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DIGESTIBILIDADE DE AMINOÁCIDOS DE PENAS SUBMETIDAS A DIFERENTES PROCESSOS EM DIETAS DE FRANGOS DE CORTE

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Digestibilidade de aminoácidos de penas submetidas a diferentes processos em dietas de frangos de corte

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RESUMO

O estudo foi conduzido para determinar a digestibilidade ileal aparente e padronizada de aminoácidos (AA) em frangos de corte alimentados com dietas contendo penas cruas, farinha de penas comercial ou penas incubadas com *Bacillus subtilis*. Um total de 384 aves fêmeas Cobb × Cobb 500 com 1 dia de idade foram alojadas aleatoriamente em 4 tratamentos com 12 repetições de 8 aves por gaiola. Os 4 tratamentos foram compostos por dietas livre de nitrogênio (DLN); DLN com 7% de penas cruas; DLN com 7% de farinha de penas e DLN com 7% de penas incubadas. A dieta experimental foi fornecida dos 18 a 23 dias de idade dos frangos. A digestibilidade ileal aparente dos AA da cisteína e glutamina não diferenciaram estatisticamente (P > 0,05) comparadas entre as fontes de penas. A digestibilidade ileal aparente da isoleucina do tratamento das penas incubadas foi 34,4% menor quando comparada com a farinha de penas e 31,9% com as penas cruas. A digestibilidade da metionina, lisina, treonina, valina, isoleucina e arginina foi pior para a digestibilidade ileal padronizada do que os AA dispensáveis. Ácido aspártico foi o AA mais digestível para a digestibilidade ileal padronizada, as diferenças da farinha de penas foi de 1,8% maior que das penas incubadas e 2,3% das penas cruas. Os resultados demonstram que as penas incubadas não aumentou a digestibilidade quando comparadas com outras fontes de penas, porém os resultados das penas cruas e farinha de penas obtiveram resultados semelhantes na digestibilidade para a maioria dos AA.

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Amino acid digestibility of feathers submitted to different process in broiler diets

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ABSTRACT

A study was conducted to determine the apparent and standard ileal digestibility of amino acids in broiler chickens fed diets containing raw feathers, commercial feather meal (FM), or feather incubated with *Bacillus subtilis.* A total of 384 slow-feathering Cobb × Cobb 500 1 d-old female chicks were randomly distributed to 4 treatments (12 replicates of 8 birds each). The 4 dietary treatments were composed by nitrogen free diet (NFD); NFD with 7% raw feather; NFD with 7% of FM and NFD with 7% of incubated feather. The experimental diets were provided from 18 to 23 d. The apparent ileal digestibility of AA of cysteine and glutamine did not differ (*P*> 0.05) according to feathers source. The apparent ileal digestibility of isoleucine in broilers fed incubated feather has a lower difference of 34.4% compared with FM and 31.9% with raw feather. The digestibility of methionine, lysine, threonine, valine, isoleucine and arginine was worst standard ileal digestibility than the dispensable AA. Aspartic acid was the most digestible AA for standard ileal digestibility, the differences of FM was 1.8% higher than incubated feather and 2.3% of raw feather. The results suggest that the incubated feather not improve the digestibility when compared with the others sources of feathers, however the digestibilities of the AA were similar for the raw feathers and the feather meal.

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RELAÇÃO DE ABREVIATURAS

AA	Aminoácidos
DLN	Dieta livre de nitrogênio
DIA	Digestibilidade ileal aparente
DIP	Digestibilidade ileal padronizada
h	Horas
min	Minutos

CAPÍTULO I

INTRODUÇÃO

A produção mundial de carne de frango foi de aproximadamente 86.100 toneladas em 2014, sendo o Brasil responsável por 14,7% deste total (USDA, 2014), evidenciando a importância deste setor para a economia brasileira. O rendimento de carcaça é de aproximadamente 70% (Holanda, 2009), sendo que as penas representam 5–7% do peso corporal (Onifade et al., 1998) resultando na produção de aproximadamente 7.400 toneladas de penas por ano no Brasil. Na busca de fontes para substituir a proteína do farelo de soja, as penas representam uma alternativa para o aproveitamento econômico deste subproduto do processamento da indústria avícola.

Na indústria, as penas são normalmente transformadas em farinha, através de um processo que utiliza temperatura e pressão elevada. O método, entretanto, não é eficiente pela baixa digestibilidade proteica da farinha de penas hidrolisadas de aproximadamente50% (Bielorai et al., 1981). De acordo com Cedrola et al. (2011), a biodegradação da queratina das penas por microrganismos representa um método alternativo, para aumentar seu valor nutricional e diminuição do impacto ambiental. Entretanto, o uso de microrganismos proteolíticos para a melhor digestão de penas é recente, e são relatados poucos trabalhos avaliando o uso desse material na dieta de frangos.

A digestibilidade ileal aparente (DIA), para ingredientes de baixo valor proteico, fornece resultados subestimados em relação à concentração de aminoácidos (AA), pois não determina a perda endógena dos mesmos. A DIA considera a alimentação de AA nas dietas fornecidas às aves mais a produção endógena, incluindo a microbiota intestinal, descamação das células epiteliais, digestivas e salivares. Entretanto, se o fluxo endógeno é mensurável, a digestibilidade pode ser corrigida para a digestibilidade ileal padronizada (DIP). Diferentes métodos vêm sendo utilizados para prever a perda endógena de AA em dietas de frangos. Nesse caso, os frangos de corte podem ser alimentados com dietas livre de nitrogênio (Furuya & Kaji, 1989), proteínas altamente digestíveis (Adedokum et al., 2008), ou alimentação peptídica – caseína hidrolisada enzimaticamente (Ravindram et al., 2004). Estes métodos têm algumas desvantagens, pois consistem em dietas incomuns e podem apresentar resultados controversos para os AA analisados.

Existem inúmeros ingredientes com alta concentração proteica. Alguns deles, como as penas, não foram estudados profundamente em relação à digestibilidade. As penas são constituídas por 90% de queratina (Bockle et al., 1995), sendo esta uma proteína insolúvel nos solventes convencionais e dificilmente digerida por enzimas, como a tripsina e pepsina (Williams et al., 1990). Essa proteína é composta por 96 AA estabilizados por fibras muito fortes, com ligações cruzadas entre pontes de hidrogênio, pontes dissulfídicas e interações hidrofóbicas organizadas em formato espiral, dificultando sua decomposição pela dificuldade da interação substrato-enzima (Grazziotin et al., 2006). Assim, para aumentar a digestibilidade e facilitar a inclusão de penas na ração, usa-se processamento baseado na temperatura e na pressão. Este processo destrói as pontes dissulfídicas, disponibilizando sítios para a ação das enzimas proteolíticas (Bielorai et al., 1981), mas todavia pode interferir o valor nutritivo da farinha de penas. Portanto, a redução das pontes dissulfídicas

parece ser indispensável para que as cadeias peptídicas tornem-se disponíveis para as proteases.

Outra abordagem visando aumentar a digestibilidade das penas pode ser a incubação direta com bactérias queratinolíticas ou proteolíticas, enzimas puras sem microrganismos ou enzimas purificadas sem bactérias. Baseado nisso, observou-se que a adição de queratinase aumenta a digestibilidade total de AA de 30 para 60% em penas cruas e 77 para 99% em farinha de penas comercial (Lee et al., 1991). No mesmo contexto, o potencial de degradação de penas da bactéria *Kocuria rosea* foi avaliada para verificar se a biomassa da bactéria contribuiria para o aumento da quantidade de alguns AA, como a lisina, histidina (deficientes em farinha de penas), ácido aspártico, metionina e alanina. Permitindo a substituição dos ingredientes proteicos por penas biodegradadas (Bertsch & Coello, 2005).

Inúmeros estudos foram realizados com intuito de avaliar a melhor bactéria para a degradação total das penas. *Bacillus subtilis* demonstrou uma completa solubilização das penas após 24 horas de incubação, baseado em aspectos visuais (Zaghloul et al., 2011).Embora as pesquisas demonstraram a completa degradação de penas pela queratinase, ainda não existem experimentos conclusivos para sua utilização na nutrição de frangos.

REVISÃO BIBLIOGRÁFICA

Formação, crescimento e estruturadas penas

A formação das penas inicia-se entre o 7º–8º dia de incubação, e ao 11° - 12° dia o embrião já se encontra coberto por plumas. À medida que a ave vai crescendo, as plumas darão lugar as penas. Estas novas penas crescem do mesmo folículo dos quais as plumas foram originadas, sendo que o centro do mesoderma forma o núcleo da pena, e do tecido epitelial serão formadas as cristas da barba. Nas barbas, as estruturas ramificadas irão induzir a formação das bárbulas, conforme a Figura 1 (Nohno et al., 1995).

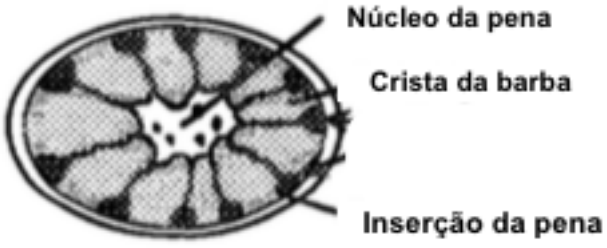

Figura 1. Corte transversal da matriz da pena.
Fonte: Nohno et al.(1995).

