UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

N,N, DIMETILGLICINA EM DIETAS PARA FRANGOS DE CORTE

DISSERTAÇÃO DE MESTRADO

Lenise Schröder Boemo

Santa Maria, RS, Brasil
2012

N,N, DIMETILGLICINA EM DIETAS PARA FRANGOS DE CORTE

Lenise Schröder Boemo

Dissertação apresentada ao Curso de Mestrado do Programa de
Pós-Graduação em Zootecnia, Área de Concentração em
Produção Animal, da Universidade Federal de Santa Maria (UFSM, RS),
Como requisito parcial para obtenção do grau de
Mestre em Zootecnia.

Orientador: Prof. Alexandre Pires Rosa

Santa Maria, RS, Brasil
2012

Boemo, Lenise Schröder
N,N dimetilglicina em dietas para frangos de corte/ por Lenise Schröder Boemo.– 2012.

Orientador: Alexandre Pires Rosa
Coorientador: Irineo Zanella
Dissertação (mestrado) – Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós-Graduação em Zootecnia, RS, 2012

1. Frangos de corte 2. Desempenho 3.Rendimento de carcaça 4. Gordura abdominal 5. Peito I. Rosa, Alexandre Pires II.Título.

CDU 636.52/.58

Ficha catalográfica elaborada por Simone G. Maisonave - CRB 10/1733
Biblioteca Central da UFSM

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Universidade Federal de Santa Maria
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Programa de Pós-Graduação em Zootecnia

A Comissão Examinadora, abaixo assinada,
aprova a Dissertação de Mestrado

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elaborada por
Lenise Schröder Boemo

como requisito parcial para obtenção do grau de
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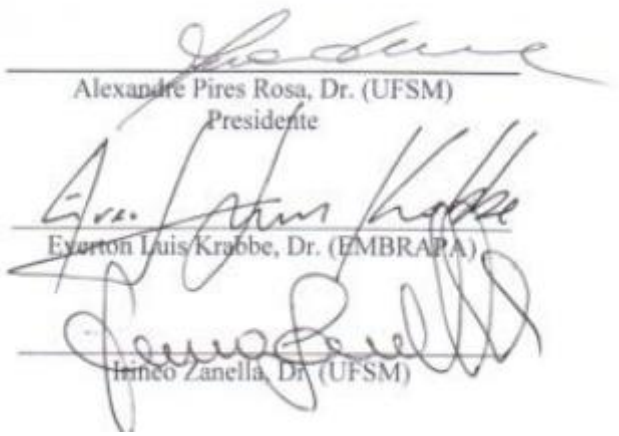
COMISSÃO EXAMINADORA



Santa Maria, 27 de fevereiro de 2012

AGRADECIMENTOS

Agradeço a Deus, pela vida e por todas as dádivas recebidas ao longo desta.

A Universidade Federal de Santa Maria, na pessoa de seus professores e funcionários, por possibilitarem as atividades de ensino, pesquisa e extensão necessárias, para o desenvolvimento de nosso pais com qualidade e comprometimento. Em especial agradeço ao meu orientador Alexandre Pires Rosa pelas horas despendidas a apoiar a realização deste trabalho, e ao professor Irineo Zanella pelo apoio e carinho ao longo desta trajetória.

Ao amor, dedicação e a compreensão de meus pais e de meu irmão Lucas Boemo, família que sempre esteve ao meu lado fornecendo apoio, amor e o incentivo para que eu pudesse continuar minha caminhada de aperfeiçoamento profissional.

Ao meu namorado, que sempre esteve ao meu lado com seu amor e carinho.

Ao Laboratório de Avicultura da Universidade Federal de Santa Maria (LAVIC), pela estrutura cedida para a condução desse estudo, e aos seus professores, funcionários e colaboradores, por possibilitarem um aprendizado constante não somente na área técnica, mas também pessoal. Em especial ao funcionário Sandro Debus pelo seu apoio e dedicação para a realização deste trabalho.

A empresa Taminco NV, Ghent, Belgium pela parceria na condução deste trabalho.

Agradeço, a todos os colegas e amigos que estiveram presentes nesse período, em especial as amigas queridas Bethy Brittes, Carolina Fantinel, Daniele Rosa e Priscila Becker.

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
Universidade Federal de Santa Maria

N,N-DIMETILGLICINA EM DIETAS PARA FRANGOS DE CORTE

AUTORA: LENISE SCHRÖDER BOEMO
ORIENTADOR: Dr. ALEXANDRE PIRES ROSA
Data e Local da Defesa: Santa Maria, 27 de fevereiro de 2012.

N, N-dimetilglicina (DMG) é um metabólito intermediário da colina no metabolismo celular da glicina, é formado nas mitocôndrias do fígado através da remoção do grupo de metil da betaína. O objetivo deste estudo foi avaliar o efeito de DMG na alimentação de frangos de corte, a fim de avaliar as características de desempenho e de carcaça. O experimento foi realizado na Universidade Federal de Santa Maria - Brasil. Foram utilizados 1.920 frangos de corte de 1 dia de idade, da linhagem Cobb-500 criados até os 42 dias de idade. As aves foram divididas aleatoriamente em três tratamentos com dez repetições de 64 aves cada. Foram utilizadas dietas com dois níveis de DMG (0 ou 1000mg de DMG/kg de dieta) e uma dieta com promotor de crescimento (AGP). As dietas foram formuladas a base de milho e farelo de soja. Aos 42 dias de idade as aves alimentadas com dieta com antibiótico tiveram maior peso corporal que as aves que receberam dieta controle (P=0,0117), mas o peso corporal e o ganho de peso das aves do grupo suplementado com antibióticos não diferiram das aves suplementadas com DMG. O consumo de ração no final do período experimental foi menor nas aves suplementadas com DMG em comparação as aves do grupo suplementado com AGP. O estudo das carcaças apresentou diferenças entre os tratamentos. Aves alimentadas com DMG tiveram melhor rendimento de carcaça (74,98%) do que as aves sem DMG (73,58%), (P=0,0195). O rendimento de peito em relação à carcaça foi 1,16% maior no grupo suplementado com DMG em relação às aves do grupo controle (P=0,0034). O percentual de gordura abdominal foi reduzido em 0,27 % nas aves do grupo DMG em relação às alimentadas com AGP (P=0,0427). Os resultados deste estudo mostram que N, N-dimetilglicina (DMG) teve efeito positivo sobre as características da carcaça de frangos de corte.

Palavras-chave: desempenho, rendimento de carcaça, gordura abdominal, peito.

ABSTRACT

Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
Universidade Federal de Santa Maria

N, N- DIMETHYLGLYCINE IN DIETS FOR BROILERS

AUTHOR: LENISE SCHRÖDER BOEMO
ADVISER: Dr. ALEXANDRE PIRES ROSA
Presentation Place and Date: Santa Maria, 27 February, 2012.

N,N-dimethylglycine (DMG) is intermediary metabolite in cellular choline to glycine metabolism, it is formed in the mitochondria of the liver by removing the group from betaine metil. The objective of this study was to determine the effect of dietary DMG in order to evaluate the performance and carcass characteristics of broilers. The experiment was carried out at the Federal University of Santa Maria – Brazil. A total of 1920 1-day-old broiler (Cobb-500) were raised until 42 days of age. Birds were randomly assigned in three treatments with ten replicate pens of 64 birds each. It was used diets with two levels (0 or 1,000mg DMG/kg feed) and a diet with growth promoter (AGP). Diets were formulated based on corn and soybean meal. At 42days of age birds fed with diet with antibiotic had higher body weight broilers fed with control diet (P=0.0117), but the body weight of the birds and the BWG of antibiotic supplemented group did not differ. Feed intake at the end of the experimental period was lower in the birds of DMG supplemented than birds of antibiotic supplemented group. The study of carcass demonstrated effect of treatments. Birds fed with N,N-dimethylglycine had better carcass yield (74.98%) than birds without DMG (73.58%) in their diets (P=0.0195). Also this product increased the percentage of breast (P=0.0034) in 1.16% in relation birds fed without DMG and decreased the percentage of abdominal fat (P=0.0427) in 0.27% in relation to birds fed with AGP. Results of this studies suggest that the N,N-dimethylglycine (DMG) had positive effect on carcass characteristics of broilers.

