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UTILIZAÇÃO DE MIX DE ENZIMAS EXÓGENAS NA ALIMENTAÇÃO DE FRANGOS DE CORTE

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Zootecnista e Mestre em Zootecnia

TESE

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UTILIZAÇÃO DE MIX DE ENZIMAS EXÓGENAS NA ALIMENTAÇÃO DE FRANGOS DE CORTE[1]

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RESUMO – Esta tese foi conduzida para avaliar os efeitos da suplementação de diferentes enzimas exógenas e suas combinações em dietas milho e farelo de soja para frangos de corte. Dois experimentos (Exp.) de digestibilidade e um experimento de desempenho foram conduzidos utilizando um total de 2616 frangos de corte, machos Cobb 500. No Exp. 1, as aves foram alimentadas com uma ração basal milho-farelo de soja ou uma dieta com 60% da ração basal milho-soja + 40% de milho. Ambas dietas foram suplementadas com níveis crescentes de beta-xilanase [0, 50, 100, 150 e 200 unidades de β-xilanase fúngica (FXU)/kg de ração], distribuídas em 10 tratamentos com 8 repetições e 6 aves cada. O Exp. 2 consistiu do fornecimento de dietas milho-soja, formuladas com ou sem fitase [1000 unidades de fitase fúngica (FYT)/kg] e suplementadas com amilase [80 kilo-Novo unidades (KNU) de alfa-amilase/kg], xilanase (100 FXU/kg) ou a combinação de amilase + xilanase, em 6 tratamentos com 8 repetições e 7 aves cada. Nestes dois estudos foi utilizada a mesma formulação da ração basal milho-soja, mesmo milho e metodologia de análises. As aves foram avaliadas dos 14 aos 25 d, com coleta de excretas de 21 a 24 d e coleta de conteúdo ileal aos 25 d. Por fim, o Exp. 3 foi realizado para avaliar o desempenho de frangos de corte e a biodisponibilidade da energia até 40 d. Foram utilizados os mesmos produtos enzimáticos, e estes foram suplementados na mesma quantidade dos estudos anteriores. Não foram observadas interações entre xilanase e as dietas no Exp. 1 ou entre carboidrases e fitase no Exp. 2. A utilização da energia e a digestibilidade da proteína bruta e matéria seca aumentaram ($P < 0,05$) com a suplementação de 100 FXU/kg de xilanase em dietas milho-soja. Frangos de corte alimentados dos 14 aos 25 d com dietas formuladas com fitase ou suplementadas com amilase + xilanase tiveram maior ganho de peso ($P < 0,05$) e menor conversão alimentar ($P < 0,05$), quando comparados aos frangos que receberam dietas sem carboidrases de 14 a 25 d. A EMA_n e a digestibilidade do amido no jejuno e no ileo aumentaram ($P < 0,05$), respectivamente, em 99 kcal/kg, 3,5% e 2,4% quando os frangos receberam dietas suplementadas com amilase + xilanase. No Exp. 3, no período de 1 a 40 d, a EMA_n estimada para GP foi 99, 83 e 136 kcal/kg e para CA foi 40, 26 e 42 kcal/kg, respectivamente para amilase, xilanase e amilase + xilanase. Resultados destes experimentos mostraram que dietas milho-soja formuladas com fitase e suplementadas com xilanase, amilase e amilase + xilanase resultaram em melhor desempenho produtivo e maior EMA_n e digestibilidade do amido para frangos de corte.

Palavras-chave: amilase, digestibilidade, energia metabolizável, frango de corte, xilanase

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UTILIZATION OF EXOGENOUS ENZYMES MIX TO FEED BROILER CHICKENS[2]

Author: Catarina Stefanello
Advisor: Sergio Luiz Vieira

ABSTRACT - This thesis was conducted to evaluate effects of different exogenous enzyme supplementation and its combinations in corn-soybean meal diets for broilers. Two experiments (Exp.) of digestibility and one experiment of performance were done using a total of 2,616 Cobb 500 male broiler chickens. In Exp. 1, birds were fed a corn-soy basal diet or a diet with 60% corn-soy basal + 40% corn. Both diets were supplemented with increasing levels of beta-xylanase [0, 50, 100, 150, and 200 fungal β-xylanase units (FXU)/kg of feed], distributed in 10 treatments with 8 replications and 6 birds each. The Exp. 2 consisted of corn-soy diets formulated without or with phytase [1,000 fungal phytase units (FYT/kg)] and supplemented with amylase [80 kilo-Novo α-amylase units (KNU)/kg] or the combination of amylase + xylanase, to 6 treatments with 8 replications and 7 birds each. In these two studies, the same corn-soy basal diet was formulated and the same corn and methodology analysis were used. Birds were evaluated from 14 to 25 d, with excreta collection from 21 to 24 d and ileal content collection at 25 d. Finally, the Exp. 3 was done to evaluate broiler growth performance and energy equivalency until 40 d. The same enzyme products supplemented at the same amount of earlier studies were used. No interactions were found between xylanase and diets in Exp. 1 or between carbohydrases and phytase in Exp. 2. Energy utilization and digestibility of crude protein and dry matter increased ($P < 0.05$) with 100 FXU/kg xylanase supplementation in corn-soy diets. Broilers fed diets from 14 to 25 d formulated whit phytase or supplemented with amylase + xylanase had higher body weight gain ($P < 0.05$) and lower feed conversion rate ($P < 0.05$) when compared to broilers fed diets without carbohydrases. The AME_n and starch digestibility in jejum and ileum increased ($P < 0.05$), respectively by 99 kcal/kg, 3.5% and 2.4% when broilers were fed diets supplemented with amylase + xylanase. In Exp. 3, from 1 to 40 d, AME_n estimated for BWG was 99, 83, and 136 kcal/kg and for FCR was 40, 26, and 42 kcal/kg, respectively for amylase, xylanase, and amylase + xylanase. Results from these experiments show that corn-soy diets having phytase and supplemented with xylanase, amylase, and amylase + xylanase led to improved growth performance, and increased AME_n and starch digestibility in broiler chickens.

Key words: amylase, digestibility, metabolizable energy, broiler, xylanase

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RELAÇÃO DE ABREVIATURAS

AA	Aminoácido
EMA	Energia metabolizável aparente
EC	Enzyme Comission
P	Fósforo
PNA	Polissacarídeos não-amídicos
TGI	Trato gastrintestinal

CAPÍTULO I

INTRODUÇÃO

Frangos de corte têm sido geneticamente selecionados para serem eficazes em converter nutrientes de origem vegetal em proteína animal (Cowieson, 2010). Ainda que a busca por alimentos alternativos seja desejável para a redução de custos na formulação, o milho e o farelo de soja são as principais fontes de energia e proteína utilizadas em rações. Amido, aminoácidos e gordura são rapidamente digeridos por frangos de corte; entretanto, devido a várias razões, parte deste conteúdo nutricional não é aproveitado, podendo apresentar limitada digestão e permanecer indigestível, o que representa perdas nutricionais para as aves (Cowieson & Adeola, 2005).

Já é bem conhecido que rações formuladas com milho e farelo de soja possuem digestibilidade alta. No entanto, estes ingredientes também possuem quantidades variáveis de ácido fítico e polissacarídeos não-amídicos (PNA), os quais estão associados à menor digestão e ao menor aproveitamento do fósforo e dos carboidratos presentes nas dietas (Bach Knudsen, 1997; Choct, 1997; Meng et al., 2005). O farelo de soja é a fonte de proteína mais utilizada na nutrição animal, apresentando baixa energia metabolizável em relação à energia bruta, principalmente, devido à presença de carboidratos não digestíveis, como a rafinose e a estaquiose. O milho possui menores quantidades de frações indigestíveis que o farelo de soja e contém o amido como principal carboidrato de reserva e também a principal fonte de energia para as rações. O amido é o principal constituinte do grão de milho, estando presente no endosperma de células vegetais na forma de grânulos insolúveis, os quais são compostos principalmente por amilose e amilopectina (Choct, 1997; Bach Knudsen, 2014).

O maior aproveitamento dos nutrientes presentes em dietas à base de milho e farelo de soja pode ser obtido através da suplementação de enzimas exógenas (Olukosi et al., 2008). A fitase tem sido amplamente utilizada na formulação de rações para frangos de corte devido à grande ênfase em pesquisas, aliada à possibilidade de melhorar a disponibilidade de nutrientes e reduzir custos de produção (Jozefiak et al., 2010; Cowieson et al., 2011). Nutricionistas e pesquisadores possuem maiores informações disponíveis sobre a atuação da fitase na degradação do fitato, bem como sua relação com o maior aproveitamento do fósforo e de outros nutrientes. Entretanto, além da fitase, outras enzimas têm sido utilizadas em rações para frangos de corte, com destaque para a suplementação de carboidrases.

Amilase, xilanase e glucanase são enzimas que podem apresentar maior demanda nos próximos anos em função das características dos ingredientes utilizados nas formulações e da eficiência que se deseja alcançar no aproveitamento dos nutrientes e energia para aves. As carboidrases exógenas são menos utilizadas em dietas milho-farelo de soja, possuindo maior demanda quando ingredientes vegetais altamente fibrosos estão disponíveis. Os produtos comerciais contendo carboidrases são suplementados em rações para aves na forma de complexos enzimáticos ou enzimas monocomponentes e atuam sobre as frações indigestíveis dos ingredientes, liberando energia e reduzindo efeitos antinutritivos. Adicionalmente, fornecem enzimas que não são secretadas pelo organismo animal e/ou potencializam a

atuação de enzimas endógenas, proporcionando um maior aproveitamento dos nutrientes (Cowieson, 2005). Uma vez que as enzimas são altamente específicas para o substrato em que atuam, existe uma ampla gama de carboidrases disponíveis comercialmente. Xilanases e glucanases possuem ênfase quando são utilizados ingredientes fibrosos e ricos em PNA como aveia, centeio e trigo (Meng et al., 2005; Francesch and Geraert, 2009). Já a alfa-amilase exógena atua sobre as ligações glicosídicas presentes nos grânulos de amido e possui maior importância quando são utilizados ingredientes ricos em amido, como o milho (Ritz et al., 1995; Gracia et al., 2003).

Muitos estudos envolvendo a utilização de enzimas exógenas têm sido conduzidos nas últimas décadas (Zanella et al., 1999; Kocher et al., 2003; Olukosi and Adeola, 2008; Olukosi et al., 2008; Williams et al., 2014; Vieira et al., 2015a,b). Alguns resultados ainda são controversos, sobretudo porque existem diferenças quanto à presença de substratos nas dietas, composição dos ingredientes, metodologias utilizadas na avaliação dos resultados e coleta de dados e também às diferenças na atividade enzimática de cada produto. Assim, todos os aspectos devem ser considerados para uma correta utilização das ferramentas disponíveis para que resultados expressivos possam ser obtidos a partir da utilização de enzimas apropriadas e que possuam estabilidade e eficácia comprovadas.

A presente tese foi conduzida para avaliar o efeito da suplementação de amilase, xilanase, ou amilase e xilanase em dietas milho-farelo de soja formuladas com ou sem fitase sobre o desempenho, aproveitamento da energia e digestibilidade de nutrientes em frangos de corte.

REVISÃO BIBLIOGRÁFICA

Milho e farelo de soja como ingredientes em rações para frangos de corte

O milho (*Zea mays*) é o ingrediente predominante em formulações de ração para aves. O valor nutricional deste ingrediente pode variar com o conteúdo de amido, óleos, proteína e fatores antinutricionais como fitato, amido resistente, PNA e inibidores de enzimas (Cowieson, 2005). O milho contribui com, aproximadamente, 65% da energia e 20% da proteína das dietas para frangos de corte (Cowieson & Adeola, 2005), sendo o amido o seu principal carboidrato de reserva e também a principal fonte de energia.

Os grãos de milho de maneira geral possuem em sua estrutura mais de 80% de carboidratos e deste total, 70 a 80% é amido, 10% a 30% são PNA e 1% a 3% são mono e oligossacarídeos (Bach Knudsen, 1997). Neste contexto, é importante descrever que o amido é um polímero semicristalino de D-glicose e está presente no endosperma de células vegetais na forma de grânulos insolúveis, os quais são compostos principalmente por amilose e amilopectina. Dentro de cada grânulo, a amilopectina forma um sistema helicoidal ramificado, onde a amilose encontra-se dispersa. A amilose é uma molécula linear de resíduos de glicose contendo cerca de 99% de suas ligações do tipo α-1,4 e 1% do tipo α-1,6. Já a amilopectina é uma molécula muito maior que a amilose, ramificada e com 95% das suas ligações do tipo α-1,4 e 5% do tipo α-1,6 (Tester et al., 2004).

A relação amilose:amilopectina da maioria dos grãos e cereais varia de 20 a 28 : 72 a 80%. Esta proporção pode variar de acordo com a espécie vegetal, variedade, condições climáticas de cultivo e grau de maturação dos grãos (Tester et al., 2004). Isto também pode exercer influência sobre a digestibilidade dos carboidratos, visto que a relação de amilose/amilopectina presente nos ingredientes possui uma correlação negativa com a digestibilidade dos mesmos, já que a amilopectina é mais facilmente digerida que a amilose (Rooney & Pflugfelder, 1986).

Também é importante destacar que o milho contém cerca de 0,9% de PNA solúveis e 6% de PNA insolúveis (Smits & Annison, 1996; Choct, 1997; Kocher et al., 2003). Os PNA predominantes no milho são arabinoxilanos, os quais são compostos basicamente por arabinoses e xiloses. Choct (2010) demonstrou que os coeficientes de digestibilidades da arabinose e da xilose para aves são de apenas 13 e 14%, respectivamente. O total de arabinoxilanos no milho foi reportado em 5,2% por Choct (1997) e os estudos de Malathi & Devegowda (2001) indicaram que a quantidade de arabinoxilanos presentes no milho foi de 5,4%.

A solubilidade dos PNA é determinada pela sua estrutura molecular primária mas também pela forma com que estes compostos estão ligados a outros componentes da parede celular (Smits & Annison, 1996). A quantidade e a proporção de frações solúveis e insolúveis de PNA também variam entre ingredientes, em que é possível observar variações de composição entre as principais publicações científicas que reportam as concentrações de cada fração (Smits & Annison, 1996; Choct, 1997; Back Knudsen, 2014). Os PNA

são macromoléculas de polímeros de açúcares simples, representados basicamente pela celulose, lignina e hemicelulose (arabinoxilanos, β-glucanos e pentosanas), são resistentes à hidrólise no trato gastrintestinal (TGI) de não-ruminantes e sua presença nos grãos e cereais é variável, pois dependem das características do vegetal e das condições de cultivo (Smits & Annison, 1996). A maioria dos PNA presentes no milho e no farelo de soja são encontrados na forma insolúvel o que, consequentemente, não aumenta a viscosidade intestinal, interferindo pouco na digestão quando comparados a outros alimentos vegetais mais fibrosos como arroz, trigo ou cevada (Bedford et al., 1991; Choct, 1997; Gracia et al., 2003).

O farelo de soja é a principal fonte proteica utilizada na formulação de rações para frangos de corte; entretanto, também contém quantidades consideráveis de carboidratos em sua composição, apresentando maiores concentrações de PNA quando comparado ao milho. Este ingrediente possui, aproximadamente, 24% de PNA totais, sendo 6% encontrados na forma solúvel e 16 a 18% na forma insolúvel, com 3,3% de arabinoxilanos (Back Knudsen, 1997). Malathi & Devegowda (2001), observaram a quantidade de PNA totais de 29%, sendo o conteúdo de celulose, pectina e arabinoxilanos de 5,2%, 6,2% e 4,2%, respectivamente. Os principais oligossacarídeos presentes no farelo de soja são a rafinose e a estaquiose, os quais variam com a quantidade de casca presente no farelo e a concentração de amido é menor do que 1% (Choct, 1997). A presença desses oligossacarídeos recebe importância porque os mesmos são considerados fatores antinutricionais, visto que estes compostos não podem ser degradados por animais não-ruminantes devido à falta de secreção da enzima α-1,6-galactosidase, o pode resultar em alteração na absorção dos nutrientes e reduzir o valor da energia metabolizável das rações (Vinjamoori et al., 2004; Vahjen et al., 2005).

A maioria das rações comerciais formuladas para frangos de corte é à base de milho e farelo de soja. Além da necessidade de conhecimento sobre os efeitos gerados pela presença de PNA nestes ingredientes, uma maior importância tem sido data à presença do fitato. Neste contexto, o fitato (mio-inositol hexafosfato) é a principal forma de armazenamento de fósforo (P) nas sementes de plantas (Prattley & Stanley, 1982). Cerca de 50 a 85% do P armazenado nos grãos de cereais está ligado ao ácido fítico e seus sais (Ravindran et al., 1995). Resumidamente, o ácido fítico interage com proteínas solúveis em pH baixo, formando agregados de inositol-6-fosfato com proteína que diminuem a digestibilidade da proteína ligada ao ácido fítico (Yu et al., 2012). A ligação de inositol-6-fosfato com a proteína também pode alterar a ionização do complexo, potencializando, assim, a capacidade para complexar com minerais, como por exemplo, cálcio, manganês, ferro e zinco (Angel et al., 2002). Também pode complexar o grupo amina de alguns aminoácidos (lisina, arginina, histidina), além de moléculas conjugadas de glicose, especialmente aquelas que constituem o amido. A formação do complexo reduz o aproveitamento dos nutrientes que estão presentes na ligação, reduzindo a disponibilidade de minerais, proteína e energia, bem como diminui a capacidade da fitase exógena em remover os grupos fosfato e disponibilizá-los para as aves.

Enzimas exógenas tem sido utilizadas comercialmente nas rações para aves com a finalidade de melhorar o aproveitamento de nutrientes e reduzir o efeito de anti-nutrientes. De acordo com os substratos presentes nos ingredientes utilizados nas formulações de ração, como é o caso dos PNA e fitato, diferentes enzimas exógenas poderão ser incluídas ou suplementadas. Dessa forma, as principais enzimas utilizadas em rações para aves são a fitase, que atua em um substrato específico, o ácido fítico e a sua forma quelatada fitato e, também as carboidrases, as quais atuam em polissacarídeos não-amídicos e no amido.

Enzimas exógenas

Enzimas são proteínas que possuem estrutura tridimensional e atuam acelerando processos químicos. Elas exercem seu efeito catalítico em condições ambientais específicas de pH, temperatura, umidade e presença de substratos. As enzimas também tornam possíveis sequências controladas de reações químicas em sistemas biológicos e voltam ao seu estado original quando a reação se completa fazendo com que pequenas quantidades de enzimas sejam necessárias, se comparadas com a concentração de substrato existente (Angel & Sorbara, 2012). Para que uma reação enzimática aconteça no TGI, condições ambientais adequadas devem existir, e estas condições são diferentes e pouco previsíveis daquelas existentes em ambientes *in vitro*, onde geralmente as enzimas são avaliadas. Como resultado, pode haver uma maior dificuldade no entendimento da atuação das enzimas em condições ambientais diferenciadas, como é o caso do processamento das dietas e do processo de digestão no TGI.