As penas das aves são formadas pelo cálamo, uma estrutura oca inserida na epiderme da ave. A raque formando o núcleo central do eixo da pena, onde são inseridas as barbas. As mesmas são divididas em estruturas ainda menores, denominadas bárbulas, que apresentam ganchos que mantém a conexão das cristas das bárbulas adjacentes, necessárias para manter a forma e estrutura da pena (Figura 2).

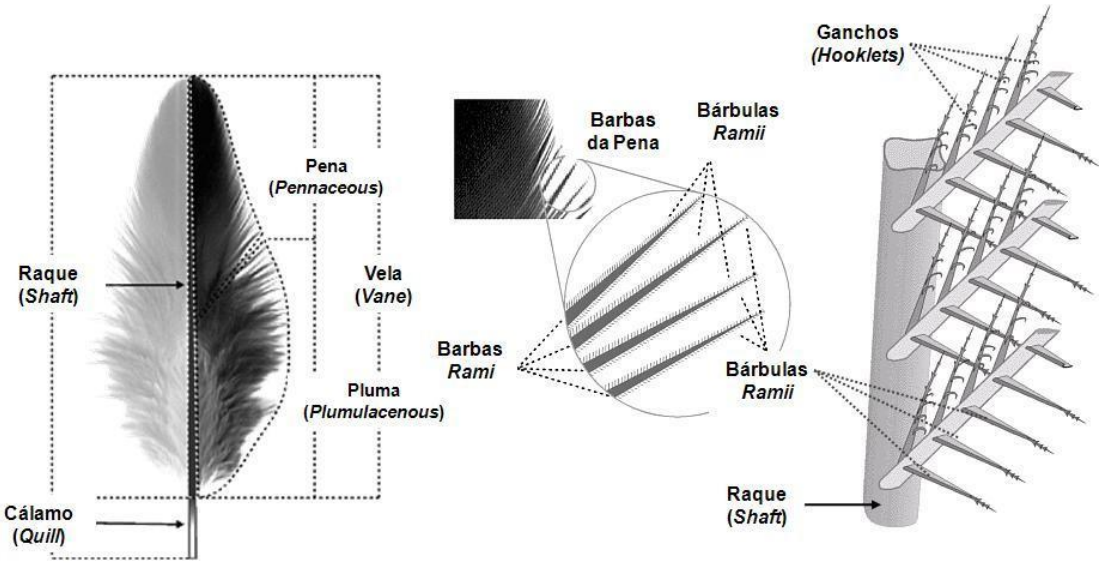

Figura 2. Anatomia da pena. Fonte: Poultry hub.

Histologicamente as penas são compostos derivados da epiderme da matriz intracelular da queratina formadas na fase embrionária (Kozák, 2011). O crescimento delas provem do folículo da pena que é caracterizado pela invaginação cilíndrica da epiderme. Sua estrutura é rígida, insolúvel e não degradada pela maioria das enzimas proteolíticas. A estabilidade mecânica e resistência a digestão proteolítica são consequências de uma compactação das cadeias proteicas em forma de α-hélice ou β-pregueada em uma cadeia polipeptídica enrolada. Ainda é encontrada a presença de grandes concentrações de ligações cruzadas de cadeias polipeptídicas, causada por uma extensiva formação das ligações dissulfídicas. As altas concentrações de cisteína facilitam a formação de pontes de cistina. Ligações de hidrogênio entre polipeptídios, as interações hidrofóbicas e a estabilidade da forma coloidal conferem uma maior resistência e força proteolítica da queratina (Onifade et al., 1998).

Composição das fontes de penas

Na Tabela 1 são apresentados os valores proteicos e aminoacídicos das penas de frango. Segundo Nascimento (2000), as penascruastêm1% de gordura, 9% de água e90% de proteínas estruturais. De acordo com Rostagno et al. (2011), a farinha de penas é constituída por 90% de matéria seca, das quais 79% de proteína bruta, 5% de gordura, 3% de extrato não nitrogenado e 4% de matéria mineral, além de ter um total de 88% de matéria orgânica.

Tabela 1. Concentração de proteínas bruta e aminoácidos totais de penas cruas e processadas (g/kg).

Proteína e AA (g/kg)	Penas cruas	Farinha de penas 207 kPa/24 min	Farinha de penas 160ºC/15 min	Farinha de penas 75%, (Rostagno et al. 2011)
Proteína	922,0	866,0	880,0	749,1
Arginina	67,6	62,5	61,0	50,7
Histidina	2,3	8,6	5,7	10,7
Isoleucina	39,4	41,3	42,3	35,6
Leucina	56,9	68,8	70,9	64,7
Lisina	15,4	22,6	18,8	23,2
Metionina	7,1	6,3	6,5	6,6
Treonina	34,5	36,5	40,2	36,6
Valina	53,0	44,0	59,6	55,6
Alanina	28,8	37,7	39,6	-
Cistina	65,8	48,7	42,9	-

Fonte: Adaptado de Onifade et al. (1998) e Rostagno et al. (2011).

Métodos de processamento das penas

Existem várias formas de processar as penas para que se tornem mais digestíveis. Uma das formas é a hidrólise por autoclave, como as que Draper et al. (1944) utilizaram para avaliar o possível aumento no valor nutricional das penas. O processo empregado não resultou em um incremento nutricional.

O processamento das penas comerciais é feita em digestores mecânicos que realizam a cocção das penas. Este apresenta uma abertura superior para o carregamento do material e uma abertura inferior para o descarregamento. No interior do digestor as penas são submetidas ao processo térmico para hidrólise. Este por sua vez, deve respeitar os limites de mínimo e máximo de temperatura evitando hidrólise incompleta ou perda excessiva de AA pela desnaturação proteica. Além destes problemas de processamento, fatores como a umidade em excesso na farinha de penas levam a multiplicação de bactérias e fungos. Concomitantemente poderão ocorrer processos de rancificação e acidificação. O processo de secagem posterior estabilizará o produto e permitirá a moagem para padronização da granulometria (Fonseca, 1991).

Scapim et al. (2003) analisaram diferentes processamentos de penas e seu impacto sobre os valores nutricionais. As variáveis avaliadas foram de diferentes tempos (30, 40, 50 e 60 minutos (min)), secagem (75,90, 105 e 120 min a 180ºC) e com a pressão constante (4 $kgf.cm^{-2}$). Os valores de proteína bruta e cinzas encontrados nas farinhas de penas foram próximos ao relatado por outros autores como Wisman et al. (1958), Fialho et al. (1984) e Albino et al. (1992a, b), porém os autores elucidam a falta de padronização dos resultados devido a matéria prima.

A queratina por ser uma macromolécula insolúvel necessita de secreção de enzimas extracelulares para a biodegradação ocorrer (Safranek & Goos, 1982). Várias bactérias degradantes de penas foram isoladas do solo e de resíduos de abatedouro de aves. Por isso, alguns estudos indicaram que a diversidade de bactérias de degradação das penas é vasta, entretanto a

maioria dos isolados são do gênero *Streptomyces* e *Bacillus* (Lin et al. 1999; Bressolier et al. 1999; Kim et al. 2001; Lucas et al., 2003; Brandelli, 2008). Porém, dentre as bactérias gram–positivas, novas bactérias foram identificadas como degradantes de penas. Algumas cepas queratinolíticas, como o *Microbacterium sp*., podem degradar as penas em até 48 h como ilustra a Figura 3.

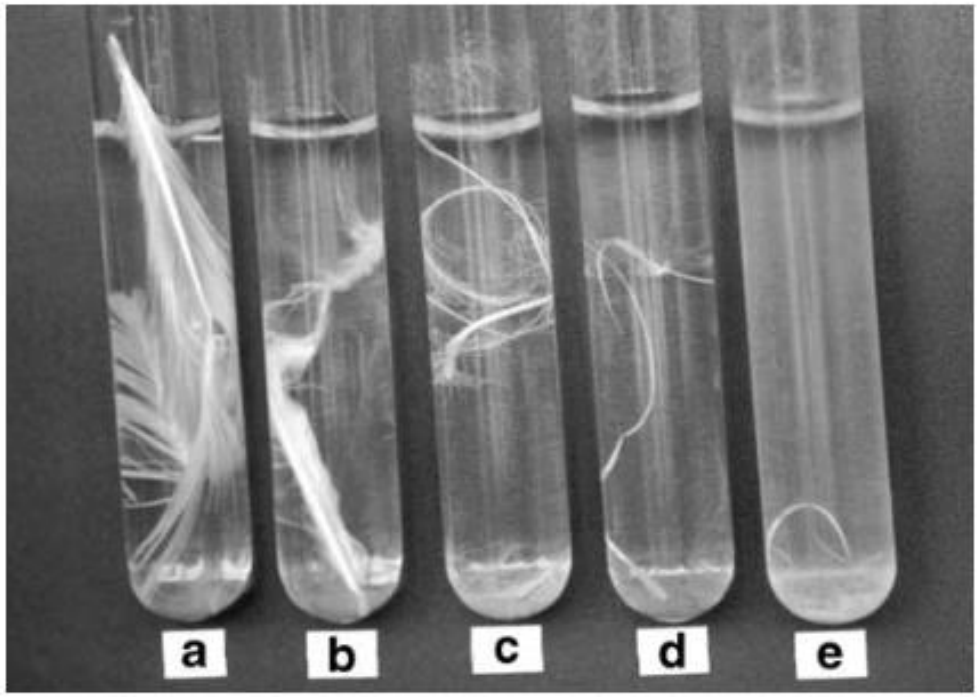

Figura 3. Degradação da pena por *Microbacterium sp*. após *a*-2, *b*-24, *c*-48, *d*-72 e *e*-96 horas. Fonte: Brandelli, (2008).