Keywords: performance; carcass yield; abdominal fat; breast

SUMÁRIO

INTRODUÇÃO

O setor avícola obteve grandes avanços nos últimos anos. Esses avanços aconteceram devido há estudos e desenvolvimento de novas tecnologias em diversas áreas da cadeia avícola, tornando a atividade de produção de aves e ovos em uma grande indústria de alimentos. Dados dos últimos 20 anos mostram que o peso corporal de frangos comerciais com 56 dias de idade dobrou de 1.600 para 3.000 g. Durante o mesmo período, o crescimento do músculo *Pectorais major* aumentou numa velocidade maior que o peso corporal (LILBURN, 1994). Nesse cenário a procura por melhores resultados é constante e sem margens para perdas produtivas.

O aumento do conhecimento da fisiologia nutricional levou ao ajuste de dietas para permitir o crescimento rápido e com alta eficiência produtiva (KALMAR, 2011). A extensão dos recentes avanços alcançados na produção de frangos de corte tem sido fornecida a partir de testes de desempenho, comparando linhagens de frangos modernas e dietas formuladas com base em novos conceitos, incluindo o uso de aminoácidos sintéticos, desenvolvimento de macro e micro elementos como potenciadores de desempenho e melhoradores das características de carcaça, visando manter a qualidade do produto final (UNI, 1998). Além disso, a vantagem econômica de obter o rendimento máximo de produto por kg de ração consumida se torna evidente tendo em vista que custo da alimentação é o principal custo variável da produção de frangos de corte.

A metionina é o primeiro aminoácido limitante em dietas de aves, baseadas em milho e farelo de soja, e sua suplementação pode melhorar o desempenho de crescimento e qualidade de carcaça de frangos de corte (XIE et al. 2007). Metionina, em forma de S-adenosilmetionina é o principal doador de radical metil no organismo, sendo que este é necessário para a biossíntese de muitas substâncias importantes envolvidas no crescimento, como creatina, carnitina, poliaminas, epinefrina, colina e melatonina (BAKER, 1991).

Autores relataram efeito poupador de metionina pela ação da colina (PESTI et al. 1981;. BAKER et al. 1983). Antes de a colina atuar como um doador de grupo metil precisa ser convertida em betaína e depois para N, N-Dimetilglicina (DMG). Saunderson e MacKinlay (1990) apresentaram dados sugerindo que a betaína pode ser um agente lipotrófico mais eficaz que a colina para aves. Há várias evidências de que a

betaína influência o crescimento e função intestinal, melhorando assim a digestibilidade dos nutrientes (EKLUND et al., 2005).

N, N-Dimetilglicina é o composto de interesse desse estudo. DMG pode estar envolvido em uma serie de processos biológicos, uma vez que é um metabólio celular intermediário da colina no metabolismo da glicina.

Friesen et al. (2007) descreve um papel de DMG como fonte de glicina para a síntese de glutationa e que este pode atuar como um doador de grupos metil. Hariganesh e Prathiba (2000) sugerem que DMG, quando administrado por via oral, possui um potencial poder sequestrador de radicais livres e antioxidante similar ao da betaína. Kalmar (2011) relata que DMG pode melhorar a absorção de nutrientes por meio do encapsulamento de nutrientes por gotículas de gordura, o que melhora a acessibilidade às enzimas digestivas e a absorção na borda em escova do intestino delgado.

Trabalhos utilizando DMG na dieta de aves são escassos. Não sendo encontrados até o momento registros na bibliografia de trabalhos utilizando DMG com frangos de corte na avicultura brasileira.

Os principais objetivos do presente estudo foram investigar o efeito da suplementação dietética com DMG sobre os parâmetros de desempenho de frangos de corte e sobre as suas características de carcaça.

CAPITULO 1
ESTUDO BIBLIOGRÁFICO

Aditivos na alimentação animal

O frango de corte comercial é hoje um dos animais que apresenta maior eficiência nutricional e rápido desenvolvimento (FURLAN, 2006).

A área da nutrição animal é muito dinâmica, sempre buscando novas estratégias para melhorar o aproveitamento dos nutrientes dietéticos, na tentativa de assegurar condições para que os animais expressem o seu potencial genético máximo de produção de carne, sem que haja acréscimos aos custos de produção e atenda as exigências do mercado consumidor (ARAÚJO et al. 2007). São várias as pesquisas realizadas na busca de alternativas que possibilitem a formulação de rações mais eficientes e econômicas, visto que a alimentação constitui o item de maior custo na produção de frango de corte (STRADA et al. 2005).

Um dos fatores que contribuem para a obtenção da alta produtividade apresentada pela indústria avícola é sem dúvida a utilização de aditivos nas dietas (ARAUJO et al. 2007). São vários os conceitos existentes para definir os aditivos utilizados nas dietas, de acordo com Brasil (2004), considera-se aditivo destinado à alimentação animal, substância, microorganismo ou produto formulado, adicionado intencionalmente a dieta, que não é utilizada normalmente como ingrediente, tenha ou não valor nutritivo e que melhore as características dos produtos destinados à alimentação animal ou dos produtos animais, melhore o desempenho dos animais sadios e atenda às necessidades nutricionais ou tenha efeito anticoccidiano.

Entre os aditivos alimentares regulamentados no Brasil, são encontrados os destinados à fins zootécnicos, que pode ser toda substância utilizada para influir positivamente na melhoria do desempenho dos animais (RENGEL, 2010).

O uso de alternativas, como os modernos produtos da biotecnologia assumem importância significativa para a avicultura industrial com o propósito de melhor os resultados de desempenho e manter a qualidade do produto final (ARAÚJO et al. 2007).

Metionina

Os aminoácidos são utilizados pelas aves para a manutenção da saúde e integridade dos tecidos e posteriormente para a produção de músculos, ovos, pele, penas e tudo que contenha especialmente um alto conteúdo de proteína (BARBOSA et al. 2002).

Basicamente, as rações avícolas são formuladas à base de milho e farelo de soja, fontes de energia e proteína, respectivamente. Entretanto, esses ingredientes não fornecem todos os aminoácidos essenciais em quantidades suficientes para um ótimo desempenho animal (AMARANTE JUNIOR et al. 2005). Até pouco tempo atráz as rações para frangos de corte eram formuladas somente para atender o valor de proteína bruta. Posteriormente, os níveis de aminoácidos totais foram utilizados para atender as necessidades proteicas dos frangos de corte (OLIVEIRA NETO et al. 2009), como também a formula de rações baseadas nos aminoácidos digestíveis.

A redução da proteína da dieta tem recebido considerável atenção na indústria de aves. A suplementação com aminoácidos industriais, principalmente metionina e lisina, tem sido comum há vários anos, permitindo considerável redução no nível de proteína bruta das dietas (GRANA, 2008). Estudos mostram que o excesso de proteína ou o imbalanço entre os aminoácidos podem comprometer o desempenho dos frangos de corte, por promover uma carga excessiva de aminoácidos na circulação sanguínea que, para serem metabolizados, exigem um gasto extra de energia, a qual é desviada da produção para os processos de excreção do nitrogênio na forma de ácido úrico (ALETOR et al. 2000).

A metionina (Figura 1) é o primeiro aminoácido limitante em rações para aves à base de milho e farelo de soja, destacando-se por participar na síntese de proteína, ser precursora da cisteína e doadora de radicais metil (WARNICK; ANDERSON, 1968). Especialmente no período de crescimento, grandes quantidades de aminoácidos sulfurosos são exigidas pelas aves. Os aminoácidos sulfurosos são os principais limitantes nas rações e, para suprir essa deficiência geralmente às dietas são suplementadas com aminoácidos sintéticos disponíveis no mercado (SILVA et al. 1999).

