EDUARDO MACHADO COSTA LIMA

EQUIVALÊNCIA DE FÓSFORO USANDO UM ESTUDO DE META-ANÁLISE E TRANSCRIPTÔMICA EM FRANGOS DE CORTE ALIMENTADOS COM FITASE

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Não-Ruminantes, para a obtenção do título de Doutor.

Dr. Paulo Borges Rodrigues
Orientador

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APROVADA em 21/11/2016

Prof. Dr. Carl J. Schmidt	University of Delaware (UDEL)
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2016

Aos meus pais, José e Maria Eulália, pelo amor, respeito e dedicação: alicerces em minha vida com quem sempre posso contar.

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Ao meu sobrinho Felipe por me mostrar outra forma de amor.

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Aos companheiros e amigos.

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DEDICO

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"A morte é algo inevitável.
Quando um homem fez algo que considera
ser seu dever com as pessoas de seu país,
ele pode descansar em paz."

Nelson Mandela

BIOGRAFIA

EDUARDO MACHADO COSTA LIMA, filho de José Costa Lima e Maria Eulália Machado Costa Lima, nasceu em 12 de janeiro de 1985, na cidade de Cachoeira de Minas, no estado de Minas Gerais.

Em junho de 2003, ingressou na Universidade Federal de Lavras, graduando-se em Zootecnia em dezembro de 2008.

Em junho de 2009, iniciou a Pós-Graduação em Zootecnia na Universidade Federal de Lavras, concentrando seus estudos na área de Nutrição de Não Ruminantes.

No dia 28 de julho de 2011 defendeu o título de Mestre em Produção e Nutrição de Não Ruminantes.

Em agosto de 2012 iniciou o Doutoramento em Produção e Nutrição de Não Ruminantes na Universidade Federal de Lavras.

Em abril de 2015 iniciou o Doutorado Sanduíche na Universidade de Delaware finalizando em dezembro do mesmo ano.

No dia 21 de novembro de 2016 defendeu o título de Doutor em Produção e Nutrição de Não-Ruminantes.

RESUMO

O presente trabalho foi desenvolvido em três etapas: Na primeira parte realizou-se um levantamento bibliográfico de trabalhos pertinentes à exigência de fósforo disponível (Pdisp) de aves de corte em dois períodos de criação (1 a 21 dias de idade, e de 1 a 42 dias de idade) e que utilizaram fitase nas dietas. Foram considerados como variáveis respostas o ganho de peso e a porcentagem de cinzas ósseas dos animais. Esses dados foram catalogados, agrupados, selecionados, filtrados e padronizados, determinando-se duas equações de equivalência do Pdisp ao se utilizar fitase nas rações. Na segunda parte, validaram-se as equações determinadas através da meta-análise (primeira parte), onde foi desenvolvido dois experimentos com frangos de corte machos, da linhagem Cobb 500, com 15 aves por repetição e cada tratamento com seis repetições. Os tratamentos consistiram em três níveis de inclusão da fitase (450/900/1350 FTU) e a redução dos respectivos valores de equivalência de fósforo disponível determinados pelas equações. Nos experimentos 1 e 2, o controle positivo foi uma ração com 0,37% de Pdisp e 0,79% de cálcio na fase de 1 à 21 dias de idade e 0,27% de Pdisp e 0,57% de cálcio na fase de 22 à 42 dias de idade. Os níveis de cálcio (Ca) foram os mesmos utilizados para todos os tratamentos, independente do uso de fitase. Na terceira parte, um experimento (3) foi conduzido com frangos de corte Ross 700 para coleta do fígado visando a análise de transcriptômica. Foram considerados como períodos de criação as fases de 1 a 21 e de 22 a 41 dias de idade, com seis repetições de 15 aves cada, onde os animais receberam rações deficientes em Pdisp e suplementadas com 500 e 1500 FTU de fitase, mais um controle positivo. Tanto a ração suplementada com fitase quanto a ração controle continham a mesma concentração de cálcio. Os valores de equivalência de fósforo determinados foram de 0,10/0,14/0,19% de Pdisp para a fase de 1 à 21 dias de idade e 0,09/0,14/0,19% para a fase de 1 à 42 dias de idade. Não houve diferenças ($P>0,05$) no ganho de peso (GP), conversão alimentar (CA) e na concentração de fosfatase alcalina (FAK) entre as aves alimentadas com a dieta controle e as aves alimentadas com os tratamentos suplementados com fitase e com níveis reduzidos de Pdisp na fase de 1 à 21 dias de idade (experimento 1). Na mesma fase, os animais alimentados com 450/900 FTU/kg considerando 0,19% de equivalência de Pdisp tiveram melhor ($P<0,05$) concentração de cinzas ósseas (CZ) na tíbia quando comparado com as aves alimentadas com a dieta controle. Mesmo resultado foi observado para as aves alimentadas com a suplementação de 450 FTU de fitase e 0,14% de equivalência de Pdisp. De 22 a 42 dias de idade, no experimento 1, não foi observado ($P>0,05$) diferença nas CZ aos 42 dias de idade, porém as aves alimentadas com a dieta controle apresentaram

pior resultado de GP, CA e FAK que as aves alimentadas com alguns tratamentos suplementados com fitase. No experimento 2, de 1 à 42 dias de idade, nenhuma diferença no GP entre o controle e os tratamento suplementados com fitase foi observada. As aves alimentadas com 450FTU/kg, considerando a equivalência de 0,19% de Pdisp, tiveram menor consumo de ração (CR) e melhor CZ comparado com aquelas alimentadas com a dieta controle. No mesmo valor de equivalência, as aves suplementadas com 1350FTU/kg tiveram menor CR e melhor CA que as alimentadas com a dieta controle. No experimento 3, no período de 1 a 21 dias de idade, as aves alimentadas com 500FTU/kg não apresentaram resultados semelhantes às aves alimentadas com a dieta controle. Aos 42 dias de idade, nenhuma houve diferença (P>0,05) entre os tratamentos suplementados com fitase (500 ou 1500 FTU/kg) e o tratamento controle. Os resultados investigativos de transcriptômica mostram uma redução na expressão de genes relacionados à translocação de moléculas pequenas no fígado das aves alimentadas com 1500 FTU/kg, comparado com as aves que receberam a dieta controle. Relacionado aos resultados dos experimentos 1 e 2, sugere-se que ambas as equações determinadas podem ser utilizadas para a determinação dos valores de equivalência de fósforo ao uso de fitase nas rações para frangos de corte e, ainda, a fitase pode influenciar a expressão de genes no fígado de frangos de corte.

Palavras-chave: Fitato. Meta-análise. Enzima. RNA. Aves.

ABSTRACT

The present study was carried out in three stages: in the first part, a bibliographical survey was carried out of pertinent assays to the requirement of available phosphorus (aP) of broilers in two breeding periods (1 to 21 days old and 1 to 42 days old) also work that used phytase in the diets. Weight gain and bone ash responses of the animals were considered as variables. These data were cataloged, grouped, selected, filtered and standardized, and two aP equivalence equations were determined when the phytase is add in feed. The second part was validated the equations obtained in the first part. Two experiments were carried out with male Cobb 500 broilers, with 15 birds per replicate and each treatment with six replicates. The treatments consisted of three levels of phytase inclusion (450/900/1350 FTU) and a reduction of the respective aP equivalence values. In the experiment 1 and 2, the positive control was formulated with 0.37% of aP and 0.79% of calcium (Ca) at 1 to 21 days old and 0.27% of aP and 0.57% of Ca at 22 to 42 days old. The same Ca levels were used for all treatments. In the third part, an experiment (3) was carried with male Ross 700 for liver transcriptomic analysis. Two periods were considered from 1 to 21 and 22 to 41 days old with six replicates of 15 birds each. The birds received aP deficient diets supplemented with 500 and 1500 FTU/kg plus a positive control. All diets had the same Ca level. The aP equivalence are 0.10/0.14/0.19% for 1 to 21 days old and 0.09/0.14/0.19% for 1 to 42 days old. No difference (P> 0.05) to weight gain (WG), feed conversion ratio (FCR) and alkaline phosphatase concentration (FAK) between the birds fed with control diet and birds fed with treatments supplemented with phytase and reduced levels of aP were observed. At the same age the birds fed with 450/900FTU/kg considering 0.19% of aP equivalence had better (P <0.05) bone ash (ASH) in tibia compared to the birds fed control diet. The same result for birds fed with 450FTU/kg and 0.14% of aP equivalence. From 22 to 42 days old, no difference was observed (P> 0.05) in the ASH, however, the birds fed control diet had worst WG, FCR and FAK results comparing with some of phyatse treatments. At experiment 2 from 1 to 42 days of age, no WG difference (P>0.05) between the birds fed control diet and phytase treatments. The birds fed with 450FTU/kg and 0.19% of aP equivalence had lower feed intake (FI) and better ASH compared to the birds fed control diet. At the same aP equivalence, birds supplemented with 1350FTU/kg had lower FI and FCR comparing with those fed control diet. In experiment 3 at 1 to 21 days old the birds fed 500FTU/kg had worst performance results comparing with the birds fed control diet. At 42 days old, no difference (P>0.05) between treatments supplemented with phytase (500 or 1500 FTU/kg) and control treatment. The

transcriptomic investigative results show a reduction in the expression of genes related to the translocation of small molecules when broilers were fed with 1500 FTU/kg, compared to the birds fed control diets. The results (experiment 1 and 2) suggest that both equations can be used for the determination of aP equivalence values for broiler chickens feeds, and also, the phytase may influence the liver gene expression of broilers.

Keywords: Phytate. Meta-analysis. Enzyme. RNA. Birds.

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1 INTRODUÇÃO

É inquestionável a posição mundial que o Brasil assume na produção avícola. Uma produção de diferentes espécies de aves com uma gama de produtos que pode suprir diferentes mercados mundiais. No quesito qualidade o país não fica atrás. Mercados exigentes como Japão, Rússia e União Européia estão entre os parceiros dos produtores brasileiros que com mão de obra especializada e utilizando de tecnologias adequadas vem expandindo no mercado interno e externo.

Nesse sentido, o emprego de tecnologias em uma produção tão intensiva quanto a avicultura se faz indispensável para que se mantenha o sistema produtivo competitivo e ajustados aos padrões de exigência dos consumidores. Isso devido ao fato do perfil dos consumidores, sejam os nacionais ou internacionais, passou por uma forte mudança nos últimos anos, tornando-os mais preocupados com a qualidade do produto e como foi produzido. Assim, a palavra sustentabilidade ganha espaço e cabe aos pesquisadores e produtores buscarem maneiras de atender essas exigências, mantendo a qualidade e a competitividade dos produtos avícolas.

Um ponto forte do apelo ambiental é a produção de dejetos e, consequentemente, a possível poluição ambiental pelos sistemas de produção animal. Somente a avicultura de corte, maior representante em volume de produção, consumiu 32,4 milhões de toneladas de ração no ano de 2015 produzindo 13,14 milhões de toneladas de carne. Mesmo considerando a alta eficiência alimentar das linhagens de aves utilizadas hoje, essa alta produção pode gerar sérios problemas ambientais devido a eliminação de poluentes. As

carcaças e a as excretas são os principais dejetos do sistema de produção avícola, e, quando não seguem destino correto, estão contribuindo não só para a poluição ambiental como também para uma produção onde não se respeita a preocupação dos consumidores em geral.

O estrume, seja cama de frango ou de galinha, quando bem manejado pode facilmente deixar de ser poluente e se tornarem coprodutos do sistema, sendo aplicados no campo como biofertilizante. Rico em nitrogênio, fósforo e potássio, pode ser utilizado com sucesso para suprir as necessidades dos principais cereais produzidos no Brasil, em especial o milho. O grande entrave é que, quando aplicado de forma inconsequente ou manejado de maneira incorreta, o esterco avícola pode contaminar o solo e, principalmente, os lençóis freáticos.

Retornando à sustentabilidade do sistema produtivo, o fósforo, além de ser um poluente em potencial, quando presente em excesso no solo, também é um nutriente caro, e sempre desperdiçado no sistema produtivo. Do fósforo total contido no milho e no farelo de soja, os dois principais ingredientes das rações no Brasil, 75% e 60% são na forma de fósforo fítico, respectivamente. O fósforo fítico não é digerido e, consequentemente, é pouco utilizado por animais monogástricos. Assim, uma forma de reduzir o potencial poluente do esterco avícola, é tornando mais eficiente a utilização dos nutrientes dos alimentos.

Assim a utilização de aditivos melhoradores da digestão de nutrientes são ferramentas utilizadas na produção avícola afim de reduzir o impacto ambiental e o custo de produção sem a perda do desempenho produtivo das aves. Enzimas exógenas auxiliam na digestão aumentando a eficiência do animal em aproveitar os nutrientes dos alimentos. Em 2015, a avicultura de corte e de postura consumiu um total de 8.300 toneladas de enzimas e, das diferentes opções de enzimas exógenas, a fitase, largamente utilizada na produção avícola, é a responsável por incrementar a digestão do fósforo fítico pelas aves de

produção.

O presente trabalho tem como objetivo a elaboração de equações de predição da utilização do fósforo em rações com diferentes concentrações de fitase para frangos de corte, empregando-se, para isso, o princípio da meta-análise sobre informações catalogadas na literatura científica. Além disso, a aplicação da técnica de transcriptômica como ferramenta investigativa, colabora para melhor elucidar as respostas fisio-metabólicas das aves quando adicionamos fitase nas rações.

2 REFERENCIAL TEÓRICO

2.1 Avicultura industrial e o meio ambiente

O primeiro ponto a ser abordado ao falar dos dejetos da avicultura industrial é a localização e concentração das granjas numa dada região, e consequentemente, o local dos insumos para a produção. O arranjo da produção avícola gera uma concentração de aves em áreas relativamente pequenas, e os insumos para a fabricação de uma ração balanceada são oriundos de outras regiões e trazem consigo os nutrientes que poderão vir causar impacto ambiental na região (OVIEDO-RONDÓN, 2008). Assim, para que o sistema continue fluindo, é essencial que seus dejetos recebam práticas adequadas de manejo e correta destinação e aplicação.

Os principais dejetos da avicultura são o esterco, efluentes, camas e aves mortas que, se não manejados corretamente podem gerar perdas econômicas ainda na própria granja. A rápida decomposição pode gerar compostos voláteis causando perda de produção e intoxicação das aves, além de ser substratos para

crescimento bacteriano.