A maioria das enzimas exógenas comercialmente disponíveis são obtidas a partir de sistemas de fermentação otimizados que dependem da utilização de bactérias ou fungos geneticamente modificados. A fitase é o aditivo enzimático padrão utilizado no sistema de produção comercial de frangos de corte. Numerosos estudos têm sido conduzidos nas últimas décadas e permitiram observar que as fitases melhoraram o aproveitamento do P da dieta, reduziram as perdas endógenas de proteína e também reduziram a excreção de P (Broz et al., 1994; Cowieson & Ravindran, 2007; Liu et al., 2008; Vieira et al., 2015a). Adicionalmente, enzimas que degradam substratos que liberam energia têm sido cada vez mais estudadas e possuem potencial emergente de uso. Estas enzimas são carboidrases exógenas, como alfa-amilase, beta-xilanase e beta-glucanase. Pesquisas têm demonstrado que carboidrases exógenas também foram eficazes para melhorar a utilização da energia e o desempenho produtivo de frangos de corte (Olukosi & Adeola, 2008; Olukosi et al., 2008; Williams et al., 2014).

Fitase

As fitases (mio-inositol hexafosfato fosfohidrolase) são hidrolases capazes de catalisar a hidrólise gradual de mio-inositol hexafosfato (ácido fítico; IP6). São classificadas no Enzyme Comission (EC) com o identificador 3.1.3 (esterase / fosfatase). As fitases relevantes para a alimentação animal são divididas em 2 subclasses (3- ou 6-fitases), dependentes de qual fosfato inicia a catálise no núcleo mio-inositol (Adeola & Cowieson, 2011). Não há

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

Os resultados positivos obtidos com a inclusão de fitase em rações para frangos provavelmente estão relacionados à presença do fósforo fítico e demais nutrientes na molécula de fitato. A fitase atua quebrando a ligação entre o fitato e minerais, liberando-os para a absorção, o que contribui para melhorar o seu aproveitamento e reduzir a excreção de P e minerais no ambiente (Sebastian et al., 1996). O aumento da digestibilidade de aminoácidos e da EMA pode ser o resultado da liberação das moléculas ligadas ao inositol hexafosfato decorrente da hidrólise realizada pela fitase e também pela redução das perdas endógenas (Cowieson et al., 2006; Selle & Ravindran, 2007). Para Cowieson et al. (2009) o fitato altera o *turnover* das células intestinais e pode causar irritação da mucosa, aumentando a produção de mucinas e, consequentemente, a perda de nitrogênio endógeno.

Frangos de corte alimentados com dietas formuladas com fitase tiveram maior ganho de peso e maior deposição de cálcio e P na tíbia quando comparados ao que receberam dietas sem fitase ou com redução de P disponível (Vieira et al., 2015a). Uma variação considerável tem sido observada nas estimativas de liberação de P reportadas quando fitases são utilizadas em dietas para aves, o que pode estar relacionado com o tipo, dose e concentração de fitase e cálcio. Nelson et al. (1968) indicaram que 50 a 100% do P ligado ao fitato em dietas milho-farelo de soja pode ser liberado com a inclusão de fitase. Simons et al. (1990) indicaram que 65% do P da dieta foi liberado pela fitase e Waldroup et al. (2000) também reportaram que 50% do P de uma dieta milho-soja foi liberado pela inclusão de fitase.

Carboidrases

As enzimas degradadoras de PNA dos alimentos também são classificadas pela União Internacional de Bioquímica e pertencem às glicosil hidrolases (EC 3.2.1.x). Esta classificação baseia-se no tipo de reação e especificidade de substrato das enzimas, por exemplo, β-glucanases hidrolisam β-glucanos, xilanases atuam sobre xilanos e amilases sobre as cadeias de glicose que compõem os grânulos de amido.

Carboidrases como xilanases, amilases e glucanases têm sido adotadas mundialmente na produção avícola. Carboidrases incluem todas as enzimas que catalisam uma redução no peso molecular de hidratos de carbono poliméricos, em que mais de 80% do mercado mundial de carboidrases é representado pela xilanase e glucanase (Adeola & Cowieson, 2011). Além destas duas enzimas, a amilase tem sido mais estudada recentemente e sobretudo quando dietas ricas em amido são fornecidas para aves. A xilanase (endo-1,4-β-xilanase), EC. 3.2.1.8, pertence à família das hidrolases e sub-familia glicosidase. Já a amilase (alfa-amilase) é classificada no EC com o identificador 3.2.1.1. Detalhes sobre a estrutura tridimensional e métodos de atuação das enzimas podem ser obtidos por intermédio da União Internacional

de Bioquímica. Informações estas que podem auxiliar a unir conhecimentos de tecnologia enzimática à nutrição animal (Adeola & Cowieson, 2011).

A principal classe de amilases suplementada nas rações atua sobre o amido e separa as cadeias α-1,4-glicosídicas entre unidades adjacentes de glicose nas cadeias lineares de amilose. O pH ótimo varia conforme os microrganismos produtores das enzimas como, por exemplo, 4,8 a 5,8 para *Aspergillus oryzae*; 5,85 a 6,0 para *Bacillus subtilis* e 5,5 a 7,0 para *B. licheniformes*. Os resultados da utilização de amilases em rações para frangos de corte são bastante variáveis e dependentes dos produtos enzimáticos, metodologias de avaliação, ingredientes presentes nas formulações, concentração dos produtos enzimáticos e idade das aves (Cowieson, 2005; Bedford & Cowieson, 2012). Alguns estudos indicaram que amilases monocomponentes ou *mix* de enzimas foram eficazes para melhorar a digestibilidade do amido, a utilização da energia, o desempenho das aves e alteraram a secreção endógena de alfa-amilase (Ritz et al., 1995; Gracia et al., 2003; Cowieson et al., 2006; Vieira et al., 2015b).

As xilanases (endoxilanases) hidrolisam as ligações β-1,4 de xilanos e uma unidade de xilanase é definida como a quantidade de enzima que libera 1 µmol de xilose por minuto em pH 5,3 a 50ºC. O mecanismo de ação da xilanase tem sido descrito como sendo associado à hidrólise de PNA de alto peso molecular em cereais, com redução da viscosidade no lumen intestinal e aumento do acesso de enzimas endógenas ao conteúdo celular (Bedford & Cowieson, 2012). Estes métodos de atuação das xilanases têm propiciado que produtos enzimáticos contendo xilanase incluam também amilase, protease e fitase, objetivando ter um maior aproveitamento da energia e nutrientes. Dessa forma, além de enzimas monocomponentes, *blends* enzimáticos também têm sido utilizados na nutrição de aves. Neste contexto, é importante ressaltar que a utilização de enzimas monocomponentes favorece o entendimento da atuação que a enzima exerce, especialmente quando se objetiva avaliar a digestibilidade de nutrientes específicos e quando se conhece os principais substratos presentes nas dietas comerciais e o resultado que se deseja obter. Entretanto, produtos enzimáticos utilizados pela indústria avícola, muitas vezes, são elaborados com diferentes enzimas, buscando otimizar a formulação a um menor custo, que pode atribuído à suplementação deste aditivo.

Pesquisas indicaram que a utilização de uma ou mais enzimas em rações à base de milho e farelo de soja para frangos de corte resultaram em melhora no desempenho, no entanto, as respostas são variáveis e dependentes da idade das aves, condições ambientais e estresse a que o animal está submetido, além de questões como a qualidade bromatológica do milho e do farelo de soja utilizados nos experimentos. Misturas de enzimas exógenas que continham várias combinações de amilases, proteases, xilanases, glucanase, celulase, mananase e pectinase adicionados à dieta milho-soja para aves foram eficazes em melhorar o desempenho produtivo (Zanella et al., 1999; Yu & Chung, 2004; Cowieson & Adeola, 2005), a energia metabolizável aparente (EMA) (Meng & Slominski, 2005), e a digestibilidade ileal da proteína e aminoácidos (AA) (Zanella et al., 1999; Meng & Slominski, 2005). Em contraste, também não foram observados efeitos significativos da suplementação de preparações enzimáticas sobre a EMA, energia digestível

ileal (Cowieson & Adeola, 2005), digestibilidade da proteína e amido (Meng & Slominski, 2005).

As carboidrases têm sido tradicionalmente utilizadas em dietas à base de trigo e estas enzimas são eficazes para reduzir os efeitos adversos de PNA sobre a viscosidade da digesta, melhorando a digestibilidade de nutrientes e o desempenho das aves (Choct, 2006). Recentemente carboidrases exógenas também estão sendo utilizadas de maneira crescente em dietas milho-soja para frangos de corte com o objetivo de reduzir custos de produção, através de melhorias no aproveitamento da EMA e da digestibilidade de AA. Os possíveis mecanismos de atuação das carboidrases em dietas para aves incluem: melhorias no acesso de enzimas endógenas aos conteúdos celulares mediante à hidrólise dos arabinoxilanos da parece celular (Kocher et al., 2003; Cowieson, 2005; Francesch & Geraert, 2009); potencializar a ação de enzimas endógenas, particularmente amilases (Ritz et al., 1995; Gracia et al., 2003); reduzir as perdas endógenas de AA e secreção de mucina (Cowieson & Bedford, 2009); reduzir fatores antinutricionais e também gerar xilo-oligômeros prebióticos que beneficiam indiretamente a digestão, aumentando a fermentação intestino e estimulando a quebra no conteúdo ileal (Cowieson, 2005). Em geral, as melhorias na EMA através da suplementação de amilase e xilanase em dietas milho-soja podem ser resultantes da combinação entre aumento da digestão e absorção das porções indigestíveis de amido e gordura da dieta e a regulação das secreções digestivas (Romero et al., 2013).

Diferentes classes de enzimas hidrolisam substratos diferentes e geram produtos diferentes, por isso um efeito maior no aumento na digestibilidade do amido, proteína / AA e gordura, com uma consequência do acúmulo energético, parece ser um resultado esperado quando diferentes enzimas são utilizadas nas rações. Embora as enzimas que se destinam a diferentes substratos não competirem entre si em termos de degradação de substrato, elas tendem a se sobrepor na digestão de nutrientes e desempenho, proporcionando resultados sub-aditivos (Cowieson & Adeola, 2005; Cowieson et al., 2006; Romero et al., 2013). Por isso, é importante salientar que os aumentos na utilização de energia a partir de alimentos vegetais por frangos de corte podem derivar de uma grande variedade de componentes com características nutricionais e a busca por novas informações sobre a atuação de enzimas exógenas em dietas para aves têm sido o objetivo de muitos estudos conduzidos nas últimas décadas.

HIPÓTESES E OBJETIVOS

Hipóteses

A suplementação de xilanase em dietas fareladas milho-farelo de soja melhora o aproveitamento da energia e de nutrientes para frangos de corte quando comparada a dietas sem a suplementação desta enzima.

O desempenho produtivo, a utilização da energia e a digestibilidade de nutrientes são melhorados quando frangos de corte são alimentados com dietas milho-farelo de soja formuladas com fitase.

A suplementação de amilase, xilanase, ou amilase em combinação com xilanase em dietas fareladas milho-farelo de soja melhora a utilização da energia, a digestibilidade do amido e de nutrientes e também o desempenho produtivo de frangos de corte quando comparada a dietas sem a suplementação destas carboidrases.

A inclusão de fitase em dietas milho-soja suplementadas com amilase e xilanase pode interferir no aproveitamento da energia e no desempenho de frangos de corte em diferentes fases de crescimento.

Objetivos

Avaliar os efeitos de níveis crescentes de β-xilanase sobre a utilização de energia e digestibilidade de nutrientes em dietas à base de milho e farelo de soja para frangos de corte. Os efeitos foram avaliados em uma dieta convencional milho-farelo de soja, em uma dieta teste que foi substituída por 40% de milho para permitir a estimativa dos efeitos interativos da xilanase e dieta, proporcionalmente.

Avaliar os efeitos de uma α-amilase monocomponente ou em combinação com uma β-xilanase sobre o desempenho produtivo, utilização da energia e digestibilidade do amido em frangos de corte alimentados com dietas milho-farelo de soja. Estes efeitos foram avaliados ambos em uma dieta livre de fitase e em uma dieta formulada com.

Avaliar os efeitos de uma α-amilase monocomponente ou em combinação com uma β-xilanase sobre o desempenho produtivo de frangos de corte alimentados com dietas milho-farelo de soja de 1 a 40 dias de idade. Os efeitos foram avaliados utilizando dietas com níveis decrescentes de energia metabolizável aparente e também foi proposta uma estimação da equivalência em energia liberada por estas enzimas.

CAPÍTULO II[1]

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METABOLISM AND NUTRITION

CORN AND XYLANASE FOR BROILERS

Energy and nutrient utilization of broiler chickens fed corn-soybean meal and corn-based diets supplemented with xylanase

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ABSTRACT A study was conducted to evaluate the effects of increased levels of a β-xylanase on energy and nutrient utilization of broiler chickens fed corn-soy diets. A total of 480 slow feathering Cobb × Cobb 500 male broilers were randomly distributed to 10 treatments having 8 replicates of 6 birds each. Birds were fed a common starter diet to d 14 post-hatch (3,050 kcal/kg AME_n, 21.7% CP, 1.05% Ca, and 0.53% nPP). The experimental diets were provided afterwards until 25 d. Two experimental diets, a conventional corn/soy-based basal diet (CS) and the basal diet where 40% of the diet was displaced by corn (CN) were fed as-is or supplemented with 50, 100, 150, or 200 fungal β-xylanase units (FXU)/kg. Dietary treatments were distributed factorially as a 2 × 5 arrangement. Samples of feed, excreta, and ileal digesta were analyzed for determination of ileal digestible energy (IDE), metabolizable energy, and total tract retention of protein and lipid. No interactions between diet and xylanase were observed. The CS diets had higher ($P < 0.05$) energy utilization and nutrient digestibility when compared to the CN diets. AME_n and IDE were improved ($P < 0.05$) by 192 and 145 kcal/kg, respectively when diets were supplemented with 100 FXU/kg xylanase. The xylanase added to the CN diet led to quadratic increases ($P < 0.05$) in IDE ($Y = -0.014x^2 + 2.570x + 3,155$; $r^2 = 0.60$) and in AME_n ($Y = -0.016x^2 + 3.982x + 3,155$; $r^2 = 0.68$). Crude protein digestibility and AME_n were linearly increased ($P < 0.05$) when xylanase was added to the CN diet. In conclusion, energy utilization, and digestibility of crude protein and dry matter increased with xylanase supplementation in corn/soy-based diets. When xylanase was tested in the CS diet, 92 and 124 FXU/kg maximized the energy release effect; however, the maximum energy response in the CN diet or corn was not achieved until 200 FXU/kg.

Key words: broiler, corn, digestibility, metabolizable energy, xylanase

INTRODUCTION

Corn and soybean meal (**SBM**) are incumbent ingredients used in the majority of commercial poultry diets worldwide and contain varying levels of non-starch polysaccharides (**NSP**). Non-starch polysaccharides are carbohydrates that can interfere with nutrient utilization by poultry (Bach Knudsen, 1997; Caffall and Mohnen, 2009). This is in part because nutrients such as starch, fat and protein are trapped within the insoluble cell wall matrix, which acts as a physical barrier that limits access for the endogenous enzyme array (Theander et al., 1989; Slominski et al., 1993). Soluble fiber can also form viscous gels within the gut and slow digestion and feed passage rate (Bedford et al., 1991).

The concentration of NSP in corn and SBM ranges from 6.8% to 9.4% and 17% to 30%, respectively (Smits and Annison, 1996; Choct, 1997; Kocher et al., 2003). The total amount of arabinoxylan is variable among ingredients but has been reported to be around 5.2% in corn (Choct, 1997) and 3.3% in SBM (Back Knudsen, 1997). Carbohydrate composition is also important to determine energy and nutrient utilization of ingredients for broilers. Thus, different substitution methods have been described to determine energy and nutrient digestibility of cereals and by-products. These methods use either one substitution level of a tested ingredient or multiple substitution inclusions by the regression method (Matterson et al., 1965; Villamide, 1996; Adeola, 2001; Adeola et al., 2010).

Adding exogenous enzymes targeting insoluble and soluble fibers may facilitate the release of nutrients encapsulated in cell walls or incorporated into the cell wall itself, resulting in improved access for digestive enzymes (Cowieson, 2005). Exogenous xylanases may hydrolyze cell wall arabinoxylans, improving the access of endogenous

enzymes to cell contents (Meng et al., 2005; Francesch and Geraert, 2009) and decrease endogenous amino acids (**AA**) losses, particularly through changes on pancreatic amylase and mucin secretion (Jiang et al., 2008; Cowieson and Bedford, 2009).

Improvements in broiler performance are often associated with increased nutrient digestibility and energy utilization (Olukosi et al., 2008). Accurate estimations of improvements in energy digestibility due to exogenous xylanase is relevant to account for the effects of these enzymes in diet formulation according to the inclusion of different ingredients. This is important also because the efficiency of energy utilization from NSP is lower than the efficiency of use of energy from fat, starch, or protein for growth, though NSP digestibility does contribute to the measured metabolizable energy in response to xylanase supplementation in some diets or ages in broiler chickens (Savory, 1992; Chwalibog, 2002).

Xylo-oligosaccharides released during the degradation of NSP by exogenous xylanase in the small intestine are fermented by the intestinal microbiota and the end products (various volatile fatty acids) are subsequently used as energy yielding substrates for broilers (Choct et al., 1996). As suggested by Cowieson and O'Neill (2013), the fatty acids have some energetic value for the host animal but perhaps more importantly, the lower pH may restrain the proliferation of putrefactive organisms, encourage the proliferation of enterocytes and may directly mediate gastric emptying, perhaps via the same infrastructure involved in the ileal brake mechanism. Additionally, if the micro biome has a central role in the effect of exogenous xylanase, it is possible that these mechanisms will be cumulative as the microflora adapt to substrate provision.

Though there are several reports in the literature on the efficacy of xylanase (or xylanase-based enzyme admixtures) on the nutritional value of corn-based diets there

are rather few that explore the relative effect on corn and SBM independently. More studies are therefore needed to evaluate if xylanase acts specifically on corn fiber or on arabinoxylan-containing carbohydrates in SBM. Furthermore, while SBM contains very low concentrations of arabinoxylan it is possible that hydrolysis of arabinoxylan in corn would indirectly influence the digestibility of SBM via gross changes to intestinal pH, passage rate and so on.

The objective of the present study was to evaluate the effects of various concentrations of an exogenous β-xylanase on energy utilization and nutrient digestibility of corn-SBM-based diets for broiler chickens. These effects were assessed both in a conventional corn-soybean meal diet and in a diet where 40% of this diet was displaced with corn to allow estimation of the interactive effects of xylanase and diet proportionality. The displacement of the corn/soy diet with pure corn facilitated extrapolation to evaluate corn independently from the rest of the diet in order to explore the possibility that xylanase efficacy may be influenced by diet composition.

MATERIALS AND METHODS

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

A total of 480 one-day-old, slow-feathering Cobb × Cobb 500 male broiler chicks, vaccinated for Marek's disease at the hatchery, with an average BW of 48 g were randomly placed into 80 wire battery cages (0.9 × 0.4 m^2). Each cage was equipped with one feeder and one drinker. Birds had ad libitum access to water and mash feeds. Average temperature was 32ºC at placement and was reduced by 1ºC every 2 d until

23ºC to provide comfort throughout the study. Lighting was continuous until d 25 post-hatch.