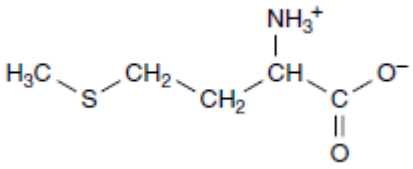


Figura 1. Representação da molécula de metionina.

A suplementação de aminoácidos sintéticos tem propiciado facilidades no ajuste das fórmulas de ração, possibilitando a obtenção dos níveis exigidos de aminoácidos essências (SCHEUERMANN et al. 1995). O nível adequado de metionina depende de vários fatores como a finalidade de produção, a genética, a idade o animal, o ambiente entre outros (VIANA et al. 2009)

A metionina, na forma de S-adenosilmetionina, é o mais importante doador do radical metil no organismo, sendo exigida para a biossíntese de muitas substâncias importantes envolvidas no crescimento, como creatina, carnitina, poliaminas, epinefrina, colina e melatonina (BAKER, 1991). Uma dieta deficiente em metionina reduz o ganho de peso, a eficiência alimentar e o teor de proteína na carcaça, além de estimular o consumo de ração, contribuindo com energia adicional e, consequentemente, ocasionando acréscimo na deposição de gordura corporal (SUMMERS et al. 1992; MORAN, 1994).

Colina

A colina é considerada um nutriente essencial ao organismo animal, porém sua classificação ainda é controversa, pois a colina não se enquadra na classificação clássica das vitaminas (BERTECHINI, 2006). Felix et al. (2009) classificam a colina como um aminoálcool quaternário essencial para a síntese de fosfolipídeos, como a lecitina e a esfingomielina. A esfingomielina é um fosfolipídio contendo um ácido graxo, um radical fosfórico, colina e um álcool aminado (POMPEU et al. 2011). De acordo com Ammerman et al. (1995) em nutrição animal a colina permanece na categoria das

vitaminas do complexo B, embora ela seja exigida em quantidades muito superiores às outras vitaminas do complexo B.

A colina é um componente dietético necessário para a função normal de todas as células. Ela e seus metabólitos, incluindo fosfolipídios, betaína e acetilcolina, asseguram a integridade estrutural e funções sinalizadoras das membranas celulares (DONAVAN et al. 1997). A colina é absorvida principalmente no intestino delgado e é exigida pelo organismo para: síntese de fosfolipídios, a formação de acetil colina, e transmetilação da homocisteína em metionina (AMMERMAN et al. 1995). A acetil colina é um importante transmissor que influencia na função do cérebro, coração, músculo, glândula adrenal, trato gastrintestinal e muitos outros órgãos (ZEISEL, 2000).

A colina é encontrada praticamente em todos os ingredientes utilizados na formulação de dietas de aves. No entanto, as fontes proteicas de origem animal são as mais ricas nesta vitamina (POMPEU et al. 2011). As exigências de colina podem ser influenciadas pelo teor de lipídeos da dieta (maiores níveis de colina em dietas ricas em lipídeos) e aminoácidos sulfurados, já que é sintetizada no fígado a partir da metionina (BARROETA et al. 2002). Níveis excessivos de proteína na dieta aumentam acentuadamente a necessidade alimentar de colina para as aves (MOLITORIS; BAKER, 1976.; KETOLA; NESHEIM, 1974).

A deficiência de colina é prontamente percebida em frangos alimentados com dieta pobre em colina, ou em seus precursores, acarretando em retardo no crescimento das aves (POMPEU, et al. 2011; AMMERMAN, et al. 1995). É difícil observar os sinais de toxicidade pelo excesso de colina na ração, uma vez que as aves toleram altos níveis – até 30.000 mg/kg de alimento (LEESON; SUMMERS, 2001).

Em função da inter-relação entre a colina, metionina e betaína, torna-se difícil estabelecer as exigências de colina, justificando as variações encontradas entre estudos (FELIX et al. 2009). Segundo o NRC (1994), as exigências de colina variam entre 750 e 1.300 mg/kg de alimento, dependendo da idade e da linhagem. Comercialmente, os níveis de colina variam de 1200 a 1800 mg/kg , com tendência de aumento dos níveis na dieta inicial e de crescimento.

Betaína

A betaína ou trimetilglicina é um produto natural, sintetizado por muitos organismos vivos (NIANG, 2005). Segundo Lima et al. (2011), a betaína é um composto metabólico, produto da oxidação da colina e que serve como doador de metilas no ciclo da adenosil-metionina à cisteína. Desta forma, ela funciona como um poupador de metionina e/ou colina nos processos metabólicos. A betaína atua na regulação do equilíbrio osmótico, sendo a única fonte doadora de grupos metil prontamente ativa (VIEIRA et al. 2001). Outros doadores, como a colina e a metionina, necessitam passar por transformações, para serem utilizados pelos animais: a colina deve ser convertida em betaína na mitocondria celular e a metionina precisa ser ativada através da síntese de S-adenosil-metionina (BETANCOURT, 1999).

Os efeitos do uso de betaína na nutrição avícola têm sido apresentados desde meados dos anos quarenta (McGINNIS et al. 1942; ALMQUIST; GRAU, 1943). De acordo com Penãflorida e Virtanen (1996), a betaína é uma alternativa competitiva para balancear rações com grandes quantidades de ingredientes vegetais, as quais poderiam apresentar problemas na qualidade da proteína e desequilíbrio em aminoácidos.

Niang (2005) relata que o interesse dos pesquisadores na utilização da betaína em dietas de frango de corte é devido às funções que esta apresenta como osmólito orgânico e como doadora de grupamentos metil. A natureza bipolar da betaína ajuda no balanço hídrico das células e como doadora de grupo metil. A betaína pode contribuir para a síntese de importantes compostos, incluindo proteína, aumentar a disponibilidade de metionina para síntese proteica e permitir o crescimento muscular. Além disso, é também conhecido que no metabolismo da betaína, há produção de glicina, um aminoácido importante na síntese de proteína que colabora para o desenvolvimento muscular.

Hruby et al. (2004) descrevem que a betaína reduz lesões associadas com coccidiose intestinal também mostra melhora no desempenho das aves e na digestibilidade quando suplementadas em aves desafiadas com coccídeos. Assim, a suplementação com betaína indiretamente reduz o suprimento nutricional à microflora gastrointestinal, devido à habilidade desta substância em melhorar a integridade intestinal e a subsequente absorção durante o desafio com coccídeos. Niang (2005)

também relata sobre o efeito osmólito da betaína que resulta em melhorias significativas na resistência do epitélio intestinal contra infecções coccídianas em frangos. Essa mesma autora ainda afirma que a betaína causa efeitos positivos sobre o desempenho de frangos de corte, melhora as características de carcaça e a digestibilidade dos nutrientes em aves infectadas ou não com coccídeos.

A betaína melhora a retenção de água corporal. Mooney et al. (1998) constataram que frangos tratados com a betaína perderam menores quantidades de água, quando expostos a estresse cíclicos de temperatura e/ou desafio por coccidiose. Remus et al. (1995) relatam que melhorias significativas na digestibilidade aparente da lisina, proteína, gordura e carotenóides foram observadas em frangos tratados com betaína sob condições desafiadoras de coccidiose.

N, N-Dimetilglicina

N, N,dimetilglicina (DMG) é um aminoácido terciário, metabólio intermediário no metabolismo da colina, formado nas mitocôndrias do fígado pela remoção do grupo metil da betaína (FRIESEN et al. 2007). A molécula foi relatada pela primeira vez em 1943 e, é atualmente, usado para uma variedade de aplicações, tendo seu uso em humanos e animais. A representação da molécula de DMG encontra-se na Figura 2.