2.1.1 Esterco de aves e cama de frango aplicados como biofertilizantes

Na década onde a sustentabilidade ganhou atenção como nunca, e que foi aplicada nos sistemas produtivos, muito resíduos de diferentes sistemas deixaram de ser resíduos para se tornarem recurso. Hoje é importante que um sistema utilize de diferentes recursos e ferramentas para manter a competividade e lucratividade.

A composição desses resíduos pode atingir concentrações significantes de nitrogênio, fósforo, potássio e minerais traço como cobre e zinco (OVIEDO-RONDÓN, 2008), cujos valores podem sofrer variações devido a diferentes fatores. Quiroga (2010) encontrou, em diferentes fazendas na Espanha, uma concentração de nitrogênio variando entre 3,93 à 7,11%, de fósforo variando entre 0,03 à 1,51% e de potássio variando entre 1,96 à 2,72%, todos os valores expressos na matéria seca. É importante ressaltar que os resíduos usados como biofertilizantes devem ser utilizados de maneira correta. Para isso, conhecer a composição química de cada um é essencial para que não gere um futuro problema ambiental nos solos onde são aplicados.

2.2 Fósforo em rações para frangos de corte

Na criação comercial de aves, o fósforo é o suplemento mineral mais dispendioso da ração. Além disso, a suplementação acima das necessidades resulta em excreção de níveis elevados, poluindo fluxos de água e rios. Por essas

razões, em muitos países existem, atualmente, sérias preocupações a respeito da presença do fósforo nas excretas e do impacto que isso causa no ambiente.

Os frangos de corte obtêm o fósforo necessário em alimentos que consomem e em compostos inorgânicos de origem geológica ou industrial, que são adicionados às rações para completar as exigências. As fontes de fósforo inorgânico para alimentação animal são os ortofosfatos produzidos pela indústria química a partir do ácido ortofosfórico. Uma das fontes mais empregadas para a suplementação das rações para animais é o fosfato bicálcico, que tem um custo elevado, representando 2,5 a 3% do custo total de uma ração (BORGES et al, 1997). A suplementação de fósforo representa o terceiro maior custo nas rações de frangos, ficando atrás apenas da proteína e energia (TEICHMANN et al., 1998).

Por outro lado, a disponibilidade do fósforo contido nos vegetais depende do teor de ácido fítico presente. Os vegetais contêm quantidades variáveis de ácido fítico, variando, portanto, a disponibilidade do fósforo presente, que normalmente é baixa, em média 30% (National Research Council – NRC, 1994). O teor de fósforo fítico pode variar de 45 a 86% do fósforo total do alimento (ROSTAGNO, 1998).

Os animais monogástricos não são capazes de hidrolisar os grupos ortofosfatos da molécula do fitato, pois não possuem a enzima fitase (PEELER, 1972). Assim, considera-se que todo o fósforo ligado à molécula de fitato é indisponível, embora haja citações de que os animais monogástricos e os próprios vegetais produzem pequena quantidade de fitase para que o fósforo fítico possa ser hidrolisado. No entanto, essa atividade fitásica é tão pequena e limitada, o que a torna insignificante (NELSON, 1967; SIMONS & VERSTEEGH, 1990).

2.2.1 Composição de fósforo nos principais alimentos para aves

Os principais alimentos utilizados na avicultura são o milho e o farelo de soja. A composição de fósforo total (Ptotal) do milho pode sofrer uma variação de 0,18-0,28% (VIEIRA et al., 2007) e para o farelo de soja de aproximadamente 0,63%, quase sem variação de acordo com Rieger et al. (2008).

Todavia, ao tratar da concentração de fósforo dos alimentos para animais monogástricos deve-se atentar ao teor de fitato ou a concentração de fósforo fítico (Pfit) que cada um apresenta. A concentração de fitato (Tabela 1) de um alimento em relação ao valor do Ptotal varia devido a origem daquele alimento (animal, vegetal ou mineral) e no caso dos vegetais, devido as variações climáticas, solo e adubação, idade da planta, cultivar utilizada, estágio de maturação dos grãos, grau de processamento entre outros fatores. A variação do teor de fitato de uma ração para aves pode chegar a 100% dependendo da qualidade e dos alimentos utilizados (RAVIDRAN, BRYDEN & KORNEGAY, 1995).

Tabela 1: Teor de fósforo de alguns alimentos utilizados na nutrição de aves.

Alimento	Teor de Fósforo (%)[1]		
	Ptotal[2]	Pdisp[3]	Pfit[4]
Milho	0,25	0,06	0,19
Farelo de Soja (45% de proteína bruta)	0,56	0,22	0,34
Soja integral tostada	0,52	0,19	0,33
Farelo de trigo	0,97	0,33	0,64
Sorgo baixo tanino	0,26	0,08	0,18
Farelo de Arroz	1,67	0,24	1,43
Farinha de Carne e Ossos (41% de Proteína bruta)	6,53	5,88	-
Farinha de vísceras de aves	2,54	2,54	-
Fosfato bicálcico	18,5	18,5	-

[1]valores na matéria natural. Adaptado de Rostagno et al. (2011). [2]Fósforo total. [3]Fósforo disponível. [4]Fósforo fítico.

De acordo com os valores apresentados na Tabela 1 pode-se perceber que alimentos de origem animal não possuem a molécula de fitato, porém o Ptotal destes alimentos pode não estar 100% disponível para as aves. Todavia, ao analisar os alimentos de origem vegetal, percebemos que estes podem possuir de 60 à 85% de Pfit, ou seja, indisponíveis para os animais monogástricos. Em média, pode-se considerar que 70% do Ptotal dos alimentos vegetais está na forma de Pfit (NRC, 1994).

2.2.2 Fitato

O fitato (Figura 1) é uma molécula presente nos grãos de cereais, legumes e sementes. É a principal forma de armazenamento de fósforo para o desenvolvimento e germinação destes vegetais (LEI; PORRES, 2003). De acordo com Almeida et al. (2003), vários nomes e símbolos são utilizados para a nomenclatura e simbologia do fitato como: ácido fítico, hexofosfato de mio-inositol, IP_6, $InsP_6$ ou $Ins(1,2,3,4,5,6)P_6$.

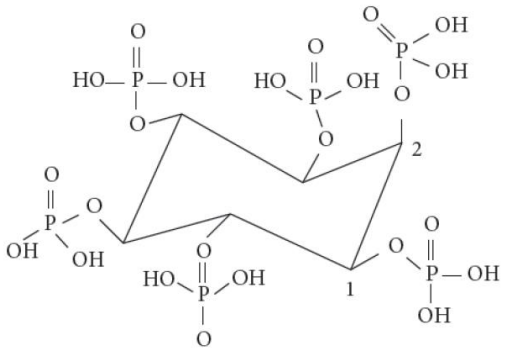

Figura 1: Estrutura mais estável energeticamente do fitato ou ácido fítico (QUIRRENBACH et al., 2009).

Almeida et al. (2003) ainda citam que a origem da molécula tem como precursor a glicose que é convertida em mio-inositol (Figura 2) e após sua completa fosforilação é transformada na molécula de fitato.

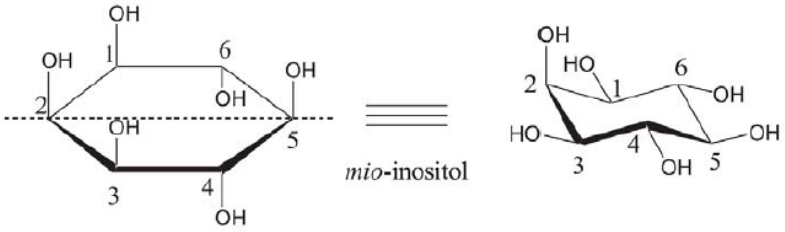

Figura 2: Estrutura do mio-inositol (ALMEIDA et al. 2003).

O fitato indisponibiliza grande parte do Ptotal dos alimentos vegetais para os animias monogástricos e, além disso, ele é considerado um fator antinutricional por se complexar com íons e moléculas. De acordo com Quirrenbach et al. (2009), o fitato pode conter até 12 cargas negativas, duas em cada um dos grupos fosfatos da molécula, e assim se complexar com minerais catiônicos, como Ca^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+} e Cu^{2+} e outros nutrientes como amido, peptídeos, aminoácidos e proteínas (KORNEGAY, 2001).

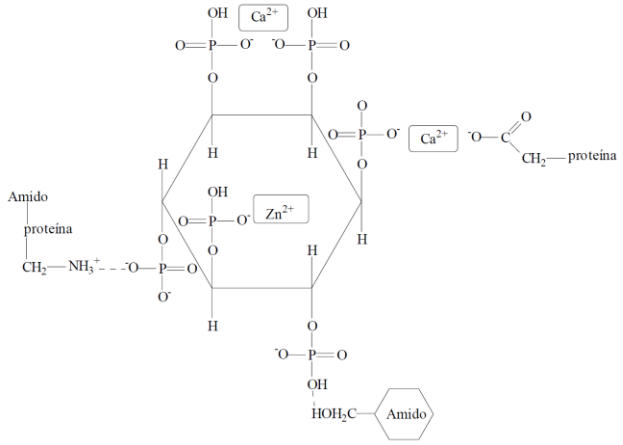

Figura 3: Nutrientes passíveis de complexação ao fitato (KORNEGAY, 2001).

O tipo de nutriente no qual o fitato irá se complexar depende do pH e da concentração do fitato, mas esses complexos, em sua maioria, são insolúveis e indisponíveis quando em condições fisiológicas normais (TORRE et al., 1991). O cálcio (Ca) tem uma grande afinidade pelo fitato, formando fosfatos de cálcio ($Ca_3(PO_4)_2$) (TAMIM; ANGEL; CHRISTMAN, 2004). Porém, destaca-se que os alimentos de origem vegetal não são ricos em Ca, portanto o Ca que irá se complexar com o fitato no trato gastrointestinal (TGI) das aves, tem origem dietética. Cerca de um terço deste Ca dietético será complexado no TGI (SELLE; COWIESON; RAVIDRAN, 2009), assim é importante que rações com diferentes teores de Pfit apresentem diferentes relações Ca:Pdisp, garantindo o desenvolvimento normal das aves.

Plumstead et al. (2008) demonstraram o efeito complexante do fitato com o Ca em um experimento com frangos de corte com 21 dias de idade que receberam rações com diferentes níveis de de Pfit e Ca, os autores concluíram que o maior nível de Pfit (0,28%) apresentou um relação Ca:Pdisp de 2,53:1 e o menor nível de Pfit (0,10%) uma relação de Ca:Pdisp de 2,34:1.

2.3 Fitase

As fitases exógenas têm sido adicionadas às rações buscando melhorar o aproveitamento do fósforo fítico por animais monogástricos. Diversas pesquisas têm comprovado os benefícios da sua utilização (SHAW et al., 2011; GOMIDE et al., 2011 e 2012; SANTOS et al., 2011; SANTOS et al., 2013; NAGATA et al., 2011; RODRIGUES et al., 2012; NOURMOHAMMADI et al., 2012).

De acordo com Ragon et al. (2008) duas das principais organizações na área química, International Union of Pure and Applied Chemistry (IUPAC) e International Union of Biochemistry (IUB), reconhecem duas classes de fitase:

3-fitases (EC 3.1.3.8) que removem o primeiro ortofosfato na posição D-3 (L-1) do fitato e as 6-fitases (EC 3.1.3.26) que iniciam a desfosforilação na posição D-4 (L-6). No entanto, a classificação das fitases segundo o pH de ação tem um importante papel na escolha da fitase a ser utilizada, já que o pH do TGI das aves varia de acordo com cada segmento. Assim, as fitases podem ser classificadas em ácidas ou alcalinas, tendo como pH ótimo de ação entre 2,5 à 6,0 e entre 6,0 à 8,0 respectivamente (GREINER; ALMINGER; CARLSSON, 2001).

Mullaney & Ullah (2003) classificaram em três tipos as fitases quando as diferenciamos de acordo com suas diferenças estruturas e catalíticas. Elas podem ser subdivididas em: histidina fosfatase ácida (HFA), fitase β-hélice (FBH) e fosfatase ácida "purple" (FAP), sendo que a maioria das fitase comerciais para a nutrição animal são as classificadas de HFA.

Alguns autores sugerem que aves adultas podem sintetizar alguma fitase intestinal. Entretanto, outros pesquisadores (MAENZ & CLASSEN, 1998; SIMONS & VERSTEEGH, 1990) afirmam que a capacidade do frango de corte para o aproveitamento do fósforo fítico é limitada e a produção de fitase endógena é insignificante.

Há vários anos, estudos têm demonstrado que o aproveitamento do fósforo fítico pode ser melhorado com a utilização de enzimas exógenas, como a fitase, que é capaz de hidrolisar o fósforo fítico, liberando outros nutrientes além do fósforo (ZENG et al., 2016; LIU et al., 2007; RUTHERFURD et al., 2004; ONYANGO et al., 2005). Santos et al. (2013) avaliaram dietas com dois níveis de fósforo (0,45 e 0,32%) e dois níveis de cálcio (0,90 e 0,76%) suplementadas ou não com fitase (0, 500, 1000 e 1500 FTU/kg) para frangos de corte (1-21 dias de idade) e concluíram que 500 FTU/kg de fitase foi suficiente para garantir o desempenho dos animais que receberam dieta com menor nível de fósforo e

cálcio.

Por outro lado, Aurelli et al (2011) avaliaram dietas com dois níveis de fósforo total (0,56% e 0,41%) suplementadas ou não com 6-fitase (0; 500; 1000; 2000 FTU/kg) na fase de 8-22 dias de idade e observaram que a adição de 500 FTU/kg foi suficiente para manter o desempenho de frangos de corte que receberam dietas com menor nível de fósforo total (0,41%). Porém, os animais que receberam 2000 FTU/kg de fitase tiveram um ganho de peso superior e uma menor excreção de fósforo, 9,7% e 66% respectivamente, comparado aos animais que receberam dieta contendo 0,56% de fósforo total sem adição de fitase.

Meneghetti et al. (2011) avaliaram altos níveis de fitase, variando de 1500 a 10000 FTU/kg para frangos de corte que receberam dietas com redução energética e nutricional e concluíram que pode ser utilizado até 4500 FTU/kg de fitase sem afetar o desempenho das aves na fase de 1-35 dias de idade. Também concluíram que altos níveis de fitase geram maior retenção de cálcio e fósforo.