Birds were allocated to 10 experimental diets with 8 replications of 6 birds each in a completely randomized design. A standard corn-SBM-based broiler starter diet was fed from 1 to 14 d (3,050 kcal/kg AME_n, 21.7% CP, 1.05% Ca, and 0.53% non-phytate P). From 14 to 25 d, broilers were fed two basal diets, an industry-standard corn-soybean meal basal diet (**CS**) or the same basal diet where 40% of the diet was displaced with corn (**CN**) as presented in Table 1. Both basal diets were supplemented with 0, 50, 100, 150, and 200 fungal β-xylanase units (**FXU**)/kg [Ronozyme WX (CT); Novozymes A/S, Bagsvaerd, Denmark]. A 2 × 5 factorial arrangement of 2 control diets and 5 xylanase supplements was used. The xylanase was a granulated heat-stable endo-xylanase from *Thermomyces lanuginosus* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism containing 1,000 FXU/g. One FXU is the amount of endo-1,4-β-xylanase which liberates 7.8 micromoles of reducing sugars (xylose equivalents) per minute from azo-wheat arabinoxylans at pH 6.0 and 50ºC. The CS diet had 1% Celite as indigestible marker (Celite, Celite Corp., Lompoc, CA).

Experimental Procedures

Excreta were collected twice daily on wax paper from 21 to 24 d being immediately mixed and pooled by cage and stored at −20ºC until analysis. Previous to calorimetry, excreta were dried in a forced air oven at 55ºC (DeLeo, Porto Alegre, Brazil) and ground to pass a 0.5-mm screen. Ileal digesta were collected from all birds at 25 d after euthanasia by electrical stunning using 45 V for 3 s. Ileal digesta were

collected from a section of intestine between Meckel's diverticulum to approximately 2 cm cranial to the ileo-cecal junction. Digesta were flushed with distilled water into plastic containers, pooled by cage, immediately frozen in liquid nitrogen, and stored in a freezer at −20ºC until lyophilized (Christ Alpha 2-4 LD Freeze Dryer, Newtown, UK).

Chemical Analysis and Calculations

Diet and freeze-dried samples of ileal digesta were ground to pass a 0.5-mm screen in a grinder (Tecnal, TE-631/2, São Paulo, Brazil). Dry matter (**DM**) analysis of samples was performed after oven drying the samples at 105ºC for 16 h (method 934.01; AOAC International, 2006). Ileal digesta, excreta, and diet samples were analyzed for gross energy using a calorimeter calibrated with benzoic acid as a standard (IKA Werke, Parr Instruments, Staufen, Germany). Calculations of ileal digestible energy (**IDE**) and AME_n were done afterwards. Crude protein (N × 6.25) was determined by the combustion method (method 968.06; AOAC International, 2006). The calculated AME was corrected to zero N retention (AME_n) using a factor of 8.22 kcal/g (Hill and Anderson, 1958). Acid insoluble ash concentration in the diets, excreta, and ileum samples was determined using the method described by Vogtmann et al. (1975), and Choct and Annison (1992). Ether extract (**EE**) in the diets and excreta samples was determined by extracting in petroleum ether using a Soxhlet apparatus for approximately 8 h (method 934.01; AOAC International, 2000).

Energy utilization and nutrient digestibility of corn were calculated by the substitution method described by Matterson et al. (1965) and Sakomura and Rostagno (2007). In this method, a corn-soybean meal-based diet was used as the reference diet, and the test diet was the diet where corn (40%) was used to dilute this reference diet.

This substitution method allowed the determination of nutrient digestibility of a tested ingredient that was used to displace the reference diet. Calculations were used to determine the digestibility of corn-soy-based diet, corn-based diet, and then extrapolated to 100% of corn.

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

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

$$AME_n \text{ (kcal/kg)} = GE_i - [GE_o \times (M_i/M_o)] - 8.22 \times \{N_i - [N_o \times M_i/M_o)]\},$$

where M_i represents the concentration of acid insoluble ash in the diet in grams per kilogram of DM; M_o represents the concentration of acid insoluble ash in the excreta and ileal digesta in grams per kilogram of DM output; E_i represents the concentration of DM, CP, energy, or EE in the diet in milligrams per kilogram of DM; and E_o represents the concentration of DM, CP, energy, or EE in the excreta and ileal digesta in milligrams per kilogram of DM. GE_i is gross energy (kcal/kg) in the diet; GE_o is the gross energy (kcal/kg) in the excreta; N_i represents nitrogen concentration in the diet, and N_o represents nitrogen concentration in the excreta in g/kg DM.

Statistical Analysis

The experimental design was a completely randomized factorial arrangement of 2 control diets (CS or CN) and 5 xylanase supplementations. Data were submitted to a 2-way ANOVA using the GLM procedure of SAS Institute (SAS, 2009). Significance was accepted at $P < 0.05$. Linear and quadratic regression equations were estimated with the

increased levels of β-xylanase supplementation on corn-soy-based diet, corn-based diet, and corn. A regression analysis was also conducted with data where corn values were extrapolated and according to increasing levels of xylanase.

RESULTS AND DISCUSSION

The corn used in this study was analyzed to contain 88.6% of DM, 3,776 kcal of GE/kg, 8,1% of CP/kg, 0.94% of crude fiber, 3,55 % of EE, 0.04% of Ca, and 0.25% of total P. Analysis of β-xylanase in the experimental diets showed that the supplemental xylanase had in-feed activity in agreement with the expected values (Table 2). Nutrient digestibility and energy values of the CS diet, CN diet, and extrapolated values for pure corn are shown in Tables 3 and 4. Ileal digestible energy was 3,278, 3,201, and 3,132 kcal/kg, for CS, CN, and corn, respectively. When diet effect was evaluated, CS diets had higher ($P < 0.01$) AME, IDE, and EE and CP digestibilities compared to the CN diet. Differences in AME and ileal digestibility of CP were found between CS and CN diets ($P < 0.01$) with values, respectively of 3,544 kcal/kg and 69.3% for CS and 3,432 kcal/kg and 62.2% for CN.

An explanation regarding the highest energy and nutrient utilization observed when broilers were fed a complete diet compared to the corn-based diet may be related to supplying all required nutrients in the CS diet in contrast with an imbalance in the CN diets. Based on calculated composition of the corn-based diet, most nutrients including calcium, phosphorus, amino acids and electrolyte balance were poorly balanced and this may have affected the digestion process, absorption of nutrients, and gastrointestinal (**GI**) flow (Cowieson and Bedford, 2009). The extrapolated calculation for corn allowed estimation of energy values, DM, CP and EE digestibilities of corn for

broilers. Digestibility of CP and EE of corn without xylanase were 63.5 and 83.4%, respectively. The IDE, AME and AME_n values of corn were 3,132, 3,366 and 3,178 kcal/kg, respectively, which compares well with the 3,381 kcal/kg AME reported by Rostagno et al. (2011), 3,340 kcal/kg AME in Lopez and Leeson (2008), and 3,3500 kcal/kg AME_n in NRC (1994). Energy utilization of corn may be influenced by the substitution method (Matterson et al., 1965; Adeola, 2001; Sakomura and Rostagno, 2007; Kong and Adeola, 2014), because the CN diet was imbalanced. However, the main objective of this study was evaluate effects of various concentrations of an exogenous β-xylanase in diets with different corn proportions and considering 100% of corn.

Evaluation of the effects of xylanase supplementation on CS diets, CN diets, and corn was another objective of this study. The effects of dietary treatments on total tract retention and ileal digestibility of nutrients by broilers are presented in Tables 3 and 4 and showed no interactions between diet and xylanase. In the present study, AME_n and IDE were improved ($P < 0.05$) by 192 and 145 kcal/kg, respectively when diets were supplemented with 100 FXU/kg xylanase. Xylanase supplementations provided an increase in all evaluated parameters when compared to the diet without xylanase ($P < 0.05$).

In the present study, xylanase supplementation at 100 FXU/kg in the corn-soy diet provided an increase ($P < 0.05$) of 145 kcal/kg, 5%, and 2.6% on IDE, CP digestibility, and EE digestibility, respectively when compared to the diet without xylanase. This response is in agreement with findings by Kalmendal and Tauson (2012) who used the same enzyme product and observed an increase of 114 kcal/kg in AME_n when 34-d-old chickens where fed diets supplemented with 200 FXU/kg. Cowieson et al. (2010) also

observed an increase of 100 kcal/kg in IDE after supplementing corn-soy diets with xylanase (8,000 β-xylanase units/kg) in 21-d-old broilers.

Regression equations of increased β-xylanase supplementation on total tract retention and ileal digestibility of CS diets, CN diets, and corn for broilers are shown in Table 5. Xylanase added to the CS diet resulted in quadratic increases ($P < 0.05$) for IDE, AME, AME_n, and CP digestibility. A maximum AME_n and IDE release was obtained with 92 and 124 FXU/kg, respectively. A maximum CP digestibility was obtained with 122 FXU/kg corresponding to 71%. However, xylanase supplementation to the CN diet led to linear increases ($P < 0.05$) in DM digestibility, AME_n, CP digestibility, and EE digestibility. Energy values, and digestibility of CP and EE increased linearly ($P < 0.05$) with xylanase supplementation and a maximum energy release was not achieved until 200 FXU/kg when broilers were fed corn-based diets. As reported by Choct (1997) and Back Knudsen (1997) the total amount of arabinoxylans is variable among ingredients and it was reported as 5.2% in corn and 3.3% in SBM. Then, based on corn and SBM inclusion, the amount of arabinoxylans was 4.0% in the CS diet and 4.5% in the CN diet. Regression analysis of data using the calculation extrapolated for corn suggested no significant effects on digestibility of DM when xylanase was used. However, energy values and digestibility of CP and EE of corn supplemented with xylanase increased linearly ($P < 0.05$).

As Table 5 shows, it seems that the improvement in energy from the CN diet with xylanase supplementation may be masked when the energy value of an ingredient is high. We also could observe based on linear equations that the amount of xylanase needed to improve digestibility and energy responses was higher in CS diets compared to CN diets. Also, there is a more optimal ME:AA ratio in the CS diet. Adding xylanase

to the CN diet would only compound this problem by increasing metabolizable energy/digestible energy further while the diet under-supplies AA. Additionally, there may be more room for improvement in the digestibility of energy in SBM than for corn. Perhaps displacing SBM with corn resulted in a reduction in the opportunity for energy digestibility improvement.

Horvatovic et al. (2015) reported that the supplementation of diets with xylanase increased diet digestibility possibly because it promoted an increase in the activity of endogenous enzymes by increasing the availability of substrates. Xylanase increases access of encapsulated nutrients to endogenous enzymes due to disruption of cell wall arabinoxylans and establish more benefical bacterial in lower GI tract through the production of xylo-oligomers (Kocher et al., 2003; Meng et al., 2005; Francesch and Geraert, 2009; O'Neill et al., 2012). Supplemental xylanase increased nutrient digestibility and also was shown to increase volatile fatty acids concentration linked to the increased flow of fermentable xylo-oligomers into the ceca (Choct et al., 1996). Furthermore, exogenous xylanases also have been related to increase nutrient digestibility via reduction in digesta viscosity and cell wall integrity, generating fermentable disaccharides, low-molecular weight polysaccharides and oligosaccharides; improving protein solubility, and decreasing endogenous losses and overcoming antinutritional factors (Cowieson and Ravindran, 2008).

The reason for the observed increase in energy utilization using xylanase may be associated with increased utilization of starch and fat from corn; protein from corn and soybean and other carbohydrates from dietary components (Batal and Parsons, 2002). Broiler chickens fed diets that are essentially adequate in all nutrients often still respond to exogenous enzyme addition (Stefanello et al., 2015, Vieira et al., 2015), suggesting

that enzyme benefits may result from changes in less tangible metrics such as appetite control, digestive physiology, immunology, or microbiology. However, if the diet is not able to provide balanced nutrients composition, energy utilization and nutrient digestibility can be affected (Bao et al., 2013).

In conclusion, the substitution method used in this study showed that AME_n, IDE, crude protein and ether extract digestibility was higher in broilers fed a complete corn-soy-based diet than an 'artificial' corn-based ration. However, the digestibility of CP and energy of corn-soy-based diets, corn-based diets, and corn increased with xylanase supplementation. When β-xylanase was tested in a corn-soy-based diet, 92 FXU/kg and 124 FXU/kg maximized its IDE and AME_n release effect; however, this maximum energy response in the corn-based diet or 100% of corn was not achieved until 200 FXU/kg.

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Table 1. Ingredient and nutrient composition of the experimental diets (as-is basis)

Item	Corn-soy-based diet (CS)	Corn-based diet (CN)[1]
Ingredients, %		
Corn	53.61	72.17
Soybean meal	36.30	21.78
Soybean oil	5.09	3.05
Dicalcium phosphate	1.83	1.10
Limestone	0.97	0.58
Salt	0.51	0.31
DL-Methionine 99%	0.29	0.17
L-Lysine HCl 76%	0.16	0.10
L-Threonine 98.5%	0.04	0.02
Choline chloride 60%	0.05	0.03
Vitamin and mineral mix[2]	0.15	0.09
Celite[3]	1.00	0.60
Calculated nutrient composition, % unless noted		
AME_n, kcal/kg	3,100	3,214
CP	21.00	15.75
Ca	0.90	0.55
Non-phytate P	0.45	0.29
Total P	0.68	0.51
Na	0.22	0.14
Choline, mg/kg	1,500	1,100
Dig. Lys[4]	1.15	0.77
Dig. TSAA	0.86	0.39
Dig. Thr	0.75	0.56
Dig. Trp	0.23	0.16
Dig. Arg	1.34	0.94
Dig. Val	0.89	1.34
Dig. Ile	0.82	0.59

[1]Corn-based diet was composed by 60% of corn-soybean meal basal diet + 40% of corn.

[2]Composition per kg of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.

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

[4]Ratios of digestible amino acids to digestible Lys were maintained at TSAA: 0.75; Thr: 0.65; Val: 0.70; Trp: 0.17; Arg: 1.08; Ile: 0.67 (Rostagno et al., 2011).

Table 2. Declared and analyzed activity of β-xylanase in the experimental diets

Treatment	Xylanase, FXU/kg[1]	
	Declared	Analyzed
Corn-soy-based diet (CS)	0	<LOD[2]
CS + 50 FXU/kg	50	64
CS + 100 FXU/kg	100	113
CS + 150 FXU/kg	150	167
CS + 200 FXU/kg	200	206
Corn-based diet (CN)[3]	0	<LOD
CN + 50 FXU/kg	50	54
CN + 100 FXU/kg	100	109
CN + 150 FXU/kg	150	172
CN + 200 FXU/kg	200	208

[1]FXU = fungal β-xylanase units per kg of feed.

[2]LOD = limit of detection.

[3]Corn-based diet was composed by 60% of corn-soybean meal basal diet + 40% of corn.

Table 3. Apparent ileal digestibility and total tract retention responses of broilers fed corn-soy-based diets and corn-based diets supplemented with β-xylanase (on DM basis)[1]

Item	Ileal digestibility		Total tract retention				
	DM, %	IDE[2], kcal/kg	DM, %	AME, kcal/kg	AME_n, kcal/kg	CP[3], %	EE[4], %
Diet							
Corn-soy-based	67.9	3,278	74.2	3,544	3,324	69.3	86.7
Corn-based[5]	64.6	3,201	72.9	3,432	3,295	62.2	83.4
Xylanase, FXU/kg[6]							
0	65.1^{b}	3,176^{b}	71.5^{b}	3,359^{b}	3,179^{b}	62.7^{b}	83.1^{b}
50	67.9^{a}	3,312^{a}	72.6^{ab}	3,451^{ab}	3,271^{ab}	64.2^{ab}	83.7^{b}
100	67.4^{a}	3,321^{a}	75.6^{a}	3,551^{a}	3,371^{a}	67.7^{a}	85.7^{ab}
150	65.7^{ab}	3,196^{ab}	74.8^{ab}	3,548^{a}	3,364^{a}	67.3^{a}	86.9^{a}
200	65.5^{ab}	3,195^{ab}	74.2^{ab}	3,530^{a}	3,362^{a}	66.8^{a}	86.6^{a}
SEM	0.49	19.36	0.40	15.36	13.61	0.60	0.44
Main effect *P*-value							
Diet	0.001	0.041	0.102	0.001	0.155	0.001	0.001
Xylanase	0.027	0.025	0.030	0.001	0.001	0.003	0.007
Diet × xylanase	0.821	0.765	0.753	0.228	0.112	0.262	0.345

[a-b]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage at the start of the experiment.

[2]IDE = ileal digestible energy.

[3]Crude protein.

[4]Ether extract.

[5]Corn-based diet was composed by 60% of corn-soybean meal basal diet + 40% of corn.

[6]FXU = fungal β-xylanase units per kg of feed.

Table 4. Energy and nutrient utilization of corn supplemented with β-xylanase (on DM basis)[1]

Item	Ileal digestibility		Total tract retention				
	DM, %	IDE[2], kcal/kg	DM, %	AME, kcal/kg	AME_n, kcal/kg	CP[3], %	EE[4], %
Xylanase, FXU/kg[5]							
0	64.7	3,132	71.5	3,366	3,178	63.5	83.5
50	65.0	3,169	72.9	3,457	3,269	65.1	83.5
100	67.6	3,187	74.6	3,538	3,359	67.6	85.9
150	65.5	3,303	74.6	3,562	3,372	67.7	86.9
200	66.9	3,322	74.8	3,572	3,386	68.6	87.0
SEM	0.38	21.94	0.43	17.17	16.56	0.45	0.43
P-value[6]							
L	0.389	0.004	0.008	0.001	0.001	0.001	0.005
Q	0.920	0.704	0.219	0.038	0.019	0.037	0.099

[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage at the start of the experiment.

[2]IDE = ileal digestible energy.

[3]Crude protein.

[4]Ether extract.

[5]FXU = fungal β-xylanase units per kg of feed.

[6]Linear (L) or quadratic (Q) effect.

Table 5. Regression equations of apparent ileal digestibility and total tract retention of nutrients and energy of corn-soy-based diets, corn-based diets, and corn supplemented with β-xylanase

Item	Regression equations[1]	*P*-value[2]	r^2	SD
Corn-soy-based diet (CS)				
IDE[3], kcal/kg DM	$Y = -0.014x^2 + 2.570x + 3,155$	0.001	0.60	89
Dry matter, %	$Y = -0.0002x^2 + 0.055x + 71.84$	0.042	0.61	1.5
AME, kcal/kg DM	$Y = -0.015x^2 + 3.687x + 3,178$	0.001	0.70	106
AME_n, kcal/kg DM	$Y = -0.016x^2 + 3.982x + 3,155$	0.001	0.68	109
Crude protein, % DM	$Y = -0.0003x^2 + 0.073x + 66.36$	0.006	0.71	1.9
Corn-based diet (CN)[4]				
Dry matter, %	$Y = 0.020x + 70.96$	0.026	0.22	4.0
AME, kcal/kg DM	$Y = 0.778x + 3,354$	0.002	0.33	116
AME_n, kcal/kg DM	$Y = 0.911x + 3,204$	0.001	0.47	107
Crude protein, % DM	$Y = 0.035x + 58.64$	0.005	0.38	4.8
Ether extract, % DM	$Y = 0.032x + 80.22$	0.001	0.46	3.8
Corn				
IDE, kcal/kg DM	$Y = 1.027x + 3,120$	0.004	0.28	139
Dry matter, %	$Y = 1.016x + 72.11$	0.007	0.17	2.7
AME, kcal/kg DM	$Y = 1.033x + 3,396$	0.001	0.47	108
AME_n, kcal/kg DM	$Y = 1.036x + 3,209$	0.001	0.51	105
Crude protein, % DM	$Y = 0.023x + 64.16$	0.001	0.34	2.8
Ether extract, % DM	$Y = 0.017x + 83.73$	0.005	0.19	2.7

[1]Regression equations for xylanase levels (0, 50, 100, 150, and 200 fungal β-xylanase units per kg of feed). The coefficient of determination (r^2) was obtained using all data.