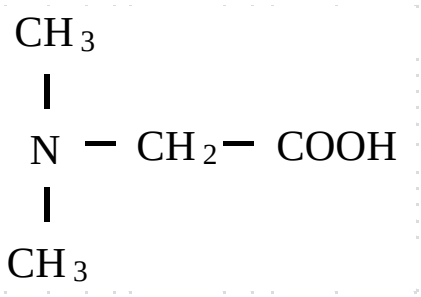


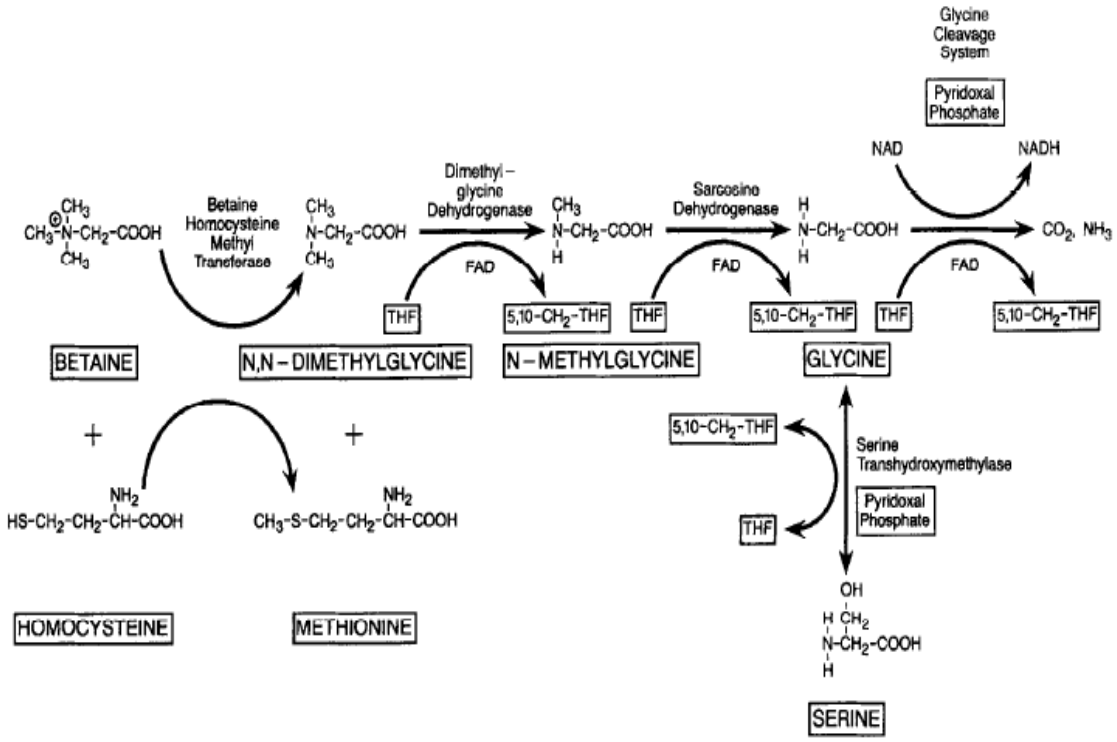
Figura 2. Representação da molécula de N,N dimetilglicina (DMG).

DMG é um componente desmetilado da betaína, que se origina pela metilação da homocisteina (LARYEA et al. 1994).

Ocorre uma série de reações até a formação da molécula de DMG a reação final é uma reação envolvendo a homocisteína como substrato que é catalisada pela betaína-homocisteina metiltransferase, que catalisa a conversão de betaína e homocisteína em N,N-dimetilglicina e metionina, respectivamente (ERICSON, 1960; McKEEVER et al. 1991). Ao lado da metionina, essa reação também oferece DMG, que por sua vez doa seus dois grupos metil para o tetrahidrofolato (Figura 3), através do qual DMG é catabolisadas em sarcosina e, posteriormente, glicina (KALMAR, 2011).

N, N-dimetilglicina é convertido em N-metilglicina, depois para glicina e finalmente para CO2 e NH3, essa reação não requer cobalamina nem folato (WAGNER, 1986.; NYHAM, 1994).

Glicina betaína é uma importante fonte de grupos metil necessário para a formação de metionina e S-adenosilmetionina (CHAMBERS; LEVER, 1996.; BARAK; BECKENHAUER; TUMA, 1996), DMG também pode desempenhar esse papel de doador de grupos metil.



Adaptado de ALLEN, R.H.; STABLER.S.P.; LINDENBAUM. J.(1993)

Figura 3. Conversão da betaína e homoscisteina a N, N-dimetilglicina e metionina.

Pesquisas com utilização de DMG

Os registros na literatura sobre pesquisas utilizando DMG na produção animal são escassos, principalmente na área de avicultura. Há registros sobre a ação de DMG em humanos e também em equinos com o objetivo de aumentar o desempenho atlético.

Cools et al. (2010) avaliaram a influência de DMG sobre a sensibilidade à insulina, glicose, metabolismo da gordura, digestibilidade dos nutrientes e desempenho reprodutivo de porcas no período peripartal. Os resultados mostraram uma melhora na digestibilidade aparente fecal da gordura e proteína, nos animais suplementados com DMG, porém sem nenhum impacto significativo sobre o desempenho reprodutivo.

Kalmar et al. (2010b) em estudo com frangos de corte, testaram a suplementação de DMG em cinco níveis (zero, 0,1; 0,2; 0,5 ou 1 g de DMG/kg de alimento) e com duas fontes de gordura, gordura animal (gordura de frango) ou gordura vegetal (óleo de soja). O estudo avaliou dados de desempenho e de analise de carcaça bem como a digestibilidade dos nutrientes e a ocorrência da síndrome ascitica. Os autores concluíram que a adição de DMG melhorou o índice de eficiência produtiva nas aves do grupo que receberam dieta com gordura de origem vegetal. As aves suplementadas com DMG independente da fonte de gordura da dieta apresentaram menor deposição de gordura abdominal, e DMG pode reduzir o estresse oxidativo e hipertensão pulmonar, mas o grau dos efeitos é modulado pelo perfil de ácidos graxos da dieta, sendo que os efeitos são mais pronunciados em uma dieta rica em ácidos graxos polinsaturados em comparação a dieta rica em gorduras saturadas e ácidos graxos monoinsaturados.

Kalmar et al (2010a) avaliaram a adição de zero e 167mg de DMG/kg de alimento na dieta de frangos de corte e seu efeito sobre a digestibilidade dos nutrientes e o desenvolvimento da síndrome de hipertensão pulmonar. O trabalho foi conduzido em condições de estresse por frio e utilizando dietas de alta energia. Os autores concluíram que DMG tem um efeito protetor na progressão para hipertensão pulmonar em frangos de corte submetidos a estresse pelo frio e dieta de alta energia, e que a suplementação de DMG resultou em uma melhoria significativa na digestibilidade fecal aparente da proteína bruta e extrativo não nitrogenado.

A tolerância, segurança alimentar e influência sobre dados de desempenho zootécnico também foram avaliados em estudo com a adição de DMG em dietas para frangos de corte, em estudo realizado por Kalmar et al. (2011) a suplementação foi de 0, 1g e 10g DMG/kg de alimento. Os autores concluíram que a suplementação de DMG na dose de 1g DMG/kg de alimento resultou em melhora de desempenho sem comprometer as características de carcaça. Os resultados da hematologia plasmática e química como o exame histopatológico de tecido de fígado, rins e coração não revelou mudanças patológicas ou indicações de efeito tóxico da suplementação de 1g DMG/kg ou de 10 vezes esta dose. A suplementação de DMG, em 1g DMG/kg de alimento não resultou em acumulo de DMG na carne, e o consumo de carne de frango ou fígado de frangos de corte alimentados com até 10g DMG/kg de alimento não aumenta a ingestão de DMG pelo consumidor. Além disso, carne de frango ou fígado de frangos de corte suplementados com a dose de 10g DMG/kg não contém uma maior quantidade de DMG que outros itens alimentares comuns, como por exemplo, o espinafre. Esse estudo revela um alto nível de tolerância e segurança para DMG em frangos de corte.

