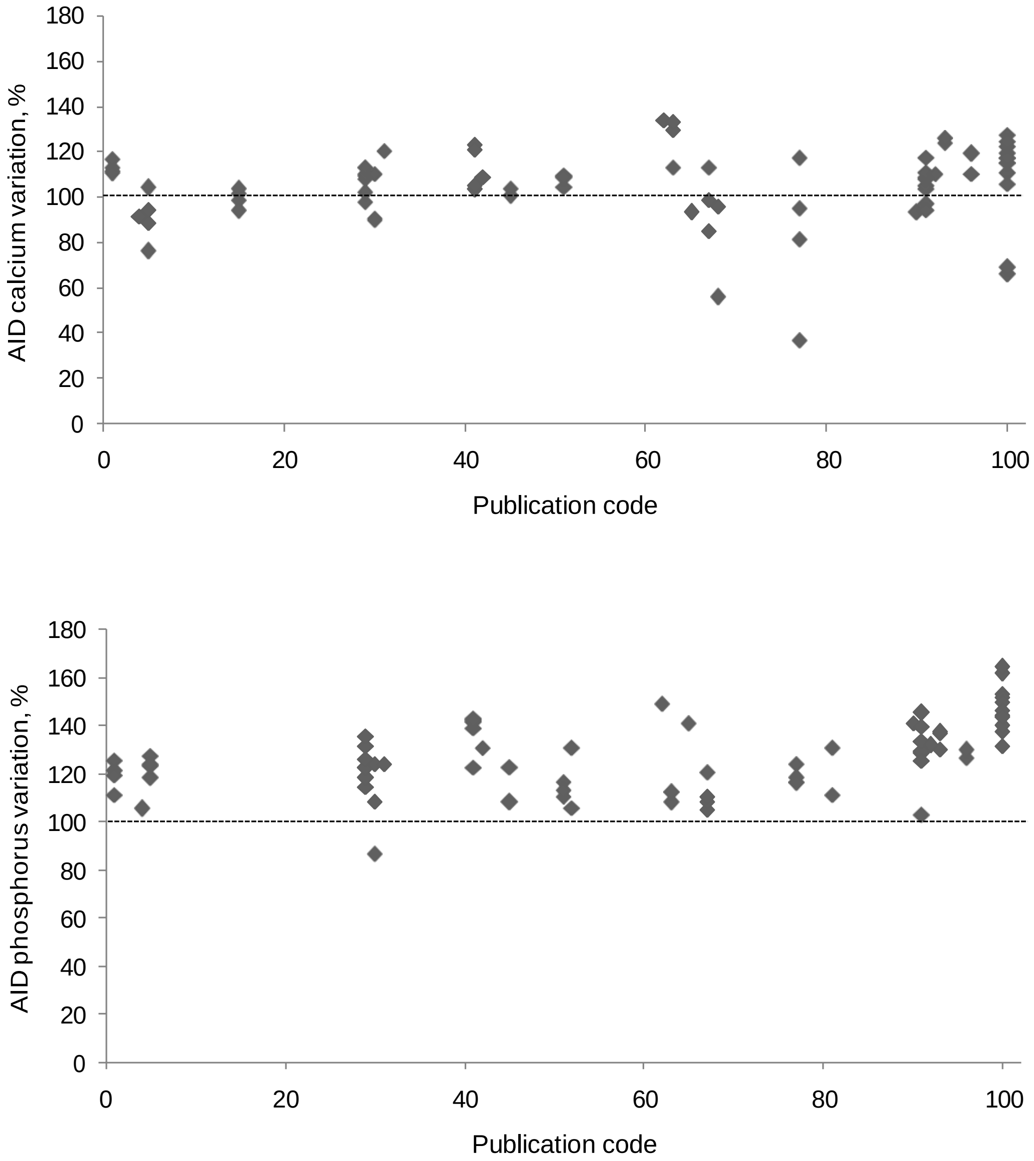


Figure 3 Apparent ileal digestibility (AID) responses of broilers fed phytase supplemented treatments relativized to the respective control treatment.