O estudo realizado por Allpress et al. (2002) avaliou a habilidade queratinolítica da bactéria *Lysobacter sp*. Os resultados indicam um alto grau de atividade queratinolítica dessa bactéria e como consequência a possibilidade de sua utilização na degradação tanto de penas como de pêlos.

Takahashi et al. (2004) verificaram o efeito do tratamento térmico nas diferentes partes da pena (barbas, raque e cálamo) que apresentam diferentes características morfológicas, utilizando a varredura diferencial de calorimetria. A desnaturação térmica demonstrou que há um menor efeito sobre as barbas do que na raque e no cálamo, resultando em heterogeneidade do produto final.

Zaghloul et al. (2011) utilizaram *Bacillus subtilis* para degradação das penas de frango. Bactérias ativas foram inoculadas em um fermentador com água e 2% de penas. A fermentação foi efetuada a 37ºC sob agitação a 700rpm e um fluxo de ar constante por 4 dias. Após 48 h já pode ser observado uma completa solubilização das penas. A atividade da protease queratinolítica no fermentador foi similar a mesma feita em laboratório. A bactéria permaneceu 100% ativa até o quinto dia de incubação. No mesmo ano, Cedrola et al. (2011) também utilizaram o *Bacillus subtilis* na obtenção de queratinase para a degradação de penas de frango. Obtiveram maior produção de queratinase no meio de pH 8. A atividade foi alta quando utilizou o inóculo por 72 horas em 1% de penas puras com suplementação de 0,1% de extrato de levedura. No

extrato enzimático, as queratinases estavam ativas no pH entre 2 e 12, com a máxima atividade no pH 10 e a temperatura de 60ºC. Nas avaliações com microscopia eletrônica foram observadas células de B. subtilis aderidas na superfície da pena após 98 h da cultura, assim como foram observados filamentos degradados das penas.

Pedersen et al. (2012), realizaram um estudo examinando quatro proteases comerciais baseadas nas bactérias: *Bacillus subtilis*, *Bacillus licheniformis, Aspergillus niger* e *Serratia proteamaculans* usadas para hidrolisar penas em diferentes condições. O grau de queratinólise foi monitorado pela quebra do grupo NH_2 usando *o*-ftalaldeído e por microscopia eletrônica. Todas as 4 proteases foram capazes de degradar as penas em pH 5,5 e 7. A hidrólise foi estimulada com a adição de reagentes como o ditiotreitol. Em geral, a protease de *B. subtilis* foi mais eficiente na degradação da queratina das penas quando comparada com as outras três proteases.

Digestibilidade ileal aparente e padronizada

Os AA são o segundo grupo de nutrientes mais caro nas dietas de frangos de corte depois da energia. Se a suplementação deles não for correta na formulação da ração, eles podem contribuir para poluição ambiental devido à excreção excessiva de nitrogênio, além de gerar custos (Nandha et al., 2013). Existem diferentes métodos para avaliar a digestibilidade dos AA dos ingredientes. Geralmente o método mais utilizado pelos pesquisadores é a análise da excreta. Esse método avalia a diferença do conteúdo de AA ingeridos pelo excretado, mas não avalia a perda metabólica, endógena e ainda sofre a influência da microbiota cecal, que modifica o perfil aminoacídico. Adicionalmente esse método não permite a separação de urina da excreta mascarando resultados (Leeson & Summers, 2001).

O método mais preciso para determinar a digestibilidade é a coleta do conteúdo ileal. Este método necessita de um indicador indigestível como o óxido de cromo (Cr_2O_3) ou cinza insolúvel ácida (CIA), que é adicionado às dietas experimentais de 1 a 2% na ração, para calcular a digestibilidade.

A digestibilidade ileal aparente (DIA) não avalia as perdas endógenas e metabólicas de AA, consequentemente leva a subestimação do conteúdo de AA aparentemente digestíveis do ingrediente (Stein et al., 2007).

A DIA determina o valor de proteínas e AA baseado na seguinte formula:

$$DIA, \% = \{[AA_i - (AA_0 \times FI/DI)]/AA_i\} \times 100,$$

Onde: AA_i = AA da dieta, (%)
AA_0= AA do conteúdo ileal, (%)
FI = indicador da dieta, (%)
DI = indicador do conteúdo ileal, (%)

A digestibilidade ileal padronizada (DIP) indica a quantidade de AA presente na proteína do ingrediente avaliado, e que foi absorvido no intestino delgado. Essa considera a perda dos AA endógenos presentes no conteúdo ileal. Para calcular a perda de AA endógeno um dos métodos preferidos é o da

dieta livre de nitrogênio (DLN), por possuir uma metodologia simples mesmo que apresente a desvantagem de não ser uma alimentação fisiológica.

Aproximadamente 85% da DLN têm como principais ingredientes o amido de milho e dextrose ou açúcar. Embora as perdas endógenas de AA teoricamente sejam independentes da composição das dietas, ainda existem dúvidas sobre a influência dos ingredientes da composição do DLN para animais em termos de determinação (Kong & Adeola, 2013).

A DLN é necessária para calcular a DIP, pois o uso da mesma resulta em perdas endógenas de AA pelos animais e pelo aporte mínimo de proteína. A DIP é calculada corrigindo a perda endógena de acordo com formula a seguir:

$$DIP\ (g/kg\ de\ MS) = (DIA + PE)/\ AA_d$$

Onde: DIA = digestibilidade ileal aparente, (%)
PE = perda endógena basal de AA em matéria seca, (g/kg)
AA_d = matéria seca do AA da dieta, (g/kg).

DIP de AA se divide em dois componentes principais: a digestibilidade ileal verdadeira dos AA e perdas específicas de AA endógenos. Em teoria a DIP de AA não tendência pela proteína bruta das dietas, pois se seguisse essa teoria as perdas específicas de AA endógenos aumentariam linearmente dependendo da proteína bruta ou teor de AA correspondendo então a digestibilidade ileal verdadeira (Zhai & Adeola, 2011).

Digestibilidade *in vitro* e *in vivo*

Geralmente o coeficiente de digestibilidade *in vitro* é realizado com pepsina. Papadoupolous (1987) avaliou a digestibilidade de três tipos de farinha de penas submetidas a diferentes tempos de industrialização, encontrando uma correlação positiva com o acréscimo do tempo de processamento. A avaliação da digestibilidade foi realizada utilizando 7 aves de 28 dias de idade, alimentadas com DLN com a adição de 12 g de farinha de penas. Concluiu que o aumento do tempo do processamento provoca a diminuição da digestibilidade dos AA, consequentemente o autor comprova que os AA limitantes são mais sensíveis a processos térmicos e os métodos de digestibilidade contrariam-se.

Bielorai et al. (1982) realizaram estudo determinando a absorção ileal de nitrogênio com amostras de farinha de penas. As dietas continham 15% de farinha de penas, balanceadas com um programa linear, usando dois valores de absorção de nitrogênio referentes ao ingrediente estudado: 55% encontrado na farinha de pena ou 85% do valor médio para alimentos padronizados. Quando a absorção de nitrogênio das farinhas de penas foi calculado com 55%, o crescimento dos frangos de 1 a 3 semanas de idade foi similar aos frangos do tratamento controle. O crescimento retardou quando a composição das dietas foi calculada usando 85% de absorção de nitrogênio para farinha de penas. O método oficial da digestibilidade da pepsina *in vitro*, usando 0,2% de pepsina, piorou os resultados do valor nutritivo das proteínas da farinha das penas.

Kim (2002) utilizou cinco tratamentos: A: penas cruas (controle); B: controle + complexo de enzimas por 24h; C: controle + hidróxido de sódio (NaOH) por 24h; D: controle + NaOH por 2h, e E: controle + NaOH por 2h + complexo enzimático por 24h. O tratamento com maior solubilidade foi o de NaOH por 24h comparado aos outros tratamentos. A digestibilidade por pepsina para o tratamento A, B, D e E foi de 4,7; 13,2; 55,8 e 59,1%, respectivamente. A digestibilidade aminoacídica feita *in vitro* demonstrou que o tratamento E foi significativamente maior que B e D, exceto para a alanina onde o D obteve resultado melhor para a digestibilidade de AA do que o tratamento B.