Em todos os estudos estão presentes a indicação dos autores sobre a necessidade de mais pesquisas sobre o DMG, para melhor elucidar o mecanismo de ação do mesmo na nutrição animal.

HIPÓTESES E OBJETIVOS

Hipóteses

A adição de N, N-Dimetilglicina afeta o desempenho zootécnico de frangos de corte no período de 1 a 42 dias de idade;

A adição de N, N-Dimetilglicina afetar as características de carcaça de frangos de corte aos 42 dias de idade.

Objetivos

Geral

O estudo teve por objetivo avaliar o efeito N, N-Dimetilglicina, suplementado a dieta de frangos de corte de 1 a 42 dias de idade, sobre as características de desempenho zootécnicas.

Específicos

- Avaliar a eficácia do aditivo sobre os índices zootécnicos de desempenho, por fase de criação;
- Analisar o rendimento de carcaça ao final do período de criação;
- Analisar o rendimento de cortes nobres e o percentual de gordura abdominal no final do período de criação;

CAPITULO 2

EFFECTS OF N, N- DIMETHYLGLYCINE IN THE PRODUCTIVE AND CARCASS QUALITY PERFORMANCE OF BROILER CHICKEN

Este capitulo é apresentado de acordo com as normas para a publicação na Revista **Poultry Science.**

N, N- DIMETHYLGLYCINE AND DIETS FOR POULTRY

Effects of N,N- dimethylglycine in the productive and carcass quality performance of broiler chicken

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ABSTRACT: N,N-dimethylglycine (DMG) is an intermediary metabolite in cellular choline to glycine metabolism. The objective of this study was to determine the effect of dietary DMG (Taminizer®D; Taminco) in order to evaluate the performance and carcass characteristics of broilers. The experiment was carried out at The Federal University of Santa Maria – Brazil. A total of 1920 1-day-old broiler (Cobb-500) were raised until 42 days of age. Birds were randomly assigned in three treatments with ten replicate pens of 64 birds each. A control diet (0 mg DMG/kg feed), a DMG supplemented diet (1,000 mg DMG/kg feed) and a diet with antibiotic growth promoter (AGP) were used. Diets were formulated to current industry standards based on corn and soybean meal. At day 42 of age birds fed the diet with AGP had higher body weight than broilers fed the control diet (P=0.0117), but the body weight and the BWG of the birds did not differ significantly. Feed intake at the end of the experimental period was lower for birds fed the DMG supplemented diet than birds of the AGP supplemented group. The study of carcass traits demonstrated treatment effects. Birds fed with DMG had better carcass yield (74.98%) than birds fed the control diet (73.58%) (P=0.0195). DMG supplementation increased the percentage of breast meat (P=0.0034) by 1.16% compared to birds fed the control and decreased the percentage of abdominal fat (P=0.0427) by 0.27% compared to birds fed the AGP diet. Results of these studies suggest that DMG has a positive effect on carcass characteristics of broilers.

Key words: performace; carcass yield; abdominal fat; breast

INTRODUCTION

The increase in poultry production and efficiency can be attributed to a parallel development of new knowledge in health, environment, genetics and nutrition. Important objectives in the meat-type broiler industry remain to improve feed conversion ratio, mortality rate and carcass yield. The increase in knowledge of nutritional physiology has led to adjustment of diets to enable rapid growth. Progress in poultry nutrition can be attributed to several factors, including the use of synthetic amino acids, development and addition of macro and micro elements in diets as performance enhancers (Uni, 1998).

Methionine is the first-limiting amino acid in poultry diets and its supplementation can improve growth performance and carcass quality of poultry (Xie et al., 2007). Methionine, in the form of S-adenosylmethionine is the major donotor of methyl radicals in the organism, required for the biosynthesis of many important substances involved in the growth, such as creatine, carnitine, polyamines, epinephrine, choline and melatonin (Baker, 1991).

Authors reported a sparing effect of choline on methionine (Pesti et al, 1981; Baker et al. 1983). Before choline acts as a methyl group donor, it needs to be converted to betaine and then to N, N-Dimethylglycine (DMG) in mitochondria.

DMG is the compound of interest in the current study. DMG is reputed to be involved in a variety of biological processes as it is an intermediary metabolite in the cellular choline and betaine metabolism. Friesen et al. (2007), for instance, describe a role of DMG as a source of glycine for glutathion synthesis. Hariganesh and Prathiba (2000) suggest free radical scavenging potential when DMG is orally administered.

Kalmar (2011) suggested that DMG might improve nutrient absorption through diminished encapsulation of nutrients by fat droplets, which enhances accessibility to digestive enzymes and the absorptive brush border of the small intestine.

The main objectives of the present trial were to investigate the effect of dietary supplementation with DMG on the performance parameters of broilers and on their carcass quality.

MATERIALS AND METHODS

This study was conducted at the Poultry Science Laboratory – LAVIC – Department of Animal Science at The Federal University of Santa Maria**.** The experimental procedures were approved and conducted under the guidelines of the local ethics committee.

Animals and experimental design

1920 male, day old broiler chicks (Cobb 500) of a commercial hatchery were used. The birds were vaccinated against Marek's disease, Infectious Bursal Disease and Avian Pox virus.

The birds were distributed in a completely randomized design, consisting of three treatments and 10 repetitions of 64 birds each. The treatments were: control diet without any supplementation (Control); control diet supplemented with 10 ppm avilamycin (APG) and control diet supplemented with 1.000mg/kg of feed DMG, DMG was added in the form of the additive Taminizer®D (Taminco NV, Ghent, Belgium), which is a preparation of N,N-dimethylglycine sodium salt (Na-DMG ≥97 %) produced by chemical synthesis.

This study was conducted in an acclimatized experimental poultry house, with east-west orientation and concrete floor. One side of the poultry house is equipped with five hoods and the other side with an evaporative cooling system.

The poultry house was 12m wide 35m long and 3m heigh, covered with steel corrugated sheet, an insulating core of EPS and textured aluminum film. The lateral walls were brick (0.47m high) with side screen (mesh 3cm) and plastic curtains.

30 boxes PVC structured cages of 4.14m² (1.8m wide by 2.3m long) were used. Each box has four drinking nipples, a tray-type feeder, for pre-initial phase, and a tubular, semi-automatic feeder (metal with plastic tray, 20kg capacity) for other phases. The heating of the birds during the initial phase is with a 150W lamp per box. Reused wood shavings from LAVIC were used as litter.

The birds were given feed and water ad libitum throughout the experimental period.

Diets

The experimental period was divided into four phases: pre starter phase (1-7 days), starter phase (8-21 days), grower phase (22 to 35 days) and finisher phase (36 to 42 days of age).

For each phase experimental mashed diets were isonutritionally formulated based on corn and soybean meal. The composition of ingredients and nutrients are presented in Table 1.

Performance

Growth performance parameters such as body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR), were recorded at the end of

each phase. Overall BWG, FI, and FCR were calculated for the whole duration of the experiment.

Mortalities were recorded daily. The Productive Efficiency Index (PI) was calculated using the following formula: PI = average daily weight gain • (100 - mortality) / feed conversion • 10. Daily body weight gain (BWD) was calculated over the total experimental period.

Carcass yield

At the end of the experiment, on day 42, three birds per pen were selected for the carcass analysis. These selected birds, with body weight within a range of 2.5% from the average weight of the unit, were selected, identified and fasted for eight hours. All birds were weighed individually and humanely euthanised by stunning followed by exsanguination. The birds were then immersed in hot water, mechanically plucked and manually eviscerated. The eviscerated carcass, chest (including pectoralis major and pectoralis minor), legs (including thigh and drumstick), back, wings and abdominal fat, were measured for each bird individually.