Sob o ponto de vista da nutrição, a viabilização técnica das enzimas exógenas é um marco importante, pois permite melhorar o aproveitamento dos nutrientes. De acordo com Viana et al. (2009), o aumento na utilização do fósforo, dos aminoácidos e da energia por meio da utilização da fitase representaria economia significativa no custo final da formulação das dietas. Entretanto, nota-se que as pesquisas relacionadas ao uso dessa enzima em rações de frangos de corte não apresentam consenso quanto à concentração de fitase a ser utilizada. Trabalhos têm mostrado efeito positivo da fitase sobre a utilização do fósforo fítico, porém com a fitase em concentrações variáveis nas rações, numa amplitude de 250 (LELIS et al., 2012) a 10.000 FTU/kg de ração (MENEGHETTI et al., 2011).

2.4 Aspectos gerais do metabolismo do cálcio e do fósforo

Os minerais possuem funções importantes no organismo dessa forma, tanto o excesso como a deficiência dos mesmos impossibilitam o máximo desenvolvimento animal. O cálcio e o fósforo são responsáveis por diversas funções orgânicas, mas a principal função é a mineralização da matriz óssea.

Segundo o National Research Council – NRC (1994), a associação desses macrominerais para a absorção é preconizada na relação de 2:1 (cálcio: fósforo), para a maioria das rações de monogástricos, com exceção de aves de postura que possuem uma exigência muito maior de cálcio devido à formação da casca do ovo. Se houver um desbalanço desses minerais, afeta a relação e o processo de absorção de ambos são influenciados.

Além disso, o esqueleto ósseo funciona como um reservatório de cálcio e o fósforo, sendo que 99% do cálcio e 80% do fósforo do organismo encontram-se nos ossos e dentes.

2.4.1 Cálcio

Além da formação e manutenção da estrutura óssea, o cálcio é demandado pelos animais para adequado crescimento e utilização eficiente dos alimentos, transmissão de impulsos nervosos, coagulação sanguínea, contração muscular, ativação de sistemas enzimáticos, envolvimento com a secreção de diferentes hormônios e formação da casca do ovo nas aves (UNDERWOOD & SUTTLE, 1999; SÁ et al., 2004). Dessa forma, a concentração de cálcio no sangue e nos tecidos deve ser mantida constante. Porém, sabe-se que ocorre um intercâmbio contínuo entre o cálcio do plasma sanguíneo e dos ossos. Portanto,

o cálcio dietético absorvido seria responsável pelo aumento na concentração desse íon na corrente sanguínea se não fosse a rápida deposição mineral no tecido ósseo (JUNQUEIRA & CARNEIRO, 2004).

Em situações de baixa concentração sanguínea, ocorre a mobilização do cálcio dos ossos para o sangue no intuito de manter o equilíbrio orgânico. A mobilização do cálcio depositado nos ossos é feita pela transferência dos íons dos cristais de hidroxiapatita para o líquido intersticial, do qual o cálcio passa para o sangue. O hormônio paratormônio (PTH), aumenta o número de osteoclastos e a reabsorção da matriz óssea, liberando fosfato de cálcio e aumentando a calcemia. Atua também sobre os rins diminuindo a excreção de fósforo e estimulando a ativação de vitamina D (McDOWELL, 1992). A secreção do paratormônio é regulada em resposta a flutuações na concentração de cálcio (PIZAURO Jr., 2002).

Outro hormônio que atua no metabolismo do cálcio para manter normal o seu nível no plasma é a calcitonina, produzido pelas células parafoliculares da tireóide, que age inibindo a reabsorção da matriz óssea e, portanto, a mobilização do cálcio. O estímulo para sua secreção é dado quando os níveis de cálcio estão elevados no sangue (MAIORKA & MACARI, 2002).

A homeostase do cálcio no fluido extracelular também sofre efeito da vitamina D, que possui papel importante no metabolismo do Ca e P, sendo que a deficiência de cálcio pode ser devido à carência desse mineral na dieta ou à falta de vitamina D, responsável pela absorção intestinal do mesmo. A vitamina D atua sobre o DNA do enterócito, induzindo a produção do RNAm, responsável pela codificação da proteína transportadora do cálcio através da membrana celular.

2.4.2 Fósforo

Assim como o cálcio, o fósforo participa de inúmeras funções no organismo, estando envolvido em várias reações metabólicas, sendo responsável juntamente com o cálcio pela formação e manutenção dos ossos. É também essencial para utilização e transferência de energia (na forma de ATP), é integrante dos ácidos nucléicos, em associação aos lipídeos forma os compostos de fosfolipídios que são os principais componentes da membrana plasmática, participa como componente ativador e constituinte de complexos co-enzimáticos como o NAD e NADP, forma o sistema tampão fosfato, visando à manutenção do equilíbrio ácido-básico e osmótico do organismo (RUNHO et al., 2001).

O fósforo é absorvido no intestino delgado por transporte ativo com gasto de energia, sendo estimulado pela vitamina D ativa e dependente de sódio. A quantidade absorvida de fósforo dependerá da fonte fornecida, da relação cálcio: fósforo, pH intestinal, vitamina D, magnésio, ferro, alumínio entre outros fatores (MAIORKA & MACARI, 2002). Após a absorção, o fósforo circula pelo corpo e é extraído do sangue para ser depositado nos ossos, podendo também ser reabsorvido dos ossos para manter níveis normais no plasma sanguíneo. Como ocorre com o cálcio, os níveis sanguíneos de fósforo também são controlados pelos hormônios calcitonina e PTH, por meio de sua relação com a forma ativa da vitamina D.

2.5 Meta-análise aplicada na pesquisa com animais

Na área animal, o emprego da meta-análise para reunir informações distintas e oriundas de diferentes condições experimentais tem sido crescente. Destaca-se que o crescente volume de publicações científicas gerado pelo

desenvolvimento das pesquisas, com conclusões obtidas em diferentes trabalhos versando sobre o mesmo tema, e algumas vezes destoantes, tem motivado os pesquisadores em compilar informações publicadas (LOVATTO & SAUVANT, 2002; HAUPTLI et al., 2007). Em vista disso, o procedimento estatístico com emprego da meta-análise, vem sendo utilizado para obtenção de uma resposta única e confiável para um conjunto de resultados publicados (GIANNOTTI, 2004).

De acordo com Lovatto et al. (2007), o emprego da meta-análise permite produzir informações úteis com custos reduzidos além de ser superior às formas tradicionais de revisão de literatura. Ela estima com maior precisão o efeito dos tratamentos, ajustando-os para a heterogeneidade experimental. O autor ainda afirma que a meta-análise exige disciplina no processo de sistematização dos resultados da pesquisa.

Jones et al. (2010) e Kerr et al. (2010) conduziram trabalhos *in vivo* com suínos, buscando ajustar equações para estimar a eficiência de liberação do fósforo rações formuladas com fitase. Entretanto, Jones et al. (2010) destacaram que a diversidade de fitases disponíveis no mercado e a ampla variação nas dosagens recomendadas para liberar quantidades similares de fósforo pelas diferentes fontes dificultam uma comparação efetiva dessas. Semelhantemente, pode-se ressaltar que nos trabalhos com frangos de corte disponíveis na literatura geralmente utilizam-se dosagens diferenciadas de fitase e, na maioria das vezes, considerando-se que uma mesma quantidade de fósforo será liberada. De acordo com Rodehutscord (2009), há necessidade de se desenvolver um protocolo padrão para estudos com animais sobre a disponibilidade de fósforo e eficiência da fitase.

2.6 Trascriptômica aplicada à pesquisa com animais

Diferentes ferramentas de biotecnologia são utilizadas afim de aprimorar e detalhar os resultados com pesquisas com animais. Análises genômicas, proteômicas, metabolômicas e trascriptômicas auxiliam na compreensão das respostas do sistema fisiológico em função das práticas zootécnicas, como conforto térmico, e às respostas ao manejo nutricional adotado (FURLAN et al., 2007).

A transcriptômica é uma técnica abrangente e normalmente utilizada para elucidar de maneira geral qual a resposta do animal quando aplicado algum fator ou condição, diferente de outras técnicas genômicas onde se procura um DNA ou RNA específico. Assim, pesquisas investigativas através da técnica de transcriptômica podem ser encontradas na literatura (LI et al., 2011; SCHMEISSER et al., 2016; COBLE et al., 2014).

No estudo de transcriptômica em aves, dependendo do tecido analisado pode-se encontrar mais de 25.000 RNA´s expressos, o que pode exigir uma análise não só estatística com também crítica, com o uso do bom senso.

2.7 Considerações finais

A utilização de um conjunto de informações disponíveis na literatura, referentes ao uso de fitase para de frangos de corte, pode tornar-se útil na otimização do uso desta enzima nas rações. Com a redução no custo da fitase pelo avanço das técnicas de biotecnologia, permite a inclusão de fitase em concentrações mais elevadas nas rações, assim a necessidade de estimar a eficiência da utilização do fósforo pelas aves. Além disso, a possibilidade de

aprofundar os efeitos da utilização da fitase exógena na alimentação de aves com a técnica de transcriptômica pode ser uma ferramenta útil para definir novas pesquisas.

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SEGUNDA PARTE

ARTICLE 1

(Drafted according to UFLA´s standards)

Available phosphorus equivalency using a meta-analysis study with weight gain and bone ash by broilers fed diets with phytase supplementation

E. M. C. Lima et al.

ABSTRACT - A meta-analysis between January 1984 to December 2014 was performed to collect the largest number of papers concerning the available phosphorus (aP) requirement and the use of phytase in feed for broilers. The weight gain (WG) and the bone ash (ASH) were considered as the variable response. The papers were cataloged, grouped, selected, filtered and standardized. The aP equivalence in response to different levels of phytase inclusion and aP supplementation to WG and ASH response was determinated using linear and quadratic regression. To validate the equations, two experiments were carried out with male Cobb 500, with 15 birds per replicate and each treatment with 6 replications. The treatments consisted of 3 levels of phytase inclusion (450/900/1350 FTU) and a reduction of the respective aP equivalence values. Experiment 1 and 2 the positive control was formulated with 0.37% of aP and 0.79% of calcium (Ca) at 1 to 21 days old and 0.27% of aP and 0.57% of Ca at 22 to 42 days old. The same Ca levels were used for all treatments. The aP equivalence when 450/900/1350FTU/kg was add are 0.10/0.14/0.19% for 1 to 21 days old and 0.09/0.14/0.19% for 1 to 42 days old. No difference (P> 0.05) to weight gain (WG), feed conversion ratio (FCR) and alkaline phosphatase concentration (FAK) between the birds fed with control diet and birds fed with treatments supplemented with phytase and reduced levels of aP.

At the same age the birds fed with 450/900FTU/kg considering 0.19% of aP equivalence had better (P <0.05) bone ash (ASH) in tibia compared to the birds fed control diet. The same result for birds fed with 450FTU/kg and 0.14% of aP equivalence. From 22 to 42 days old, no difference was observed (P> 0.05) in the ASH, however, the birds fed control diet had worst WG, FCR and FAK results comparing with some of phyatse treatments. At experiment 2 from 1 to 42 days of age, no WG difference (P>0.05) between the birds fed control diet and phytase treatments. The birds fed with 450FTU/kg and 0.19% of aP equivalence had lower feed intake (FI) and better ASH compared to the birds fed control diet. At the same aP equivalence, birds supplemented with 1350FTU/kg had lower FI and FCR comparing with those fed control diet. In conclusion, equations can be used for the determination of aP equivalence values for broiler chickens feeds.

Keywords: Phytate. Meta-analysis. Enzyme. Birds.

Introduction

For several years, exogenous phytases have been added to broilers feed seeking to improve the use of phytic phosphorus for monogastric animals. The research has proven the benefits of its use (SHAW et al., 2011; GOMIDE et al., 2012; SANTOS et al., 2011; SANTOS et al., 2013; NAGATA et al., 2011; NOURMOHAMMADI et al., 2012).

Jones et al. (2010) and Kerr et al. (2010) conducted *in vivo* studies with pigs, trying to adjust equations to estimate the release efficiency of phosphorus diets with phytase. However, Jones et al. (2010) pointed out that the diversity of exogenous phytases and the wide variation in dosages recommended to release

similar amounts of phosphorus by different sources difficult an effective comparison of these. According with Rodehutscord (2009), there is need to develop a standard protocol for animal studies on phosphorus availability and efficiency of phytase. Similarly, Bedford & Cowieson (2009) highlight the importance of considering, in the formulation, not only the phytic acid content of the diet, but also the relative solubility of this phytate, appropriately modifying the values of the phytase matrix.

In animals studding, the use of meta-analysis to gather different information and from different experimental conditions has been increasing. It is noteworthy that the growing volume of scientific publications generated by the development of research, with findings obtained in different works dealing on the same subject, and sometimes dissonant, has motivated researchers to compile published information (LOVATTO & SAUVANT, 2002; HAUPTLI et al., 2007). Before this, the statistical procedure using the meta-analysis has been used to obtain a unique and reliable response to a set of published results (GIANNOTTI, 2004).

Thus, the possibility to predict the phosphorus liberation using different concentrations and sources of phytase in broiler diets, using the principle of meta-analysis, cataloging information in the scientific literature, can constitute an important alternative and enable the use of exogenous phytase with higher frequency and accuracy, related to availability of phosphorus to birds when used in different concentrations and sources. In this context, this project came to establish, from information collected in the literature, new equations to estimate with efficiency and accuracy, the use of phosphorus for broilers receiving diets supplemented with phytase.

Material e methods

Meta-analysis, conversion, data correction and equivalency equations

The information used to estimate the predict equations of phosphorus utilization for broilers were related to cataloging results in weight gain and bone ash from papers published from January 1984 to December 2014. The articles concerning about the available phosphorus requirement also related to the use of phytase in broiler´s feed, between the phases 1 to 21 and 1 to 42 days of age.

The data were derived from extensive literature review, conducted via "Periódicos Capes", to include as much as possible studies in Brazil and international journals with significant Journal Impact Factor like the Poultry Science, Journal of Applied Poultry Research, British Poultry Science, among others. The study was standardized by using keywords 9 (Phytase, 3-phosphato, 6-phosphato, broiler, birds, phosphorus, digestibility, nutrient, and enzyme) they are used two by two, totalizing 36 combinations.

