Universidade Federal do Rio Grande do Sul
Faculdade de Agronomia
Programa de Pós-Graduação em Zootecnia

Efeito do ácido linoleico conjugado e da luteína no desempenho e na resposta imune de frangos de corte

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EFEITO DO ÁCIDO LINOLEICO CONJUGADO E DA LUTEÍNA NO DESEMPENHO E NA RESPOSTA IMUNE DE FRANGOS DE CORTE[1]

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Orientadora: Prof. Dr[a]. Andréa Machado Leal Ribeiro

RESUMO

Tanto a luteína quanto o ácido linoleico conjugado (CLA) são nutrientes que já demonstraram efeitos benéficos na modulação do sistema imune em diferentes modelos experimentais, porém o uso de ambos em conjunto ainda não foi explorado. O CLA é incorporado nas membranas celulares e hipotetiza-se que a luteína, com seu potencial antioxidante, possa proteger a estrutura do CLA em situações de estresse oxidativo. Outra razão para se pensar na interação entre estes nutrientes é que já foram observados de forma individual, efeitos modulatórios do CLA e da luteína sobre receptores celulares que atuam em conjunto e possuem papel chave na regulação da resposta imune (receptores PPAR e RXR). Objetivou-se com o presente estudo avaliar o efeito da suplementação dietética de CLA e luteína no desempenho e na resposta imune de frangos de corte de 1 a 22 dias de idade. Foram testados 3 níveis de inclusão de CLA (0, 1 e 2%) em conjunto com luteína (0 e 50 mg/kg) na presença ou ausência de desafio imunológico com LPS. O CLA e a luteína apresentaram efeitos imunomodulatórios positivos, porém, não foi observada interação entre ambos os nutrientes para as avaliações relacionadas ao sistema imune. O CLA, adicionado em 2% na dieta, elevou a produção de IgY em resposta ao estímulo com albumina de soro bovino (BSA) e também foi capaz de aumentar a expressão de RXRα no fígado. A luteína diminuiu o óxido nítrico plasmático e também a expressão de TLR-4 no baço e de IL-1β e IL-12 no fígado apesar de ter aumentado a expressão de TLR-4 hepática. O desafio com LPS estimulou a resposta inflamatória aguda, evidenciado pela queda no ganho de peso, pelo aumento da relação fígado:peso corporal, pelo aumento na expressão de IL-1β e IL-12 hepáticos e diminuição na expressão de PPARα no duodeno e fígado e de PPARγ e RXRα no baço. Entretanto, nem a luteína e nem o CLA foram capazes de reverter ou atenuar os efeitos causados pelo desafio com LPS. Para desempenho, uma forte interação entre CLA e luteína foi observada, de forma que a suplementação com 1 ou 2% de CLA pioraram o peso corporal, o ganho de peso e a eficiência alimentar de 1 a 20 dias de idade, mas estes efeitos foram revertidos quando a luteína foi incluída na dieta com 1% de CLA. Concluiu-se que o CLA teve efeito benéfico sobre a resposta imune humoral, na dependência da sua dose. A luteína se mostrou como nutriente anti-inflamatório e também capaz de reverter o efeito negativo da inclusão dietética de 1% de CLA sobre o desempenho de frangos de corte.
Palavras-chave: CLA, desempenho, frango de corte, imunidade, LPS, luteína

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[1]Tese de Doutorado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (121 p.). Março, 2015.

EFFECTS OF CONJUGATED LINOLEIC ACID AND LUTEIN ON THE GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKENS[1]

Author: MSc. Mariana Lemos de Moraes
Advisor: Prof. Dr. Andréa Machado Leal Ribeiro