Carcass percentage was calculated as the ratio between the eviscerated carcass and live BW after fasting. The weight percentages of chest, legs, back, wings and abdominal fat were calculated as a percentage of eviscerated carcass weight. Abdominal fat included the fat that can be manually excised from the abdominal cavity.

Statistical analysis

Data were submitted to variance analysis using the General Linear Model procedure (Statistical Analysis System, 2009), according to the general model: $Yij = \mu +$ you $+ \varepsilon$ ij where Yij is the observed dependent variable, μ is the general average, you is

the effect of treatment (control, AGP or DMG), and ε_{ij} is the residual random error. Means were compared by Tukey test at a 5% level of significance.

RESULTS

Performance features

The main objectives of the current study were to evaluate the effect of dietary supplementation with DMG (1.000mg/kg of feed) on the performance parameters of broilers and on their carcass quality. Results for body weight (BW), body weight gain (BWG), feed conversion ratio (FCR), daily feed intake (FI), and daily body weight gain (BWGD) in the experimental period are presented in Table 2 and Table 3.

Performances were not significantly influenced by treatments in the pre-starter and starter phases.

In the growth phase (22-35 days) there was significant difference in feed intake ($P = 0.0130$), where birds fed AGP diets showed higher feed intake than to the birds control group and birds of DMG supplemented group . In the same period the other production indices were not affected by treatments. In a trial with broilers of 15 to 40 days old fed a diet supplemented with 167mg DMG/kg, Kalmar et al (2010a) did not find significant influences in the body weight, feed intake, daily growth and feed conversion in relation to the birds of the control group.

Considering the whole experimental period feed intake was lower in the DMG supplemented birds than in the AGP group. Cools et al. (2010), in a swine study in the peripartal period, showed that short-term supplementation of DMG had an emulsifying effect resulting in an improved nutrient digestibility. These beneficial effects of nutrient digestibility in swine probably could reduce the feed intake.

At 42 days of age birds fed with AGP diet had higher body weight broilers fed with control diet (P=0.011), but the body weight of the birds and the BWG of AGP group did not differ (table 2 and 3). In the study of 36 to 42 days others parameters were not affected by different treatments (P=0.05).

Birds submitted to the treatments of 1 to 42 days of age had different BWG and BWGD at the end of experimental period (P=0.011), where birds of AGP group showed higher BWG and BWGD than to the birds of the control group. Kalmar et al. (2011) evaluated the tolerance and safety of dietary DMG in broilers chickens of 1 to 39 days. The tested levels of Na-DMG were 0, 1 and 10g Na-DMG/kg of diet. Supplementation with 1g Na-DMG/kg feed resulted in a better FCR ratio than with the control diet. According to the authors the DMG content in excreta was found to be similar between broilers fed a control diet and 1 g Na-DMG/kg feed, indicating a high efficiency of absorption, as described by Cupp and Tracy (2003).

The authors described technical performance was not significantly influenced by the addition of DMG in the 10g Na-DMG/kg of diet. This indicates, on the one hand, a high tolerance range for DMG in broilers and, on the other hand that a dose of 10g Na-DMG/kg is above the optimal dose to improve broiler performance.

In our trial FCR and PI were not affected by treatments throughout the duration of the experiment (P>0.05). These results are in contradiction with with Kalmar (2011), that in three trials performed in different broiler strains reared under common conditions, evaluated that although FCR widely varied between trials, supplementation with DMG at a dose of 1,000 mg Na-DMG/kg feed resulted in all trials in an improved feed efficiency. Moreover, finishing BW and efficiency index were increased.

Irrespective of trial site, FCR, finishing BW and production value were improved by DMG use.

Kalmar et al. (2010b) evaluated DMG in broiler diets from 1 to 42 days of age. Five different doses of DMG (Taminizer® D, Taminco, Belgium) were tested in broiler diets with one of two fat sources. Tested levels of Na-DMG were 0 (control), 0.1, 0.2, 0.5 and 1 g/kg diet. The added fat source was either animal fat (chicken fat) or vegetal fat (soybean oil). Supplementation of DMG resulted in a significant linear improvement of production value in the vegetal fat groups. Irrespective of dietary fat source finishing weight, average daily feed intake, average daily growth, feed conversion ratio and production value were not significantly affected by DMG.

Mortality and viability showed no differences between diets in our trial. Results similar were found by Kalmar (2011) and Kalmar (2010b).

Carcass yield

The studies of carcass demonstrated effect of treatments and are presented in Table 4.

The carcass yield related with live body weight was 1.86% higher in birds supplemented with DMG than control birds (P=0.019). The carcass yield of birds supplemented with AGP were similar to the control birds or DMG birds. Kalmar et al. (2010b) demonstrated DMG improved slaughter traits by a decline in abdominal fat and a tendency to linearly increased meat yield in the range from 0 to 1,000 mg Na-DMG/kg feed.

The back, legs and abdominal fat did not differ between the treatments, but birds fed with control diet had bigger back (%) related to the carcass yield than birds fed with

AGP and DMG (P=0.006). Kalmar (2011) found slaughter performances similar between treatments, except for breast meat yield which was higher in DMG groups compared to the control. In a meta-analysis by Kalmar (2011) involving three studies with broiler chickens fed with DMG concluded that slaughter performance revealed similar carcass and total meat yield between diets, but both meat yield to abdominal fat ratio and breast meat yield were higher in DMG groups compared to the control.

The birds of the DMG group had a lower percentage of abdominal fat when compared to the birds of the AGP group (P=0.042). This effect can be attributed to DMG, which is a naturally occurring tertiary amino acid in the intermediary metabolism of betaine in living organisms. Betaine, the direct precursor of DMG, has been demonstrated to reduce the expression of lipogenic enzymes through altered DNA-methylation (Huang et al., 2008). Several authors reported significantly reduced abdominal fat pad, whereas breast meat yield increased in broiler chickens (McDevitt et al., 2000; Waldroup et al., 2006) fed betaine supplemented diets. Kalmar (2011) describes that abdominal fat deposition in broiler chickens tends to decrease when fed a DMG supplemented diet. In a trial with broilers fed with animal fat (chicken fat) or vegetable fat (soybean oil) and supplemented with DMG Kalmar et al (2010b) shows DMG supplementation resulted in less deposition of dietary fat into abdominal depot tissue and hence promoted deposition as lean tissue.

Eviscerated carcass yield of the DMG supplemented group was significantly higher than the control group. The birds fed with diet DMG and AGP had similar breast meat weight, back: carcass and breast: carcass ratios significantly higher than the control group results. Birds in the control group had higher wings of the birds of the antibiotic group (P=0.029).

Associating the lowest percentage of abdominal fat and highest percentage of breast in relation to carcass can be concluded that DMG supplementation promotes a greater deposition of lean tissue. McDevitt et al., (2000) reports that mode of action of betaine as 'carcass modifier' appears to be related to its methyl group donor properties, thereby attributing to a leaner carcass due to a higher availability of methionine and cysteine for protein deposition in birds fed betaine supplemented diets. Independent of dietary fat source, supplementation with DMG tended to linearly increase meat yield and significantly, linearly lowered both abdominal adipose tissue (Kalmar et al 2010b).

Yuan et al. 2008 reports that the deposition of fat in adipose tissue depends on the availability of plasma lipoproteins originating from the diet or produced in the liver. Kalmar et al. (2010b) suggested that DMG promotes enhanced hepatic fatty acid metabolism and results in increased utilization of dietary fat as an energy source. As a consequence, less body fat is deposited and the protein is less used for energy and this promotes growth of lean tissue.

The carcass quality in broilers is related to the balance of nutrients, where the balance of these components promotes better development of lean tissue, thereby preventing excessive fat deposition.

CONCLUSION

The present study demonstrated that DMG promoted a decrease in feed intake without affecting other parameters of performance. Promoted an increase in carcass and breast yield associated with a decrease in abdominal fat, which shows the action of DMG as improving carcass quality of broilers.

More studies are needed to better elucidate the mechanism of action of DMG in the nutrition of broilers.