One hundred seventy tree (173) articles were cataloged (1296 and 907 observations of weight gain and bone ash respectively) and the selected containing at least three additional phosphorus and / or 3 phytase levels including the level "zero". After selecting, the data were depurated and separated by the type of phytase (3 or 6-phytase), as well as additional data of phosphorus (aP) followed the experimental reference (3 or 6-phytase).

Because the chemistry of the ingredients used in each experiment the compositions of feed was transformed into a standard, and available phosphorus concentrations, phytic phosphorus and calcium from each feed were recalculated according to the food composition values presented in Brasilian Tables to Poultry and Swine (Rostagno et al. 2011). For the calculation of additional

phosphorus values considered in each experiment, the basal diet deficient in available phosphorus to be zero (0%) of additional phosphorus and other levels supplemental phosphorus calculated as a function of phosphorus derived from inorganic source. However, weight gain and bone ash information were transformed in a common metric as performed by and Vasquez & Pesti (1997).

Levels of supplemental phosphorus and phytase were considered independent variables in preparing the response equations for weight gain and bone ash. A correction was made to remove the secondary effects: sex, mean age and lineage of birds used in the experiments also the number of replication, experimental diets used, phytic phosphorus and calcium levels, as well as the interaction between these two latter, whose data were corrected for these effects. Through the SAS 9.1 software the data were corrected by the statistical model:

$$Yijk = \mu + Si + Ij + Ek + Ll + Rm + Dn + Po + Cp + PCop + eijklmnopq$$

Where:

Yijklmnop = q observation value (weight gain or bone ash), from birds that received feed considering the secondary effects:

μ = overall average;

Si = sex i effect;

Ij = age j effect;

Ek = phytase sourse k effect;

Ll = lineage l effect;

Rm = replication numbers m effect;

Dn = used food n effect;

Po = phytic phosphorus level effect;

Cp = calcium level effect;

PCop = phytic phosphorus and calcium level interaction effect;

eijklmnopq = associated error in each observation.

The corrected weight gain or bone ash values (Ycorrected) were obtained by adding the overall average of observations over the estimated error for each observation, as described:

Ycorrected (ijklmnopq) = μ + eijklmnopq

Wherein:

Yijklmnopq = weight gain or bone ash correted observations, from birds that received feed considering the secondary effects;

μ = general average of the observations;

eijklmnopq = associated error to each observation.

The linear and quadratic regression of the data were made by R software. The regressions were matched according to the response variable and type of phytase used. Thus obtained the supplemental phosphorus equivalent in response to the inclusion of different level of phytase in the equation.

Validation

Two trials were carried out in the Poultry Sector of the Department of Animal Science of the Federal University of Lavras, Minas Gerais, Brazil, to validate the additional phosphorus equivalency equations in broiler´s diets when supplemented with phytase. The experiments were carried with male Cobb500® that were weighed, homogenized and randomly distributed in 6 replications with 15 birds in 10 treatments in a randomized block design (RBD). This study was fully approved by the Ethics Committee on Animal Use of the Federal University of Lavras (CEUA / UFLA).

In both trials were used two experimental periods, 1 to 21 and 22 to 42 days old following the equations of equivalence. In the first trial the animals received the experimental treatments until 21 days old and between 22 to 42

days old the control diet was offered. In trial 2 the birds received the treatments until the age of slaughter (42 days old) considering two periods. The nutritional needs were determined by the average of the requirement value for each proposed period, as well as Ca and aP requirements were calculated using the equations proposed in Brazilian Tables For Poultry and Swine (ROSTAGNO et al., 2011).

The performance was evaluated (feed consumption, weight gain and feed conversion), the bone ash and the serum alkaline phosphatase concentration at 21 (trial 1) and 42 days old (both trials). Two birds per replication were sacrificed by cervical dislocation and the left tibia and the serum were collected. The tibias were degreased in ethyl ether and incinerated at 600 °C as described by AOAC (2005) to determine the % bone ash. Alkaline phosphatase was evaluated using a commercial kit (K019. Bioclin) by the pull of the serum from the two birds in the same replication. The absorbance determined at Multiskan GO (Thermo Fisher Scientific).

Statistical analysis was performed by using the “Multcomp package” of the R software (R Core Team, 2015). The treatments were compared with the control by the Dunnett test.

Results

The linear and quadratic regression determined with data concerning about the additional inorganic phosphorus and the phytase inclusion is shown in Tables 1 and 2.

Table 1. Linear and quadratic regression of 1 to 21 days old for 3 and 6-phytase and additional phosphorus to weight gain and bone ash response.

	3-Phytase (Phy3)	R^2
WG	0.0001 Phy3+0.803	0.28
WG	-0.000000169711 $Phy3^2$+0.00034906 Phy3+0.77205	0.38
ASH	0.00009 Phy3+0.8193	0.18
ASH	-0.00000011986 $Phy3^2$+0.00023404 Phy3+0.80553	0.23
	Additional Phosphorus (adP) with 3-Phytase	
WG	1.0505 adP+0.72285	0.45
WG	-4.3627 adP^2+2.4286 adP+0.6611	0.59
ASH	0.84353 adP+0.76884	0.55
ASH	-2.66453 adP^2+1.70365 adP+0.73174	0.65
	6-Phytase (Phy6)	
WG	0.0001 Phy6+0.8006	0.27
WG	-0.00000011573 $Phy6^2$+0.00026577 Phy6+0.79273	0.29
ASH*	0.00008 Phy6+0.84	0.17
ASH	-0.0000000489377 $Phy6^2$+0.00014043 Phy6+0.83131	0.18
	Additional Phosphorus (adP) with 6-Phytase	
WG	0.98164 adP +0.70681	0.32
WG	-3.9276 adP^2+2.2598 adP +0.6638	0.40
ASH	0.70894 adP +0.78758	0.42
ASH*	-1.97241 adP^2+1.35476 adP +0.76095	0.47

WG – weight gain; ASH – bone ash; *Regression used to determined the equations of equivalence to the validation.

Table 2. Linear and quadratic regression of 1 to 42 days old for 3 and 6-phytase and additional phosphorus to weight gain and bone ash response.

	3-Phytase (Phy3)	R2
WG	0.0001 Phy3+0.8193	0.25
WG	-0.00000013933 $Phy3^2$+0.00029797 Phy3+0.79784	0.33
ASH	0.00007 Phy3+0.8595	0.15
ASH	-0.000000100831 $Phy3^2$+0.00018424 Phy3+0.846	0.21
	Additional Phosphorus (adP) with 3-Phytase	
WG	0.84143 adP +0.75595	0.39
WG	-3.44214 adP^2+2.01768 adP +0.70291	0.52
ASH	0.66862 adP +0.79793	0.48
ASH	-2.51851 adP^2+1.5407 adP +0.75718	0.61

6-Phytase (Phy6)		
WG	0.0001 Phy6+0.8	0.25
WG	-0.000000123693 $Phy6^2$+0.00028599 Phy6+0.78532	0.30
ASH*	0.00008498 Phy6+0.85536	0.22
ASH	-0.0000000732641 $Phy6^2$+0.00017506 Phy6+0.84188	0.25
Additional Phosphorus (adP) with 6-Phytase		
WG	0.76976 adP +0.75875	0.31
WG	-4.02795 adP^2+2.08926 adP +0.70394	0.45
ASH	0.71347 adP +0.81265	0.44
ASH*	-2.03187 adP^2+1.33978 adP +0.78797	0.50

WG – weight gain; ASH – bone ash; *Regression used to determined the equations of equivalence to the validation.

To validate this study, were used the equation determinate by the bone ash response at 1-21 days old and 1-42 days old using a 6-phytase. Thus, in the trial 1 the equivalence values that were reduced form the control diet were 0.10/0.14/0.19% of aP, according with the equation:

$$adP = \frac{-1.35476+\sqrt{1.8354-7.88964 \times(0.00008Phy6+0.07905)}}{-3.94482}$$

In trial 2 the equivalence to the same phytase levels were 0.09/0.14/0.19% of aP, according with the equation:

$$adP = \frac{-1.33978+\sqrt{1.795-8.12748 \times(0.000085Phy6+0.0674)}}{-4.06374}$$

The diets composition were presented in Table 3. The food nutrients composition were considerated from The Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2011). The treatments were adjusted with the addition of inorganic phosphate, limestone, kaolin and phytase. It was considered 0.37% and 0.27% of aP and 0.79% and 0.57% of Ca to control diets at 1 to 21 and 22 to 42 days old, respectively. The other treatments were considered the equivalence of phosphorus estimated by the inclusion of 450/900/1350 FYT/kg of feed using a 6-Phytase (*Aspergillus oryzae*), considering the bone ash response.

Table 3. Diets composition for trial 1 and 2 at the two experimental period.

Ingredients (kg)	Trial 1		Trial 2	
	1 – 21 days old	22 – 42 days old	1 – 21 days old	22 – 42 days old
Corn 7.88% CP	56,196	62.030	56,196	62.030
Soy bean meal 45%	37.270	30.280	37.270	30.280
Refined soya oil	2.511	3.80	2.511	3.80
DL-methionine	0.235	0.176	0.235	0.176
L-lysine HCl (78%)	0.092	0.076	0.092	0.076
Salt	0.495	0.449	0.495	0.449
Mineral Supl.[1]	0.050	0.05	0.050	0.05
Vitaminic Supl.[2]	0.045	0.03	0.045	0.03
Anticoccidial	0.05	0.05	0.05	0.05
Choline chloride	0.05	0.05	0.05	0.05
Growth promoter[3]	0.005	0.005	0.005	0.005
Dicalcium Phosphate	1.375/0.835/0.618/0.347	0.896	1.375/0.875/0.618/0.347	0.896/0.409/0.139/0.0
Graund Limestone	0.920/1.271/1.411/1.588	0.687	0.920/1.233/1.411/1.588	0.687/1.004/1.180/1.350
Kaolin	0.705/0.894/0.971/1.065	1.415	0.705/0.890/0.971/1.065	1.415/1.586/1.681/1.649
Total	100,000	100,000	100,000	100,000
		Energy and nutrientes (%)		
Crude Protein	21.5	18.75	21.5	18.75
ME[4] (kcal/kg)	2975	3125	2975	3125
Sodium	0.215	0.198	0.215	0.198
Lysine	1.242	1.044	1.242	1.044
Methionine+cystine	0.895	0.762	0.895	0.762
Tryptophan	0.215	0.188	0.215	0.188
Threonine	0.808	0.679	0.808	0.679
Calcium	0.79	0.57	0.79	0.57
Available phosphorus	0.37/0.27/0.23/0.18	0.27	0.37/0.28/0.23/0.18	0.27/0.18/0.13/0.08
Phytase[5] (FTU/kg)	0/450/900/1350	0	0/450/900/1350	0/450/900/1350

[1]Per kg: Mn: 156,000mg; Fe:96,000mg; Zn: 110,000mg; Cu: 20,000mg; I: 1,400mg; Se: 360mg. [2]Per kg: A: 20,000.000UI; D3: 5,000.000UI; E: 40,500UI; K3: 4,800mg; B1: 3,000mg; B2: 12,000mg; B6: 6,000mg; B12: 28,000mcg; Biotina: 60mg; Folic acid: 1,600mg; Nicotinic acid: 87,000mg; Pantotenic acid: 29,000mg; BHT: 5,000mg. [3]It was removed in the last week. [4]Metabolizable Energy [5]6-phytase with 10,000 FTU/kg of concentration.

The ingredients analyzed had different nutrients concentration comparing with the Brazilian Table for Poultry and Swine (Table 4).

Table 4. Literature values and used food composition.

Nutrients[1] (%)	Corn		Soy bean meal	
	Analyzed	Rostagno et al. (2011)	Analyzed	Rostagno et al. (2011)
P	0.23	0.25	0.62	0.56
aP	0.05	0.06	0.22	0.22
pP	0.18	0.19	0.40	0.34
Ca	0.01	0.03	0.30	0.24
CP	7.20	7.88	43.93	45.22
GE (kcal/kg)	3986	3940	4237	4090

[1]Phosphorus – P; Available phosphorus – aP; Phytic phosphorus – pP; Calcium – Ca; Crude protein – CP; Gross energy – GE. Dicalcium phosphate with 19.05% of aP and 28.83% of Ca. Ground limestone with 39.90% of Ca.

The performance results for both trials were shown at Table 5. In trial 1, at 1 to 21 days old, the birds fed with 1350 FTU/kg considering 0.10% of phosphorus equivalence had higher ($P<0.05$) FI comparing with the birds fed with control diet (without phytase inclusion). For the ASH results, the birds fed with 450 FTU/kg considering 0.14% of phosphorus equivalence and 450/900 FTU/kg considering 0.19% of phosphorus equivalence had higher bone ash concentration comparing with the birds fed with control diet. No significant difference ($P>0.05$) for WG, FCR or FAK were found between the phytase treatment comparing with the control by the Dunnett test.

At 22 to 42 days old, no significant difference ($P>0.05$) for the ASH results for the birds fed with the control diet comparing with thebirds fed with the phytase treatment. The birds fed with 1350 FTU/kg at 0.10% of phosphorus equivalence, also the bird fed with 900 FTU/kg at 0.19% of phosphorus equivalence had higher FI comparing with the control. However, the birds fed with 450 FTU/kg at 0.10% of phosphorus equivalence had lower FI comparing with the birds fed with control diet.

Table 5. Feed intake (FI), weight gain (WG), feed conversion ratio (FCR), alkaline phosphatase (FAK) and bone ash (ASH) results in broilers fed diets supplemented with phytase or not according to the additional phosphorus equivalence determined by prediction equation for the period 1 to 21 days old (Trail 1) and 1 to 42 days old (Trail 2).