ABSTRACT

Both lutein and conjugated linoleic acid (CLA) have beneficial effects on the modulation of the immune system. However, the simultaneous supplementation of both lutein and CLA has not yet been examined. CLA is incorporated into cell membranes, and it is hypothesized that lutein, as an antioxidant, protects CLA structure during oxidative stress. Additionally, an examination of the interaction of lutein and CLA is vital because individual effects of CLA and lutein on nuclear receptors that work together and have important roles in the immune response have been observed (PPAR and RXR receptors). The objective of this study was to evaluate the effects of dietary CLA and lutein supplementation on the performance and immune response of 1- to 22-d-old broiler chickens. Three CLA inclusion levels (0, 1 and 2%) and two lutein levels (0 and 50 mg/kg) were tested in the presence and absence of LPS immune challenge. Lutein and CLA showed positive immunomodulatory effects, but no interaction between these nutrients on the immune system was observed. A 2% CLA supplementation increased plasmatic IgY anti-BSA production and hepatic RXRα expression. Lutein decreased plasmatic nitric oxide and TLR-4 in the spleen and IL-1β and IL-12 in the liver in addition to increasing hepatic TLR-4. LPS challenge effectively promoted an acute inflammatory response, as illustrated by decreased body weight gain, increased liver:body weight ratio, increased expression of hepatic IL-1β and IL-12, decreased expression of PPARα in the duodenum and liver and decreased expression of PPARγ and RXRα in the spleen. However, neither lutein nor CLA reversed or attenuated the effects of the LPS challenge. A strong interaction between CLA and lutein was observed on performance: CLA supplementation at 1 or 2% decreased body weight, body weight gain and feed efficiency from 1 to 20 d old. However, these negative effects were reversed when lutein was included in the 1% CLA diet. In conclusion, CLA improved the humoral immune response (depending on CLA dose). Lutein was anti-inflammatory and could reverse the negative effects of dietary 1% CLA supplementation on broiler chicken performance.
Key words: broiler, CLA, immunity, LPS challenge, lutein, performance

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[2]Doctoral thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil (121 p.). March 2015.

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RELAÇÃO DE ABREVIATURAS E SÍMBOLOS

ANOVA	analysis of variance
BSA	albumina de soro bovino; bovine serum albumin
BW	body weight
BWG	body weight gain
cDNA	complementary DNA
CLA	ácido linoleico conjugado; conjugated linoleic acid
COX	cicloxigenase, cyclooxygenase
CP	crude protein
Ct	cycle threshold
d	day
ELISA	enzyme-linked immunosorbent antibody assay
E_R	reference PCR amplification efficiency
E_S	sample PCR amplification efficiency
FI	feed intake
g	gram
G:F	gain-to-feed ratio
GLM	general linear model
h	hour
Ig	imunoglobulina; immunoglobulin
IL	interleucina; interleukin
INF	interferon
iNOS	óxido nítrico sintetase; nitric oxide synthase
IU	international units
IκB-α	proteína inibitória de NF-κB
kcal	kilocalorie
kg	quilograma; kilogram
LPS	lipopolissacarídeo; lipopolysaccharide
LT	leucotrieno
M	molar
M1	macrófagos classicamente ativados
M2	macrófagos alternativamente ativados
ME	metabolizable energy
mg	miligrama, miligram
min	Minute
mL	Militer

mRNA	messenger ribonucleic acid
n	number of observations
NF-kB	fator nuclear kappa B; nuclear factor kappa B
nm	nanometer
NO	óxido nítrico, nitric oxide
NRC	National Research Council
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PG	prostaglandina; prostaglandin
PPAR	peroxisome proliferator X receptor; receptores ativados por proliferadores de peroxissoma
ppm	parte por milhão; part per million
PPRE	elementos responsivos aos proliferadores de peroxissoma; peroxisome proliferator response element
PUFA	ácidos graxos poli-insaturados; poliinsaturated fat acids
R^2	coefficient of determination, multiple
ROS	espécies reativas de oxigênio
RT-qPCR	reverse transcription - real time quantitative polymerase chain reaction; transcriptase reversa - reação em cadeia da polimerase quantitativa em tempo real
RXR	receptor de retinoide X; retinoic acid X receptor
s	second
SAS	Statistical Analisis System
SD	standard deviation
SE	standard error
SEM	standard error of a mean
SRBC	sheep red blood cells
TGF	fator de transcrição de crescimento
TLR	receptor tipo “toll”, toll like receptor
TNF	fator de necrose tumoral; tumor necrosis factor
vs.	versus
µ	micro

CAPÍTULO I

1. INTRODUÇÃO

Ao longo do tempo, a forma de se produzir animais para a alimentação humana vem sofrendo intensas modificações, sempre motivada por novos desafios. Com o deslocamento populacional em direção aos centros urbanos, os animais de produção, que antes eram criados em pequena escala para consumo próprio ou para abastecer pequenos centros comerciais, passaram a ser produzidos em sistemas intensivos e cada vez mais tecnificados. Nesses modelos, a concentração de animais por unidade de espaço é muito maior que no cenário anterior e a introdução de um agente patogênico no lote pode ter consequências catastróficas. Sendo assim, difundiu-se o uso de antibióticos promotores de crescimento com o objetivo de manter infecções subclínicas controladas, porém barreiras vêm sendo empregadas a essa prática. Nos últimos anos, não apenas por causa dessas restrições, a busca por nutrientes que tenham propriedades funcionais tem movimentado o mercado. Há interesse por nutrientes capazes de modular a resposta vacinal aumentando a produção de anticorpos protetores e também por aqueles que atuem em outras situações de estimulação do sistema imune. Nutrientes que sejam capazes de se incorporar na carne e tragam efeito benéfico para o consumidor também vem recebendo maior atenção.