ACKNOWLEDGEMENTS

The authors are gratefully for the support provided by Taminco NV, Ghent, Belgium.

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Table 1: Composition of experimental diets and nutritional

Ingredients	Control diet			
	Pre-starter (1-7d)	Starter (8-21d)	Grower (22-35d)	Finisher (36-42d)
Corn	57.25	57.66	57.97	60.44
Soybean meal	35.82	34.70	33.56	31.30
Soybean oil	2.50	3.20	4.50	4.79
Dicalcium phosphate	1.82	1.83	1.67	1.58
Limestone	1.04	0.91	0.89	0.84
Salt	0.40	0.40	0.40	0.40
Vit. & Min. Premix[1]	0.50	0.50	0.50	0.50
L-lysine	0.15	0.13	0.12	0.07
DL-methionine	0.17	0.18	0.19	0.07
L-threonine	0	0.01	0.03	0.01
Inert	0.35	0.48	0.16	0
Calculated composition				
Crude protein (%)	22.00	21.50	21.00	20.00
Metabolizable energy (Kcal/kg)	3000	3050	3150	3200
Calcium (%)	1.00	0.95	0.90	0.85
Available phosphorous (%)	0.45	0.45	0.42	0.40
Lysine (%)	1.36	1.25	1.21	1.10
Total sulphur AA (%)	0.91	0.90	0.90	0.76
Methionine (%)	0.57	0.57	0.57	0.44
Threonine (%)	0.82	0.82	0.82	0.77
Tryptophan(%)	0.22	0.22	0.21	0.19
Phenylalanine (%)	1.05	1.25	0.99	0.95
Valine (%)	1.00	0.98	0.95	0.91

[1] Supplying per kg of diet: Vitamin A, 2,200,000 UI; Vitamin E, 5,000 UI; Vitamin $D_{3,}$ 500,000 UI; Vitamin $K_{3,}$ 660 mg; Nicotinic Acid, 5,560 mg; Vitamin $B_{1,}$ 440 mg; Vitamin $B_{12,}$ 3,600 mcg; Vitamin $B_{2,}$ 1,150 mg; Vitamin $B_{6,}$ 926 mg; Folic Acid, 250 mg; Biotin 36 mg; Choline, 60,000 mg; Panthothenic Acid 3,600 mg; Cu, 1,600 mg; Fe, 9,998 mg; I, 88 mg; Mg, 11,993 mg; Se, 40 mg; Zn, 10,996 mg; Methionine, 297,000 mg; Lysine, 78,000 mg; Coccidiostat, 1,200 mg.

Table 2: Effects of dietary supplementation with N,N-dimethylglycine (DMG) on technical performance in broilers[1]

Treatments	PHASES															
	Pre-Starter (1 - 7 days)				Starter (8 - 21 days)				Grower (22 - 35 days)				Finisher (36 - 42 days)			
	BW (7 d)	BWG (g)	FI (g)	FCR (g:g)	BW (21 d)	BWG (g)	FI (g)	FCR (g:g)	BW (35 d)	BWG (g)	FI (g)	FCR (g:g)	BW (42 d)	BWG (g)	FI (g)	FCR (g:g)
Control	130.08	86.05	134.40	1.56	778.04	647.96	965.62	1.49	1938.13	1160.09	2060.60 b	1.78	2564.29 b	626.16	1287.03	2.06
Antibiotic	132.05	88.13	133.95	1.52	775.92	643.87	964.13	1.50	1957.50	1181.58	2092.57 a	1.77	2603.35 a	645.85	1304.06	2.02
DMG	132.64	88.54	132.64	1.50	781.89	649.25	964.72	1.49	1945.22	1163.33	2060.32 b	1.77	2579.75 ab	634.53	1285.59	2.03
Means	131.59	87.57	133.66	1.52	778.62	647.03	964.82	1.49	1946.95	1168.33	2071.16	1.77	2582.46	635.51	1292.22	2.04
SEM	0.83	0.84	0.81	0.01	3.08	2.91	1.88	0.00	6.09	6.11	5.36	0.00	5.63	6.17	4.58	0.01
P-value	0.438	0.448	0.670	0.149	0.739	0.747	0.952	0.706	0.437	0.312	0.013	0.965	0.011	0.440	0.190	0.670

[a-b] Means within a row, not sharing a common superscript, are significantly different ($P \leq 0.05$)

[1]Data represent means from 10 replicates per treatment

* Pooled SEM, n = 30

Table 3: Effects of dietary supplementation with N,N-dimethylglycine (DMG) on technical performance in broilers in overall period[1]

	PHASE											
	Overall (1 - 42 days)											
Treatments	BWD (g)		BWG (g)		FI (g)		FCR (g:g)		Mortality (%)	PI		
Control	60.00	B	2520.25	b	4447.64	ab	1.76		5.92	302.08		
Antibiotic	60.94	A	2559.44	a	4494.71	a	1.76		6.07	302.56		
DMG	60.37	Ab	2535.66	ab	4443.26	b	1.75		5.91	302.28		
Means	60.44		2538.45		4461.87		1.76		5.96	302.31		
SEM	0.13		5.64		8.81		0.00		0.72	3.62		
P-value	0.011		0.011		0.025		0.334		0.998	0.871		

[a-b] Means within a row, not sharing a common superscript, are significantly different ($P \leq 0.05$)
[1]Data represent means from 10 replicates per treatment
* Pooled SEM, n = 30

Table 4: Carcass characteristics of birds, male, cobb 500 strain at 42 days old [1]

Treatments	Bird Weight	Carcass		Chest		Legs		Back		Wings		Abdominal Fat	
	(g)	(g)	(%)	(g)	% of cacass	(g)	% of cacass	(g)	% of cacass	(g)	% of cacass	(g)	% of cacass
Control	2519.87	1854.23	73.58b	659.70b	35.53b	528.06	28.49	436.93	23.57a	191.76a	10.35	37.76	2.03ab
Antibiotic	2547.47	1884.23	73.96ab	696,23a	36.94a	528.50	28.05	426.26	22.61b	193.76ab	10.28	39.46	2.09a
DMG	2518.50	1888.80	74.98a	693.43a	36.69a	532.13	28.18	431.06	22.81b	197.70b	10.47	34.46	1.82b
Means	2528.68	1881.30	74.17	683.12	36.38	529.56	28.24	431.41	22.99	194.40	10.36	37.22	1.98
SEM	6.98	7.60	0.20	5.02	0.14	2.63	0.10	2.92	0.12	0.93	0.04	0.94	0.04
P-value	0.156	0.124	0.019	0.003	0.003	0.791	0.334	0.333	0.006	0.029	0.220	0.086	0.042

[a-b] Means within a row, not sharing a common superscript, are significantly different ($P \leq 0.05$)

[1]Data represent means from 30 replicates per treatment

* Pooled SEM, n = 90

CONCLUSÕES

O presente estudo demonstrou que N,N-dimetilglicina promoveu uma redução no consumo de ração, sem afetar outros parâmetros de desempenho em comparação as aves que receberam suplementação com AGP.

N,N-dimetilglicina promoveu um aumento no rendimento de carcaça e de peito associado a diminuição de gordura abdominal, o que demonstra uma ação de DMG como melhorador da qualidade da carcaça de frangos de corte.

Mais estudos são necessários para melhor elucidar o mecanismo de ação da DMG na nutrição de frangos de corte

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ANEXOS

Anexo A- Instrução aos autores para publicação na Revista Poultry Science

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After a manuscript is submitted electronically, the editorial office informs the appropriate section editor, who 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 section editor may recommend rejection or acceptance at this point, after which the manuscript and reviewer comments are made available to the editor-in-chief for a final decision. More commonly, the manuscript will be sent back to the corresponding author for revision according to the guidelines of the reviewers. Authors have 6 weeks to complete the revision, which shall be returned to the section editor. Failure to return the manuscript within 6 weeks will cause the paper to be purged from the files. Purged manuscripts may be reconsidered, but they will have to be processed as new manuscripts.