Trial 1

Phosphorus equivalence (%)	Phytase (FTU/kg)	1 to 21 days old					22 to 42 days old				
		FI (g)	WG (g)	FCR (g/g)	FAK (U/L)	ASH (%)	FI (g)	WG (g)	FCR (g/g)	FAK (U/L)	ASH (%)
0.0¥	0	1124	860.8	1.31	418	47.73	3844	2024.5	1.90	331	52.41
0.10	450	1135	859.3	1.32	451	49.12	3722*	2101.6	1.78*	261	53.33
	900	1114	862.7	1.29	403	48.05	3892	2152.6	1.81	271	52.65
	1350	1150*	903.5	1.27	386	47.28	3963*	2201.5*	1.80	268	53.27
0.14	450	1124	859.3	1.31	481	50.54*	3908	2224.9*	1.76*	232*	52.15
	900	1139	872.5	1.31	444	49.02	3863	2246.0*	1.72*	330	53.82
	1350	1133	906.7	1.25	432	47.76	3862	2177.1*	1.76*	251*	52.00
0.19	450	1116	817.1	1.37	447	52.10*	3850	2148.9	1.79	243*	50.31
	900	1112	851.9	1.31	440	50.81*	3957*	2211.2*	1.79	255	51.3
	1350	1133	859.0	1.32	381	49.37	3910	2177.8*	1.79	277	52.52

Trial 2 – 1 to 42 days old

		FI (g)	WG (g)	FCR (g/g)	FAK (U/L)	ASH (%)
0.0¥	0	5012	2957	1.69	252	51.62
0.09	450	4972	3098	1.61	246	52.01
	900	4995	2991	1.67	290	52.41
	1350	5032	3070	1.64	257	51.50
0.14	450	5010	3001	1.67	288	52.52
	900	5103	3070	1.66	292	52.11
	1350	4910	2998	1.64	338*	51.59
0.19	450	4689*	2835	1.65	273	55.70*
	900	4936	3006	1.64	223	53.32
	1350	4782*	3121	1.53*	227	53.40

*Means are different of positive control (0 FTU/kg of phytase) by the Dunnett test (0.05). ¥ Control treatment with 0.37% and 0.27% of available phosphorus at 1 to 21 and 22 to 42 days old respectively.

At 22 to 42 days old, the birds fed with 1350 FTU/kg at 0.10%, 450/900/1350 FTU/kg at 0.14% and 900/1350 FTU/kg at 0.19% of phosphorus equivalence had higher WG comparing with the birds fed with control diet. Also, the birds fed with 450 FTU/kg at 0.10% and 450/900/1350 FTU/kg at 0.14% of phosphorus equivalence, were observed better FCR (Lower) comparing with the birds fed with control diet.

As well, the birds fed with 450/1350 FTU/kg at 0.14% and 450 FTU/kg at 0.19% of phosphorus equivalence had lowed serum FAK concentration than the birds feed with control diets.

At 1 to 42 days old for Trial 2, no significant difference ($P>0.05$) for the WG results from birds fed with phytase treatment comparing with the birds fed with control diets. However, the birds fed with 450/1350 FTU/kg at 0.19% of phosphorus equivalence had lower FI comparing with the birds fed with control diet. At the same phosphorus equivalence, the bird supplemented with 1350 FTU/kg had better FCR comparing with the birds fed with control diet. Also, the birds supplemented with 450 FTU/kg had higher bone ash concentration comparing with the birds fed with control diet.

Only the birds fed with 1350 FTU/kg at 0.14% of phosphorus equivalence had a higher serum FAK concentration comparing with the birds fed with control diets.

Discussion

Jendza et al. (2006) in concluding 500 to 1000 FTU/kg of a 6-phytase found equivalency values of 0.0716% and 0.0916% of inorganic phosphorus (iP) in response to weight gain for broilers in the period from 1 to 42 days old. Higher iP equivalency (0.08 and 0.12%) was found by this work. Bigger

difference when compared in response to bone ash (0.10 and 0.15% of iP), to the equation applied to the validation of this work step.

However, Vieira et al. (2015) found similar iP equivalency comparing with this work. Testing a 6-phytase for tibia ash response, they found 0.098% and 0.168% of equivalence when broilers with 8 to 25 days were fed with 500 and 1000 FTU/kg of phytase. The author also relate that 500 FTU/kg of phytase was insufficient to guarantee the same positive control FI and BW of broilers fed with low aP diet at 8 to 25 days old.

Wu et al. (2014) observed a lower FI in animals fed aP deficient diet supplemented with 500 FYT/kg of feed with a 6-phytase compared to the positive control.

According to Zeng et al. (2016) phytase is not only available pP, but also amino acids and minerals, and these nutrients can result in different performance responses by birds. Thus, Souza et al. (2015) tested the inclusion of 500 FYT/kg of a phytase from *Escherichia coli* in diet with reduced level of different nutrients and found better FI, WG and FCR in animals that received feed low aP and Ca diet supplemented with phytase comparing with positive control. Also, no difference between the FI, WG and FCR average from birds fed with low nutrients (amino acids, aP, Ca and energy) comparing the positive control, that show the improvement of nutrients avaibility when the phytic acid was breached by phytase added.

Midilli et al. (2014), supplemented diets with 500 FTU/kg of feed (0.31% of aP) at 1 to 21 days old, not observed statistical difference in the WG compared to the control (0.43% of aP). The same result was observed in this work on Trial 1 at 1 to 21 days old and Trial 2 at 1 to 42 days old. The FCR was not affected at the same period in Trails 1 and 2. Similar results were found by Lalpanmawia et al. (2014) that found no differences in FCR from birds fed diets

supplemented with 500 FYT/kg at 21 and 42 days of age.

In general, looking at the overall average of the treatments supplemented with phytase in Trails 1 and 2, phytase was able to sustain the aP deficiency in diets and maintain the animal performance.

In both Trials, the phytase supplementation was sufficient maintain the bone deposition in relation to the control. Liu et al. (2014) found higher ash concentration on fingers of chickens fed diets supplemented with 1000 FYT/kg of feed at 27 days of age observed the same. The difference in Ca:aP ratios of the feed can influence the birds performance how was demonstrated by Plumstead et al. (2008).

Walk et al. (2014) found the same tibia ash in broilers at 1 to 21 days old fed with a negative control (0.30% of aP and 0.82% of Ca) and supplied with 500, 1000 and 1500 FYT/kg of phytase, also, was similar with the positive control (0.45 % of aP and 0.98% of Ca).

According to Kramer (1989) the alkaline phosphatase levels in serum of broilers tend to be smaller with advancing age, which can be numerically referred to in this work. Magnago et al. (2015) observed a linear effect on lower response of the alkaline phosphatase to the increasing levels of phytase (0, 300, 600; 1200 FTU/kg of feed) in serum of pigs at 90 days of age. Similar results founded by Bilal et al. (2015), that fed broilers with diet supplemented with 600 FT/kg of phytase and observed that phytase decrease the FAK serum level, also, the increased aP diet level influenced the FAK generating lower concentrations.

Conclusion

Both equations that was validate can be used to determinate the phosphorus equivalence in diets for broilers on the overall period, guarantee the bone quality in broilers in the periods 1-21 and 22-42 days of age.

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ARTICLE 2
(Drafted according to UFLA´s standards)

Transcriptome analysis of liver of broilers fed diets supplemented with phytase decrease of several transmembrane transporters

E. M. C. Lima et al.

ABSTRACT - Many results already show how phytase increase the nutrients digestion, but not enough results related if phytase could change gene expression. Thus, an experiment was developed with male Ross 708 and housed in starter batteries until 3 week of age when the chickens were transferred to grower batteries for the remainder of the 5 week study. Diets in mash form and water was offered *ad libitum*. A basal diet was formulated with reduction of 0.1 of available phosphorus (aP) level and reduced calcium (Ca) level, that was corrected with dicalcium phosphate, limestone and/or phytase addition to supplied the nutrients requirements in the periods. The treatments consisting in a positive control (0.46% and 0.38% of aP at starter and grower feed respectively) and two levels of phytase inclusion (500 and 1500FTU/kg of feed). A phytase from a commercial source, from *Aspergillus niger* with 5000 units per gram was used in this study. The performance was evaluated at 21 and 41 days old. At 41 days old, one bird/replicate from the positive control and 1500FTU treatment was euthanized by cervical dislocation and the liver was collected to RNA isolation. At 21 days old, the birds fed with low aP and supplemented with 1500 FTU/kg of phytase has no difference in the WG and the FI with the control group (0 FTU/kg). At 22 to 41 days old, or in the complete experiment period (1 to 41 days old) no difference was observed for all parameters ($P>0.05$) showing that the lowest level of phytase was sufficient to guarantee the same control performance. Minerals and peptide transporters were repressed in liver from

birds that received feed supplemented with 1500 FTU/kg of phytase. Zinc-transporters stands out between others. The investigative transcriptomic technique show that phytase had influence in liver gene expression, but more analysis are necessary to confirm this result.

Keywords: Phytate, chickens, transcriptomic, enzyme.

INTRODUCTION

The phytic acid is a major storage form of myo-inositol, phosphate as well as, several mineral cations, all needed to sustain seedling development. Phytic acid absorption by the digestive track depends largely on microbial phytase activity, which is essentially lacking in nonruminant animals, including humans (GREINER & KONIETZNY, 2010). Futhermore, the intact phytic acid molecule will interfere with absorption of nutritionally important minerals such as iron, zinc and magnesium, resulting in suboptimal animal weight gain and categorized the phitic acid as an anti-nutritional component in many maize-based foods and feeds (SOUSA et al., 2015).

During the past decade, the inclusion of exogenous phytase in poultry diets has increased remarkably, mainly in response to heightened concerns over phosphorus (P) pollution of the environment (CATALAN et al., 2016). The capacity of this feed enzyme to release phytate-bound P and reduce P excretion is now well documented (SOUSA et al., 2015; ZELLER et al., 2015; OLUKOSI & FRU-NJI, 2014; SELLE et al., 2009). Effectively, phytase is an alternative, economical P source and, as global phosphate reserves are not renewable, this is beneficial to their preservation.

As know the sequencing, assembly, and annotation of the chicken

genome (International Chicken Genome Sequencing Consortion, 2004) was an important step to basic and applied research (BURT, 2007; DODGSON, 2007). And the understanding about the effect causing from feed on metabolism, phisiology and immunology through of biogenomics techniques come to amplify and get directions to animal nutrition research. Cogburn et al. (2007) highlights the importance of genomic, proteomic and metabolomic studies to understanding the complexity biological systems and how this knowledge can help to materialize as phenotypes.

Therefore, this study has the purpose to check the phytase effect on performance and the liver mRNA expression (RNA-seq) on broilers fed with diets supplemented with phytase.

METHODS

Animals

This study was carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health recommendations, also approved by the Committee on the Ethics of Animal Experiments of the University of Delaware.

A Trail was development with male Ross 708 that was hatched at the Charles C. Allen Jr. Biotechnology Laboratory, University of Delaware, Newark - DE. Birds were sexed and housed in Petersime start batteries until 3 week of age when the chickens were transferred to grower batteries for the remainder of the 5 week study. Chickens received 23 hours of light and 1 hours of dark. The temperature was 90°F at the first week than decreased 5°F per week until 4th week when the temperature was fixed at 68°F until the end. Diets in mash form and water was offered *ad libitum*.

A starter and grower basal diets (Table 1) was formulated with reduction of 0.1 of available phosphorus (aP) level and reduced calcium (Ca) level. The treatments consisting in a positive control (0.46% and 0.38% of aP at starter and grower feed respectively) and two levels of phytase inclusion (500 and 1500FTU/kg of feed) with 0.1 of aP reduction from positive control. The aP and Ca from positive control were corrected with dicalcium phosphate and limestone addition to supplied the nutrients requirements.

Table 1. Feed ingredients and nutrient composition of the control diets.

*Ingredients	Starter	Grower
Corn meal	58.441	65.848
Soybean meal(48% of CP)	35.577	27.180
Soy oil	2.613	3.660
Phosphate dicalcium	1.260	1,430
Ground limestone	1.106	0.980
Salt	0.463	0.415
L-lysine	0.028	0.020
DL-methionine	0.213	0.167
Choline Cl 60%	0.100	0.100
VIT – Premix[2]	0.100	0.100
MIN – Premix[2]	0.050	0.050
Selenium premix	0.050	0.050
Total	**1.000**	**1.000**
Calculated composition[1]		
EM (kcal/kg)	3050	3200
PB %	22.250	18.900
Ca(%)	0.920	0.770
P aval. (%)	0.460	0.380
Na (%)	0.200	0.180
Met + cys (%)	0.910	0.780
Lys (%)	1.220	0.990

*None growth promoter or coccidiostatic was used. [1]Nutrients requirements from the Ross 708 Nutrition Spefications (2014), means values for 1 to 21 days old and 22 to 42 days old. [2]From a commercial source.

. Each treatment has 6 replicate with 10 birds each until the 3rd week when was reduced to 6 birds each replicate to fit at the grower batteries. A phytase from a commercial source, from *Aspergillus niger* with 5000 units per gram was used in this study.

At 21 and 41 days old the feed and the birds were weighed and the performance calculated for each period. Also, at 41 days old, one bird/replicate from the control and 1500FTU treatment was euthanized by cervical dislocation and the liver was collected and immediately frozen in liquid nitrogen and stored at -80°C until RNA isolation.

RNA-seq

Aproximately 30mg from the liver was used to total RNA extraction from 12 samples from 41 days old male Ross 708 (6 from control diet and 6 from 1500FTU diet) using the Qiagen RNasy Mini Kit as per the manufacturer´s instructions. RNA concentration was determined by Nanodrop ND-100 spectrophotometer and the quality of the RNA by Agilent 2100 Bioanalyzer.

With magnetic oligo-dt beads, mRNA was purified from total RNA extracts. Using the mRNA, the cDNA were synthesized using the Ilumina TruSeq RNA Sample Preparation Kit (Ilumina, San Diego, California, USA) as per the manufacturer's instructions.

The libraries were sequenced using the HiSeq 2000 Sequencing System (Ilumina) at the Delaware Biotechnology Institute´s Sequencing and Genotyping Center, Newark – Delaware, USA. All libraries were sequenced to a depth of 10 millions reads per library approximately.

Statistical analyses were performed using JMP 9.0 software. The ratio of reads per kilobase of gene per million mapped reads (RPKM) between the

control and the 1500FTU treatment and performed a log_2 transformation was determined, then the identification of differentially expressed genes, functional clustering and pathway analysis using DAVID, eGIFT, KEGG Path way Mapper.

RESULTS AND DISCUTION

Performance

At 21 days old, the birds fed with low aP and supplemented with 1500 FTU/kg of phytase has no difference in the WG and the FI with the control group (0 FTU/kg). The birds fed with 500 FTU/kg (Table 2) did not observe the same results, and the same were observed by Wu et al. (2014), that fed broilers at 1 to 21 days old with 500 FTU/kg of phytase and they found lower FI comparing with the positive control. However, Midilli et al. (2014), found no difference in BW and FI at 21 days old and 42 days old between a positive control and 500 FTU/kg of phytase supplementation.