Neste contexto, a nutrição passou a ser vista como uma atraente ferramenta de modulação do sistema imune com o intuído de manter o animal saudável e evitar, ou ao menos atenuar, os efeitos negativos causados por desafios imunológicos. O conhecimento das necessidades nutricionais que maximizam o desempenho produtivo e reprodutivo, além de prevenir deficiências, já é bastante maduro. Entretanto, muito ainda se estuda a fim de definir quais são os níveis de inclusão e a possibilidade de interação dos diferentes nutrientes com atividades imunomodulatórias.

Entender os mecanismos fisiológicos e metabólicos pelos quais os nutrientes afetam a imunidade permite a formulação de dietas de que otimizem a resistência a doenças e o desempenho zootécnico de animais em situações práticas de produção. É diante deste cenário que o presente trabalho foi desenhado, com o objetivo de avaliar os efeitos da suplementação dietética com luteína e com ácido linoleico conjugado (CLA), bem como a possibilidade de interação entre ambos, no desempenho e na resposta imune de frangos de corte.

2. REVISÃO BIBLIOGRÁFICA

2. 1 A resposta inflamatória de fase aguda

A resposta inflamatória é um mecanismo de defesa do organismo em reação a danos em um tecido vivo. O estímulo pode ser desencadeado por infecção bacteriana, trauma físico, fratura óssea, neoplasma, queimadura, entre outros (Davison et al., 2008). A reação inflamatória tem por objetivo eliminar o foco da inflamação, prevenir o aumento de dano tecidual e restaurar a homeostase do hospedeiro. As reações do organismo que ocorrem imediatamente após o dano tecidual são conhecidas como reposta inflamatória de fase aguda (Asasi et al., 2013) e geralmente causam febre, sonolência, letargia e anorexia (Klasing, 1998).

A maioria das respostas comportamentais decorrentes de uma infecção podem ser mimetizadas em aves através da inoculação de lipopolissacarídeos bacterianos (LPS; Klasing et al., 1987; Johnson et al., 1993). O estímulo inicia quando os LPS se ligam a receptores de membrana conhecidos como tipo *toll* (TLR-4, do inglês *toll like receptors type-4*). Esta ligação estimula os macrófagos a desencadearem uma cascata de reações com a finalidade de produzir substâncias pró-inflamatórias que ajam no combate dos organismos invasores. Primeiramente, os macrófagos estimulados com LPS causam uma rápida ativação da enzima NOX2 NADPH oxidase, que atua transferindo elétrons do NADPH para moléculas de oxigênio (O_2) gerando o radical superóxido (O_2^-). O superóxido, por sua vez, pode ser oxidado, formando o radical peróxido (H_2O_2; Rafi et al., 2007; Mengoni et al., 2010; Hadad & Levy, 2012). Ambos radicais são espécies reativas de oxigênio (ROS) e ativam o fator nuclear kappa B (NF-κB). Este é um ponto chave da estimulação da resposta inflamatória, uma vez que o NF-κB que responde elevando a produção de potentes sinalizadores pró-inflamatórios como o fator de necrose tumoral-α (TNF-α) e a interleucina-1β (IL-1β). Além disso, o NF-κB também aumenta a expressão das enzimas cicloxigenase-2 (COX-2), óxido nítrico sintetase (iNOS) e NOX2 NADPH, responsáveis pela produção de prostaglandina E_2 (PGE_2), óxido nítrico (NO) e superóxido, respectivamente (Lee et al., 2003; Kim et al., 2008). Embora estes mediadores sejam importantes para a defesa do organismo (Baran et al., 1996), quando liberados em excesso, causam um estresse oxidativo que culmina em danos de variadas proporções nas células saudáveis do próprio hospedeiro (Tidball, 2005; Barnham et al., 2004).

Os efeitos negativos causadas pelos mediadores pró-inflamatórios para o hospedeiro podem ser combatidos por agentes que diminuam a produção ou ação dos primeiros. São exemplos os inibidores de COX-2, anticorpos anti-TNF-α, antioxidantes, e ainda, mediadores que inibam a transcrição de NF-κB e a expressão de TLR-4 (Leach et al., 1998; Ritchlin et al., 2003). Um ponto chave no controle desses dois últimos mecanismos são os receptores nucleares que atuam no sentido de minimizar a intensidade da resposta inflamatória e são conhecidos como receptores ativados por proliferadores de peroxissoma (PPAR, abreviatura do termo em inglês *peroxisome proliferator-activated receptor;* Dasu et al., 2009).