Section editors handle all initial correspondence with authors during the review process. The editor-inchief will notify the author of the final decision to accept or reject. Rejected manuscripts can be resubmitted only with an invitation from the section editor or editor-inchief.

Revised versions of previously rejected manuscripts are treated as new submissions. Therefore, authors must complete a new Manuscript Submission and Copyright Release Form.

PRODUCTION OF PROOFS

Accepted manuscripts are forwarded by the editor-inchief to the editorial office for technical editing and typesetting. At this point the technical editor may contact the authors for missing information or figure revisions. The manuscript is then typeset, figures reproduced, and author
proofs prepared.

Proofs

Author proofs of all manuscripts will be provided to the corresponding author. Author proofs should be read carefully and checked against the typed manuscript, because the responsibility for proofreading is with the author(s). Corrections may be returned by fax, mail, or e-mail. For faxed or mailed corrections, changes to the proof should be made neatly and clearly in the margins of the proof. If extensive editing is required, corrections should be provided on a separate sheet of paper with a symbol indicating location on the proof. Changes sent by e-mail to the technical editor must indicate page, column, and line numbers for each correction to be made on the proof. Corrections can also be marked using the note and highlight tools to indicate necessary changes. Author alterations to copy exceeding 10% of the cost of composition will be charged to the author.

Editor queries should be answered on the galley proofs; failure to do so may delay publication. Proof corrections should be made and returned to the technical editor within 48 hours of receipt.

Publication Charges and Offprints *Poultry Science* has two options available for the publication of articles: conventional page charges and Open Access (**OA**). ***OA.*** For authors who wish to publish their papers OA (freely available to everyone when the issue is posted online), authors will pay the OA fee when proofs are returned to the editorial office. Charges for OA are $2,400 if at least one author is a current professional member of PSA; the charge is $3,100 when no author is a professional member of PSA.

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When the galley proof is sent, the author is asked to complete an offprint order requesting the number of offprints desired and the name of the institution, agency, or individual responsible for publication charges.

MANUSCRIPT PREPARATION:

STYLE AND FORM

General

Papers must be written in English. The text and all supporting materials must use American spelling and usage as given in *The American Heritage Dictionary, Webster's Third International Dictionary*, or the *Oxford American English Dictionary.* Authors should follow the style and form recommended in *Scientific Style and Format. The CBE Manual for Authors, Editors, and Publishers.* 6th ed. Council of Biology Editors Style Manual Committee. Cambridge Univ. Press, Cambridge, UK.

Authors should prepare the main text, tables, and figure captions in MS Word. Details on figure preparation and file formats are provided in the Figures section of these instructions.

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 symbols palette available in this font. Complex math should be entered using MathType from Design Science (http://www.dessci.com). Equations created using the new Equation Builder feature in Microsoft Word 2007 may not be compatible with earlier versions of Word or other software used in our journal composition system. Tables and figures should be placed in separate sections at the end of the manuscript

(not placed in the text). Failure to follow these instructions may result in an immediate rejection of the manuscript.

Headings

Major Headings. Major headings are centered (except ABSTRACT), all capitals, boldface, and consist of ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), ACKNOWLEDGMENTS (optional), APPENDIX (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 boldface 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 have no abbreviations, and numbers must be given in words rather than in numerals (e.g., One-Day-Old Broilers).

Under the title, names of authors should be typed with initial capital letters and a space between initials (e.g., T. E. Smith). Affiliations will be footnoted using the following symbols: *, †, ‡, §, #, ‖ , and be placed below the author names. Do not give authors' titles, positions, or degrees. Numbered footnotes may be used to provide supplementary information, such as present address, acknowledgment of grants, and experiment station or journal series number. The corresponding author should be indicated with a numbered footnote (e.g.,1Corresponding author: myname@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 convenience for 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). Authors should consult a current "Subject Index " in *Poultry Science* for additional
key words. Key words should be formatted as follows:
Key words: . . .

Introduction

The Introduction, while brief, should provide the reader 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 numbers 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 references.

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. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as appropriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phosphorus 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 equivalents:
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 dl-α-tocopherol

In the instance of vitamin D3, cholecalciferol is the acceptable 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. Statistical 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. Reference to a statistical package without reporting the sources 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 analysis should justify the interpretations and conclusions.

When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments 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 chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use timesequence 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 example, 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 procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals 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 measurement 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 variable, 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 arrangement in 5 randomized complete blocks consisting of initial BW." Note that **a factorial arrangement is not a design**; 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 error 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 reporting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of experimental 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 observations 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* statistic.

Unbalanced factorial data can present special problems.

Accordingly, it is well to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases. Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by Bonferroni *t* statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged.

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 arrangements, 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 important 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 traditionally have been reserved for $P < 0.05$ and $P < 0.01$, respectively; 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 decimal. 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" relationship 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 contained in the data is sacrificed. In most cases, 2 or 3 significant digits (not decimal places) are sufficient.

Results and Discussion

Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Results 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.

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.

Review copies shall have authors' institutions omitted.

Appendix

A technical Appendix, if desired, shall follow the Discussion section or Acknowledgments, if present. The Appendix may contain supplementary material, explanations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel computer 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, refer 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 unpublished work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and unpublished data must not be included in the References section.

References Section. To be listed in the References section, papers must be published or accepted for publication.

Manuscripts submitted for publication can be cited as "personal communication" or "unpublished data" in the text.

Citation of abstracts, conference proceedings, and other 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 listed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the authors' names. As with text citations, two or more publications 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 abbreviations given in journals database of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/query. 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 increased 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 challenge with Marek's disease virus. Avian Dis. 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. Regist. 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. http://www.emedicine.com/neuro/topic 20.htm Accessed Feb. 2006. El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, assignee. 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 electronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gainesville. 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 feature 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 problems).

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 placement 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 superscripts 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 identified 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 publication. 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. Symbols 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 descriptor 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, 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 apparent only when the figure is reproduced in grayscale.

• ***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 caption or with a scale bar on the figure. Reduction for publication can make a magnification power designation (e.g., 100×) inappropriate.

• ***Caption.*** The caption should provide sufficient information 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 readable at final publication size.

Color Figures. Submitted color images should be at least 300 ppi. The cost to publish each color figure is $995; 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. The form “Color Charge Agreement” is available on the journal web site (http://ps.fass.org) and should be completed and returned to PSA Headquarters upon submission.

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 scientific 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 abbreviations 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 (or the updated list at http://ps.fass.org/) 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 editors 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 abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations 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, abbreviations shall be defined within each of these units of the manuscript.

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 abbreviated 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

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 immunoglobin

i.m. intramuscular

i.p. intraperitoneal

IU international units

i.v. intravenous

kb kilobase pairs

kDa kilodalton

L liter*

L:D hours light:hours darkness in a photoperiod

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
R2 coefficient of determination, multiple
RFLP restriction fragment length polymorphism
RH relative humidity
RIA radioimmunoassay
RNA ribonucleic acid
rpm revolutions per minute
s second
s.c. subcutaneous
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., in vitro, in vivo, in situ, a priori). However, genus and species 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 written 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 sequences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submission of sequence data to one of the databases. The following statement should appear as a footnote to the title on the title page of the manuscript. "The nucleotide sequence data reported in this paper have been submitted to GenBank Submission (Mail Stop K710, Los Alamos National Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN."

Publication of the description of molecular clones is assumed 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 cameraready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow *Poultry Science* guidelines.

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 numbered 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 (Online)

The following information is available online and 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://ps.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

Figure and Table Preparation Guidelines. Current detailed information on figure and table preparation can be found at http://ps.fass.org/misc/ifora.dtl

Manuscript Central Instructions. Manuscripts are submitted online (http://mc.manuscriptcentral.com/psa). Full user instructions for using the Manuscript Central system are available on the Mansuscript Central home page.