Table 2. Performance results at 1 to 21, 22 to 41 and 1 to 42 days old from broilers that received feed supplemented with phytase or not.

Phytase level (FYT/kg of feed)	BW (g)	FI (g/bird/day)	WG (g/day)	FCR (g/g)
	1 to 21 days old			
0	856A	52A	39A	1.33AB
500	772B	47B	35B	1.34B
1500	848A	51A	39A	1.31A
	22 to 42 days old			
0	2776	165	91	1.81
500	2663	159	90	1.76
1500	2777	167	92	1.82
	1 to 42 days old			
0	2776	108	65	1.66
500	2663	103	62	1.64
1500	2777	109	65	1.67

BW – Body weight; FI – Feed intake; WG – Weight gain; FCR – Feed convertion ratio. Means with different letters differ by Tukey test ($P<0.05$) at the same column.

Vieira et al. (2015) test low aP diets (0.14% of aP) supplemented with 500 or 1000 FTU/kg of phytase. They observed that 500FTU was not sufficient to guarantee the WG and FI from broilers fed 8 to 25 days old comparing with the positive control (0.42% of aP). On the other hand, the broilers fed with 1000 FTU/kg had the same positive control performance.

Both phytase level supplied enough aP to get similar FCR with the control group, however, the birds fed with 1500 FTU/kg of phytase has lower FCR comparing with 500 FTU/kg level at 1 to 21 days old period.

At the next period, 22 to 41 days old, or in the complete experiment period (1 to 41 days old) no difference was observed for all parameters ($P>0.05$) showing that the lowest level of phytase was sufficient to guarantee the same control performance (Table 2). Catalan et al. (2016) founded similar results when they fed broilers at 22 to 32 days old with 500 FTU/kg of phyatse from *Aspergillus niger*. No difference was observed for BW, FI, WG or FCR.

The literature results and the results of this work show that phytase improve the phytic phosphorus availability for monogastric animals, but indicate that the aP level reducement in diet could be different according the period. The digestive tract maturing is very important in nutrient digestion and absorption, thus, comparing the worst birds performance at 1 to 21 period, supplemented 500 FTU/kg of phytase, the birds recover their performance at the end of time production. As weel, the phytase supplementation in diet with low nutrients level can provide amino acid, others minerals and energy (ZENG et al., 2016; SOUZA et al., 2015; VIANA et al., 2009).

Also, the concentration of phytic phosphorus in diets and the Ca:aP ratio can determine the amount absorption of this two important minerals, changing the ideal Ca:aP ratio and the phytase efficiency (PLUMSTEAD et al., 2008).

RNA-seq

Comparing the liver from positive control *versus* 1500 FTU/kg of phytase inclusion at 41 days old, more than 29,000 genes were analyzed for the expression level. From this total genes, 340 genes has the P<0.05. We detect that in general, the significant difference was genes correlated with transmembrane transporters (Figure 1). Minerals and peptide transporters were repressed in liver from birds that received feed supplemented with 1500 FTU/kg of phytase. Zinc-transporters stands out between others.

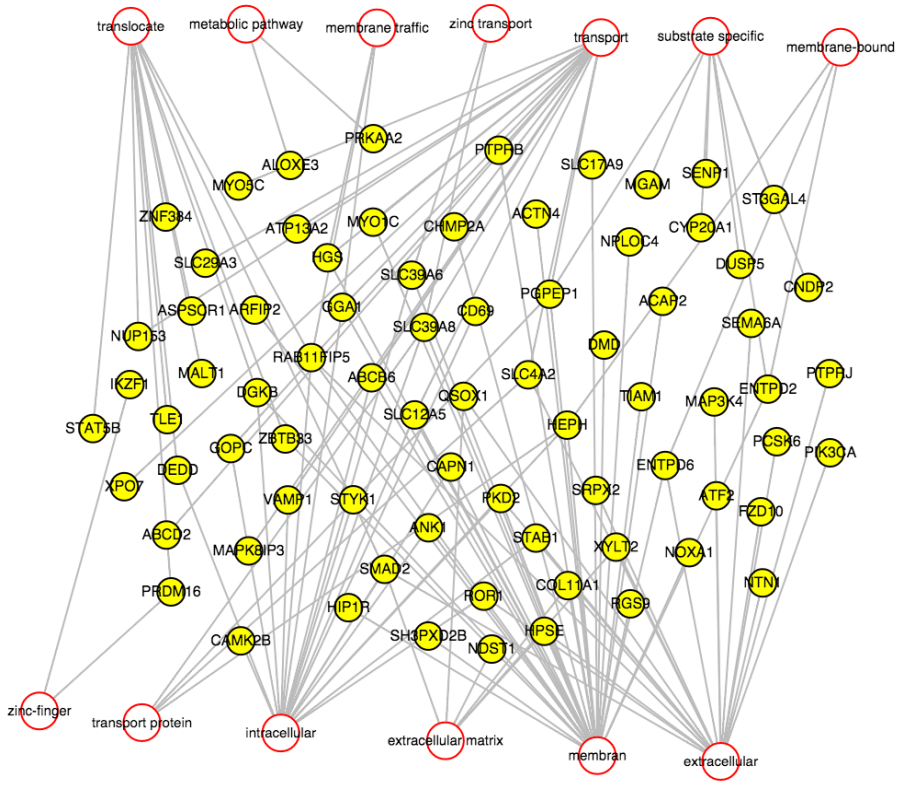
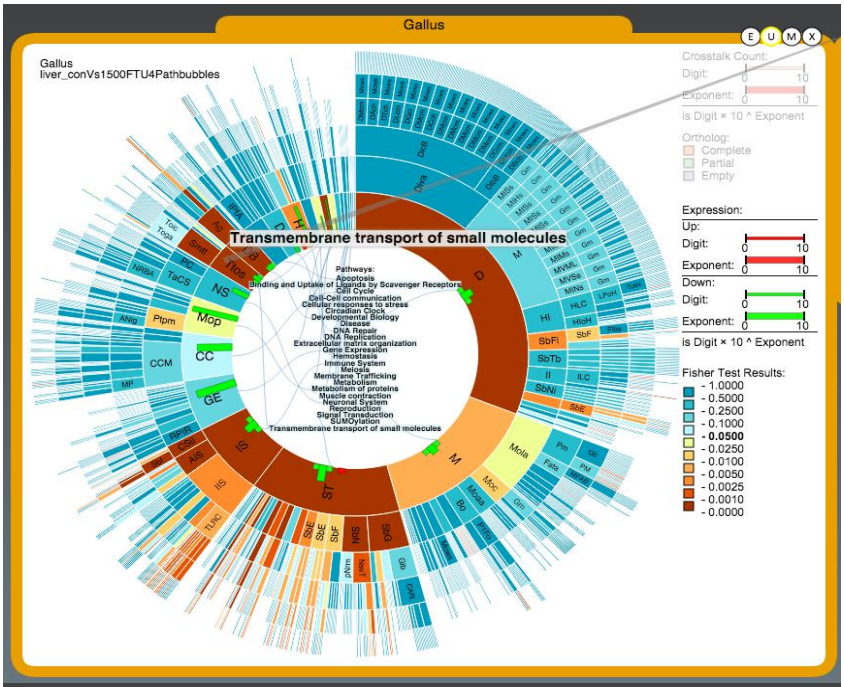


Figure 1. Cytoscape output from WebGIVI of some of significant genes correlated with transmembrane transporters of ions and minerals.

Was used the Path Rings (Figure 2) to correlate all pathways that each gene can actuate.



Transmembrane transport of small molecules

symbol	gene_id	regulation	ratio(log2 based)	crossTalk	rateLimit
TRPM6	100859603	Down	-2.66903	0	0
BEST4	771694	Down	-1.84128	0	0
HEPH	422168	Down	-1.69289	5	0
SLC4A2	395809	Down	-1.60750	4	0
ABCB6	425721	Down	-1.38848	3	0
SLC24A3	421244	Down	-1.38159	4	0
SLC39A8	430411	Down	-1.30764	6	0
ATP6V0A2	395473	Down	-1.23831	9	0
ANO8	429912	Down	-1.22244	0	0
SLC29A3	423717	Down	-1.13253	4	0
SLC12A5	777252	Down	-1.12789	4	0
NUP153	420848	Down	-1.05259	23	0
SLC39A6	420984	Down	-0.97128	6	0
ABCD2	417694	Down	-0.96727	3	0
CLCN5	422285	Down	-0.86897	0	0

Figure 2. Screen capture from Path Rings with the transmembrane transporter genes, positive control *versus* 1500FTU/kg of phytase supplementation.

Several zinc-transporters on liver were significant lower at phytase treatment, and the zinc transmembrane transporters (Zinc-T) expression levels depend of the animal physiology and nutritionally status (COUSINS & McMAHON, 2000). The zinc available in the diet and post-absorption can increase or decrease some of the Zinc-T expression, thus, it is a hypothesis that phytase action on the phytic acid liberated the complexed zinc to absorption on the enterocyte.

Jou et al. (2009) evaluated defficients zinc (Zn) diets for rats, and suggested that lower zinc level diet increase the Zn absortion, reduce the Zn losses and increased Zn mobilization from liver, corroborating with this investigative results founded on this work.

Phytic acid can complex with different molecules (KORNAGAY et al., 2001). However, it is impossible determinate the amount of each complexed nutrient because the ambient, cultivar and others factors have influence on the phytic acid concentration on plant grains.

In addition, Jozefiak et al. (2010) evaluated the IGF-1 (Insuline-like growth factor 1) gene expression on liver from broilers fed with diet supplemented with 100 FTU/kg of phytase, and with 0.08 of P value and the phytase supplementation reduce 10% of the IGF-1 gene expression. The author points out that more research must done to elucidate the phytase influence on genes expression.

In a exploratory transcriptomic analysis in muscle tissue, Schmeisser et al. (2016) found similar result with this work where phytase change the genes expression. The author evaluated the genes expression on *Pectoralis major* from broilers fed diet with phytase, however the phytase influence on genes expression involved in the development of the tissue pathways, that was upregulated.

On the literature, some research evaluating the phytase effect on intestinal nutrients transporter gene expression (HUBER et al., 2015; VIGORS et al., 2014) in broilers and pigs could be found. Also the high temperature exposure effect on gene expression in different tissues (COBLE et al., 2014; HAO & GU, 2014; GU, HAO & WANG, 2012; LI et al., 2011). Those works indicate that the genomics analysis is an important tool to help the understanding on animal response by different ambient and nutritional conditions.

In conclusion, the exogenous phytase is a good nutritional tool to increase the available nutrients for broilers. The phytase guarantee the broilers performance fed with low aP diets, also others nutrients. The investigative by RNA-seq show that phytase reduced severals liver transmembrane transporters and how future researches are necessary to elucidate more the phytase action.

The RNA-seq analysis on liver tissue showed good start for investigative nutritional research, indicate a large spectrum of consequences when an additive was used on broilers feed.

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Table 1. References used in meta-analysis of weight gain data in studies conducted with additional phosphorus and/or 3-phytase at 1 to 21 days old with up to 1500 FTU/kg of phytase results.

Article	Exp.	Reference	Additional P Level* (%)	Phytase Level* (FTU/kg)
1	1	Timmons et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 225; 450; 675; 900; 1125; 1350
1	2	Timmons et al. (2004)	0.0; 0.050; 0.100; 0.150	--
2	3	Ribeiro et al. (2003)	0.0; 0.079; 0.157	--
3	4	Viveiros et al. (2002)	0.0; 0.144; 0.250	--
4	5	Simons et al. (1990)	0.0; 0.152; 0.304	0; 250; 500; 750; 1000; 1500
4	6	Simons et al. (1990)	0.0; 0.152; 0.304	0; 375; 750; 1500
5	7	Wu et al. (2004)	0.0; 0.057; 0.121; 0.180	0; 500; 1000; 1500
6	8	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 500; 1000
6	9	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	--
7	10	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
7	11	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	--
7	12	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
7	13	Augspurger et al. (2003)	0.0; 0.050; 0.100	--
8	14	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
8	15	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
8	16	Payne et al. (2005)	--	0; 300; 500; 750
9	17	Pillai et al. (2006)	0.0; 0.052; 0.102; 0.151	0; 250; 500; 1000
10	18	Laurentiz et al. (2009)	0.0; 0.082; 0.161; 0.241	0; 500; 1000
11	19	Leytem et al. (2008)	0.0; 0.152; 0.155	--
12	20	Denbow et al. (1998)	0.0; 0.080; 0.160; 0.240	0; 400; 800; 1200
13	21	Ibrahim et al. (1999)	0.0; 0.061; 0.122; 0.185	0; 300; 600; 900
14	22	Driver et al. (2005)	0.0; 0.040; 0.149; 0.257	--
15	23	Jiang et al. (2011)	0.0; 0.074; 0.148; 0.222; 0.296; 0.370; 0.444	0; 250; 500; 750
16	24	Waldroup et al. (2000)	0.0; 0.049; 0.082; 0.131; 0.181; 0.230; 0.280; 0.329; 0.379	--
17	25	Kornegay et al. (1996)	0.0; 0.070; 0.140; 0.250	0; 200; 400; 600; 800; 1000; 1200
18	26	Karimi et al. (2011)	0.0; 0.056; 0.109; 0.165; 0.218; 0.274	--
19	27	Adedokun et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.380	--
20	28	Yonemochi et al. (2001)	0.0; 0.075; 0.150	0; 125; 250; 500
21	29	Nourmohammadi et al. (2012)	--	0; 500; 1000

22	30	Hassanabadi et al. (2007)	--	0; 300; 600
23	31	Hassanabadi et al. (2008a)	--	0; 250; 500; 750; 1000; 1250
24	32	Hassanabadi et al. (2008b)	--	0; 250; 500; 750; 1000
25	33	Bahadoran et al. (2011)	--	0; 500; 1000
26	34	Brunelli et al. (2012)	--	0; 750; 1500
27	35	Jondreville et al. (2007)	--	0; 250; 500; 750; 1000
28	36	Brenes et al. (2003)	--	0; 200; 400; 600
29	37	Zhou et al. (2008)	--	0; 500; 750
30	38	Mondal et al. (2007)	--	0; 250; 500
31	39	Conte et al. (2003)	--	0; 400; 800; 1200
32	40	Ravindran et al. (2001)	--	0; 125; 250; 375; 500; 750; 1000
33	41	Silversides et al. (2004)	--	0; 150; 450; 1250
34	42	Shirley & Edwards (2003)	--	0; 93.75; 187.5; 375; 750; 1500
35	43	Hall et al. (2003)	--	0; 750; 1500
36	44	Qian et al. (1997)	--	0; 300; 600; 900
37	45	Zhang et al. (2000)	--	0; 250; 500
38	46	Rama Rao et al. (1999)	--	0; 250; 500
39	47	Donato et al. (2011)	--	0; 600; 1200
Total Observations (n)			**115**	**175**

*The observations could be repeated.