O PPAR é expresso em diversas células do sistema imune,

principalmente nas apresentadoras de antígeno como macrófagos e células dendríticas (Szatmari et al., 2006; Wang et al., 2014). Os macrófagos podem assumir diferentes fenótipos conforme a situação em que se encontram. Após um estímulo inflamatório, assumem a forma de "classicamente ativados", conhecida também como forma M1, e promovem a fagocitose de organismos estranhos e o aumento na produção de citocinas pró-inflamatórias, ROS e óxido nítrico (Benoit et al., 2008). Por sua vez, os macrófagos "alternativamente ativados" ou M2, têm elevada expressão de PPAR e atuam no sentido de controlar a intensidade da resposta inflamatória. Eles são estimulados pelas interleucinas 4, 13 e 10 e pelo fator de transcrição de crescimento-β (TGF-β; Benoit et al., 2008; Mosser & Zhang, 2008; Martinez et al., 2009).

Os PPARs controlam a expressão não só de genes envolvidos com a inflamação, mas também aqueles envolvidos com o metabolismo lipídico (Youssef & Badr, 2011). Três isotipos já foram identificados, PPARα (Issemann & Green, 1990), PPARβ/δ, e PPARγ (Dreyer et al., 1992). A diferença principal entre eles é a sua distribuição nos tecidos e seus ligantes específicos (Berger & Moller, 2002), porém o processo de transcrição gênica é idêntico para todos (Wang et al., 2014). Depois de se ligarem a um ligante de PPAR, estes receptores se unem a um receptor de retinoide X (RXRα, β ou γ, abreviatura do termo em inglês *retinoid X receptor*), também ativado por um ligante de RXR, sofrem mudanças na sua conformação e, em conjunto, formam heterodímeros (Feige et al., 2005). O heterodímero PPAR-RXR se liga a elementos responsivos aos proliferadores de peroxissoma (PPRE, do inglês *peroxisome proliferator response element*) na região promotora dos genes alvo e o processo de transcrição é então iniciado (Gearing et al., 1993; Feige & Auwerx, 2007; Mandard & Patsouris, 2013). Entre os ligantes de PPAR estão os ácidos graxos e os eicosanoides (Forman et al., 1997; Kliewer et al., 1997; Lalloyer & Staels, 2010), e de RXR, os carotenoides (Nikawa et al., 1995; Selvaraj & Klasing, 2006; Selvaraj et al., 2010) e também ácidos graxos (Steineger et al., 1998; Lengqvist et al., 2004).

Um dos mecanismos mais efetivos para a atividade anti-inflamatória do PPAR está relacionado com a supressão da cascata de reações desencadeadas pelo NF-κB (Mandard & Patsouris, 2013). O PPAR é capaz de aumentar a expressão da proteína inibitória (IκB-α) que suprime a transcrição de NF-κB e, consequentemente, também de TNFα, IL-1β, COX-2, iNOS e NOX2 NADPH (Stienstra et al., 2007; Mandard & Patsouris, 2013). Porém, durante a resposta inflamatória, a expressão de PPAR é fortemente reduzida pela IL-1β. A redução na expressão de PPAR em resposta a exposição a LPS foi relatada em diversos órgãos, como fígado, coração, rins e tecido adiposo (Lu et al., 2006; Beigneux et al., 2007; Mansouri et al., 2008). Neste momento, a disponibilidade de ligantes específicos que ativem o PPAR é ponto determinante para a sua atividade. Já foi demonstrado que na presença deles, o aumento na expressão de PPAR amenizou a produção de citocinas pró-inflamatórias em ratos (Liu et al., 2005), em suínos (Fan et al., 2010), em aves (Selvaraj & Klasing, 2010) e em camundongos (Qu et al., 2012), diminuindo os efeitos causados por desafios com LPS.