[2]Linear (L) or quadratic (Q) effect ($P < 0.05$).

[3]IDE = ileal digestible energy.

[4]Corn-based diet was composed by 60% of corn-soybean meal basal diet + 40% of corn.

CAPÍTULO III[1]

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METABOLISM AND NUTRITION

STARCH AND CARBOHYDRASE FOR BROILERS

Starch digestibility, energy utilization and growth performance of broilers fed corn-soybean basal diets supplemented with enzymes

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ABSTRACT A study was conducted to evaluate the effects of dietary α-amylase and β-xylanase supplementation of corn-soy diets formulated with or without supplemental phytase on growth performance, energy utilization and starch digestibility in broiler chickens. A total of 336 slow feathering, Cobb × Cobb 500 male broilers were randomly distributed to 6 treatments having 8 replicates of 7 birds each. Birds were fed a common starter diet to d 14 post hatch (3,050 kcal/kg AME_n, 21.7% CP, 1.05% Ca, and 0.53% nPP). The experimental diets were provided afterwards until 25 d. A 2 × 3 factorial arrangement of 2 control diets (Basal = corn-soy diet without added phytase or PHY = corn-soy diet formulated with 1,000 phytase units/kg) and 3 carbohydrase supplementations (0, 80 kilo-Novo α-amylase units/kg, or 80 kilo-Novo α-amylase units/kg + 100 fungal β-xylanase units/kg) was used from 14 to 25 d. Excreta were collected from 21 to 24 d and all birds were euthanized at 25 d for jejunum and ileum content collection. Samples of feed, excreta, jejunal and ileal digesta were analyzed for determination of total tract retention and ileal apparent digestibility. No interactions between diet and carbohydrase were observed. Broilers fed diets formulated with phytase or supplemented with amylase + xylanase had higher BWG and lower FCR ($P < 0.05$) when compared with birds fed diets without carbohydrases. Relative to the basal diet, AME_n was increased ($P < 0.01$) by 70 kcal/kg and 99 kcal/kg when birds were fed the diet supplemented with amylase and amylase + xylanase, respectively. Starch digestibility in the jejunum and ileum was increased ($P < 0.05$) by 3.5% and 2.4% respectively when birds were fed the diet supplemented with amylase + xylanase. Results from this experiment show that corn-soy diets having phytase and supplemented with amylase and xylanase led to increased growth performance, AME_n, and starch

digestibility in broilers. Furthermore, the efficacy of exogenous amylase and xylanase was independent of the presence of microbial phytase.

Key words: amylase, broiler, metabolizable energy, starch, xylanase

INTRODUCTION

Nutrient and energy digestibility of plant feedstuffs by poultry is limited by the proportion of their components for which there are no corresponding endogenous digestive secretions. This is the case of the non-starch polysaccharides that are present in and within the cell walls of soybean meal (SBM) and corn (Choct, 1997; Bach Knudsen, 1997; Huisman et al., 1998; Caffall and Mohnen, 2009). They are either indigestible (Graham and Aman, 1991) or of very low digestibility when fed to poultry (Slominski and Campbell, 1990; Kocher et al., 2003). Therefore, the energy derived from them is restricted by a lower organic combustion of these components as well as by the physical barrier represented by the cell wall itself that prevents enzyme access to substrates (Theander et al., 1989; Slominski et al., 1993) such as starch or protein.

Non-starch polysaccharides can increase digesta viscosity and this has been related to a reduction in starch, protein and fat digestibility (Bedford et al., 1991; Meng et al., 2005). However, corn-soy diets may not contain sufficient concentrations of high-molecular weight soluble polysaccharides to increase intestinal viscosity to a point that is detrimental to nutrient utilization by poultry (Gracia et al., 2003; Bach Knudsen, 2014), particularly when compared with diets containing wheat, rye, and barley (Englyst, 1989; Bedford et al., 1991; Meng and Slominski, 2005). Starch degradability itself is affected by the proportion of amylose: amylopectin, being higher as more of the latter exists in the molecule (Moran, 1982). Lately the term resistant starch has been increasingly referred in the literature to describe starch that escapes digestion in the small intestine (Englyst et al., 1982). This is variable in corn and other plant seeds and can significantly influence the AME_n content of these feedstuffs (Tester et al., 2004).

Phytate (myo-inositol hexaphosphate) is the main form of stored phosphorus (P) in plant seeds (Prattley and Stanley, 1982). Besides the limitation in the use of P by poultry, phytate has also been reported to reduce energy utilization (Rutherfurd et al., 2004; Ravindran et al., 2006). Phytate and lower inositol phosphates can be hydrolyzed by the enzyme phytase (myo-inositol hexaphosphate phosphohydrolase), which has become a standard enzymatic additive in poultry diets (Nelson et al., 1968; Jozefiak et al., 2010; Cowieson et al., 2011).

Studies with supplemental enzymes targeting the degradation of substrates that release energy for poultry have been increasing. Exogenous carbohydrases such as xylanases, amylases and glucanases, have been reported to improve energy utilization and the performance of broilers (Olukosi et al., 2008; Olukosi and Adeola, 2008; Williams et al., 2014). These enzymes may improve the access of endogenous enzymes to cell contents due to hydrolysis of cell wall arabinoxylans (Kocher et al., 2003; Meng et al., 2005; Francesch and Geraert, 2009) as well as to augment endogenous amylase in young animals (Ritz et al., 1995; Gracia et al., 2003). Decreases in endogenous amino acids (AA) losses may also contribute to the beneficial effects of supplemental enzymes, possibly associated with a reduction in the antinutritional effect of some polysaccharides and/or through feedback mechanisms that reduce the need for endogenous enzyme synthesis and secretion (Jiang et al., 2008; Cowieson and Bedford, 2009). Finally, some exogenous carbohydrases (notably the xylanase family) may create pre-biotic xylo-oligomers that benefit digestion indirectly via increased fermentation in the hindgut and stimulation of the ileal brake mechanism (Cowieson, 2005).

As increasing numbers of enzyme products become available, the assembly of strategically optimized admixtures becomes a challenge in terms of targeting the

complex mixture of components that have low digestibility. Whilst different enzyme classes hydrolyze different substrates and generate different products, a net effect on the increase in the digestibility of starch, protein/AA and fat with a cumulative energetic consequence seems to be an expected outcome of their action. While enzymes that target different substrates do not compete in terms of substrate degradation itself, they tend to overlap in nutrient digestion and performance, delivering sub-additive outcomes (Cowieson and Adeola, 2005; Cowieson et al., 2006; Romero et al., 2013). Increases in energy utilization from plant feedstuffs by broilers may derive from a large variety of components; however, since starch is quantitatively the largest energy supplier in broiler diets, any improvement in its degradation would lead to a significant benefit.

The objective of the present study was to evaluate the effects of an α-amylase alone or in combination with a β-xylanase on growth performance, energy utilization and starch digestibility of broiler chickens fed corn-SBM-based diets. These effects were assessed both in a phytase-free diet and in a diet that included phytase appropriately formulated with the associated displacement of inorganic phosphate in order to explore the possibility that carbohydrase efficacy may be muted by the presence of phytase.

MATERIALS AND METHODS

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

Bird Husbandry

A total of 336 one-day-old, slow-feathering Cobb × Cobb 500 male broiler chicks, vaccinated for Marek's disease at the hatchery, with an average BW of 47 g were randomly placed in 48 wire cages (0.9 × 0.4 m^2). Each cage was equipped with one feeder and one drinker. Birds had ad libitum access to water and mash feeds. Average temperature was 32°C at placement being reduced by 1°C every 2 d until 23°C to provide comfort throughout the study. Lighting was continuous until d 25 post hatch.

Diets and Experimental Design

Birds were allocated to 6 experimental diets with 8 replications of 7 birds each in a completely randomized design. A standard corn-SBM-based broiler starter was fed from 1 to 14 d (3,050 kcal/kg AME_n, 21.7% CP, 1.05% Ca, and 0.53% non-phytate P) whereas the experimental diets are presented in Table 1. A 2 × 3 factorial arrangement of 2 control diets (Basal = corn-soy basal diet without added phytase or PHY = corn-soy diet formulated with phytase) and 3 carbohydrase supplements (0, α-amylase, or α-amylase + β-xylanase) were provided from 14 to 25 d. The supplemental enzymes used in the present study are commercially available (Novozymes A/S, Bagsvaerd, Denmark). Their inclusion per kg of diet were: phytase [Ronozyme HiPhos (GT)] 1,000 phytase units (FYT), alpha amylase [Ronozyme HiStarch (CT)] 80 kilo-Novo alpha amylase units (KNU), and beta xylanase [Ronozyme WX (CT)] 100 fungal xylanase units (FXU). All experimental diets had 1% Celite as indigestible marker (Celite, Celite Corp., Lompoc, CA).

The phytase product used was a 6-phytase produced by the expression of synthetic genes incorporated into *Aspergillus oryzae* and contained 10,000 FYT/g. One FTU is

defined as the quantity of enzyme required to liberate 1 µmol of inorganic P per minute, at pH 5.5, from an excess of 15 µM of sodium phytate at 37°C. The α-amylase was a granulated enzyme preparation produced by submerged fermentation of *Bacillus amyloliquefaciens* and contained 600 KNU/g. One KNU is the amount of enzyme that releases in a two step reaction, 6 µmol p-nitrophenol per minute from 1.86 mM ethyledene-G7-p-nitrophenyl-maltoheptaoside at pH 7.0 and 37ºC. The β-xylanase was a granulated heat-stable endo-xylanase from *Thermomyces lanuginosus* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism containing 1,000 FXU/g. One FXU is the amount of endo-1,4- β-xylanase which liberates 7.8 micromoles of reducing sugars (xylose equivalents) per minute from azo-wheat arabinoxylans at pH 6.0 and 50ºC.

Experimental Procedures

Chicks were individually weighed into groups of 7 birds per cage before placement. Bird weights, averaged by cage, were recorded at 14 and 25 d. Body weight gain (BWG), feed intake (FI) and FCR corrected for the weight of dead birds were determined between those dates.

Excreta were collected twice daily on wax paper from 21 to 24 d being immediately mixed and pooled by cage and stored at −20°C until analysis. Previous to calorimetry, excreta were dried in a forced air oven at 55°C (DeLeo, Porto Alegre, RS) and ground to pass through a 0.5-mm screen. Intestinal contents were collected from all birds at 25 d after euthanasia by electrical stunning using 45 V for 3 s. Jejunal digesta was collected from segment between 2 cm proximal to the duodeno-jejunal junction and the Meckel's diverticulum. Ileal digesta was collected from the Meckel's diverticulum

to approximately 2 cm cranial to the ileo-cecal junction. Jejunal and ileal contents from all birds were flushed with distilled water into plastic containers, pooled by cage, immediately frozen in liquid nitrogen, and stored in a freezer at −20°C until lyophilized (Christ Alpha 2-4 LD Freeze Dryer, Newtown, UK).

Chemical Analysis and Calculations

Diet and freeze-dried samples of jejunal and ileal contents were ground to pass through a 0.5-mm screen in a grinder. Excreta samples were dried in a forced air oven at 55ºC and ground to pass through a 0.5-mm screen in a grinder (Tecnal, TE-631/2, São Paulo, SP). Dry matter (DM) analysis of samples was performed after oven drying the samples at 105°C for 16 h (method 934.01; AOAC International, 2006). Ileal digesta, excreta, and diet samples were analyzed for gross energy using a calorimeter calibrated with benzoic acid as a standard (IKA Werke, Parr Instruments, Staufen, Germany). Calculations of ileal digestible energy (IDE) and AME_n were done afterwards. Crude protein (N × 6.25) was determined by combustion method (method 968.06; AOAC International, 2006). The calculated AME was corrected to zero N retention (AME_n) using a factor of 8.22 kcal/g (Hill and Anderson, 1958). Acid insoluble ash concentration in the diets, excreta, jejunum and ileum samples were determined using the method described by Vogtmann et al. (1975), and Choct and Annison (1992). Starch analyzes were done using the method 996.11 of AOAC International (2000).

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

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

$$AME_n\ (kcal/kg) = GE_i - [GE_o \times (M_i/M_o)] - 8.22 \times \{N_i - [N_o \times M_i/M_o)]\},$$

where M_i represents the concentration of acid insoluble ash in the diet in grams per kilogram of DM; M_o represents the concentration of acid insoluble ash in the excreta, jejunal and ileal digesta in grams per kilogram of DM output; E_i represents the concentration of DM, CP, energy, or starch in the diet in milligrams per kilogram of DM; and E_o represents the concentration of DM, CP, and energy in the excreta and ileal digesta, or starch in the jejunal and ileal digesta in milligrams per kilogram of DM. GE_i is gross energy (kcal/kg) in the diet; GE_o is the gross energy (kcal/kg) in the excreta; N_i represents nitrogen concentration in the diet, and N_o represents nitrogen concentration in the excreta in g/kg DM.

Statistical Analysis

Normality and homoscedasticity of the data were verified by the Shapiro-Wilk test (Shapiro, 1965). The experimental design was a completely randomized factorial arrangement of 2 control diets (without or with phytase) × 3 carbohydrase supplementations. Data were submitted to a 2-way ANOVA using the GLM procedure of SAS Institute (SAS, 2009). Significance was accepted at $P < 0.05$ and mean differences were separated using Tukey's HSD test (Tukey, 1991).

RESULTS AND DISCUSSION

Analyses of α-amylase, β-xylanase and phytase in the experimental diets showed that the supplemental enzymes had in-feed activities in agreement with the expected values (Table 2). The effects of dietary treatments on broiler performance are presented

in Table 3 showing no interactions between diet and carbohydrase. Feed intake and mortality were not affected by dietary treatments. In general, birds fed diets supplemented with amylase + xylanase had lower FCR and increased BWG than those fed the diets without carbohydrase ($P < 0.05$). Broilers fed the PHY diets also had higher BWG ($P < 0.05$) and lower FCR compared to birds fed the basal diet without enzymes.

A combination of precise phosphorus nutrition and addition of proper levels of microbial phytase is expected to optimize broiler performance while reducing the reliance on inorganic phosphorus sources through improving utilization of phytate-bound P from the diet (Nelson, 1967; Karimi et al., 2013). Studies have reported improvements in performance when phytase was used in chickens (Żyła et al., 2000; Onyango et al., 2005). However, the effect of enzymes targeting alternative substrates on performance of broilers is conflicting (Olukosi et al., 2008). In many studies, xylanase and amylase, when added to diets separately, have resulted in improved broiler performance when fed corn-SBM diets (Gracia et al., 2003; Jiang et al., 2008; Williams et al., 2014). Other authors found no effect supplementing various carbohydrases on growth performance (Kocher et al., 2003; Singh et al., 2012). Information on simultaneous effects of dietary supplementation of amylase-xylanase admixtures with or without phytase in broilers is scarce.

Effects of phytase and carbohydrases supplementation on total tract retention and ileal digestibility of broiler chickens are shown in Table 4. There were no interactions between diet and carbohydrase for ileal digestibility and total tract retention in 25-d-old broilers. Differences in AME_n and ileal digestibility of DM were found between PHY

and basal diets ($P < 0.01$) with values, respectively of 3,483 kcal/kg and 65.2% for PHY and 3,412 kcal/kg and 63.4% for basal.

Phytase has been shown to improve digestibility and retention of P in chickens (Żyła et al., 2000; Dilger et al., 2004; Juanpere et al., 2005; Onyango et al., 2005). The use of phytase may also be relevant for the so-called extra-phosphoric effects such as beneficial effects on amino acid retention, net energy and myo-inositol release. It is known that part of the beneficial effect of microbial phytase in poultry may be derived from generation of myo-inositol through a phytase-initiated enzymatic cascade that results in the complete dephosphorylation of dietary phytate (Jozefiak et al., 2010; Cowieson et al., 2011).

Phytate limits the efficacy of digestive enzymes after forming indigestible complexes especially with Ca and Zn (Singh and Krikorian, 1982; Matyka et al., 1990). Because phytase is able to hydrolyze phytate, phytase is expected to improve the digestibility of nutrients in general. Additionally, mechanisms by which phytase may improve ME have been related with the reduced endogenous energy and AA flow (Cowieson and Ravindran, 2007), reduced integrity of fibrous complexes, and improved capacity for active transport of nutrients from the gut associated with Na (Cowieson et al., 2004; Liu et al., 2008).

In the present study, AME_n was improved ($P < 0.01$) by 70 and 99 kcal/kg when amylase and amylase + xylanase were supplemented, respectively. Amylase and xylanase supplementation provided an increase in AME_n of 2.8% when compared to the diet without carbohydrases ($P < 0.01$). This response is in agreement with findings by Rutherfurd et al. (2007) that observed an increase of 2.3% in AME_n after supplementing corn-SBM diets with carbohydrases (80 kilo-Novo α-amylase units/kg, 140 β-glucanase

units/kg, and 100 β-xylanase units/kg) in 28-d-old broilers. Improvements in the AME_n for broilers fed corn-SBM-based diets containing amylase is likely to be attributed to an increase in starch digestibility. A shift in the site of starch digestibility from caudal to proximal gastrointestinal segments could also have occurred (Svihus, 2014). Svihus (2006) observed that AME and total tract starch digestibility for individual birds were correlated (r = 0.984).

Increased starch digestibility when xylanase and amylase are supplemented may occur as a result of α-amylase activity in parallel with degradation of soluble and non-starch polysaccharides to free sugars, such as arabinose and xylose (Choct et al., 2004). Xylanase may increase access of cell contents to endogenous enzymes due to hydrolysis of cell wall arabinoxylans and also reduce the antinutritional effect of some polysaccharides (Kocher et al., 2003; Meng et al., 2005; Francesch and Geraert, 2009). Furthermore, other researchers found increased digestibility of starch in the small intestine by the addition of exogenous enzyme products into corn-SBM diets, leading to enhanced energy availability to birds (Zanella et al., 1999; Yu and Chung, 2004; Meng and Slominski, 2005).

The digestibility of starch in the jejunum and ileum for broilers fed the different enzyme combinations are presented in Table 5. No interactions between diet and carbohydrase were observed on starch digestibility in 25-d-old broilers. The digestibility of starch in the jejunum and ileum was higher ($P < 0.01$) for broilers fed PHY or diets supplemented with amylase + xylanase. The starch digestibility in the jejunum and ileum was increased ($P < 0.05$) by 3.5% and 2.4%, respectively in broilers fed amylase + xylanase diet compared to broilers fed the diet without carbohydrases.