Table 2: References used in meta-analysis of bone ash data in studies conducted with additional phosphorus and/or 3-phytase at 1 to 21 days old with up to 1500 FTU/kg of phytase results.

Article	Exp.	Reference	Additional P Level* (%)	Phytase Level* (FTU/kg)
1	1	Timmons et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 225; 450; 675; 900; 1125; 1350
1	2	Timmons et al. (2004)	0.0; 0.050; 0.100; 0.150	--
2	3	Viveiros et al. (2002)	0.0; 0.144; 0.251	--
3	4	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 500; 1000
3	5	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	--
4	6	Augspurger et al. (2003)	0.0; 0.050; 0.100	--
4	7	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
4	8	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	--
4	9	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000

5	10	Banks et al. (2004)	0.0; 0.07; 0.14; 0.2	--
6	11	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
6	12	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
6	13	Payne et al. (2005)	--	0; 300; 500; 750
7	14	Pillai et al. (2006)	0.0; 0.052; 0.102; 0.151	0; 250; 500; 1000
8	15	Ribeiro et al. (2003)	0.0; 0.079; 0.157	--
9	16	Denbow et al. (1998)	0.0; 0.080; 0.160; 0.240	0; 400; 800; 1200
10	17	Driver et al. (2005)	0.0; 0.040; 0.149; 0.257	--
11	18	Ibrahim et al. (1999)	0.0; 0.061; 0.122; 0.185	0; 300; 600; 900
12	19	Jiang et al. (2011)	0.0; 0.074; 0.148; 0.222; 0.296; 0.37; 0.444	0; 250; 500; 750
13	20	Waldroup et al. (2000)	0.0; 0.049; 0.082; 0.131; 0.181; 0.230; 0.280; 0.329; 0.379	--
14	21	Kornegay et al. (1996)	0.0; 0.070; 0.140; 0.250	0; 200; 400; 600; 800; 1000; 1200
15	22	Karimi et al. (2011)	0.0; 0.056; 0.109; 0.165; 0.218; 0.274	--
16	23	Adedokun et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.380	--
17	25	Conte et al. (2003)	--	0; 400; 800; 1200
19	26	Silversides et al. (2004)	--	0; 150; 450; 1250
20	27	Shirley & Edwards (2003)	--	0; 93.75; 187.5; 375; 750; 1500
21	28	Teichmann et al. (1998)	--	0; 300; 600; 900
22	29	Zhou et al. (2008)	--	0; 500; 750
23	30	Ravindran et al. (2001)	--	0; 125; 250; 375; 500; 750; 1000
24	31	Hassanabadi et al. (2007)	--	0; 300; 600
25	32	Rama Rao et al. (1999)	--	0; 250; 500
26	33	Brenes et al. (2003)	--	0; 200; 400; 600
27	34	Zhang et al. (2000)	--	0; 250; 500
28	35	Qian et al. (1997)	--	0; 300; 600; 900
29	36	Hall et al. (2003)	--	0; 750; 1500
Total Observations (n)			**99**	**111**

*The observations could be repeated.

Table 3: References used in meta-analysis of weight gain data in studies conducted with additional phosphorus and/or 6-phytase at 1 to 21 days old with up to 1500 FTU/kg of phytase results.

Article	Exp.	Reference	Additional P Level* (%)	Phytase Level* (FTU/kg)
1	1	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 500; 1000
1	2	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	--
2	3	Augspurger et al. (2003)	0.0; 0.050; 0.100	--
2	4	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
2	5	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000; 1500
2	6	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
3	7	Coppedge et al. (2011)	--	0; 150; 200; 250
4	8	Francesh & Gehaert et al. (2009)	0.0; 0.057; 0.225	--
5	9	Onyango et al. (2005)	0.0; 0.075; 0.150; 0.370	0; 500; 1000
6	10	Onyango et al. (2004)	0.0; 0.111; 0.37	--
7	11	Persia & Saylor (2006)	0.0; 0.059; 0.118; 0.177; 0.237; 0.296;	--
7	12	Persia & Saylor (2006)	0.0; 0.080; 0.168; 0.237; 0.316; 0.395	--
8	13	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 300; 500; 750
8	14	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	--
9	15	Pereira et al. (2012)	0.0; 0.050; 0.010	--
10	16	Pillai et al. (2006)	0.0; 0.052; 0.109; 0.151	0; 250; 500; 750; 1000
10	17	Pillai et al. (2006)	0.0; 0.052; 0.102; 0.151	0; 250; 500; 1000
11	18	Santos et al. (2008)	0.0; 0.012; 0.082; 0.223	0; 500; 750; 1000
12	19	Shaw et al. (2011)	0.0; 0.079; 0.158	0; 500; 1000
13	20	Jendza et al. (2006)	0.0; 0.059; 0.120; 0.180	0; 250; 500; 750; 1000
14	21	Adedokun et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.380	0; 500; 1000
15	22	Shaw et al. (2010)	0.0; 0.99; 0.201	0; 500; 750
16	23	Walk et al. (2011)	0.0; 0.01; 0.13	--
17	24	Aureli et al. (2011)	--	0; 500; 1000
18	25	Cowieson et al. (2006)	--	0; 150; 300; 600; 1200
19	26	Cowieson & Adeola (2005)	--	0; 500; 1000
20	27	Pirgozliev et al. (2008)	--	0; 250; 500
21	28	Dilger et al. (2004)	--	0; 500; 1000
21	29	Dilger et al. (2004)	--	0; 500; 750; 1000
22	30	Santos et al. (2013)	--	0; 500; 1500
22	31	Santos et al. (2013)	--	0; 500; 1000; 1500
23	32	Fukayama et al. (2008)	--	0; 500; 750; 1000
24	33	Liu et al. (2010)	--	0; 500; 1000
25	34	Liu et al. (2009)	--	0; 500; 1000
26	35	Liu et al. (2008)	--	0; 500; 1000

27	36	Guo et al. (2009)	--	0; 500; 1000
28	37	Naves et al. (2014)	--	0; 750; 1500
28	38	Naves et al. (2014)	--	0; 750; 1500
29	39	Naves et al. (2014)	--	0; 750; 1500
30	40	Silversides et al. (2004)	--	0; 150; 450; 1250
31	41	Timmons et al. (2008)	--	0; 250; 275; 500; 550; 1000; 1100
32	42	Woyengo et al. (2010)	--	0; 500; 600; 700
32	43	Woyengo et al. (2010)	--	0; 500; 600; 700
33	44	Woyengo et al. (2008)	--	0; 250; 500
34	45	Tejedor et al. (2001)	--	0; 500; 750
Total Observations (n)			**99**	**144**

*The observations could be repeated.

Table 4: References used in meta-analysis of bone ash data in studies conducted with additional phosphorus and/or 6-phytase at 1 to 21 days old with up to 1500 FTU/kg of phytase results.

Article	Exp.	Reference	Additional P Level* (%)	Phytase Level* (FTU/kg)
1	1	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 500; 1000
1	2	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	--
2	3	Augspurger et al. (2003)	0.0; 0.050; 0.100	--
2	4	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
2	5	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000; 1500
2	6	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
3	7	Coppedge et al. (2011)	0.0; 0.057; 0.114; 0.171	0; 150; 200; 250
4	8	Francesh & Gehaert (2009)	0.0; 0.057; 0.225	--
5	9	Lelis et al. (2010)	0.0; 0.031; 0.132	--
6	10	Onyango et al. (2005)	0.0; 0.075; 0.150; 0.370	0; 500; 1000
6	11	Onyango et al. (2004)	0.0; 0.111; 0.37	--
7	12	Persia & Saylor (2006)	0.0; 0.059; 0.118; 0.177; 0.237; 0.296;	--
7	13	Persia & Saylor (2006)	0.0; 0.080; 0.168; 0.237; 0.316; 0.395	--
8	14	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
8	15	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
8	16	Payne et al. (2005)	--	0; 300; 500; 750
9	17	Pereira et al. (2012)	0.0; 0.049; 0.098	--
10	18	Pillai et al. (2006)	0.0; 0.052; 0.109; 0.151	0; 250; 500; 750; 1000
10	19	Pillai et al. (2006)	0.0; 0.052; 0.102; 0.151	0; 250; 500; 1000
11	20	Jendza (2006)	0.0; 0.059; 0.120; 0.180	0; 250; 500; 750; 1000
12	21	Adedokun et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.380	0; 500; 1000

13	22	Shaw et al. (2011)	0.0; 0.079; 0.159	0; 500; 1000
14	23	Aureli et al. (2011)	--	0; 500; 1000
15		Timmons et al. (2008)	--	0; 250; 275; 500; 550; 1000; 1100
16	24	Fukayama et al. (2008)	--	0; 500; 750; 1000
17	25	Cowieson et al. (2006)	--	0; 150; 300; 600; 1200
18	26	Dilger et al. (2004)	--	0; 500; 750; 1000
19	27	Santos (2013a)	--	0; 500; 1500
19	27	Santos (2013b)	--	0; 500; 1000; 1500
20	28	Guo et al. (2009)	--	0; 500; 1000
21	29	Silversides et al. (2004)	--	0; 150; 450; 1250
22	30	Naves et al. (2014)	--	0; 750; 1500
22	31	Naves et al. (2014)	--	0; 750; 1500
23	32	Naves et al. (2014)	--	0; 750; 1500
24	33	Woyengo et al. (2010)	--	0; 500; 600; 700
24	34	Woyengo et al. (2010)	--	0; 500; 600; 700
25	35	Woyengo et al. (2008)	--	0; 250; 500
Total Observations (n)			**99**	**144**

*The observations could be repeated.

Table 5. References used in meta-analysis of weight gain data in studies conducted with additional phosphorus and/or 3-phytase at 1 to 42 days old with up to 1500 FTU/kg of phytase results.

Article	Exp.	Reference	Additional P Level* (%)	Phytase Level* (FTU/kg)
1	1	Timmons et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 225; 450; 675; 900; 1125; 1350
1	2	Timmons et al. (2004)	0.0; 0.050; 0.100; 0.150	--
2	3	Ribeiro et al. (2003)	0.0; 0.079; 0.157	--
3	4	Viveiros et al. (2002)	0.0; 0.144; 0.250	--
3	5	Viveiros et al. (2002)	0.0; 0.138; 0.249	--
4	6	Simons et al. (1990)	0.0; 0.152; 0.304	0; 250; 500; 750; 1000; 1500
4	7	Simons et al. (1990)	0.0; 0.152; 0.304	0; 375; 750; 1500
5	8	Wu et al. (2004)	0.0; 0.057; 0.121; 0.180	0; 500; 1000; 1500
5	9	Wu et al. (2004)	0.0; 0.057; 0.116; 0.161	0; 500; 1000; 1500
5	10	Wu et al. (2004)	0.0; 0.057; 0.116; 0.161	0; 500; 1000; 1500
6	11	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 500; 1000
6	12	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	--
7	13	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
7	14	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	--
7	15	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
7	16	Augspurger et al. (2003)	0.0; 0.050; 0.100	--

8	17	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
8	18	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
8	19	Payne et al. (2005)	--	0; 300; 500; 750
9	20	Pillai et al. (2006)	0.0; 0.052; 0.102; 0.151	0; 250; 500; 1000
10	21	Laurentiz et al. (2009)	0.0; 0.082; 0.161; 0.241	0; 500; 1000
11	22	Leytem et al. (2008)	0.0; 0.152; 0.155	--
12	23	Denbow et al. (1998)	0.0; 0.080; 0.160; 0.240	0; 400; 800; 1200
13	24	Ibrahim et al. (1999)	0.0; 0.061; 0.122; 0.185	0; 300; 600; 900
14	25	Driver et al. (2005)	0.0; 0.040; 0.149; 0.257	--
15	26	Jiang et al. (2011)	0.0; 0.074; 0.148; 0.222; 0.296; 0.370; 0.444	0; 250; 500; 750
15	27	Jiang et al. (2011)	0.0; 0.080; 0.160; 0.240; 0.320; 0.400; 0.480	0; 200; 400; 600
16	28	Waldroup et al. (2000)	0.0; 0.049; 0.082; 0.131; 0.181; 0.230; 0.280; 0.329; 0.379	--
17	29	Kornegay et al. (1996)	0.0; 0.070; 0.140; 0.250	0; 200; 400; 600; 800; 1000; 1200
18	30	Karimi et al. (2011)	0.0; 0.053; 0.109; 0.163; 0.218; 0.274	--
18	31	Karimi et al. (2011)	0.0; 0.056; 0.109; 0.165; 0.218; 0.274	--
19	32	Adedokun et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.380	--
20	33	Yonemochi et al. (2001)	0.0; 0.075; 0.150	0; 125; 250; 500
21	34	Bank et al.(2004)	0.0; 0.070; 0.140; 0.200	--
22	35	Milica et al. (2012)	0.0; 0.151; 0.392	--
22	36	Milica et al. (2012)	0.0; 0.148; 0.388	--
23	37	Teixeira et al. (2013)	0.0; 0.100; 0.200	0 500; 1000; 1500
24	38	Yan et al. (2001)	0.0; 0.050; 0.100; 0.150; 0.200; 0.250; 0.300; 0.350	--
25	39	Yan et al. (2003)	0.0; 0.050; 0.100; 0.150; 0.200; 0.250	--
26	40	Teichmann et al. (1998)	--	0; 300; 600; 900
27	41	Nourmohammadi et al. (2012)	--	0; 500; 1000
27	42	Nourmohammadi et al. (2012)	--	0; 500; 1000
28	43	Hassanabadi et al. (2007)	--	0; 300; 600
29	44	Hassanabadi et al. (2008a)	--	0; 250; 500; 750; 1000; 1250
30	45	Hassanabadi et al. (2008b)	--	0; 250; 500; 750; 1000
31	46	Bahadoran et al. (2011)	--	0; 500; 1000
31	47	Bahadoran et al. (2011)	0.0; 0.090; 0.170	0; 500; 1000
32	48	Brunelli et al. (2012)	--	0; 750; 1500
32	49	Brunelli et al. (2012)	--	0; 750; 1500

33	50	Brenes et al. (2003)	--	0; 200; 400; 600
34	51	Zhou et al. (2008)	--	0; 500; 750
34	52	Zhou et al. (2008)	--	0; 500; 750
35	53	Mondal et al. (2007)	--	0; 250; 500
36	54	Conte et al. (2003)	--	0; 400; 800; 1200
37	55	Ravindran et al. (2001)	--	0; 125; 250; 375; 500; 750; 1000
38	56	Silversides et al. (2004)	--	0; 150; 450; 1250
39	57	Shirley & Edwards (2003)	--	0; 93.75; 187.5; 375; 750; 1500
40	58	Catalá-regori et al. (2006)	--	0; 400; 600
40	59	Catalá-regori et al. (2006)	--	0; 400; 600
41	60	Cabahug et al. (1999)	--	0; 400;800
41	61	Cabahug et al. (1999)	--	0; 400;800
41	62	Cabahug et al. (1999)	--	0; 400;800
42	63	Hall et al. (2003)	--	0; 750; 1500
43	64	Qian et al. (1997)	--	0; 300; 600; 900
44	65	Zhang et al. (2000)	--	0; 250; 500
45	66	Rama Rao et al. (1999)	--	0; 250; 500
46	67	Donato et al. (2011)	--	0; 600; 1200
Total Observations (n)			**172**	**203**

*The observations could be repeated.