Morris & Balloun (1972) realizaram cinco processos de farinha de penas com pressões, tempos e agitações diferentes. No primeiro experimento os tratamentos substituíram todo o farelo de soja da dieta de milho e soja, representado 22% de proteína no total da dieta. Não houve suplementação de AA. A farinha de penas com maior pressão e tempo obteve o melhor ganho de peso e conversão alimentar. No experimento 2, o objetivo era avaliar o desempenho dos animais, e desta vez a suplementação de farinha de penas foram de 2,5 e 5% dentro da dieta basal de milho e soja. Os resultados demonstram que os tratamentos mantiveram o mesmo desempenho que o da dieta basal até a 5ª semana. Não houve diferença significativa entre os níveis de 2,5 e 5%, porém o nível de 5% diminui o crescimento lentamente. O tratamento de menor pressão e tempo não acompanhou o crescimento dos outros tratamentos. O terceiro experimento realizado foi comparando os tempos diferentes com níveis de proteína de 5 ou 7,5% em dietas basais de 22, 20, 18 e 16% de base proteica. Para ambos os tratamentos, quando o nível de 7,5% foi usado diminuiu o ganho de peso. A suplementação de metionina e lisina não foram necessárias quando a farinha de penas foi fornecida 2,5% na dieta basal. Estes dois AA deveriam ser suplementados na dieta quando as penas foram fornecidas a 5% ou mais na dieta basal. Farinha de penas processadas com agitação intermitente e altas temperaturas ou por longos períodos do que o padrão tenderam a aumentar a resposta biológica dos frangos. Frangos com mais de 4 semanas de idade utilizaram farinha de penas mais eficientemente.

Grazziotin et al. (2006) avaliaram digestibilidade das penas degradadas por bactérias queratinolíticas. Foram utilizados como tratamentos 60 g de penas em meio de cultura de *Vibrio sp*. por 7 dias e o sobrenadante da cultura da bactéria com 10 g de penas por 5 dias. As composições aminoacídica dos tratamentos apresentaram deficiência de metionina, lisina e histidina. A digestão *in vitro* realizada com pepsina e pancreatina foram avaliadas. A digestibilidade do tratamento sobrenadante de bactérias foi similar ao da caseína e da proteína da soja. O tratamento com o meio de cultura de *Vibrio sp*. apresentou conteúdo de baixo valor proteico e uma quantidade relativamente alta de cinzas.

Além da *Vibrio sp*., outras bactérias foram estudadas com o mesmo intuito de digestibilidade, como foi o caso da *Bacillus licheniformis*. Onde, Williams et al. (1991) realizaram um estudo no qual trataram as penas em condições aeróbias e anaeróbias com a adição do *B. licheniformis.* A inclusão de 25% nas dietas de frangos demonstrou que penas tratadas em condições anaeróbias resultaram no aumento do peso vivo de 6,9 e 19,3% superior do

que os tratamentos com penas fermentadas aerobicamente e dieta controle, respectivamente. Dessa forma, ficou evidente o potencial nutricional do uso de bactérias queratinolíticas como o *Bacillus licheniformis* na degradação de penas (Williams et al., 1991).

Além dos estudos *in vitro*, Latshaw et al. (1994) comparam três diferentes tratamento de penas preparadas em hidrólise continua e 1 amostra em hidrólise em parcelas. O vapor da pressão usado nos processos foi de 207, 310 e 414 kPa na hidrólise continua e 283 kPa para a hidrólise parcelada. Cinco galos foram alimentados forçadamente com 30g de cada tratamento das farinha de penas para avaliar a digestibilidade. A média do coeficiente de digestibilidade dos AA foi de 0,72 para as pressões de 207 e 310 kPa e 0,66 para as pressões de 414 e 283 kPa das farinhas de penas. Segundo os autores, os resultados sugerem que os processamentos necessitam ter condições apropriadas de pressão e agitação, pois quando a pressão foi maior (414 kPa) e a agitação não foi constante a digestibilidade diminuiu.

O uso de dietas contendo penas também foi estudado em perus, por Eissler & Firman (1996), em dois experimentos avaliando o efeito da farinha de penas e a mesma em combinação com outras farinhas de subprodutos sobre desempenho de perus do 10º dia até a 19º semana de idade. No primeiro experimento, os perus receberam dietas com níveis de farinha de penas entre 0 e 14%%. Quando a inclusão de farinha de penas foi maior que 4% não foram obtidos efeitos sobre o ganho de peso e conversão alimentar. No mesmo trabalho realizaram um experimento com tratamento de dietas formuladas com 0 a 6% de farinha de penas e o outro tratamento com dietas contento 6% de farinha de penas mais 4%de farinha de sangue, peixe, carne e osso ou farinha dos subprodutos de frangos. Os animais que receberam a dieta de farinha de penas mais a farinha de sangue apresentaram ganho de peso menor do que perus alimentados com 2, 4 e 6% de farinha de penas na dieta.

Sulabo et al. (2013) realizaram uma pesquisa com DLN empregando 4 diferentes tipos de farinha de penas com a adição de sangue no processamento. Os tratamentos com a adição de sangue apresentaram a DIA dos AA limitantes (isoleucina, leucina, lisina, fenilalanina e valina) melhor que a farinha de penas sem a adição de sangue.

Baseado em todos os estudos citados anteriormente, ainda existem lacunas a serem estudados em relação às penas. Pesquisas em relação às penas cruas são dificilmente encontrados na literatura, além de estudos com a digestibilidade de todos os ingredientes tendo como fonte a pena. Portanto, este trabalho contribuirá para sanar esses problemas.

HIPÓTESES E OBJETIVOS

Hipóteses

O processo de incubação aumenta a disponibilidade dos nutrientes contidos nas penas.

O uso de penas incubadas com *Bacillus subtilis* confere maiores valores de digestibilidade para frangos de corte do que o emprego de penas cruas ou penas hidrolisadas.

Penas incubadas com *Bacillus subtilis* representam um alimento alternativo e viável nutricionalmente.

Objetivos

Determinar a digestibilidade ileal padronizada dos aminoácidos de penas de frangos de corte avaliando a diferença entre três processos de tratamentos: nenhum, hidrólise em digestor e incubadas com *Bacillus subtilis.*

CAPÍTULO II

[1]Artigo elaborado conforme as normas da revista The Journal of Applied Poultry Research (JAPR)

Running title: Digestibility of feathers

Apparent and standard ileal digestibility of amino acids of raw feathers, feather meal, and feather post incubation with a mix of *Bacillus subtilis* strains for broiler chickens

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SUMMARY

A study was conducted to determine the apparent and standard ileal digestibility of amino acids in broiler chickens fed diets containing raw feathers, commercial feather meal, or feather incubated with *Bacillus subtilis.* A total of 384 slow-feathering Cobb × Cobb 500 1 d-old female chicks were randomly distributed to 4 treatments (12 replicates of 8 birds each). The 4 dietary treatments were composed by nitrogen free diet **(NFD)**; NFD with 7% raw feather; NFD with 7% of FM and NFD with 7% of incubated feather. The experimental diets were provided from 18 to 23 d. The AID of AA of Cys and Glu did not differ ($P > 0.05$) according to feathers source. The AID of Ile in broilers fed incubated feather has a lower difference of 34.4% compared with FM and 31.9% with raw feather. The digestibility of Met, Lys, Thr, Val, Ile and Arg showed worst SID than the dispensable AA. Asp was the most digestible AA for SID, the differences of FM was 1.8% higher than incubated feather and 2.3% of raw feather. The results suggest that the incubated feather do not improved the digestibility when compared with the others sources of feathers.

DESCRIPTION OF PROBLEM

The global poultry industry is increasing every year, as well the concerning about the waste produced during the whole chicken process, especially the pollutants generated at the slaughterhouse [1]. Since the poultry feathers, may represent 10% of the total chicken weight, the feathers total volume can reach 7.7×10^8 kg/year [2]. However, the absence of feathers accumulation in the environment shows the existence of microorganism that consumes this product [3].

Feathers have been subject of study between nutritionists, due to their low price and the protein composition [4]. In this context, the cost for feather meal (**FM**) production show the limiting of nutritional improvement that seems justifiable the usage of different bioresources optimization [1]. Some researchers are trying to find the best way to improve nutritional value for feathers using an environmental friendly technology.

Since the digestibility of different ingredient was not deeply studied as feathers, them weight is constituted by 90% of keratin [8], the proteins are insoluble in conventional solvents and hardly digestible by digestive enzymes, such as trypsin and pepsin [9]. These proteins are composed of 96 AA stabilized by hard fibers, with cross-linked hydrogen bonds, disulfide bridges, and hydrophobic interactions arranged in spiral [2]. Thus, to improve the digestibility and facilitate the inclusion into the feed, the FM industry has adopted heat and pressure processes. This destroys disulfide bridges, allowing the proteolytic enzymes to act on the protein molecule [10]; however, can change the nutritive value of FM. The reason for the difficult decomposition of feathers is the disulfide bridges that become a compact structure hard to make interaction between subtract-enzyme. Thus, the reduction of disulfide bridges seems to be indispensable to peptide chains be available to proteases.

Another method used to improve the digestibility of feathers could be the direct incubation of feathers with keratinolytic or proteolytic bacteria; pure enzyme free of microorganism, or a purified enzyme without bacteria. Based on this, it have been demonstrated that the addition of keratinase improved the total digestibility of AA from 30 to 66% on raw feather, and 77% to 99% on commercial FM [11]. At the same context, the potential of *Kocuria rosea* bacteria on degradation of feathers was tested to verify if the bacteria biomass could contribute to increase the amount of some AA, as Lys, His (deficient in FM), asparagine, methionine and alanine. Thereby with a better quality of protein, it is possible to decrease common protein ingredients switching for biodegraded feathers [4].