Starch digestibility was higher in the ileum than in the jejunum. The disappearance of starch evaluated through the difference between percentage of starch in the distal ileum and distal jejunum was 6.4% higher in broilers ($P < 0.05$) fed the amylase + xylanase diet compared to broilers fed the diet without carbohydrases. Zanella et al. (1999) reported that the digestion of starch in the small intestine is incomplete and continues in the lower gut as a result of microbial fermentation. Kaczmarek et al. (2014) demonstrated that starch digestibility in birds fed a corn-SBM diet was similar in the ileum and excreta collection, although no effect of amylase was observed. Furthermore, Weurding et al. (2001) showed that rapid starch digestion may lead to the same extent of starch digestion as gradual starch digestion, but the amount of starch digested at specific sites of the small intestine (jejunum and ileum) differs, and differences in these digestion sites may have metabolic consequences that affect feed utilization.

Englyst et al. (1982) introduced the term resistant starch, which was defined as total starch minus digested starch. An appreciable amount of intact starch granules have been observed by microscopic analysis in the ileal digesta collected from birds, from 4 to 21 d for starch digestibility below 86% (Noy and Sklan, 1995; Bedford and Autio, 1996). These results suggested that secretion of pancreatic amylase from the immature pancreas during the post hatching period might retard intestinal starch digestion, and consequently limit early growth. Chickens are considered well able to digest high starch diets; however, physiological differences according to age and carbohydrase supplementation have influenced performance of broilers when the starter phase is compared to the finisher period. Thus, as corn is included at higher levels in the finisher

diets for broilers, the ratio of non-starch polysaccharides and starch increases when compared to the started feed (Svihus, 2014).

It is possible that the very high feed intake of the modern fast-growing broiler chickens may cause limitations for starch digestion, which could allow for exogenous amylase to be effective. Additionally, xylanase may increase access to entrapped nutrient components by destroying some fractions of the plant cell walls of grains, allowing α-amylase access to starch fractions (Kocher et al., 2003; D'Alfonso, 2005; Leslie et al., 2007). Furthermore, the data show improved benefit to the growing broiler chickens with addition of exogenous amylase and xylanase or phytase as measured by energy utilization and starch digestibility.

In conclusion, corn-SBM-based diets formulated with 1,000 phytase units/kg or supplemented with 80 kilo-Novo α-amylase units/kg and 100 fungal β-xylanase units/kg had a beneficial impact on BWG and FCR of broilers. AME_n was also improved when birds were fed amylase + xylanase diet compared to the diet without carbohydrases. Finally, the digestibility of starch in the jejunum was lower than in the ileum, and corn-SBM-based diets formulated with phytase or supplemented with amylase + xylanase improved starch utilization.

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Table 6. Ingredient and nutrient composition of the experimental diets (as-is basis)

Item	Basal diet	Phytase diet (PHY)
Ingredients, %		
Corn	53.61	54.97
Soybean meal	36.30	36.09
Soybean oil	5.09	4.63
Dicalcium phosphate	1.83	1.01
Limestone	0.97	1.11
Salt	0.51	0.51
DL-Methionine 99%	0.29	0.30
L-Lysine HCl 76%	0.16	0.15
L-Threonine 98.5%	0.04	0.03
Choline chloride 60%	0.05	0.05
Vitamin and mineral mix[1]	0.15	0.15
Celite[2]	1.00	1.00
Phytase[3]	0.00	0.01
Calculated nutrient composition, % unless noted		
AME_n, kcal/kg	3,100	3,100
CP	21.00	21.00
Ca	0.90	0.75
Non-phytate P	0.45	0.30
Total P	0.68	0.53
Na	0.22	0.22
Choline, mg/kg	1,500	1,500
Dig. Lys[4]	1.15	1.15
Dig. TSAA	0.86	0.86
Dig. Thr	0.75	0.75
Dig. Trp	0.23	0.23
Dig. Arg	1.34	1.34
Dig. Val	0.89	0.89
Dig. Ile	0.82	0.82

[1]Composition per kg of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.

[2]Insoluble marker (Celite, Celite Corp., Lompoc, CA).

[3]Ronozyme HiPhos (GT) with 10,000 FYT/g (Novozymes A/S, Bagsvaerd, Denmark).

[4]Ratios of digestible amino acids to digestible Lys were maintained at TSAA: 0.75; Thr: 0.65; Val: 0.70; Trp: 0.17; Arg: 1.08; Ile: 0.67 (Rostagno et al., 2011).

Table 7. Declared and analyzed activities of amylase, xylanase and phytase in the experimental diets[1]

Treatment	Amylase, KNU/kg		Xylanase, FXU/kg[3]		Phytase, FYT/kg[4]	
	Declared	Analyzed	Declared	Analyzed	Declared	Analyzed
Basal[5]	0	<LOD[6]	0	<LOD	0	<LOD
Basal + amylase	80	90	0	<LOD	0	<LOD
Basal + amylase + xylanase	80	85	100	108	0	<LOD
PHY[7]	0	<LOD	0	<LOD	1,000	1,055
PHY + amylase	80	86	0	<LOD	1,000	1,083
PHY + amylase + xylanase	80	88	100	103	1,000	1,049

[1]Enzyme activity are expressed as the quantity of product added in the feed.
[2]KNU = kilo-Novo α-amylase units per kg of feed.
[3]FXU = fungal β-xylanase units per kg of feed.
[4]FYT = phytase units per kg of feed.
[5]Corn-soy diet without added phytase.
[6]LOD = limit of detection.
[7]PHY = corn-soy diet formulated with 1,000 phytase units/kg.

Table 8. Growth performance of broilers (from 14 to 25 d) fed corn-soybean meal-based diets with or without phytase and supplemented or not with amylase or amylase + xylanase[1]

Item	BW gain, g	Feed intake, g	FCR[2]
Diet			
Basal[3]	1,036	1,136	1.097
PHY[4]	1,055	1,126	1.067
Carbohydrase			
0	1,032[b]	1,144	1.109[a]
Amylase[5]	1,046[ab]	1,125	1.076[ab]
Amylase + xylanase[6]	1,060[a]	1,126	1.062[b]
SEM	4.126	17.603	0.008
Main effect *P*-value			
Diet	0.014	0.497	0.031
Carbohydrase	0.015	0.420	0.020
Diet × Carbohydrase	0.802	0.187	0.184

[a-b]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Means were obtained from 8 replicate cages of 7 birds per replicate cage at the start of the experiment.

[2]Feed conversion ratio corrected for the weight of dead birds.

[3]Corn-soy diet without added phytase.

[4]PHY = corn-soy diet formulated with 1,000 phytase units/kg.

[5]Supplemented with 80 kilo-Novo α-amylase units/kg.

[6]Supplemented with 80 kilo-Novo α-amylase units/kg + 100 fungal β-xylanase units/kg.

Table 9. Energy and nutrient utilization response of broilers fed corn-soybean meal-based diets with or without phytase and supplemented or not with amylase or amylase + xylanase[1]

Item	Apparent ileal digestibility			Total tract retention	
	DM, %	IDE[2], kcal/kg DM	CP, %	DM, %	AME_n, kcal/kg DM
Diet					
Basal[3]	63.4	3,187	80.1	70.2	3,412
PHY[4]	65.2	3,267	81.9	71.2	3,483
Carbohydrase					
0	62.9[b]	3,150	80.7	69.6[b]	3,391[b]
Amylase[5]	64.4[ab]	3,234	81.8	70.9[ab]	3,461[a]
Amylase + xylanase[6]	65.6[a]	3,297	82.1	71.5[a]	3,490[a]
SEM	0.576	18.798	0.269	0.266	12.392
Main effect *P*-value					
Diet	0.019	0.121	0.122	0.046	0.001
Carbohydrase	0.026	0.168	0.102	0.009	0.001
Diet × Carbohydrase	0.675	0.970	0.860	0.489	0.971

[a-b]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Means were obtained from 8 replicate cages of 7 birds per replicate cage.

[2]IDE = ileal digestible energy.

[3]Corn-soy diet without added phytase.

[4]PHY = corn-soy diet formulated with 1,000 phytase units/kg.

[5]Supplemented with 80 kilo-Novo α-amylase units/kg.

[6]Supplemented with 80 kilo-Novo α-amylase units/kg + 100 fungal β-xylanase units/kg.

Table 10. Digestibility and disappearance of starch (%) in 25 d broilers fed corn-soybean meal-based diets with or without phytase and supplemented or not with amylase or amylase + xylanase[1]

Item	Jejunum	Ileum	Disappearance[2]
Diet			
Basal[3]	78.5	89.8	29.6
PHY[4]	79.2	91.3	32.6
Carbohydrase			
0	77.0[b]	89.6[b]	28.5[b]
Amylase[5]	79.2[b]	90.2[ab]	30.0[ab]
Amylase + xylanase[6]	80.5[a]	92.0[a]	34.9[a]
SEM	0.367	0.347	1.070
Main effect *P*-value			
Diet	0.046	0.014	0.106
Carbohydrase	0.001	0.013	0.018
Diet × Carbohydrase	0.104	0.208	0.981

[a-b]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Means were obtained from 8 replicate cages of 7 birds per replicate cage.

[2]Amount of starch present in the jejunum and digested until the distal ileum (%).

[3]Corn-soy diet without added phytase.

[4]PHY = corn-soy diet formulated with 1,000 phytase units/kg.

[5]Supplemented with 80 kilo-Novo α-amylase units/kg.

[6]Supplemented with 80 kilo-Novo α-amylase units/kg + 100 fungal β-xylanase units/kg.

CAPÍTULO IV[1]

[1] Artigo nas normas da revista *Animal Production Science*.

Effects of α-amylase and β-xylanase supplementation on growth performance and metabolizable energy of broiler chickens fed corn-soy diets

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Abstract. A study was conducted to evaluate the effects of dietary α-amylase and β-xylanase, in single or combined supplementation, on growth performance of broiler chickens fed corn-soy diets. A total of 1,800 slow feathering, Cobb × Cobb 500 male broilers were randomly distributed into 8 treatments with 9 replicates of 25 birds each. Broilers were fed starter (1 to 21 d) and finisher diets (22 to 40 d) with a positive control (PC, with 12.77 and 13.27 MJ/kg of AME_n, respectively); increases in AME_n of 0.21 MJ/kg (PC + 0.21) and 0.42 MJ/kg (PC + 0.42), and reductions in AME_n of 0.21 MJ/kg (PC – 0.21) and 0.42 MJ/kg (NC, negative control). The NC diet was supplemented with α-amylase (80 kilo-Novo α-amylase units/kg), β-xylanase (100 fungal β-xylanase units/kg), and both enzymes combined at the same supplementation levels. Broilers fed the PC, PC + 0.21 and PC + 0.42 diets had lower FCR and higher BWG when compared to the NC diet ($P < 0.05$). Regressing performance responses to AME_n levels showed only linear significative adjustments ($P < 0.05$). These were equated and solved for X in linear equations at different enzyme supplementations. Corresponding AME_n estimates for BWG and FCR from 1 to 40 d were, respectively 0.41, 0.35, and 0.57 and 0.21, 0.11, and 0.18 MJ/kg for amylase, β-xylanase and amylase + β-xylanase. In conclusion, supplementing corn-soy diets with α-amylase and β-xylanase led to increased dietary energy yields. A marked difference occurred in favor of α-amylase when compared to β-xylanase supplementation whereas adding both enzymes in the same feed generated similar AME_n to the single addition of α-amylase.

Additional keywords: amylase, broiler, metabolizable energy, performance, xylanase.

Introduction

Energy and nutrient utilization of feed ingredients by broilers depends on their carbohydrate composition (Cowieson *et al.* 2010). Starch is quantitatively the main energy-yielding source for poultry from cereal grains (Svihus 2014) representing approximately 690 g/kg of corn composition (Bach Knudsen 1997). On the other hand, non-starch polysaccharides (NSP) in corn ranges from 6.8% to 9.4% whereas in SBM variation is from 17% to 30% (Smits and Annison 1996; Choct 1997; Kocher *et al.* 2003). Among the soluble fibers, arabinoxylan is the highest in corn, reaching up to 5.2% (Choct 1997); however, in SBM its proportion is lower (around 3.3%) (Back Knudsen 1997).

Xylanases have been shown to have the capacity to reduce the nutrient encapsulating effects of the fibrous cell wall and to reduce viscosity associated with soluble arabinoxylans and glucans (Romero *et al.* 2014). The expected action mode of xylanases is predominately associated with the hydrolysis of high molecular weight NSP in cereals as well as ileal brake, peptide YY, and gastric residency of feed (Bedford and Cowieson 2012). Xylanases partially hydrolyze arabinoxylans leading to increased fermentative activity in the caecum resulting in volatile fatty acids production, which, in turn, once absorbed, stimulate peptide YY feedback mechanisms that delays gastric emptying (Choct *et al.* 1996; Cowieson and O'Neill 2013). Additionally, in a conventional corn-soy diet, there is up to 1.88 MJ/kg of energy available for utilization via exogenous enzymes, including 37% from undigested starch (Cowieson *et al.* 2010). Although chicks are adapted to starch-based diets soon after hatch, the high feed intake of modern broilers may produce a physiological limitation to starch digestion (Sklan and Noy 2003). Thus, α-amylase supplementation in corn-based diets may improve

starch digestibility, energy utilization, and performance of broilers (Ritz *et al*. 1995; Gracia *et al*. 2003).

Substantial research has been conducted using supplemental carbohydrases in poultry feeds. Starch stores are intracellular and, therefore, may benefit from exogenous enzyme supplementation targeting the degradation of cell wall as well as augmenting endogenous amylase. For this reason, broiler diets supplemented with enzymes capable of degrading cell wall polysaccharides may allow pancreatic enzymes access to nutrients trapped within the cell (Cowieson 2005). An additional benefit of cell wall degradation is the release of oligosaccharides and monosaccharides that could either be directly absorbed or degraded by the intestinal microflora to provide volatile fatty acids for the animal to utilize as energy (Cowieson and O'Neill 2013).

Chickens are considered well able to digest high starch diets; however, physiological differences according to age and carbohydrase supplementation have increased performance of broilers when the starter phase is compared to the finisher period (Svihus 2014). Though exogenous xylanase and amylase target different substrates and do not compete in terms of substrate degradation itself, they tend to overlap in nutrient digestion and performance effects, delivering sub-additive outcomes (Cowieson and Adeola 2005; Cowieson *et al.* 2006; Romero *et al.* 2013). However, whilst fully additive or synergistic effects of xylanase and amylase may be unlikely it is possible that biologically and economically significant effects of this combination will be apparent.

Improvements in energy utilization and availability of nutrients from feed ingredients are closely associated with increased broiler performance (Olukosi *et al.* 2008). Supplemental enzymes may be incorporated into poultry feed also to reduce feed

cost while obtaining the same weight gain and feed efficiency (Tahir *et al.* 2005; Woyengo *et al.* 2010), and to lessen the environmental impact of animal production by minimizing nutrient excretion, particularly phosphorus and nitrogen in the manure (Patterson *et al*. 1998; Selle and Ravindran 2007). The value of dietary feed enzymes is not easy to set because they increase nutrient utilization and thereby change nutrient density of the feed (Zanella *et al.* 1999). Whenever feed efficiency and/or energy level of feed is changed, the corresponding bird performance as well as feed costs per kg of gain also change (Zou *et al.* 2006).

The objective of the present study was to evaluate the effects of an α-amylase alone or in combination with a β-xylanase on growth performance of broiler chickens fed corn-SBM-based diets from 1 to 40 d. The effects were assessed using diets with decreased AME_n levels and an estimation of the equivalence in AME_n of these enzymes were also proposed.

Materials and methods

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

Birds and experimental diets

A total of 1,800 one-day-old, slow-feathering Cobb × Cobb 500 male broiler chicks, vaccinated for Marek's disease at the hatchery and averaging 44 ± SD g were randomly placed in 72 floor pens (1.65 × 1.65 m; 9.2 birds/m^2; 25 birds per pen). Bedding was of rice hulls and pens were equipped with a 15 kg capacity tube feeder and 3 nipple drinkers. Average temperature was 32ºC at placement being reduced by 1ºC

every 2 d until 23ºC to provide comfort throughout the study with the use of thermostatically controlled heaters, fans and foggers. Lighting was continuous until 7 d of age, with a 14L:10D cycle used afterwards. Birds had *ad libitum* access to water and mash feeds.

Birds were allocated to 8 experimental diets with 9 replications in a completely randomized design using a 2 phases feeding program (starter from 1 to 21 d and finisher from 22 to 40 d). The dietary treatments were formulated to contain typical Brazilian industry nutrient levels (Table 1), but AME_n was variable. The dietary 8 treatments consisted of: a positive control diet (PC, with standard AME_n); two diets with increased AME_n of 0.21 MJ/kg (PC + 0.21) and 0.42 MJ/kg (PC + 0.42), and two diets with AME_n reduction of 0.21 MJ/kg (PC – 0.21) and 0.42 MJ/kg (NC, negative control diet). The NC diet was supplemented with amylase, xylanase, or amylase + xylanase. The supplemental enzymes used in the present study are commercially available (Novozymes A/S, Bagsvaerd, Denmark). Their inclusions per kg of diet were: alpha-amylase [Ronozyme HiStarch (CT)] 80 kilo-Novo α-amylase units (KNU) and beta-xylanase [Ronozyme WX (CT)] 100 fungal β-xylanase units (FXU). All diets were formulated with 1,000 fungal phytase units (FYT) per kg [Ronozyme HiPhos (GT)].

The β-xylanase was a granulated heat-stable endo-xylanase from *Thermomyces lanuginosus* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism containing 1,000 FXU/g. One FXU is the amount of endo-1,4-β-xylanase which liberates 7.8 micromoles of reducing sugars (xylose equivalents) per minute from azo-wheat arabinoxylans at pH 6.0 and 50ºC. The α-amylase was a granulated enzyme preparation produced by submerged fermentation of *Bacillus licheniformis* and contained 600 KNU/g. One KNU is the amount of enzyme that

releases in a two-step reaction, 6 µmol p-nitrophenol per minute from 1.86 mM ethyledene-G7-p-nitrophenyl-maltoheptaoside at pH 7.0 and 37ºC.

Different amounts of NC and PC + 0.42, diets with the lowest and highest formulated AME_n, respectively, were mixed to obtain treatments with intermediate AME_n levels. From 1 to 21 d, broilers were fed experimental diets having 12.35; 12.56; 12.77; 12.98, and 13.19 MJ/kg of AME_n for NC; PC – 0.21; PC; PC + 0.21, and PC + 0.42, respectively. From 22 to 40 d, broilers were fed experimental diets having 12.85; 13.06; 13.27; 13.48, and 13.69 MJ/kg of AME_n for NC; PC – 0.21; PC; PC + 0.21, and PC + 0.42, respectively.