Table 6. References used in meta-analysis of bone ash data in studies conducted with additional phosphorus and/or 3-phytase at 1 to 42 days old with up to 1500 FTU/kg of phytase results.

Article	Exp.	Reference	Additional P Level* (%)	Phytase Level* (FTU/kg)
1	1	Timmons et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 225; 450; 675; 900; 1125; 1350
1	2	Timmons et al. (2004)	0.0; 0.050; 0.100; 0.150	--
2	3	Viveiros et al. (2002)	0.0; 0.144; 0.251	--
2	4	Viveiros et al. (2002)	0.0; 0.138; 0.249	
3	5	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 500; 1000
3	6	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	--
4	7	Augspurger et al. (2003)	0.0; 0.050; 0.100	--
4	8	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
4	9	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	--
4	10	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
5	11	Banks et al. (2004)	0.0; 0.07; 0.14; 0.2	--
6	12	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
6	13	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
6	14	Payne et al. (2005)	--	0; 300; 500; 750
7	15	Pillai et al. (2006)	0.0; 0.052; 0.102; 0.151	0; 250; 500; 1000

8	16	Ribeiro et al. (2003)	0.0; 0.079; 0.157	--
9	17	Denbow et al. (1998)	0.0; 0.080; 0.160; 0.240	0; 400; 800; 1200
10	18	Driver et al. (2005)	0.0; 0.040; 0.149; 0.257	--
11	19	Ibrahim et al. (1999)	0.0; 0.061; 0.122; 0.185	0; 300; 600; 900
12	20	Jiang et al. (2011)	0.0; 0.074; 0.148; 0.222; 0.296; 0.37; 0.444	0; 250; 500; 750
12	21	Jiang et al. (2011)	0.0; 0.080; 0.160; 0.240; 0.320; 0.400; 0.480	0; 200; 400; 600
13	22	Waldroup et al. (2000)	0.0;0.049;0.082;0.131; 0.181; 0.230; 0.280; 0.329; 0.379	--
14	23	Kornegay et al. (1996)	0.0; 0.070; 0.140; 0.250	--
15	24	Karimi et al. (2011)	0.0; 0.053; 0.109; 0.163; 0.218; 0.274	--
15	25	Karimi et al. (2011)	0.0; 0.056; 0.109; 0.165; 0.218; 0.274	--
16	26	Adedokun et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.380	--
17	27	Yan et al. (2001)	0.0; 0.050; 0.100; 0.150; 0.200; 0.250; 0.300; 0.350	
18	28	Yan et al. (2003)	0.0; 0.050; 0.100; 0.150; 0.200; 0.250	
19	29	Bahadoran et al. (2011)	0.0; 0.090; 0.170	0; 500; 1000
20	30	Nourmohammadi et al. (2012)	--	0; 500; 1000
21	31	Catalá-Gregori et al. (2006)	--	0; 400; 600
21	32	Catalá-Gregori et al. (2006)	--	0; 400; 600
21	33	Catalá-Gregori et al. (2006)	--	0; 400; 600
21	34	Catalá-Gregori et al. (2006)	--	0; 400; 600
22	35	Conte et al. (2003)	--	0; 400; 800; 1200
23	36	Silversides et al. (2004)	--	0; 150; 450; 1250
24	37	Shirley & Edwards (2003)	--	0; 93.75; 187.5; 375; 750; 1500
25	38	Teichmann et al. (1998)	--	0; 300; 600; 900
26	39	Teichmann et al. (1998)	--	0; 300; 600; 900
27	40	Zhou et al. (2008)	--	0; 500; 750
27	41	Zhou et al. (2008)	--	0; 500; 750
28	42	Ravindran et al. (2001)	--	0; 125; 250; 375; 500; 750; 1000
29	43	Hassanabadi et al. (2007)	--	0; 300; 600
30	44	Rama Rao et al. (1999)	--	0; 250; 500
31	45	Brenes et al. (2003)	--	0; 200; 400; 600
32	46	Zhang et al. (2000)	--	0; 250; 500
33	47	Qian et al. (1997)	--	0; 300; 600; 900
34	48	Hall et al. (2003)	--	0; 750; 1500
Total Observations (n)			**137**	**128**

*The observations could be repeated.

Table 7. References used in meta-analysis of weight gain data in studies conducted with additional phosphorus and/or 6-phytase at 1 to 42 days old with up to 1500 FTU/kg of phytase results.

Article	Exp.	Reference	Additional P Level* (%)	Phytase Level* (FTU/kg)
1	1	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	0; 500; 1000
1	2	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	--
2	3	Augspurger et al. (2003)	0.0; 0.050; 0.100	--
2	4	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
2	5	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000; 1500
2	6	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
3	7	Coppedge et al. (2011)	--	0; 150; 200; 250
4	8	Francesh & Gehaert et al. (2009)	0.0; 0.057; 0.225	--
4	9	Francesh & Gehaert et al. (2009)	0.0; 0.056; 0.223	--
5	10	Onyango et al. (2005)	0.0; 0.075; 0.150; 0.370	0; 500; 1000
6	11	Onyango et al. (2004)	0.0; 0.111; 0.37	--
7	12	Persia & Saylor (2006)	0.0; 0.059; 0.118; 0.177; 0.237; 0.296;	--
8	13	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 300; 500; 750
8	14	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	--
9	15	Pereira et al. (2012)	0.0; 0.050; 0.010	--
10	16	Pillai et al. (2006)	0.0; 0.052; 0.109; 0.151	0; 250; 500; 750; 1000
10	17	Pillai et al. (2006)	0.0; 0.052; 0.102; 0.151	0; 250; 500; 1000
11	18	Santos et al. (2008)	0.0; 0.012; 0.082; 0.223	--
11	19	Santos et al. (2008)	0.0; 0.074; 0.213	0; 500; 750; 1000
12	20	Shaw et al. (2011)	0.0; 0.079; 0.158	--
13	21	Jendza et al. (2006)	0.0; 0.059; 0.120; 0.180	--
13	22	Jendza et al. (2006)	0.0; 0.060; 0.120; 0.180	0; 250; 500; 750; 1000
14	23	Adedokun et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.380	0; 500; 1000
15	24	Shaw et al. (2010)	0.0; 0.99; 0.201	0; 500; 750
16	25	Han et al. (2009)	0.0; 0.083; 0.160; 0.249	0; 125; 250; 500; 1000
17	26	Manangi & Coon (2008)	0.0; 0.053; 0.106; 0.165; 0.223; 0.276; 0.339; 0.413	0; 250; 500; 750; 1000; 1500
17	27	Manangi & Coon (2008)	0.0; 0.053; 0.106; 0.165; 0.223; 0.276; 0.339; 0.413	--
18	28	Manangi et al. (2008)	--	0; 250; 500; 750; 1000; 1500
19	29	Timmons et al. (2008)	--	0; 250; 275; 500; 550; 1000; 1100
20	30	Walk et al. (2011)	--	--
21	31	Aureli et al. (2011)	--	0; 500; 1000

22	32	Cowieson et al. (2006)	--	0; 150; 300; 600; 1200
23	33	Cowieson & Adeola (2005)	--	0; 500; 1000
24	34	Pirgozliev et al. (2008)	--	0; 250; 500
25	35	Dilger et al. (2004)	--	0; 500; 1000
25	36	Dilger et al. (2004)	--	0; 500; 750; 1000
25	37	Dilger et al. (2004)	--	0; 500; 750; 1000
26	38	Santos et al. (2013a)	--	0; 500; 1500
27	39	Santos et al. (2013b)	--	0; 500; 1000; 1500
27	40	Santos et al. (2013b)	--	0; 500; 1000; 1500
28	31	Fukayama et al. (2008)	--	0; 500; 750; 1000
29	42	Liu et al. (2010)	--	0; 500; 1000
29	43	Liu et al. (2010)	--	0; 500; 1000
30	44	Liu et al. (2009)	--	0; 500; 1000
31	45	Liu et al. (2008)	--	0; 500; 1000
32	46	Guo et al. (2009)	--	0; 500; 1000
33	47	Guo et al. (2009)	--	0; 500; 1000
34	48	Naves et al. (2014)	--	0; 750; 1500
34	49	Naves et al. (2014)	--	0; 750; 1500
35	50	Naves et al. (2014)	--	0; 750; 1500
36	51	Silversides et al. (2004)	--	0; 150; 450; 1250
37	52	Woyengo et al. (2010)	--	0; 500; 600; 700
37	53	Woyengo et al. (2010)	--	0; 500; 600; 700
38	54	Woyengo et al. (2008)	--	0; 250; 500
39	55	Tejedor et al. (2001)	--	0; 500; 750
Total Observations (n)			**113**	**170**

*The observations could be repeated.

Table 8. References used in meta-analysis of bone ash data in studies conducted with additional phosphorus and/or 6-phytase at 1 to 42 days old with up to 1500 FTU/kg of phytase results.

Article	**Exp.**	**Reference**	**Additional P Level* (%)**	**Phytase Level* (FTU/kg)**
1	1	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	--
1	2	Augspurger & Baker (2004)	0.0; 0.050; 0.100; 0.150; 0.200	--
2	3	Augspurger et al. (2003)	0.0; 0.050; 0.100	--
2	4	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
2	5	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000; 1500
2	6	Augspurger et al. (2003)	0.0; 0.050; 0.100; 0.150	0; 500; 1000
3	7	Coppedge et al. (2011)	0.0; 0.057; 0.114; 0.171	0; 150; 200; 250
4	8	Francesh & Gehaert (2009)	0.0; 0.057; 0.225	--
4	9	Francesh & Gehaert (2009)	0.0; 0.055; 0.223	--
5	10	Lelis et al. (2010)	0.0; 0.031; 0.132	0; 250; 500

6	11	Onyango et al. (2005)	0.0; 0.075; 0.150; 0.370	0; 500; 1000
6	12	Onyango et al. (2004)	0.0; 0.111; 0.37	--
7	13	Persia & Saylor (2006)	0.0; 0.059; 0.118; 0.177; 0.237; 0.296;	--
7	14	Persia & Saylor (2006)	0.0; 0.080; 0.168; 0.237; 0.316; 0.395	--
8	15	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
8	16	Payne et al. (2005)	0.0; 0.059; 0.109; 0.160	0; 100; 200; 300
8	17	Payne et al. (2005)	--	0; 300; 500; 750
9	18	Pereira et al. (2012)	0.0; 0.049; 0.098	--
10	19	Pillai et al. (2006)	0.0; 0.052; 0.109; 0.151	0; 250; 500; 750; 1000
10	20	Pillai et al. (2006)	0.0; 0.052; 0.102; 0.151	0; 250; 500; 1000
11	21	Jendza (2006)	0.0; 0.059; 0.120; 0.180	--
11	22	Jendza (2006)	0.0; 0.060; 0.120; 0.180	0; 250; 500; 750; 1000
12	23	Adedokun et al. (2004)	0.0; 0.050; 0.100; 0.150; 0.380	0; 500; 1000
13	24	Shaw et al. (2011)	0.0; 0.079; 0.159	0; 500; 1000
14	25	Han et al. (2009)	0.0; 0.083; 0.160; 0.249	0; 125; 250; 500; 1000
15	26	Santos et al. (2008)	0.0; 0.074; 0.213	0; 500; 750; 1000
16	27	Aureli et al. (2011)	--	0; 500; 1000
17	28	Timmons et al. (2008)	--	0; 250; 275; 500; 550; 1000; 1100
18	29	Fukayama et al. (2008)	--	0; 500; 750; 1000
19	30	Cowieson et al. (2006)	--	0; 150; 300; 600; 1200
20	31	Dilger et al. (2004)	--	0; 500; 750; 1000
20	32	Dilger et al. (2004)	--	0; 500; 750; 1000
21	33	Santos (2013a)	--	0; 500; 1500
22	34	Santos (2013b)	--	0; 500; 1000; 1500
23	35	Guo et al. (2009)	--	0; 500; 1000
23	36	Guo et al. (2009)	--	0; 500; 1000
24	37	Silversides et al. (2004)	--	0; 150; 450; 1250
25	38	Naves et al. (2014)	--	0; 750; 1500
25	39	Naves et al. (2014)	--	0; 750; 1500
26	40	Naves et al. (2014)	--	0; 750; 1500
27	41	Woyengo et al. (2010)	--	0; 500; 600; 700
27	42	Woyengo et al. (2010)	--	0; 500; 600; 700
28	43	Woyengo et al. (2008)	--	0; 250; 500
Total Observations (n)			**100**	**143**

*The observations could be repeated.

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