Few studies were reported with the proposal to obtain a preparation of bacteria*Bacillus subtilis*, among them was observed the complete solubilization of feathers after 24 hours of incubation, based on visual aspects [12]. Therefore, the results showed a great keratinase potential, but any study was conducted to evaluation of incubated feathers with *Bacillus subtilis* in chicken nutrition. Thus, the objective of this current study was to evaluate the apparent and standard ileal digestibility of AA on raw feather, feather incubated with *Bacillus subtilis*, and FM for broiler chickens.

MATERIALS AND METHODS

All the procedures used in this study were approved by the Ethic Committee of Animals Use of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Bird Husbandry

A total of 384 one-day-old, slow-feathering Cobb × Cobb 500 female broiler chicks, vaccinated for Marek's disease at the hatchery, were randomly placed in 48 wire cages (0.9 × 0.4 m^2) with 8 birds per cage. Each cage was equipped with one feeder and one drinker. Birds had *ad libitum* access to water and mash feed. Average temperature was 32°C at placement and reduced by 1°C every 2 d until 23°C to provide comfort throughout the study. Fluorescent lighting was continuous until 23 d.

Diets

Birds were allocated to 4 experimental diets, with 12 replicate cages in a completely randomized design. Birds were fed a standard broiler starter diet from d 1 to 18 posthatch. The composition of experimental diets formulated to be nitrogen-free is presented in Table 1. Birds were fed with the experimental diets from 18 to 23 d. The 4 dietary treatments were composed by NFD; 93% NDF + 7% raw feathers; 93% NDF + 7% incubated feathers, and 93% NDF + 7% FM.

Treatments Preparation

The feathers sources came from the same batch of the slaughter house and was use to treatments process.

The FM was devoid of blood and acquired from the industry, obtained after cooking process in an intern press digester with the inside pressure of 3.0 kgf/cm^3, for an approximate time of 20 minutes. The feather inside the cooker attained a temperature of 133ºC necessary for

the particle hydrolyses. The drying was held in a rotary drum drier, where the material came into contact with air heated to 350ºC, for 15 minutes. After the drying, the feather material was grinded in a hammer mill screen size of 2 mm.

The raw feathers were obtained from a slaughter and all the impurities from the feathers were manually taken, and afterward the feathers were washed with running water. Following washing, the materials were dried in a forced air oven at 60ºC until it had lost 92-94% of its moisture. After dried, the feathers were grounded to obtain similar granulometry to the commercial meal feather. One part of the grounded feathers were stored in an appropriated place for treatment with raw feather included in the diet; the other part was separated to be mixed with the *Bacillus subtilis* digestion.

The digestion of raw feather was done to obtain incubated feathers product. A stainless steel bioreactor with constant movement of 9 rotations per minute and a capacity of 1,080 L was used. The bacteria for feather digestion was prepared with 150 L water, 350 g NaCl, 400 g yeast extract, 50 g peptone and 4 L of 10,000 units of *Bacillus subtilis*/mL culture, amounting about 4×10^7CFU. All components were incubated for 24 h to multiplication culture. After, 2% of raw feathers based on dry matter were added in these bacteria culture and incubated for 7 days. During the digestion process, the temperature was maintained between 30-35ºC. At the end, the material obtained was immediately frozen and were dried in a forced air oven at 60ºC until it was reduced to about 85% of the original weight.

Experimental Procedures

At the beginning, with 18 d old, all birds were individually weighed and groups of 8 birds were housed in each cage. At d 23, all the birds were slaughtered by the desensitization electronarcosis and bloody. The ileum collection method was used for the determination of AA digestibility. The birds were stimulated to feed consumption two hours before the slaughter to assure the presence of digesta at the ileum. For the ileum content collection, the portion of the small intestine from Meckel's diverticulum to approximately 4 cm proximal to the ileo-cecal

junction was flushed with distilled water. The content was immediately frozen with liquid nitrogen and later freeze-dried and submitted for analyses of AA, for this the proteins was hydrolyzed with HCl 6 N during 24 h at 110ºC. The AA were opened during the acid hydrolyze and was react with the phenylisothiocyanate (PITC), separed by HPLC in reverse phase with ultraviolet (UV) detector with 254 nm. The column was a Waters Pico-Tag® for free AA (3.9 x 300mm), the temperature was held at 43ºC, the flow was 1.00 ml/min, stop time at 27 min with solvent A at 100% and B when get to 27 min was 100% of concentration.

The AA analyses were done with the ileum content, feed and ingredients. Dry matter content was determined by drying the samples of the feed and ileal digesta at 100ºC for 24h [14]. Acid insoluble ash (indigestible marker – Celite®) concentration in the diets, ileum samples were determined using the method described by Vogtmann et al. [15], and Choct and Annison [16]. Subsequently the necessary chemical analyses were performed to determine the indigestible factor and the AA levels in the test diet and digesta samples, apparent AA digestibility coefficients (AID) were calculated using the following equation [17]:

$$\text{AID, \%} = \{[AA_i - (AA_0 \times FI/DI)]/AA_i\} \times 100,$$

where AA_i and AA_0 are the AA concentration of diet and ileal digesta (%); FI is the feed indicator (%) and DI is the digesta indicator (%). Standard ileal AA digestibility (**SID**) for the treatments was calculated for the correct AA endogenous loss using the following formula by Lemme et al. [18], and Adedokum et al. [19]:

$$\text{SID (g/kg of DM)} = (\text{AID} + \text{basal endogenous AA losses})/ AA_d,$$

where the AID is presented in percentage; basal endogenous AA losses in grams per kilogram of DM; AA_d is the DM of diet AA in grams per kilogram.

Statistical analyses

Data were submitted to a 2-way ANOVA using the GLM procedure of SAS Institute [20]. Significance was accepted at $P \leq 0.05$ and mean differences were separated using Tukey's HSD test [21].

RESULTS AND DISCUSSION

The composition of AA in the experimental diets is shown in Table 2. There were no differences between treatments for mortality ($P>0.05$), and broilers remained healthy and consumed their daily feed allowances. The results of AID of AA are presented in Table 3. The AID for dry matter was higher ($P<0.001$) in broilers fed FM diets than raw feathers, although, this digestibility did not increase when the birds were fed with the incubated feathers diet. The results of AID of DM of FM were5.1 and 9.3% higher than raw feather and incubated feather, respectively. Additionally, the AID of His, Ile, Leu, Met and total AA of the raw feather and FM were higher ($P< 0.05$), compared with incubated feather thus means that the treatment with *Bacillus subtilis* on feathers did not increase the digestibility. The AID of Ile in broilers fed incubated feather was 45.6%, and this is very low compared with 80% in FM and 77.5% in raw feather, but this contrast with Bendegan et al. [22] that found Ile the most digestible AA for FM, also the least digestible AA (39.4%) for them was the Asp been the opposite in this present study (96.4%). The AID of Arg, Cys, and Glu did not differ by feather sources ($P> 0.05$).

Papadopoulos et al. [23] observed that Lys and Phe from FM and incubated feather lose 31.2% of the AID when compared with raw feathers. These authors also reported that this was a result of the heat process to these ingredients. However, the digestibility of Ala increased in 16.2 and 12.7% of FM and incubated, respectively compared with the raw feather.

The results for SID was obtained by the endogenous losses from the NFD provide from Table 4. The AID results showed a shorter AA digestibility range for raw feather (93.1 to 74.0%) compared with FM (96.4 to 50.5%) either with the incubated (94.7 to 45.6%). The SID of AA in broiler chickens fed NFD replaced with different sources of feathers is shown in Table

5. Similar results were observed for SID and AID; however, the variation was shorter, 27.2, 17.4, and 38.5% in feather meal, raw, and incubated feathers, respectively. Sulabo et al. [24] did a comparison of SID for pigs between 4 treatments using FM, that the Lys and Met is not well digestible for pigs, similarly of the results noticed in this research.

The least digestible AID of AA for raw feather was Val (74.0%), for FM was Phe (50.5%), and for incubated feather was Ile (45.6%), and the lowest values of SID follow the AID 77.3% in Val, 69.7% in Phe and 56.6% in Ile for raw, meal and incubated feather, respectively, even for AID or SID the results shows the essential AA for the broiler developing. In contrast, the higher AID was for Asp in all the treatments, this also happened for SID but the results of SID was higher than the AID.

The results for the standardized digestibility elucidated that FM digestibility were higher than the other sources, as well as previously reported for AID. One of the factors, which could result to the lower digestibility for incubated feathers, can be the fact of making with in large scale, because when it is made in lab all the feather can be dissolve, and the AA content increased as reported by Zaghloul et al. [14]. Another problem that Latshaw et al. [25] observed was using four different processing for raw feather, some AA as Ala, Leu, His, Asp, Lys, and Val were found lower compared to the raw feathers. The digestion coefficients was higher (0.72) for the processing with 202 and 322 kPa using continuous hydrolyzation than 428 kPa with continuous hydrolization and batch processed been the 0.66 this confirm the steam and pressure processing can affect on the nutritional value. Likewise, the heat process may cause the formation of new cross-linkage inside of protein molecules, which causes the arrangement of new AA as well as lanthionine, lysinalanine and ornithialanine [26]. This means that the drying and pressure method could change the results for the incubated feather or even for better results of feather meal.