Chicks were individually weighed into groups of 25 birds per pen before placement. Bird weights, averaged by pen were recorded on 1, 7, 14, 21, 28, 35, and 40 days of age. Body weight gain (BWG), feed intake (FI) and FCR corrected for the weight of dead birds were determined weekly and from 1 to 21 d, 22 to 40 d, and 1 to 40 d on a pen-basis. Bird weight was recorded following mortality.

Statistical analyses

Data were analyzed using the GLM procedure of SAS Institute (SAS 2009). Significance was accepted at $P < 0.05$. Data were submitted to a one-way ANOVA and mean differences were separated using Tukey's HSD test (Tukey 1991). An attempt to estimate AME_n provided by the supplemented enzymes was done using the methodology reported by Adedokun *et al.* (2004) and Jendza *et al.* (2006) to estimate P equivalence from phytase. Shortly, linear and quadratic effects of decreasing AME_n were tested for the diets not supplemented with enzymes. The corresponding AME_n for obtained BWG and FCR at each enzyme supplemental levels allowed estimations of

added improvements in AME_n provided by the enzyme at any point of the curve. Regression equations of AME_n and supplemental enzymes (based on formulated values) for a particular response variable were equated and solved for X in quadratic or linear equations.

Results and discussion

Analyses of commercial enzymes added to the experimental diets showed in-feed activities in agreement with the expected values (Table 2). Growth performance was increased when birds were fed the PC + 0.42 in a two-phases feeding program (Table 3). There were no effects of the treatments on mortality (overall grand mean = 1.67%). Broilers fed the PC + 0.42 diet had higher BWG ($P < 0.05$) from 1 to 21 d and from 1 to 40 d than birds fed the NC diet; however the PC + 0.42 was not different than the PC diet. The PC, PC + 0.21 and PC + 0.42 diets had lower FCR ($P < 0.05$) from 22 to 40 d when compared to the NC diet. No differences between PC and NC + amylase or NC + xylanase + amylase were observed on BWG and FCR in the cumulative periods.

The current study showed that growth performance of broilers was more impacted by dietary treatments for broilers until 28 d. A possibility regarding this result is because chicks have been considered rapidly adapted to starch digestion when fed at hatch, although researchers have shown that the total tract starch digestibility in modern fast-growing broiler chickens dropped from 5 to 7 d and was restored to normal high level at 14 d in corn diets (Thomas *et al.* 2008; Svihus 2014). As recently reported by Stefanello (2015), 25-d-old broilers fed corn-soy starter diets supplemented with 80 KNU/kg amylase + 100 FXU/kg xylanase had an increase of 3.5% and 2.4% on starch digestibility in the jejunum and ileum, respectively. Increased starch digestibility when

xylanase and amylase are supplemented seems to have resulted from α-amylase activity in parallel with degradation of soluble and NSP to free sugars, such as arabinose and xylose (Choct *et al.* 2004). Xylanase may increase access of cell contents to endogenous enzymes due to hydrolysis of cell wall arabinoxylans and also reduce the antinutritional effect of some polysaccharides (Kocher *et al.* 2003; Meng *et al.* 2005; Francesch and Geraert 2009).

It has been suggested that it is conceivable that the introduction of xylanase immediately post-hatch results in the gradual emergence of a distal gut microbial community that has an advantageous profile for nutrient recovery and gastrointestinal (GI) tract integrity (Cowieson and O'Neill 2013). Cowieson *et al.* (2010) found that the removal of 2% vegetable oil from a broiler starter diet in order to accommodate the anticipated energy effects of supplemental xylanase resulted in a reduction of 4% in energy utilization. These authors speculated that the removal of dietary fat altered the rate of passage of feed in the GI tract, perhaps mediated via gastric residency. Hence, dietary interventions that are commonly used in enzyme trials, such as dilution of fat, may alter the residency of feed in the gastric gut and, therefore, impair feed digestibility. Finally, the ileal brake mechanism may play an important role in the efficacy of xylanase. Modern broilers may have a feed passage rate that is too rapid for optimal digestibility of nutrients and thus some decrease in transit rate may be beneficial (Croom *et al.* 1999).

Current recommendations of enzymes that are based on ME systems, in particular for enzymes targeting the digestion of fiber such as xylanase and β-glucanase, may be overestimating the net energy contribution that birds can use for growth. The metabolic use of energy substrates in response to the addition of exogenous enzymes, as well as

the interaction of these enzymes, dietary ingredients, and the composition of microbial populations in chickens require further study (Romero *et al.* 2014). These authors also reported that results from digestibility studies are not always an indicative of animal growth or feed efficiency; however, frequently higher responses on nutrient digestibility and energy utilization using exogenous enzymes have been related to the highest broiler performance (Olukosi *et al*. 2008; Stefanello *et al.* 2015). Stefanello *et al.* (2015) observed that AME_n was improved by 0.29 and 0.41 MJ/kg when amylase and amylase + xylanase, respectively, were supplemented in diets for 25-d-old broilers, and an increase of 2.6% on BWG was observed.

Results of the current study support the previous findings such that the negative effect of reducing energy in broiler diets can be partially recovered through the supplementation of exogenous carbohydrases. The observed benefit on FCR within individual dietary phases was observed in the starter phase. The early improvements indicate a positive benefit in young broilers that do not yet have a fully functional GI tract or a mature microflora (Sklan and Noy 2003). This response is in agreement with findings by Stefanello *et al.* (2015) who evaluated growth performance, energy, and nutrient utilization of 25-d-old broilers fed a corn-soy basal diet supplemented with amylase and amylase + xylanase. These authors observed an increase of 2.8% in AME_n and a beneficial impact on BWG, FCR and starch digestibility of broilers supplementing corn-soy diets with amylase + xylanase. Furthermore, Meng and Slominski (2005) reported that a higher corn inclusion level used in finisher phases resulted in relative lower starch digestibility due to the higher NSP contents, suggesting that NSP-degrading enzymes potentially improve starch digestibility in corn-based diets.

Significant differences between low and high energy levels were also observed in this present growth performance experiment. Bao *et al.* (2013) also reported that is generally accepted that ME improvements in response to exogenous enzymes in lower energy diets are greater than the diets with higher levels. Sorbara *et al.* (2009) showed that the use of an α-amylase-β-glucanase complex or β-xylanase during the finisher phase using a NC corn-SBM diet with 0.50 MJ/kg less than the PC diet, improved BWG, especially in broilers fed 80 KNU/kg + 100 FXU/kg when compared to the NC diet. Considering the whole period of our study, FCR was 5.0% lower in broilers fed the PC diet than those fed the NC, and these results are also in agreement with Sorbara *et al.* (2009) that found FCR 3.5% lower in the PC than in the NC diet.

Table 4 shows that BWG and FCR linearly fit to AME_n reductions in the diets without enzyme supplementation ($P < 0.05$), but no effects were observed on feed intake in all weekly and cumulative evaluations. Linear adjustments ($P < 0.05$) for BWG and FCR were obtained from placement to the end of the study when birds were fed amylase, xylanase, or the combination.

Apparent metabolizable energy equivalence was calculated using BWG and FCR of the birds fed the different AME_n levels compared with the responses obtained when AME_n was reduced in diet without enzyme supplementation. These equivalences varied with age and carbohydrases supplementation in the feed, reaching the lowest values in the starter phase and highest in the finisher. Estimates for the entire period were 0.41, 0.35, and 0.57 MJ/kg for BWG and 0.17, 0.11, and 0.18 MJ/kg for FCR when birds were fed amylase, xylanase, and amylase + xylanase, respectively.

Equivalences in AME_n obtained in the present study were 0.41 MJ/kg for BWG and 0.17 MJ/kg for FCR when broilers were fed the NC + amylase diet from 1 to 40 d.

Vieira *et al.* (2015) found similar results when broilers were fed corn-SBM diets supplemented with an α-amylase-β-glucanase complex containing 80 KNU/kg also until 40 d. These authors observed that equivalence estimations were 0.23 MJ/kg for BWG and 0.41 MJ AME_n/kg for FCR. There are no reports in which the AME_n equivalences have been determined in diets supplemented with xylanase or amylase + xylanase for broiler chickens.

Broiler chickens fed diets that are essentially adequate in all nutrients often still respond to exogenous enzyme addition, suggesting that enzyme benefits may result from changes in less tangible metrics such as appetite control, digestive physiology, immunology, or microbiology (Bao *et al.* 2013). No differences between dietary treatments were observed on FI in the current study, and this is in accordance with Vieira *et al.* (2015) that had no different results when birds were fed corn-SBM diets with AME_n reduction (-0.50 MJ/kg) and supplemented with an α-amylase-β-xylanase complex. However, Kocher *et al.* (2003) reported that improvements in nutrient availability with carbohydrase supplementation often result in reduced FI, as a consequence of greater energy availability, leading to improved feed efficiency.

In conclusion, growth performance was improved when birds were fed corn-soy diets with higher energy levels. Broilers fed the NC diet with 0.42 MJ/kg of AME_n reduction and supplemented with 80 KNU/kg + 100 FXU/kg in the starter phase had improved body weight gain and feed conversion ratio. Apparent metabolizable energy estimates for the entire period were 0.41, 0.35, and 0.57 MJ/kg for BWG; and 0.17, 0.11, and 0.18 MJ/kg for FCR when broilers were fed amylase, xylanase, and amylase + xylanase, respectively.

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Table 1. Ingredient and nutrient composition of the experimental diets (as-is basis)

Ingredients, g/kg	Starter (1 to 21 d)		Finisher (22 to 40 d)	
	NC[A]	PC + 0.42[B]	NC	PC + 0.42
Corn	536.5	541.2	577.2	583.7
Soybean meal	379.0	378.1	331.0	330.0
Soybean oil	25.7	46.9	36.6	57.1
Dicalcium phosphate	8.7	8.7	5.6	5.6
Limestone	13.3	13.3	11.8	11.8
Salt	4.7	4.7	4.5	4.5
DL-Methionine 990 g/kg	2.7	2.7	2.4	2.4
L-Lysine HCl 760 g/kg	1.4	1.4	1.7	1.7
L-Threonine 985 g/kg	0.4	0.4	0.4	0.4
Choline chloride 600 g/kg	0.8	0.8	1.0	1.0
Vitamin and mineral mix[C]	1.5	1.5	1.5	1.5
Kaolin	25.0	0.0	26.0	0.0
Phytase[D]	0.1	0.1	0.1	0.1
Calculated nutrient composition				
AME_n, MJ/kg	12.35	13.19	12.85	13.69
CP, g/kg	223.8	223.8	204.1	204.2
Ca, g/kg	8.9	8.9	7.6	7.6
Non-phytate P, g/kg	4.4	4.4	3.7	3.7
Total P, g/kg	5.2	5.2	4.6	4.6
Na, g/kg	2.0	2.0	2.0	2.0
Chloride, g/kg	3.5	3.5	3.5	3.5
Dig. Lys, g/kg[E]	12.3	12.3	11.3	11.3
Dig. TSAA, g/kg	9.0	9.0	8.3	8.3
Dig. Thr, g/kg	7.7	7.7	7.2	7.2
Dig. Trp, g/kg	2.4	2.4	2.1	2.1
Dig. Arg, g/kg	14.1	14.1	12.7	12.7
Dig. Val, g/kg	9.3	9.3	8.5	8.5
Dig. Ile, g/kg	8.9	8.9	8.1	8.1

[A]NC = negative control was the positive control with a reduction of 0.42 MJ/kg AME_n.

[B]PC + 0.42 = positive control + 0.42 MJ/kg AME_n.

[C]Composition per kg of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; sodium monensin 40%, 120 mg, and avilamycin, 10 mg (Elanco Animal Health, Greenfield, IN).

[D]Ronozyme HiPhos (GT) with 10,000 FYT/g (Novozymes A/S, Bagsvaerd, Denmark).

[E]Ratios of digestible amino acids to digestible Lys were maintained at TSAA: 0.75; Thr: 0.65; Val: 0.70; Trp: 0.17; Arg: 1.08; Ile: 0.67 (Rostagno et al., 2011).

Table 2. Declared and analyzed activities of amylase, xylanase and phytase in the experimental diets

Treatment	Amylase, KNU/kg[A]			Xylanase, FXU/kg[B]			Phytase, FYT/kg[C]		
	Declared	Analyzed		Declared	Analyzed		Declared	Analyzed	
		Starter	Finisher		Starter	Finisher		Starter	Finisher
NC[D]	0	<LOD[E]	<LOD	0	<LOD	<LOD	1,000	1,023	1,042
PC – 0.21 MJ/kg	0	<LOD	<LOD	0	<LOD	<LOD	1,000	1,123	1,077
PC[F]	0	<LOD	<LOD	0	<LOD	<LOD	1,000	1,025	1,069
PC + 0.21 MJ/kg	0	<LOD	<LOD	0	<LOD	<LOD	1,000	1,056	1,064
PC + 0.42 MJ/kg	0	<LOD	<LOD	0	<LOD	<LOD	1,000	1,067	1,099
NC + amylase	80	85	88	0	<LOD	<LOD	1,000	1,050	1,030
NC + xylanase	80	89	84	100	106	109	1,000	1,087	1,045
NC + amylase + xylanase	80	88	86	100	103	111	1,000	1,083	1,047

[A]KNU = kilo-Novo α-amylase units per kg of feed.
[B]FXU = fungal β-xylanase units per kg of feed.
[C]FYT = phytase units per kg of feed.
[D]NC = negative control was the positive control with a reduction of 0.42 MJ/kg AME_n.
[E]LOD = limit of detection.
[F]PC = positive control.

Table 3. Growth performance of broilers fed diets with different AME_n and supplemented with carbohydrases

Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test. n.s., not significant ($P > 0.05$). s.e.m., standard error of the mean

Parameter	1 to 21 d		22 to 40 d		1 to 40 d	
	BWG,[A] g	FCR[B]	BWG, g	FCR	BWG, g	FCR
NC[C]	978c	1.316a	1,912	1.535a	2,889b	1.450a
PC – 0.21 MJ/kg	995bc	1.299a	1,913	1.518ab	2,897b	1.420abcd
PC[D]	1,006ab	1.284ab	1,919	1.484bc	2,924ab	1.381bcd
PC + 0.21 MJ/kg	1,018ab	1.258bc	1,936	1.467c	2,968ab	1.374cd
PC + 0.42 MJ/kg	1,029a	1.232c	1,952	1.453c	2,983a	1.368d
NC + amylase[E]	995bc	1.299a	1,940	1.516ab	2,931ab	1.433ab
NC + xylanase[F]	998bc	1.302a	1,932	1.532a	2,923ab	1.444a
NC + amylase + xylanase[G]	997bc	1.295a	1,946	1.516ab	2,950ab	1.432abc
Mean[H]	1,002	1.285	1,931	1.501	2,933	1.413
s.e.m	2.604	0.004	6.178	0.004	8.021	0.006
P-value	<0.001	<0.001	n.s.	<0.001	<0.036	<0.001

[A]BW gain.
[B]Feed conversion ratio corrected for the weight of dead birds.
[C]NC = negative control was the positive control with a reduction of 0.42 MJ/kg AME_n.
[D]PC = positive control.
[E]Supplemented with 80 kilo-Novo α-amylase units/kg.
[F]Supplemented with 100 fungal β-xylanase units/kg.
[G]Supplemented with 80 kilo-Novo α-amylase units/kg + 100 fungal β-xylanase units/kg.
[H]Means were obtained from 9 replicate pens of 25 birds per replicate pen.

Table 4. Regression equations of increasing levels of AME_n

Parameter	Regression equations[A]	r^2	*P*-value[B]	AME_n estimations, MJ/kg[C]		
				Amylase[D]	Xylanase[E]	Amylase + xylanase[F]
BWG[G] 1 to 21 d, g	Y = 59.31x + 980.33	0.5010	<0.001	0.28	0.25	0.30
BWG 22 to 40 d, g	Y = 49.63x + 1,905	0.1840	<0.051	0.70	0.54	0.82
BWG 1 to 40 d, g	Y = 122.80x + 2,880	0.2654	<0.003	0.41	0.35	0.57
FCR[H] 1 to 21 d, g	Y = -0.099x + 1.319	0.6851	<0.001	0.20	0.21	0.24
FCR 22 to 40 d, g	Y = -0.102x + 1.534	0.5494	<0.001	0.15	0.07	0.21
FCR 1 to 40 d, g	Y = -0.100x + 1.441	0.3689	<0.001	0.21	0.11	0.18

[A]Regression equations for AME_n levels from 1 to 21 d (12.35; 12.56; 12.77; 12.98, and 13.19 MJ/kg) and from 22 to 40 d (12.85; 13.06; 13.27; 13.48, and 13.69 MJ/kg). The coefficient of determination (r^2) was obtained using all data.
[B]Linear effect ($P < 0.05$).
[C]Determined based on response of the means to graded addition of energy for each parameter. The difference between the levels of AME_n (0; 0.21; 0.42; 0.63, and 0.84 MJ/kg) was used to obtain the relative bioequivalence.
[D]Supplemented with 80 kilo-Novo α-amylase units/kg.
[E]Supplemented with 100 fungal β-xylanase units/kg.
[F]Supplemented with 80 kilo-Novo α-amylase units/kg + 100 fungal β-xylanase units/kg.
[G]Body weight gain.
[H]Feed conversion ratio corrected for the weight of dead birds.

CAPÍTULO V

CONSIDERAÇÕES FINAIS

O método de substituição utilizado neste estudo demonstrou que a digestibilidade da proteína bruta e extrato etéreo, bem como a utilização da energia foram maiores em frangos de corte alimentados com dietas milho-soja quando comparadas à dieta teste com maior inclusão de milho. A digestibilidade da proteína bruta e a energia das dietas milho-soja, dieta teste à base de milho ou 100% milho foram maiores com a suplementação de beta-xilanase.

Frangos de corte alimentados com dietas milho-farelo de soja formuladas com fitase ou suplementadas com beta-xilanase e alfa-amilase tiveram maior ganho de peso e menor conversão alimentar que as aves que receberam dietas sem enzimas. A energia metabolizável aparente também aumentou quando os frangos receberam dietas suplementadas com amilase + xilanase comparada à dieta sem carboidrases. A digestibilidade do amido no jejuno foi menor que no íleo e dietas milho-soja formuladas com fitase ou suplementadas com amilase + xilanase melhoraram a digestibilidade do amido.

O fornecimento de dietas milho-soja formuladas com níveis crescentes de energia proporcionou melhoria no desempenho dos frangos, com aumento no ganho de peso e diminuição da conversão alimentar.

Estudos realizados para avaliar a digestibilidade de nutrientes e a utilização da energia em dietas suplementadas com carboidrases podem receber maior ênfase mediante a avaliação concomitante do desempenho produtivo dos frangos de corte, pois possibilitam também visualizar reais ganhos resultantes da produção avícola. Avaliar a degradação de substratos específicos através da suplementação enzimática também se faz importante para entender as respostas obtidas e o modo de ação dos produtos enzimáticos. Isto recebe maior importância no estudo de enzimas monocomponentes e, por exemplo, quando xilanases são adicionadas em rações, visto que atuam em diferentes porções de polissacarídeos e contribuem de maneiras distintas com os resultados de digestibilidade. Pesquisas prévias objetivando avaliar a atuação de enzimas monocomponentes são importantes devido a suas respostas e contribuem para o maior entendimento quando combinações de enzimas são suplementadas em dietas para frangos de corte.