Complementary material

Table 1 *Description of the broilers database*

Reference		Genetic line	Animals (n)	Microbial phytase origin
First author	Year			
Ao	2007	*	864	*
Centeno	2007	Cobb	240	*Aspergillus niger*
Olukosi	2007	Ross	600	*Escherichia coli*
Panda	2007	*	270	*Peniophora lycii*
Pirgozliev	2007	Ross	408	*E. coli*
Da Silva	2008	Cobb	250	*P. lycii*
Ebrahimnezhad	2008	Ross	420	*A. niger*
Ebrahimnezhad	2008	Ross	360	*A. niger*
Liebert	2008	Cobb	240	*Hansenula polymorpha*
Liem	2008	Cobb	240	*A. niger*
Manangi	2008	Cobb	400	*E. coli*
Nyannor	2008	*	288	*E. coli*
Oliveira	2008	Cobb	330	*A. niger*
Pirgozliev	2008	Ross	480	*E. coli*
Powell	2008	Ross	1960	*A. niger*
Surek	2008	Ross	200	*A. niger*
Timmons	2008	Ross	2816	*P. lycii*
Amerah	2009	Ross	192	*E. coli*
Assuena	2009	Ross	960	*A. niger*
Attia	2009	Arbor Acres	210	*E. coli*
Guo	2009	Arbor Acres	540	*E. coli*
Han	2009	Arbor Acres	480	*Trichoderma reesei*
Han	2009	Arbor Acres	240	*Trichoderma reesei*
Laurentiz	2009	*	1200	*A. niger*
Nagata	2009	Cobb	900	*P. lycii*
Omar	2009	Cobb	200	*
Cardoso Júnior	2009	Cobb	1404	*P. lycii*
El-Sherbiny	2010	Ross	500	*
Lelis	2010	Ross	350	*E. coli*
Pirgozliev	2010	Ross	150	*E. coli*
Shaw	2010	Ross	960	*E. coli* *P. lycii*
Tiwari	2010	Cobb	250	*E. coli*
Woyengo	2010	Ross	144	*E. coli*
Woyengo	2010	Ross	324	*E. coli*
Akyurek	2011	Ross	140	*A. niger*
Aureli	2011	Ross	480	*P. lycii*
Donato	2011	Ross	2160	*A. niger*
Dos Santos	2011	Cobb	6030	*P. lycii*
Dos Santos	2011	Cobb	2340	*P. lycii*
Junqueira	2011	Ross	960	*A. niger*
Meneghetti	2011	Cobb	1848	*Citrobacter braakii* *E. coli*
Nagata	2011	Ross	150	*P. lycii*

Nagata	2011	Ross	150	*P. lycii*
Nezhad	2011	Ross	360	*A. niger*
Pirgozliev	2011	Ross	48	*E. coli*
Powell	2011	Ross	288	*A. niger*
Shaw	2011	Ross	384	*C. braakii*
Brunelli	2012	*	300	*A. niger*
Gomide	2012	Cobb	840	*P. lycii*
Green	2012	Ross	480	*E. coli*
Lelis	2012	Ross	250	*E. coli*
Namini	2012	Ross	160	*A. niger*
Oliveira	2012	Cobb	875	*P. lycii*
Pirgozliev	2012	Ross	360	*E. coli*
Rousseau	2012	Ross	144	*A. niger*
Tang	2012	*	384	*Yersinia frederiksenii*
Walk	2012	Ross	728	*E. coli*
Adeola	2013	Ross	768	*E. coli*
Beiki	2013	Ross	480	*A. niger*
Chen	2013	Arbor Acres	*	*
Donato	2013	Ross	108	*A. niger*
Gehring	2013	Ross	1152	*E. coli*
Jiang	2013	Arbor Acres	540	*A. niger*
Karimi	2013	Cobb	735	*E. coli*
Olukosi	2013	Ross	496	*P. lycii*
Santos	2013	Arbor Acres	768	*E. coli*
Amer	2014	Ross	300	*
Amerah	2014	Ross	384	*Buttiauxella* spp.
Cowieson	2014	Ross	1040	*P. lycii*
Elkhalil	2014	Cobb	70	*A. niger*
Lalpanmawia	2014	Cobb	192	*E. coli*
Naves	2014	Cobb	120	*Aspergillus orizae*
Olukosi	2014	*	288	*E. coli*
Roy	2014	Cobb	200	*E. coli*
De Sousa	2015	Cobb	1120	*E. coli*
Delezie	2015	Ross	1260	*E. coli*
Kiarie	2015	Ross	420	*Buttiauxella* spp.
Liu	2015	Ross	240	*Buttiauxella* spp.
Naves	2015		304	*C. braakii* *E. coli* *P. lycii*
Stefanello	2015	Cobb	336	*P. lycii*
Zeller	2015	Ross	180	*E. coli*
Attia	2016	Hubbard	336	*E. coli* *P. lycii*
Borda-Molina	2016	Ross	1064	*E. coli*
Ganaatparast-Rashti	2016	Arian	320	*A. niger*
Manobhavan	2016	Cobb	168	*E. coli*
Schmeisser	2016	Ross	120	*C. braakii*
Farhadi	2017	Ross	660	*E. coli*
Gautier	2017	Ross	240	*E. coli*