In conclusion, the FM and the raw feather had the similar results for the AID and SID o AA. The inclusion of the *Bacillus subtilis* to degradation of feathers decreases the digestibility of AA when compared with the other two sources of feathers, the availability of the process

with the bacteria should be studied more about the economic and environmental availability because of the amount of water used to degradation.

CONCLUSIONS AND APPLICATIONS

1. The FM and the raw feathers sources presented higher digestibility of AA compared with incubated feathers.
2. The incubation of feathers with *Bacillus subtitlis* did not increase the digestibility of AA.
3. Other researches using the raw feather with some addition of protease should increase the digestibility.

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Table 1. Nitrogen free experimental diet

Ingredients	Inclusion, %
Corn starch	78.06
Sugar	6.72
Soybean oil	5.00
Solka floc[1]	5.00
Dicalcium phosphate	2.57
Limestone	1.00
Salt	0.50
Vitamin and mineral mix[2]	0.15
Celite[3]	1.00

[1]Purificade cellulose, International Fiber Corp., North Tonawanda, NY.
[2]Composition by kg of feed: vit. A, 8,000 UI; vit. D_3, 2,000 UI; vit. E, 30 UI; vit. 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]Insoluble marker (Celite, Celite Corp., Lompoc, CA).

Table 2. Amino acids composition of the experimental diets, g/100g

Item	Dietary treatments			
	Free nitrogen	7% raw feathers	7% feather meal	7% incubated
Indispensable AA				
Arginine	0.00	0.07	0.05	0.07
Histidine	0.01	0.01	0.01	0.01
Isoleucine	0.00	0.02	0.01	0.01
Leucine	0.03	0.04	0.04	0.03
Lysine	0.00	0.01	0.00	0.00
Methionine	0.00	0.01	0.01	0.00
Phenylalanine	0.01	0.04	0.01	0.01
Threonine	0.00	0.04	0.03	0.04
Valine	0.00	0.03	0.02	0.02
Dispensable AA				
Alanine	0.01	0.02	0.07	0.07
Aspartic acid	0.05	0.08	0.17	0.17
Cystine	0.00	0.02	0.01	0.00
Glutamic acid	0.01	0.12	0.07	0.08
Glycine	0.00	0.07	0.05	0.05
Serine	0.01	0.18	0.10	0.12
Tyrosine	0.03	0.02	0.04	0.01
Total AA[1]	0.18	0.86	0.72	0.72

[1]Representing 17 amino acids

Table 3. Apparent ileal digestibility of amino acids in the different feathers diets, %

Item	Dietary treatments			Mean	SEM	*P*-value
	7% raw	7% meal	7% incubated			
DM	78.15[b]	83.29[a]	74.00[c]	80.72	0.566	0.0001
Indispensable AA						
Arginine	87.58	87.79	83.94	84.44	1.243	0.0634
Histidine	85.44[a]	86.47[a]	76.24[b]	82.72	0.588	0.0001
Isoleucine	77.49[a]	80.00[a]	45.64[b]	67.71	2.204	0.0001
Leucine	83.60[a]	85.04[a]	75.66[b]	81.43	1.986	0.0046
Lysine	87.36[a]	68.77[b]	76.92[b]	77.68	2.470	0.0001
Methionine	90.06[a]	87.54[a]	78.41[b]	85.33	1.031	0.0001
Phenylalanine	82.31[a]	50.51[b]	51.92[b]	61.58	2.031	0.0001
Threonine	81.40[ab]	84.20[a]	76.58[b]	80.72	1.449	0.0030
Valine	74.01[b]	81.45[a]	51.51[c]	69.99	1.697	0.0001
Dispensable AA						
Alanine	75.84[b]	92.06[a]	88.54[a]	85.48	1.492	0.0001
Aspartic acid	93.11[b]	96.43[a]	94.67[ab]	94.74	0.769	0.0173
Cystine	79.17	80.58	75.64	78.46	3.035	0.5026
Glutamic acid	86.88	88.68	88.61	88.05	2.021	0.7750
Glycine	75.50[ab]	79.79[a]	70.90[b]	75.39	2.282	0.0341
Serine	82.48[ab]	84.79[a]	76.47[b]	81.24	1.863	0.0106
Tyrosine	85.83[b]	93.23[a]	81.23[c]	86.76	1.089	0.0001
Total AA[1]	84.14[a]	84.99[a]	78.82[b]	82.66	1.229	0.0024

[a-c] different letters same row different by Tukey test ($P<0.05$).
[1]Representing 17 amino acids.

Table 4. Endogenous amino acids losses at the ileal digesta of broilers fed a nitrogen-free diet, g/kg

Item	Endogenous loss, g/kg of DM
Indispensable AA	
Arginine	0.265
Histidine	0.079
Isoleucine	0.103
Leucine	0.126
Lysine	0.078
Methionine	0.103
Phenylalanine	0.159
Threonine	0.314
Valine	0.173
Dispensable AA	
Alanine	0.183
Aspartic acid	0.218
Cystine	0.060
Glutamic acid	0.468
Glycine	0.234
Serine	0.666
Tyrosine	0.118
Total AA[1]	4.178

[1]Representing 17 amino acids.

Table 5. Standardized ileal digestibility of amino acid in the feathers diets, %

Item	Dietary treatments			Mean	SEM	*P*-value
	7% raw	7% meal	7% incubated			
Indispensable AA						
Arginine	89.13^{a}	89.67^{a}	86.10^{b}	89.40	0.753	0.0045
Histidine	90.97^{b}	93.81^{a}	85.51^{c}	92.39	0.387	0.0001
Isoleucine	80.79^{a}	83.35^{a}	56.59^{b}	82.07	1.605	0.0001
Leucine	85.16^{a}	87.46^{a}	79.55^{b}	86.31	0.797	0.0001
Lysine	88.19^{a}	78.24^{b}	84.24^{ab}	83.21	2.697	0.0448
Methionine	92.30	89.38	85.11	90.84	2.679	0.1788
Phenylalanine	84.58^{a}	69.73^{b}	65.68^{c}	77.16	1.037	0.0001
Threonine	85.23^{b}	88.06^{a}	84.11^{b}	86.64	0.779	0.0036
Valine	77.30^{b}	87.93^{a}	61.61^{c}	82.61	1.379	0.0001
Dispensable AA						
Alanine	82.37^{c}	93.72^{a}	89.46^{b}	88.05	1.189	0.0001
Aspartic acid	94.62^{b}	96.93^{a}	95.11^{ab}	95.78	0.643	0.0398
Cystine	82.14	83.07	81.17	82.60	2.407	0.8570
Glutamic acid	89.58	91.13	89.96	90.35	1.232	0.6544
Glycine	78.62^{a}	81.81^{a}	73.89^{b}	80.21	1.031	0.0001
Serine	83.01^{ab}	85.81^{a}	79.70^{b}	84.45	1.101	0.0001
Tyrosine	88.93^{b}	94.88^{a}	87.57^{b}	91.90	0.604	0.0020
Total AA[1]	86.63^{a}	88.31^{a}	84.33^{b}	87.47	0.572	0.0001

a-c different letters same row different by Tukey test ($P<0.05$).
[1] Representing 17 amino acids.

CAPÍTULO III

CONSIDERAÇÕES FINAIS

Este estudo comparou a digestibilidade ileal aparente e padronizada dos AA de penas incubadas com *Bacillus subtilis,* penas cruas e farinha de penas comerciais e os melhores resultados obtidos foram das farinha de penas.Evidenciados pelo resultado da DIP dos AA essências como a histidina, treonina, valina, arginina, histidina, isoleucina e leucina.Portanto, a farinha de penas continua sendo o melhor subproduto depenas a ser adicionada na ração.

A incubação de penas por *Bacillus subtilis* não representou um alimento alternativo para substituir fontes proteicas, pois a viabilidade nutricional foi insuficiente. As penas cruas por sua vez apresentaram uma melhor digestibilidade de AA, contrariando alguns autores citados anteriormente. Os seus resultados foram equivalentes aos resultados de digestibilidade da farinha de penas.

Na produção de penas incubadas com *Bacillus subtilis* alguns fatores devem ser considerados como interferentes na qualidade. Um dos aspectos é referente a presença de impurezas, tais como pele, bico, cabeças e patas, mas não são inerentes ao processo de produção industrial, podendo ser um interferente na eficiência do *Bacillus subtilis.* O segundo aspecto relevante está no processo. Neste, o emprego de grandes quantidades de água implicam em uma posterior secagem com impactos econômicos e ambientais.

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APÊNDICES

Apêndice 1. Normas para publicação de artigos no periódico Journal of Applied Poultry Research

Journal of Applied Poultry Research
Instructions to Authors
Editorial Policies and Procedures

The mission of *Journal of Applied Poultry Research* (JAPR) is to provide practical, reliable, andtimely information to those whose livelihoods are derived from the commercial production of poultryand those whose research benefits this sector; address topics of near-term application based on appropriatelydesigned studies and critical observations; encourage scientific approaches to practicalproblem solving; and present information comprehensible to a broad readership.