Vale ressaltar que as enzimas exógenas podem atuar melhorando o aproveitamento de nutrientes, desde que estes estejam presentes nos ingredientes que compõem as dietas para aves. As metodologias utilizadas para avaliar a digestibilidade de nutrientes e a utilização da energia também podem influenciar os resultados quando enzimas são suplementadas em rações. Portanto, deve-se buscar um maior conhecimento sobre outros fatores que também exercem influência nas respostas, como atividade enzimática e a presença de substrato ou composição dos ingredientes utilizados nas formulações.

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APÊNDICES

Apêndice 1. Instruções para publicação na revista Poultry Science

POULTRY SCIENCE INSTRUCTIONS TO AUTHORS [1]

Editorial Policies and Procedures

Poultry Science publishes the results of fundamental and applied research concerning poultry, poultry products, and avian species in general. Submitted manuscripts shall provide new facts or confirmatory data. Papers dealing with experimental design, teaching, extension endeavors, or those of historical or biographical interest may also be appropriate. A limited number of review papers will be considered for publication if they contribute significant additional knowledge, or synthesis of knowledge, to a subject area. Papers that have been, or are scheduled to be, published elsewhere will not be accepted. Publication of a preliminary report, such as an abstract, does not preclude consideration of a complete report for publication as long as it has not been published in full in a proceedings or similar scientific publication; appropriate identification of previously published preliminary reports should be provided in a title page footnote. Translation of an article into other languages for publication requires approval by the editor-in-chief. Opinions or views expressed in papers published by *Poultry Science* are those of the author(s) and do not necessarily represent the opinion of the Poultry Science Association or the editor-in-chief.

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For information on the scientific content of the journal, contact the editor-in-chief, Dr. Tom Porter, Department of Animal and Avian Sciences, University of Maryland, College Park, Building 142, College Park, MD 20742; e-mail: ps-editor@umd.edu.

For assistance with ScholarOne Manuscripts, manu- script submission, supplemental files, copyright forms, or other information, contact Nes Diaz, Oxford University Press, 198 Madison Ave., New York, NY 10016 (nes.diaz@oup.com).

Care and Use of Animals

Authors must make it clear that experiments were con- ducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and lab- oratory techniques. Experiments shall be conducted in accordance with the principles and specific guidelines pre- sented in *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 3rd edition, 2010 (Association Headquarters, Champaign, IL 61820); and, if applicable, *Guide for the Care and Use of Laboratory Animals* (United States Department of Human Health and Services, Na- tional Institutes of Health, Publication Number ISBN 0-309-05377-3, 1996); or *Guide to the Care and Use of Experi- mental Animals,* 2nd ed. Volume 1, 1993 (Canadian Coun- cil on Animal Care). Methods of killing experimental ani- mals must be described in the text. In describing surgical procedures, the type and dosage of the anesthetic agent must be specified. Intra-abdominal and intrathoracic in- vasive surgery requires anesthesia. This includes capon- ization. The editor-in-chief of *Poultry Science* may refuse to publish manuscripts that are not compatible with these guides. If rejected solely on that basis, however, the paper may be

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Types of Articles

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

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

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The section editor assigns two reviewers, at least one of whom is an associate editor. Each reviewer has 3 weeks to review the manuscript, after which his or her comments are forwarded to the section editor. The sec- tion editor may recommend rejection or acceptance at this point, after which the manuscript

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General

Papers must be written in English. The text and all sup- porting materials must use American spelling and usage as given in *The American Heritage Dictionary, Webster's Third New International Dictionary,* or the *Oxford Ameri- can English Dictionary.* Authors should follow the style and form recommended in *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers.* 2006. 7th ed. Style Manual Committee, Council of Science Editors, Reston, VA.
Authors should prepare their manuscripts with Microboldface and italic. Text that follows a first subheading should be in a new paragraph.
Second Subheadings. Second subheadings begin the first line of a paragraph. They are indented, boldface, italic, and followed by a period. The first letter of each important word should be capitalized. The text follows immediately after the final period of the subheading.

Title Page

The title page shall begin with a running head (short title) of not more than 45 characters. The running head is centered, is in all capital letters, and shall appear on the top of the title page. No abbreviations should be used.
The title of the paper must be in boldface; the first letter of the article title and proper names are capitalized, and the remainder of the title is lowercase. The title must not have abbreviations.
Under the title, names of authors should be typed (first name or initial, middle initial, last name). Affili- ations will be footnoted using the following symbols:
*, †, ‡, §, #, ‖, and be placed below the author names. Do not give authors' titles, positions, or degrees. Num- bered footnotes may be used to provide supplementary information, such as present address, acknowledgment of grants, and experiment station or journal series num- ber. The corresponding author should be indicated with 1 soft Word and upload them using the fewest files pos a numbered footnote (e.g., Corresponding author: mysible to facilitate the review and editing process.

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

Preparing the Manuscript File

Manuscripts should be typed double-spaced, with lines and pages numbered consecutively, using Times New Roman font at 12 points. All special characters (e.g., Greek, math, symbols) should be inserted using the sym- bols palette available in this font. Complex math should be entered using MathType from Design Science (http:// www.dessci.com). Tables and figures should be placed in separate sections at the end of the manuscript (not placed within the text). Failure to follow these instructions may result in an immediate rejection of the manuscript.

Headings

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

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

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

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

Abbreviations

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

Abstract

The Abstract disseminates scientific information through abstracting journals and through conveniencefor the readers. The Abstract, consisting of not more than 325 words, appears at the beginning of the manuscript with the word ABSTRACT without a following period. It must summarize the major objectives,

methods, results, conclusions, and practical applications of the research. The Abstract must consist of complete sentences and use of abbreviations should be limited. References to other work and footnotes are not permitted. The Abstract and Key Words must be on a separate sheet of paper.

Key Words

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

Introduction

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

Materials and Methods

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

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

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

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

Vitamin A

1 IU = 0.3 µg of all-*trans* retinol
1 IU = 0.344 µg of retinyl acetate
1 IU = 0.552 µg of retinyl palmitate

1 IU = 0.60 µg of β-carotene
Vitamin E
1 IU = 1 mg of dl-α-tocopheryl acetate
1 IU = 0.91 mg of dl-α-tocopherol
1 IU = 0.67 mg of d-α-tocopherol
In the instance of vitamin D3, cholecalciferol is the ac- ceptable term on the basis that 1 IU of vitamin D3 = 0.025 µg of cholecalciferol. The sources of vitamins A and E must be specified in parentheses immediately following the stated concentrations.

Statistical Analysis. Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statisti- cal methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Refer- ence to a statistical package without reporting the sourc- es of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical anal- ysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar ex- periments separately.
The experimental unit is the smallest unit to which an individual treatment is imposed. For group-fed animals, the group of animals in the pen is the experimental unit; therefore, groups must be replicated. Repeated chemi- cal analyses of the same sample usually do not consti- tute independent experimental units. Measurements on the same experimental unit over time also are not inde- pendent and must not be considered as independent ex- perimental units. For analysis of time effects, use time- sequence analysis.
Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. For ex- ample, it is recommended that percentage data between 0 and 20 and between 80 and 100 be subjected to arc sin transformation prior to analysis. Most statistical pro- cedures are based on the assumption that experimental units have been assigned to treatments at random. If ani- mals are stratified by ancestry or weight or if some other initial measurement should be accounted for, the model should include a blocking factor, or the initial measure- ment should be included as a covariate.
A parameter [mean (µ), variance (σ2)], which defines or describes a population, is estimated by a statistic (x, s2). The term **parameter** is not appropriate to describe a vari- able, observation, trait, characteristic, or measurement taken in an experiment.
Standard designs are adequately described by name and size (e.g., “a randomized complete block design with 6 treatments in 5 blocks”). For a factorial set of treatments, an adequate description might be as follows: “Total sulfur amino acids at 0.70 or 0.80% of the diet and Lys at 1.10, 1.20, or 1.30%

of the diet were used in a 2 × 3 factorial ar- rangement in 5 randomized complete blocks consisting of initial BW." Note that **a factorial arrangement is not a de- sign**; the term "design" refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted).

Standard deviation refers to the variability in a sample or a population. The standard error (calculated from er- ror variance) is the estimated sampling error of a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not "statistically significant" is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by "±" to a number implies that the second value is its standard error (not its standard deviation). Adequate re- porting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of ex- perimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of ob- servations or the heterogeneity of the error variance is to be emphasized. Presenting individual standard errors clutters the presentation and can mislead readers.

For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator of each *F* sta- tistic. Unbalanced factorial data can present special prob- lems. Accordingly, it is well to state how the computing was done and how the parameters were estimated. Ap- proximations should be accompanied by cautions con- cerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was de- signed; they should form the basis for comparing treat- ment means. Nonorthogonal contrasts may be evalu- ated by Bonferroni *t* statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally ar- ranged. Fixed-range, pairwise, multiple-comparison tests should be used only to compare means of treatments that are unstructured or not related. Least squares means are the correct means to use for all data, but arithmetic means are identical to least squares means unless the design is unbalanced or contains missing values or an adjustment is being made for a covariate. In factorial treatment ar- rangements, means for main effects should be presented when important interactions are not present. However, means for individual treatment combinations also should be provided in table or text so that future researchers may combine data from several experiments to detect impor- tant interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

The terms significant and highly significant tradition- ally have been reserved for $P < 0.05$ and $P < 0.01$, re- spectively; however, reporting the *P*-value is preferred to the use of these terms. For example, use ". . . there was a difference ($P < 0.05$) between control and treated samples" rather than ". . . there was a significant ($P < 0.05$) difference between control and treated samples." When available, the observed significance level (e.g., $P = 0.027$) should be presented rather than merely $P < 0.05$ or $P < 0.01$, thereby allowing the reader to decide what to reject. Other probability (α) levels may be discussed if properly qualified so that the reader is not misled. Do not report *P*-values to more than 3 places after the deci- mal. Regardless of the probability level used, failure to reject a hypothesis should be based on the relative consequences of type I and II errors. A "nonsignificant" rela- tionship should not be interpreted to suggest the absence of a relationship. An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of $P > 0.05$ to declare nonsignificance, such as indicating that a difference is not significant at $P > 0.05$ and subsequently declaring another difference significant (or a tendency) at $P < 0.09$. In addition, readers may incorrectly interpret the use of $P > 0.05$ as the probability of a β error, not an α error.

Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information con- tained in the data is sacrificed. Significant digits in data reported should be restricted to 3 beyond the decimal point, unless warranted by the use of specific methods.

Results and Discussion

Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Re- sults section shall contain only the results and summary of the author's experiments; there should be no literature comparisons. Those comparisons should appear in the Discussion section. Manuscripts reporting sequence data must have GenBank accession numbers prior to submit- ting. One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experi- ments are adequately replicated. The results of experi- ments must be replicated, either by replicating treatments within experiments or by repeating experiments.

Acknowledgments

An Acknowledgments section, if desired, shall follow the Discussion section. Acknowledgments of individuals should include affiliations but not titles, such as Dr., Mr., or Ms. Affiliations shall include institution, city, and state.

Appendix

A technical Appendix, if desired, shall follow the Dis- cussion section or Acknowledgments, if present. The Appendix may contain supplementary material, expla- nations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel com- puter programs or

mathematical computations would be appropriate. The Appendix will not be a repository for raw data.

References

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

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

Citation of abstracts, conference proceedings, and oth- er works that have not been peer reviewed is strongly discouraged unless essential to the paper. Abstract and proceedings references are not apropriate citations in the Materials and Methods section of a paper.

In the References section, references shall first be list- ed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the au- thors' names. As with text citations, two or more publi- cations by the same author or set of authors in the same year shall be differentiated by adding lowercase letters

after the date. The dates for papers with the same first author that would be abbreviated in the text as et al., even though the second and subsequent authors differ, shall also be differentiated by letters. All authors' names must appear in the Reference section. Journals shall be abbreviated according to the conventional ISO abbrevia- tions given in journals database of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/que- ry.fcgi?db=journals). One-word titles must be spelled out. Inclusive page numbers must be provided. Sample references are given below. Consult recent issues of *Poultry Science* for examples not included below.

Article:

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

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

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

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

Tables
Tables must be created using the MS Word table fea- ture and inserted in the manuscript after the references section. When possible, tables should be organized to fit across the page without running broadside. Be aware of the dimensions of the printed page when planning tables (use of more than 15 columns will create layout prob- lems). Place the table number and title on the same line above the table. The table title does not require a period. Do not use vertical lines and use few horizontal lines. Use of bold and italic typefaces in the table body should be done sparingly; such use must be defined in a footnote. Each table must be on a separate page. To facilitate place- ment of all tables into the manuscript file (just after the references) authors should use “section breaks” rather than “page breaks” at the end of the manuscript (before the tables) and between tables.
Units of measure for each variable must be indicated. Papers with several tables must use consistent format. All columns must have appropriate headings. Abbreviations not found on the inside front cover of the journal must be defined in each table and must match those used in the text. Footnotes to tables should be marked by superscript numbers. Each footnote should begin a new line.
Superscript letters shall be used for the separation of means in the body of the table and explanatory footnotes must be provided [i.e., “Means within a row

lacking a common superscript differ ($P < 0.05$)."]; other significant *P*-values may be specified. Comparison of means within rows and columns should be indicated by different series of superscripts (e.g., a,b, . . . in rows; x–z . . . in columns) The first alphabetical letter in the series (e.g., a or A) shall be used to indicate the largest mean. Lowercase super- scripts indicate $P \leq 0.05$. Uppercase letters indicate $P \leq 0.01$ or less.
Probability values may be indicated as follows: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, and $\dagger P \leq 0.10$. Consult a recent issue of *Poultry Science* for examples of tables.

Figures
To facilitate review, figures should be placed at the end of the manuscript (separated by section breaks). Each figure should be placed on a separate page, and identi- fied by the manuscript number and the figure number. A figure with multiple panels or parts should appear on one page (e.g., if Figure 1 has parts a, b, and c, place all of these on the same page). Figure captions should be typed (double spaced) on a separate page.
• ***Figure Size.*** Prepare figures at final size for publi- cation. Figures should be prepared to fit one column (8.9 cm wide), 2 columns (14 cm wide), or full-page width (19 cm wide).
• ***Font Size.*** Ensure that all type within the figure and axis labels are readable at final publication size. A minimum type size of 8 points (after reduction) should be used.
• ***Fonts.*** Use Helvetica or Times New Roman. Sym- bols may be inserted using the Symbol palette in Times New Roman.
• ***Line Weight.*** For line graphs, use a minimum stroke weight of 1 point for all lines. If multiple lines are to be distinguished, use solid, long-dash, short-dash, and dotted lines. Avoid the use of color, gray, or shaded lines, as these will not reproduce well. Lines with different symbols for the data points may also be used to distinguish curves.
• ***Axis Labels.*** Each axis should have a description and a unit. Units may be separated from the de- scriptor by a comma or parentheses, and should be consistent within a manuscript.
• ***Shading and Fill Patterns.*** For bar charts, use dif- ferent fill patterns if needed (e.g., black, white, gray, diagonal stripes). Avoid the use of multiple shades of gray, as they will not be easily distinguishable in print.
• ***Symbols.*** Identify curves and data points using the following symbols only: □, ■, ○, ●, ▲, ▼, n, ,, e, r, +, or ×. Symbols should be defined in a key on the figure if possible.
• ***File Formats.*** Figures can be submitted in Word, PDF, EPS, TIFF, and JPEG. Avoid PowerPoint files and other formats. For the best printed quality, line art should be prepared at 600 ppi. Grayscale and color images and photomicrographs should be at least 300 ppi.
• ***Grayscale Figures.*** If figures are to be reproduced in grayscale (black and white), submit in grayscale. Often color will mask contrast problems that are ap- parent only when the figure is reproduced in gray- scale.
• ***Color Figures.*** If figures are to appear in color in the print journal, files must be submitted in CMYK color (not RGB).

• ***Photomicrographs.*** Photomicrographs must have their unmagnified size designated, either in the cap- tion or with a scale bar on the figure. Reduction for publication can make a magnification power desig- nation (e.g., 100×) inappropriate.
• ***Caption.*** The caption should provide sufficient in- formation that the figure can be understood with excessive reference to the text. All author-derived abbreviations used in the figure should be defined in the caption.
• ***General Tips.*** Avoid the use of three-dimensional bar charts, unless essential to the presentation of the data. Use the simplest shading scheme possible to present the data clearly. Ensure that data, symbols, axis labels, lines, and key are clear and easily read- able at final publication size.
Color Figures. Submitted color images should be at least 300 ppi. The cost to publish each color figure is $600; a surcharge for color reprints ordered will be assessed. Authors must agree in writing to bear the costs of color production after acceptance and prior to publication of the paper.

Miscellaneous Usage Notes
Abbreviations. Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD). A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the scien- tific field. Abbreviations may be used in heads within the paper, if they have been first defined within the text. The inside back cover of every issue of the journal lists ab- breviations that can be used without definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and edi- tors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used less than three times must be spelled out in full rather than abbreviated. All terms are to be spelled out in full with the abbrevia-tion following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbre- viations shall be used consistently thereafter, rather than the full term.
The abstract, text, each table, and each figure must be understood independently of each other. Therefore, ab- breviations shall be defined within each of these units of the manuscript.
EST expressed sequence tag g gram
g gravity
G guanine
GAT glutamic acid-alanine-tyrosine
G:F gain-to-feed ratio
GLM general linear model
h hour
HEPES *N*-2-hydroxyethyl piperazine-*N*′-ethane-sulfonic acid
HPLC high-performance (high-pressure) liquid chromatography

ICU international chick units
Ig immunoglobulin
IL interleukin
IU international units
kb kilobase pairs
kDa kilodalton
L liter*
L:D hours light:hours darkness in a photoperiod (e.g., 23L:1D)
m meter
µ micro
M molar
MAS marker-assisted selection
ME metabolizable energy
MEn nitrogen-corrected metabolizable energy
MHC major histocompatibility complex
mRNA messenger ribonucleic acid
min minute
mo month
MS mean square
n number of observations
N normal
NAD nicotinamide adenine dinucleotide
NADH reduced nicotinamide adenine dinucleotide
NRC National Research Council
NS not significant
PAGE polyacrylamide gel electrophoresis
PBS phosphate-buffered saline
PCR polymerase chain reaction
pfu plaque-forming units
QTL quantitative trait loci
r correlation coefficient
r2 coefficient of determination, simple 2
R coefficient of determination, multiple

Plural abbreviations do not require “s.” Chemical symbols and three-letter abbreviations for amino acids do not need definition. Units of measure, except those in the standard *Poultry Science* abbreviation list, should be ab- breviated as listed in the *CRC Handbook for Chemistry and Physics* (CRC Press, 2000 Corporate Blvd., Boca Raton, FL 33431) and do not need to be defined.