Lee	2017	Cobb	2,376	*E. coli*
McCormick	2017	Ross	720	*E. coli*
Pieniazek	2017	Cobb	2,336	*E. coli*
Schramm	2017	Cobb	1,120	*P. lycii*
Momeneh	2018	Ross	936	*E. coli*
Sommerfeld	2018	Ross	1,064	*E. coli*
Ajith	2018	Cobb	90	*Aspergillus foetidus*
Jiang	2018	Ross	192	*E. coli*
Li	2018	Cobb	648	*Buttiauxella* spp.
Nardelli	2018	Cobb	630	*A. niger* *C. braakii*
Ribeiro	2018	Ross	792	*C. braakii*
Babatunde	2019	Cobb	64	*P. lycii*
Dessimoni	2019	Cobb	896	*E. coli*
Lee	2019	Cobb	2,970	*E. coli*

CAPÍTULO IV

4. Considerações finais

As aves e os suínos, pertencentes a duas classes distintas do reino animal, são animais monogástricos e algumas vezes são estudados em conjunto. Possuem similaridades quanto ao processo produtivo, que é intensivo e quanto à formulação das dietas e os ingredientes e aditivos utilizados. Por outro lado, ao realizarmos um experimento, a condução é completamente diferente. Suínos podem se tornar animais enormes. Os 42 dias de experimento com frangos de corte podem significar até 42 kg a mais para um suíno.

A avaliação da digestibilidade ileal em frangos de corte requer a eutanásia e a coleta do conteúdo de alimento diretamente do íleo. Geralmente, mais de uma ave compõe a unidade experimental, então se houver algum erro, ele pode ser diluído. No caso dos suínos, é necessário um procedimento cirúrgico, com recuperação lenta e um grande período de adaptação – além de ser um trabalho delicado, é mais caro. Dessa forma, a avaliação da digestibilidade total é uma alternativa.

Este trabalho refletiu as diferenças entre as duas espécies. A disponibilidade de dados para cada uma das respostas coletadas foi distinta. Os trabalhos com aves são mais completos, e esse é um ponto importante quando observamos o comportamento dos resultados. Ainda, percebemos que a fitase continua cumprindo o seu papel em reduzir a eliminação de fósforo no ambiente e, conforme novas gerações de enzimas são criadas, melhores são os resultados. Associada aos números gerados, uma análise de ciclo de vida seria o segundo passo após este estudo, e todas as respostas provenientes desta meta-análise poderiam ser aproveitadas.

Já sabemos que a enzima funciona, mas temos uma infinidade de fatores que podemos avaliar, com as características que queremos e com a espécie animal da nossa escolha, utilizando a ferramenta da meta-análise. Mas também é importante que façamos experimentos para preencher as lacunas de dados cuja extensão só percebemos ao formular as bases de dados.

Por fim, precisamos fortalecer a consciência de que o meio ambiente não está ao nosso dispor. Muitos dos recursos disponíveis para a produção de aves e suínos são finitos e precisam de alternativas cada vez mais eficientes em períodos de tempo mais curtos, e, portanto o papel da ciência e da pesquisa é fundamental.

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5. Vita

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