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Authors must make it clear that experiments were conducted in a manner that avoided unnecessarydiscomfort to the animals by the use of proper management and laboratory techniques. Experimentsshall be conducted in accordance with the principles and specific guidelines presented in *Guidelinesfor the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, 1st revisededition, 1999 (Association Headquarters, 2441 Village Green Place, Champaign, IL 61822); and, ifapplicable, *Guide for the Care and Use of Laboratory Animals* (United States Department of HumanHealth and Services, National Institutes of Health, Publication Number ISBN 0-309-05377-3, 1996);or *Guide to the Care and Use of Experimental Animals*, 2nd ed., Vol. 1, 1993 (Canadian Council onAnimal Care). Methods of killing experimental animals must be described in the text. In describingsurgical procedures, the type and dosage of the anesthetic agent must be specified. Intra-abdominal orintra-thoracic invasive surgery requires anesthesia. This includes caponization. The editor-in-chief ofJAPR may refuse to publish manuscripts that are not compatible with these guides. If rejected solelyon that basis, however, the paper may be resubmitted

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Field Reports. Field reports will be published when adequate background is available and conclusionscan be supported by quantifiable laboratory or diagnostic results. The manuscript should followthe format outlined in the Style and Form. It should include a section titled Field Report in which theobservations are explained and discussed under subheadings of Materials and Methods and Resultsand Discussion. Authors are encouraged to include subheadings for all major areas in this section.

Review Articles. Articles submitted to this section may cover new developments in a field, describethe evolution of a currently accepted management practice, propose changes in managementbased on current research, or describe procedures. Clear distinctions should be made between firmlyestablished practices and unresolved questions. Articles should begin with a concise description of thetopic, followed by a critical evaluation of the important references. Review articles, whether solicitedor unsolicited, will be subject to a stringent review process.Review articles should follow the general format outlined in the Style and Form when appropriateand include brief subheadings to separate main ideas. The title page should use the appropriate formatand include a summary and statement of primary audience. Review articles may include tables, figures,and photographs. A Conclusions and Applications section should be included in most cases.

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Letters and Commentaries. The journal accepts letters, book reviews, and other free-form communications(used to correct errors, provide clarification, or offer other points of view on pertinentissues). Submissions may be edited in consultation with the author.

SUBMISSION OF MANUSCRIPTS

Authors should submit their papers online to our Web-based submission and review system(http://mc.manuscriptcentral.com/psa). Detailed instructions for submitting electronically are providedonline at that site. Authors who are unable to submit online should contact the editorial office(jeremyh@assochq.org) for assistance.

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The journal uses a two-stage review process. All manuscripts will first receive a preliminary reviewto ensure appropriateness for the journal. The second review will be a more detailed scrutinyby individuals knowledgeable in the specific subject area of the paper. Additional examination of themanuscript will be made by the editors.The review process will be stringent. Names of authors will be made known to reviewers; reviewersmay contact the authors directly with questions, suggestions, and comments if such contact willimprove the paper or streamline the review process. The subject editors will handle all initial correspondencewith authors during the review process; the editor-in-chief will notify the author of thefinal decision to accept or reject.

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Accepted manuscripts are forwarded to the editorial department for preparation for typesetting. Atthis point, a technical editor may contact the authors for missing information or table or figure revisions.

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Author proofs of all manuscripts will be sent to the corresponding author indicated on the titlepage of the manuscript. Proofs should be read carefully, because the responsibility for proofreadingis with the authors.

Corrections to the proof should be made neatly and clearly in the margins of the proof. Galleyproofs should be faxed (217-378-4083) to PSA headquarters. Proofs should be corrected and returnedwithin 3 working days.

Editor queries appear in the text, within brackets and in boldface type. Queries should be answeredon the galley proofs; failure to do so may delay publication.

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Two options are available for the publication of articles in this journal: conventional page chargesand Open Access (**OA**).

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MANUSCRIPT PREPARATION: STYLE AND FORM

Preparing the Manuscript File

Manuscripts should be submitted in Microsoft Word 2003 and should be double-spaced with linesand pages numbered consecutively using Times New Roman font at 12 points. Files created in Office2007 should be saved down to Office 2003 before submission for compatibility with our compositionsoftware. All special characters (e.g., Greek, math, symbols) should be inserted using the symbolspalette available in this font. Complex math should be entered using MathType or another equationeditor. Tables and figures should be placed in separate sections at the end of the manuscripts(not placed in the text). Failure to follow these instructions may result in immediate rejection of themanuscript.

Metric or English units (or both) are acceptable. Authors should use units appropriate for the intendedaudience. Energy content of feeds will be expressed as calories.

Headings

Major Headings. Major headings are centered, boldface, in all capital letters, and consist of SUMMARY,DESCRIPTION OF PROBLEM, MATERIALS AND METHODS, RESULTS AND DISCUSSION,CONCLUSIONS AND APPLICATIONS, and REFERENCES AND NOTES.

Major headings in review articles, field reports, and symposium articles may vary from those listedhere, but should include SUMMARY, CONCLUSIONS AND APPLICATIONS, and REFERENCESAND NOTES.

First Subheadings. First subheadings are boldface and italic, on a separate line beginning at theleft margin, and have the first letter of each important word capitalized. Text that follows a first subheadingshould 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 is capitalized. Thetext follows immediately after the final period of the subheading.

Title Page

• The title should be indicative of the content. It should capture the interest of all who mightbenefit from information in the manuscript. However, the length of the title should be kept toa minimum.

• Address and affiliation of authors should be included. Indicate to whom correspondence shouldbe directed by means of a footnote, with the notation "Corresponding author: (e-mail address)"

at the bottom of the title page. • List 3 to 8 key words or phrases to identify the most important subjects covered by the paper.

• The running title should be 30 characters or less, including spaces.

• Statement of primary audience: To determine appropriateness for the journal and to assist inselecting reviewers, the author should indicate clearly what sector(s) within the poultry community(e.g., flock supervisors, nutritionists, quality assurance personnel, researchers, plantmanagers, veterinarians) could most benefit from the content of this article.

Summary

The Summary (12 to 16 lines) is not an abstract. It is intended to give readers with diverse backgroundsa general appreciation of the manuscript contents. It should be written so that even those notdirectly interested in the topic will enjoy reading at least this section to keep abreast of areas otherthan their own. This section should not include details of materials and methods or a detailed reviewof the results. Keep the summary free-flowing, giving the reader a general, not specific, idea of whatthe study revealed. Do not include reference citations in the summary.

Description of Problem

This section will acquaint the reader with the problem, citing field experiences where appropriate.Readability is of utmost importance. Detailed literature reviews may not be appropriate for this section.

A more extensive citation of references should be included in the Results and Discussion or Referencesand Notes section. This section should end with a statement of the objective(s) of the study.

Materials and Methods

The author(s) should clearly establish in the Materials and Methods section why the problemwas approached in a particular way. The rationale for including each treatment should be clearlystated. Detailed laboratory and bird management procedures should be described in the Referencesand Notes section and not in the Materials and Methods section. Sources of stock, equipment, andmaterials should be listed in the References and Notes section and not in the text or a footnote.

A brief statement of the statistical methods should be included, with more detailed descriptionsplaced in the References and Notes section.

In manuscripts using several treatments, a description of treatments should be included asTable 1.

Results and Discussion

This section begins with observed results and their interpretation. Descriptive subheadings mayprecede all major paragraphs and changes in subject emphasis. This section should discuss specificallyhow findings address the problem described in the Description of Problem section and how theyare related to published works.

Statements regarding statistically significant differences between treatments in results should beincluded in the text, tables, and figures. Statements regarding differences should be avoided unlessthey are supported by statistical analyses and meet the stated level of probability (e.g., $P < 0.05$).

Conclusions and Applications

Conclusions and recommendations of the author(s) should be listed numerically. Each statementshould be clear, concise, and without discussion. Authors are encouraged to summarize their significantfindings, to identify further research needs, and to describe the constraints, economics, andother factors associated with using the results in scientific or commercial applications. Do not includereferences in this section.

References and Notes (with Acknowledgments)

References and notes should be cited in text, by number within an editorial bracket (e.g., [1]). Inthe References and Notes section, citations should be listed in the order they appear and are numberedin the text (not alphabetically). Authors are encouraged to use reference management software(e.g., EndNote or Reference Manager) to facilitate renumbering or inserting references by the editoror inserting references during the revision process. Manuscripts may be returned to authors *beforereview* for renumbering of references if not cited in numerical order. Include details such as statisticalanalysis; detailed procedures; sources of birds, instruments, or items; details of designed instruments;a literature review; and other tangential matters.

Cite acknowledgments at the end of this section in a subsection called *Acknowledgments*. Theseentries are not numbered.

Tables

Number tables consecutively according to the citation in the text. Tables must be created using theMS Word table feature and inserted in the manuscript after the references section. Each table must beplaced on a separate page and must have a clear descriptive heading so that the meaning of the datawill be understandable without reference to the text. Indicate footnotes to tables with numbers, beginningwith 1. Statistical notation should be made with lowercase and uppercase superscript letters orwith asterisks, as appropriate. Statistical notation should place the superscript “a” on the largest mean.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 the journal for examples of

tables.

Figures

• ***Figure Size***. Prepare figures at the final size for publication. Figures should be created at thefinal publication size of 8.9 cm wide (1 column), 14 cm wide (2 column), or 19 cm wide (fullpage width).