The following abbreviations may be used without definition in *Poultry Science.*
A adenine
ADG average daily gain
ADFI average daily feed intake
AME apparent metabolizable energy
AMEn nitrogen-corrected apparent metabolizable energy
ANOVA analysis of variance
B cell bursal-derived, bursal-equivalent derived cell

bp base pairs
BSA bovine serum albumin
BW body weight
C cytosine
cDNA complementary DNA
cfu colony-forming units
CI confidence interval
CP crude protein
cpm counts per minute
CV coefficient of variation
d day
df degrees of freedom
DM dry matter
DNA deoxyribonucleic acid
EDTA ethylenediaminetetraacetate
ELISA enzyme-linked immunosorbent antibody assay
RFLP restriction fragment length polymorphism
RH relative humidity
RIA radioimmunoassay
RNA ribonucleic acid
rpm revolutions per minute
s second
SD standard deviation
SDS sodium dodecyl sulfate
SE standard error
SEM standard error of the mean
SRBC sheep red blood cells
SNP single nucleotide polymorphism
T thymine
TBA thiobarbituric acid
T cell thymic-derived cell
TME true metabolizable energy
TMEn nitrogen-corrected true metabolizable energy
Tris tris(hydroxymethyl)aminomethane
TSAA total sulfur amino acids
U uridine
USDA United States Department of Agriculture
UV ultraviolet
vol/vol volume to volume
vs. versus
wt/vol weight to volume
wt/wt weight to weight
wk week
yr year
*Also capitalized with any combination, e.g., mL.

International Words and Phrases. Non-English words in common usage (defined in recent editions of standard dictionaries) will not appear in italics (e.g., invitro, in vivo, in situ, a priori). However, genus and spe- cies of plants,

animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. German nouns shall begin with capital letters.

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

Number Style. Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits. Measures must be in the metric system; however, US equivalents may be given in parentheses. *Poultry Science* requires that measures of energy be given in calories rather than joules, but the equivalent in joules may be shown in parentheses or in a footnote to tables. Units of measure not preceded by numbers must be writ- ten out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract and in the body of the paper at first use. Units of measure for feed conversion or feed efficiency shall be provided (i.e., g:g).

Nucleotide Sequences. Nucleotide sequence data must relate to poultry or poultry pathogens and must complement biological data published in the same or a companion paper. If sequences are excessively long, it is suggested that the most relevant sections of the data be published in *Poultry Science* and the remaining se- quences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submis- sion of sequence data to one of the databases. The fol- lowing statement should appear as a footnote to the title on the title page of the manuscript. “The nucleotide se- quence data reported in this paper have been submitted to GenBank Submission (Mail Stop K710, Los Alamos Na- tional Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN.”

Publication of the description of molecular clones is as- sumed by the editors to place them in the public sector. Therefore, they shall be made available to other scientists for research purposes.

Nucleotide sequences must be submitted as camera- ready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow *Poultry Science* guidelines.

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

General Usage. Note that “and/or” is not permitted; choose the more appropriate meaning or use “x or y or both.”

Use the slant line only when it means “per” with num- bered units of measure or “divided by” in equations. Use only one slant line in a given expression (e.g., g/d per chick). The slant line may not be used to indicate ratios or mixtures.

Use “to” instead of a hyphen to indicate a range.

Insert spaces around all signs (except slant lines) of operation (=, –, +, ×, >, or <, etc.) when these signs occur between two items.

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

Restrict the use of “while” and “since” to meanings related to time. Appropriate substitutes include “and,” “but,” or “whereas” for “while” and “because” or “although” for “since.”
Leading (initial) zeros should be used with numbers less than 1 (e.g., 0.01).
Commas should be used in numbers greater than 999.
Registered (®) and trademark (™) symbols should not be used, unless as part of an article title in the References section. Trademarked product names should be capitalized.

Supplemental Information
The following information is available online and up- dated regularly. Please refer to these pages when prepar- ing a manuscript for submission.
Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online: http://www.oxfordjournals.org/our_journals/ps/for _authors/index.html
SI Units. The following site (National Institute of Standards and Technology) provides a comprehensive guide to SI units and usage: http://physics.nist.gov/Pubs/SP811/ contents.html
Figure Preparation Guidelines. Current detailed information on figure preparation can be found at http:// www.oxfordjournals.org/for_authors/figures.html
ScholarOne Manuscripts Instructions. Manuscripts are submitted online (http://mc04.manuscriptcentral. com/ps). Full user instructions for using the ScholarOne Manuscripts system are available on the ScholarOne Manuscripts home page.

Apêndice 2. Instruções para publicação na revista Animal Production Science

ANIMAL PRODUCTION SCIENCE*:*
Instructions to Authors[1]

Editorial Policies and Procedures
Author Instructions
All manuscripts should be submitted via ScholarOne Manuscripts.
Animal Production Science welcomes the submission of articles presenting original and significant research that are within the journal´s scope.

Journal policy and scope
Research papers in Animal Production Science focus on improving livestock and food production, and on the social and economic issues that influence primary producers. The journal is predominantly concerned with domesticated animals (beef cattle, dairy cows, sheep, pigs, goats and poultry); however, contributions on horses and wild animals may be published where relevant. Animal Production Science publishes original research papers, critical review articles, and viewpoints; it does not publish technical and research notes, or short communications.
High quality original contributions are encouraged on: animal breeding and genetics animal nutrition and reproduction livestock farming systems, sustainability and natural resource management meat science and consumer acceptability behaviour, health and welfare feed quality and nutritional value biopharmaceuticals derived from animals.
The subject scope extends from the molecular level through to the role of animals in farming systems. The target readership is animal scientists, and administrators and policymakers who interface with this discipline.

Review papers
Prestigious, invited reviews are commissioned from authors who are world leaders in the animal sciences. Reviews should summarise a body of knowledge and, from it, formulate ideas and recommendations which would be useful to international research community. If you are interested in preparing a Review article, please discuss the subject matter with the EditorinChief or the appropriate Associate Editor.

Perspective
A perspective is a pithy (but balanced) opinion piece about current or future directions in animal science. A perspective can critically assess current scientific topics or report on future issues that may arise from the discipline. The intent is to stimulate discussion and possible rethinking of current views in the animal sciences. Perspectives that address interdisciplinary research areas with relevance to a broader audience are of particular interest to the Editors. The Perspective should be accompanied by an abstract and generally range from 1000 to 4000 words; tables and figures can be included.

Editorials
Editorials are usually commissioned. Editorials are opinion pieces which reflect on papers previously or currently published in Animal Production Science, or on issues of general interest to the animal sciences community. They should be written in a crisp, lively style. They should have a maximum of 800 words, and not more than 5 references.

Comment papers
A brief comment or critique on a paper recently published in Animal Production Science. No abstract required. Authors of the original paper will be invited to submit a response.

Licence to publish
Submission of a paper is taken to mean that the results reported have not been published and are not being considered for publication elsewhere. A summary of the findings in the proceedings of a conference or in an extension article is not necessarily regarded as prior publication. However, if substantial parts of the data, such as those in Tables and Figures, have been published before, the inclusion of extra peripheral data does not alter the judgment that the paper is not new.
The Editor assumes that all authors of a multiauthored paper have agreed to its submission. For details regarding copyright, please see Copyright/Licence to Publish.

Open access
Authors may choose to publish their paper Open Access on payment of a publication fee. See Open Access for more details.
Citing personal communications and statistical software Citation of submitted manuscripts, unpublished data and personal communications should be avoided but if essential, they should be cited parenthetically in the text thus (e.g. PA Smith, pers. comm.). In such cases, the authors must obtain permission from the data owner to quote his or her unpublished work. Likewise, any statistical software used to process your data should be cited in brackets in the text, providing the name and version of the package and the name, city, state and country of the company that produced it.

Animal experimentation
Experiments involving animals are expected to have been conducted in accordance with the guidelines set out in the joint publication of the National Health and Medical Research Council of Australia, CSIRO and the Australian Agricultural Council entitled ´Code of Practice for the Care and Use of Animals for Experimental Purposes´ (National Health and Medical Research Council: Canberra, 1997). Editors will take account of animal welfare issues and reserve the right not to publish.

Preparing your manuscript
All authors should read at least one book on scientific writing. The titles of some suitable books are listed at the end of these notes. The work should be

presented concisely and clearly in English. Introductory material, including a review of the literature, should not exceed that necessary to indicate the reason for the work and the essential background. However, a short statement explaining the broader relevance of the study can be helpful to readers. Sufficient experimental detail should be given to enable the work to be repeated, and the discussion should focus on the significance of the results.
Poorly prepared or unnecessarily lengthy manuscripts have less prospect of being accepted. Authors should note the layout of headings, references, Tables and Figures in the latest issues of the Journal and follow the Journal style. Strict observance of these and the following requirements will shorten the interval between submission and publication.

Title
The title should be concise and informative and contain all keywords necessary to facilitate retrieval by modern searching techniques. Additional keywords not already contained in the title or abstract may be listed beneath the abstract. A short title of less than 50 letter spaces, to be used as a running head at the top of the printed page, should be supplied. The title, author(s), address(es) and short title should comprise a separate title page.

Summary text for the Table of Contents
This is a threesentence paragraph of 50 to 80 words written for interested nonexperts, such as journalists, teachers, government workers, etc. The text should be free from scientific jargon, and written at the level of an article in a science magazine. Your first sentence should engage the reader, convincing them that this is an important area. The second sentence should introduce the problem addressed in the paper, and state your main discovery. The final sentence should describe how the results fit into the bigger picture (i.e. implications or impact of the discovery).

Abstract
The abstract (preferably less than 250 words) should state concisely the scope of the work and the principal findings and should not just recapitulate the results. It should be complete enough for direct use by abstracting services. Acronyms and references should be avoided.
Please suggest 36 keywords, noting that all words in the title and abstract are already considered to be keywords.
Keyword should list alternative spellings, e.g. defense for defence, aluminum for aluminium etc.

References
References are cited by the author and date (Harvard system); they are not numbered. All references in the text must be listed at the end of the paper, with the names of authors arranged alphabetically; all entries in this list must correspond to references in the text. In the text, the names of 2 coauthors are linked by ´and´; for 3 or more, the first author´s name is followed by ´et al.´. Where more than one reference is cited in the text, they should be listed chronologically. No editorial responsibility can be taken for the accuracy of the

references. The titles of papers and the first and last page numbers must be included for all references. Papers that have not been accepted for publication cannot be included in the list of references and must be cited in the text as ´unpublished data´ or ´personal communication´; the use of such citations is discouraged. Authors should refer to the latest issues of the Journal for the style used in citing references in books and other literature. Full titles of periodicals must be given.
Examples of common references can be found in the ´Style guide for references.
Use of referencing software. To obtain the style file for this journal, please go to the following websites.
If using ´Reference Manager´, visit http://www.refman.com/support/rmoutputstyles.asp.
If using ´ProCite´, visit http://www.procite.com/support/pcoutputstyles.asp.
If using ´EndNote*´ software, visit http://www.endnote.com/support/enstyles.asp.
*You will find the style file under the ´Agriculture´ category, listed as Animal Production Science.

Units
The SI system of units should be used for exact measurements of physical quantities and, where appropriate, elsewhere. The double solidus must not be used in complex groupings of units (i.e. use mg/sheep.day, not mg/sheep/day or mg sheep day). This Journal uses the abbreviation ´L´ for litre; ´mL´ for millilitre. When using nonstandard abbreviations, define the abbreviation where it first occurs in the text.
Spell out numbers lower than 10 unless accompanied by a unit, e.g. 2 mm, 15 mm, two plants, 15 plants, but 2 out of 15 plants. Do not leave a space between a numeral and %, ‰ or C.
Formulae should be carefully typed with symbols correctly aligned and adequately spaced. If special symbols must be handwritten, they should be inserted with care and identified by pencilled notes in the margin. Judicious use should be made of the solidus to avoid 2 mathematical expressions wherever possible and especially in the running text. Each long formula should be displayed on a separate line with at least 1 line of space above and below.

Tables
Tables must be numbered with Arabic numerals and each must be accompanied by a title. A headnote containing material relevant to the whole Table should start on a new line.
Tables should be arranged with regard to the dimensions of the Journal columns (8 by 21 cm), and the number of columns in the Table should be kept to a minimum. Excessive subdivision of column headings is undesirable and long headings should be avoided by the use of explanatory notes which should be incorporated into the headnote. The first letter, only, of headings should be capitalised.
The symbol of unit of measurement should be placed in parentheses beneath the column heading. The prefixes for units should be chosen to avoid an

excessive number of digits in the body of the Table or a scaling factor should be added to the heading. Footnotes should be kept to a minimum and be reserved for specific items in the columns.
Horizontal rules should be inserted only above and below column headings and at the foot of the Table. Vertical rules should not be used. Each Table must be referred to in the text, and the preferred position of the Table in the text should be indicated by a note in the margin.
Short tables can frequently be incorporated into the text as a sentence or as a brief untitled tabulation. Only in exceptional circumstances will the presentation of essentially the same data in both a Table and a Figure be permitted: where adequate, the Figure should be used.

Figures and computer graphics
Lettering should be in sanserif type (Helvetica or Arial type 1 font) with the first letter of the first word and proper names capitalised. The xheight after reduction should be 1.21.3 mm. Thus for the preferred reductions of graphs to 30, 40 or 50% of linear dimensions, the initial xheight of lettering should be 4, 3 or 2.5 mm respectively. Symbols and grid marks should be the same respective sizes, and curves and axes should then be either 0.8, 0.7 or 0.6 mm thick respectively.
Proportionally smaller sizes of type, symbols, grid marks and curve thicknesses should be used for lesser reductions. The following symbols are readily available and should be used: . The symbols + or × should be avoided. Explanations of symbols should be given in the caption to the figure, and lettering of graphs should be kept to a minimum. If information is given in a caption instead of a legend describe the lines and symbols in words (e.g. solid lines, dashed lines, dotanddash lines, open circles, solid circles, striped bars, crosshatched bars and so forth).

Photographs
Photographs must be of the highest quality, with a full range of tones and of good contrast. Before being mounted, photographs must be trimmed squarely to exclude features not relevant to the paper and be separated from neighbouring photographs by uniform spaces that will be 2 mm wide after reduction. Lettering should be in a transfer lettering sanserif type (Helvetica font) and contrast with its background; thus, white lettering should be used on dark backgrounds. The size of lettering should be such that the xheight after reduction is 1.5l2 mm. A scale bar must be inserted on each photomicrograph and electron micrograph. Important features to which attention has been drawn in the text should be indicated (i.e. by coded upper case letters and/or arrows). Colour photographs will be accepted if they are essential, but the cost of production must be borne by the author.

Statistical evaluation of results
Manuscripts must contain a clear and concise description of the experimental design used; with sufficient detail such that, in the case where analysis of variance or regression models are to be used in the statistical evaluation, the reader is quite clear as to how the error term was estimated. The statistical tests

should be briefly described and, if necessary, supported by references. Numbers of individuals, mean values and measures of variability should be stated. It should be made clear whether the standard deviation or the standard error has been given.

Nomenclature

The nomenclature of compounds such as amino acids, carbohydrates, lipids, steroids and vitamins should follow the recommendations of the IUPACIUB Commission on Biochemical Nomenclature. Other biologically active compounds, such as metabolic inhibitors, plant growth regulators and buffers should be referred to once by their correct chemical name (which is in accordance with IUPAC Rules of Chemical Nomenclature) and then by their most widely accepted common name. For pesticides, the latest issue of ´Pesticides Synonyms and Chemical Names´ (Australian Government Publishing Service: Canberra) should be followed. Where there is no common name, trade names or letter abbreviations of the chemical may be used. The first letter of a trade name must be capitalised.

Submission of research manuscripts

To submit your paper, please use our online journal management system ScholarOne Manuscripts, which can be reached directly through this link or from the link on the journal´s homepage. If a firsttime user, register via the ´Register here´ link, or use your existing username and password to log in. Then click on the ´Author Centre´ link and proceed.

A covering letter must accompany the submission and should include the name, address, fax and telephone numbers, and email address of the corresponding author. The letter should also contain a statement justifying why the work should be considered for publication in the journal, and that the manuscript has not been published or simultaneously submitted for publication elsewhere. Suggestions of possible referees are welcome.

Post acceptance of manuscript

When asked to submit production files, please provide the Production Editor with the original figure files separately from the manuscript, and in highest resolution.

Ensure that figures are in their original file format (i.e. Photoshop, Adobe Illustrator, Excel, CorelDraw, SigmaPlot, etc.) rather than embedded in a Word document or converted to a derived format. However, if your figures are in a format that we do not accept, highquality highresolution PostScript or PDF files are acceptable. Sending files in more than one format is fine; we will use the format that will reproduce the best.

Scanned photographs must be saved as .tif files; all supplied .tif files must be compatible with Adobe Photoshop, which is the preferred program. If figures are prepared in a ´paint´ program, line art should be saved at 600 dpi, and greyscale or colour images should be saved at 300 dpi. Electronic photographic work should be submitted at the intended print size (85 mm wide for one column and up to a page width of 175 mm) (on CDROM if necessary). These will be returned after use if requested at the time of submission.

Colour photographs will be accepted if they are essential but the cost of colour reproduction on the printed copy must be borne by the author. The Production Editor will provide an estimate of the cost with the page proofs. Colour figures must be supplied in CMYK, not RGB, format.

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Approximately two weeks after the paper is accepted, the corresponding author will receive an edited MSWord document that has undergone formatting and copyediting. Questions from the Production Editor should be answered. Minor corrections can be made at this stage. The paper is then typeset, and page proofs sent to the corresponding author for checking prior to publication. At this stage only essential alterations and correction of typesetting errors may be undertaken.

Excessive author alterations will be charged back to the author. Reprint order forms and prices are sent with the proofs and should be returned to the Production Editor with the proofs.

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Style guide for references

Journal article

Hubick KT, Farquhar GD, Shorter R (1986) Correlation between wateruse efficiency and carbon isotope discrimination in diverse peanut (Arachis) germplasm. Australian Journal of Plant Physiology 13, 803816.

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VITA

Catarina Stefanello, filha de Nelci S. Stefanello e Anesia Rita Osmari Stefanello nasceu em Nova Palma, Rio Grande do Sul, no dia 25 de novembro de 1985. Cursou o ensino fundamental na Escola Estadual de Ensino Fundamental Padre João Zanella e o ensino médio na Escola Estadual de Educação Básica Tiradentes em Nova Palma, RS. Em 2005 ingressou no Curso de Zootecnia da Universidade Federal de Santa Maria, Santa Maria, RS, obtendo o Grau de Zootecnista em 2009. Iniciou, em março de 2010, o Mestrado em Zootecnia, área de concentração Produção Animal, na Universidade Estadual de Maringá, Maringá, PR, realizando estudos na área de nutrição de não-ruminantes. Obteve o título de mestre em Zootecnia em março de 2012. Em abril de 2012, ingressou no curso de Doutorado em Zootecnia, área de Produção Animal pelo Programa de Pós-Graduação em Zootecnia na Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, desenvolvendo o trabalho de tese sobre a utilização de enzimas exógenas em dietas para frangos de corte. Realizou pesquisas durante 12 meses no Departamento de Animal Science na Purdue University, West Lafayette, Indiana, USA, através do programa doutorado-sanduíche da CAPES, no período de agosto de 2014 a julho de 2015, trabalhando com avaliação de ingredientes e exigências nutricionais para aves e suínos. Submeteu-se à banca de defesa de Tese em março de 2016 pela Universidade Federal do Rio Grande do Sul em Porto Alegre, RS.