• ***Font Size***. Ensure that all type within the figure and axis labels is readable at the final publicationsize. A minimum type size of 8 points (after reduction) should be used.

• ***Fonts***. Use Helvetica or Times New Roman. Symbols may be inserted using the Symbol palettein Times New Roman.

• ***Line Weight***. For line graphs, use a minimum stroke weight of 1 point for all lines. If multiplelines are to be distinguished, use solid, long dash, short dash, and dotted lines. Avoid the use ofcolor, gray, or shaded lines because these will not reproduce well. Lines with different symbolsfor 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 thedescriptor by a comma or parentheses, and should be consistent within a manuscript.

• ***Shading and Fill Patterns***. For bar charts, use different fill patterns if needed (e.g., black,white, gray, diagonal stripes). Avoid the use of multiple shades of gray because they will not beeasily distinguishable in print.

• ***Symbols***. Identify curves and data points using the following symbols only: □, ■, ◯, ●, ▲, ▼,△, ▽, ◊, ♦, +, 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 Power-Point 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 ingrayscale. Often color will mask contrast problems that are apparent only when the figure isreproduced in grayscale.

• ***Color Figures***. If figures are to appear in color in the print journal, files must be submitted inCMYK color (not RGB).

• ***Photomicrographs***. Photomicrographs must have their unmagnified size designated, either inthe caption or with a scale bar on the figure. Reduction for publication can make a magnificationpower designation (e.g., 100×) inappropriate.

• ***Caption***. The caption should provide sufficient information so that the figure can be understoodwithout excessive reference to the text. All author-derived abbreviations used in the figureshould be defined in the caption.

• ***General Tips***. Avoid the use of 3-dimensional bar charts unless essential to the presentation ofthe data. Use the simplest shading scheme possible to present the data clearly. Ensure that data,symbols, axis labels, lines, and the key are clear and easily readable at the final publicationsize.

Color Images. The cost to publish in color is $995 per figure; there is also a surcharge for coloroffprints. The corresponding author should complete a Color Charge Agreement form, available onthe journal Web site (http://japr.fass.org/misc/ifora.dtl) and should fax (217-378-4083) that form tothe JAPR editorial office when a manuscript with color figures is accepted for publication.

Sample References

NOTE: The headings that appear above the following sample references and notes are for clarificationin these instructions, but they are not used in an actual paper, except for *Acknowledgments*.

Journal Article

Dansky, L. M., and F. W. Hill. 1952. Application of the chromic oxide indicator method to balance studies with growing chicks. J. Nutr. 47:449–459.

Snow, J. L., M. W. Douglas, and C. M. Parsons. 2003. Phytase effects on amino acid

digestibility in molted laying hens. Poult. Sci. 82:474–477.
Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, with and without prior vaccination, to challenge with Marek's disease virus. Avian Dis. doi:10.1637/7498-010306.R1

Monograph

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Dissertation

Heskett, E. A. 2003. Efficacy of a recombinant herpes virus of turkeys vector vaccine, expressing genes to Newcastle disease virus and Marek's disease virus, in chickens and turkeys against exotic Newcastle disease virus challenge. PhD Diss. Univ. Florida, Gainesville.

Trade Publication

Wilgus, H. S. 1973. Temperature-programmed feeding schedules and other means of conserving protein in market turkey production. Feedstuffs 45(27):27–31.

Book or Chapter in Book

AOAC International. 2007. Official Methods of Analysis of AOAC International. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.
Whittow, G. C. 1976. Regulation of body temperature. Pages 146–173 in Avian Physiology. P. D. Sturkie, ed. Springer-Verlag, New York, NY.

Proceedings

Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Pages 3–5 in Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium, MD. Univ. Maryland, College Park.

Federal Register

USDA, Plant and Animal Health Inspection Service. 2004. Blood and tissue collections at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Regist. 69:10137–10151.

Laboratory Procedure

The extract was added to 30 mL of hexane, made to 100 mL with 10% aqueous Na2SO4.

Personal Communication

Wilson, H. R. 2005. Univ. Florida, Gainesville. Personal communication.

Proprietary Product

Incubator, Petersime, Zulte, Belgium. Avizyme TX, Finnfeed International, Marlborough, Wiltshire, UK. Thymol, 99% purity, Acros Organics, Geel, Belgium.

Statistical Procedure

If a note has an embedded reference, the reference is cited by number (as in the text) or parenthetically within the note:

Data were analyzed by ANOVA with flock as the independent variable. When differences among flocks were significant, means were separated using Duncan's multiple range test (SAS User's Guide, 2001, Version 8 ed., SAS Institute Inc., Cary, NC). Pearson product-moment correlation coefficients were calculated between average percentage cracks from each flock recorded every week and average values for egg-specific gravity, breaking strength, percentage shell, shell thickness, and shell weight per unit of surface area. Significance implies $P < 0.05$.

Statistical Software

SAS User's Guide. 2001. Version 8 ed. SAS Inst. Inc., Cary, NC.

US Patent

El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, assignee. US Pat. No. 6,766,767. ***Web Site***
Dyro, F. M. 2005. Arsenic. WebMD. http://www.emedicine.com/neuro/topic20.htmAccessed Feb. 2006.

Acknowledgments

The advice and technical assistance of Thomas Jones (affiliation, location) are acknowledged.

Abbreviations

The following abbreviations may be used without definition in the *Journal of Applied Poultry Research*. Plurals do not require "s". Chemical symbols and 3-letter abbreviations for amino acids do not need definition. Other abbreviations should be defined at first use in the summary and the main text, as well as in each table or figure in which they appear. Abbreviations are boldface at first use in the main text. Abbreviations should not be used in the manuscript title, running title, or to begin a paragraph or sentence. They can be used in section headings if previously defined. This list appears inside the back cover of each issue of the journal.

ADF	acid detergent fiber
ADFI	average daily feed intake
ADG	average daily gain
AME	apparent metabolizable energy
AMEn	nitrogen-corrected apparent metabolizable energy
ANOVA	analysis of variance
BSA	bovine serum albumin
BW	body weight
°C	Celsius
cDNA	complementary DNA
CF	crude fiber
cfu	colony-forming units (following a numeral)
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
EE	ether extract
°F	Fahrenheit
FCR	feed conversion ratio
FE	feed efficiency
ft	foot
g	gram
gal	gallon
G:F	gain-to-feed ratio
GLM	general linear model
h	hour
HEPES	*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid
HPLC	high-performance (high-pressure) liquid chromatography
ICU	international chick units
Ig	immunoglobulin
IL	interleukin
i.m.	intramuscular
in.	inch
i.p.	intraperitoneal
IU	international units
i.v.	intravenous

kcal	kilocalorie
L	liter (also capitalized with any combination, e.g., mL)
lb	pound
L:D	hours of light:hours of darkness in a photoperiod
LSD	least significant difference
m	meter
µ	micro
M	molar
ME	metabolizable energy
MEn	nitrogen-corrected metabolizable energy
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
min	minute
mo	month
MS	mean squares
n	number of observations
N	normal
NAD	nicotinamide adenine dinucleotide
NADH	reduced form of NAD
NDF	neutral detergent fiber
NRC	National Research Council
NS	not significant
PBS	phosphate-buffered saline
ppm	parts per million
r	correlation coefficient
r2	coefficient of determination, simple
R2	coefficient of determination, multiple
RH	relative humidity
RIA	radioimmunoassay
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
s.c.	subcutaneous
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SNP	single nucleotide polymorphism
SRBC	sheep red blood cells
TBA	thiobarbituric acid
T	cell thymic-derived cell
TME	true metabolizable energy
TMEn	nitrogen-corrected true metabolizable energy
TSAA	total sulfur amino acids
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

Supplemental Information (Online)

The following information is available online and is updated regularly. Please refer to these pages when preparing a manuscript for submission.

Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online (http://japr.fass.org/misc/ifora.dtl).

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

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VITA

Barbara de Almeida Mallmann, filha de Carlos Augusto Mallmann e Ezilda Jacobsen de Almeida, nasceu em Santa Maria, RS, em 5 de abril de 1988. Cursou o ensino fundamental no Colégio Riachuelo, em Santa Maria - RS. Concluiu o ensino médio no Colégio Santa-mariense Objetivo também em Santa Maria - RS. Em 2006 ingressou no Curso de Medicina Veterinária da Universidade Federal de Pelotas, RS, onde realizou estágios na área de patologia, nutrição e reprodução em aves. Em 2008 à 2010 participou do Grupo de Estudos em Aves e Suínos de Pelotas(GEASPEL), em 2010 teve a oportunidade de fazer estágios na Alemanha nas áreas ruminantes, reprodução e veterinária preventiva. Em 2011 até 2012 participou do Laboratório de Reprodução de Aves (LABRA). Realizou o estágio final obrigatório na University of New England (UNE) na Austrália, exercendo atividades com experimentação na avicultura. Obteve o Grau de Médica Veterinária em agosto de 2012. Ingressou, em abril de 2013, o curso de mestrado na área de nutrição animal no programa de pós-graduação da Zootecnia na Universidade Federal do Rio Grande do Sul sob orientação do Prof. Sergio Vieira. Foi submetida à banca de defesa de Dissertação em março de 2015 pela Universidade Federal do Rio Grande do Sul em Porto Alegre, RS.