2. 2 A resposta imune humoral

A resposta imune humoral é o tipo de resposta que atua no líquido extracelular impedindo a penetração dos patógenos nos tecidos. A resposta é formada após linfócitos B entrarem em contato com os antígenos reconhecidos como estranhos ao organismo. Após o estímulo, os linfócitos B se proliferam e se diferenciam em plasmócitos secretores de imunoglobulinas. As imunoglobulinas inativam os antígenos por diversos mecanismos que incluem opsonização, fixação do sistema complemento, neutralização, aglutinação e precipitação (Erf, 2004). As classes de imunoglobulinas conhecidas para aves são: IgM, IgY (correspondente a IgG de mamíferos), e IgA. A IgM é a primeira a se elevar em nível sistêmico após uma estimulação e é classe responsável pela resposta primária a antígenos. A IgY é a principal imunoglobulina da resposta secundária a bactérias e vírus e é ela que permanece no soro em certo nível após uma resposta humoral ter sido montada. A IgA está concentrada principalmente nas secreções exócrinas conferindo imunidade local (Davison et al., 2008).

As interleucinas fazem parte de uma ampla categoria de pequenas proteínas com atividade na sinalização celular, conhecidas por citocinas. Possuem um papel estimulatório da resposta imune humoral, a IL-2 e a IL-10. A IL-2 é uma citocina que induz a proliferação de linfócitos T e age também sobre os linfócitos B estimulando a produção de anticorpos (Kawano & Noma, 1996). A IL-10 é conhecida principalmente por regular a intensidade da resposta inflamatória já que atua inibindo a expressão de vários mediadores pró-inflamatórios e suprimindo a ativação das células T. Em contraste com os efeitos inibitórios, a IL-10 estimula a diferenciação de linfócitos B e aumenta a produção de anticorpos (Akdis et al., 2011).

2. 3 A resposta imune e o desempenho zootécnico

Pensando em animais de produção, a resposta imune gera perdas de desempenho não só pela diminuição do consumo alimentar, mas também por outros eventos que induzem a ineficiência metabólica (Klasing & Barnes, 1988). Nessas situações, o organismo do animal reage de forma a dar suporte para o sistema imune, através da disponibilização dos nutrientes dietéticos, catabolismo muscular e aceleração da lipólise (Klasing & Johnstone, 1991). Sendo assim, os animais respondendo a um estímulo com uma inflamação aguda, passam de um estado anabólico para catabólico diminuindo o crescimento e a eficiência alimentar. Jiang et al. (2010) observaram queda de 22,5% no ganho de peso de frangos de corte desafiados com LPS comparado ao grupo não desafiado que tinha livre acesso à dieta. A redução no crescimento também foi notada com a equalização do consumo de aves não desafiadas ao nível do grupo desafiado com LPS, porém não tão intensamente, e permitiu concluir que apenas 59% da queda de desempenho é decorrente da redução no consumo enquanto 41% é devido a outros fatores, provavelmente associados ao metabolismo durante a resposta imune.

É preciso salientar que apesar do sistema imune ter seus requerimentos nutricionais aumentados durante a resposta inflamatória aguda, as exigências totais de energia e de proteína do animal estão diminuídas, uma vez que as funções reprodutivas e o crescimento ficam temporariamente

deprimidos (Klasing & Barnes 1988; Klasing & Leshchinsky, 1999). Klasing & Barnes (1988) avaliaram os requerimentos nutricionais de lisina e metionina de frangos de corte desafiados com imunógenos. A queda de desempenho decorrente do desafio foi menor nas aves alimentadas com dietas deficientes nesses aminoácidos quando comparadas às das aves recebendo dietas com níveis suficiente ou acima das exigências preconizadas para aves saudáveis. Também foi observado que apenas as aves desafiadas que tinham o aporte suficiente ou acima das exigências tiveram aumento na atividade de IL-1β plasmática. A IL-1β causa os efeitos de sonolência, letargia, e anorexia (Klasing, 1994), o que permite concluir que as aves recebendo dietas aminoácido-deficientes tiveram menor expressão de estresse imunológico.

Apesar dos requerimentos nutricionais estarem diminuídos na fase de resposta inflamatória aguda, logo após o animal se recuperar do desafio imunológico, eles estarão aumentados e recebendo uma dieta adequada, o animal apresenta maior taxa de crescimento quanto comparado a animais que não diminuíram o consumo alimentar (Doyle & Leeson, 2001). Este conceito de crescimento compensatório é também outra estratégia para atenuar as perdas de produção decorrentes da resposta inflamatória aguda. Trabalhando com leitões desafiados com LPS de *E. Coli*, Moraes et al. (2012) observaram acentuada queda no ganho de peso no 3^{o} dia após a inoculação. O processo foi acompanhado até no 7^{o} dia pós-desafio, quando os animais já tinham superado o potencial de ganho do grupo controle, porém não atingiram o mesmo peso final.

2. 4 Modulação da resposta imune pela nutrição

Para evitar, ou pelo menos minimizar, os efeitos negativos consequentes da resposta imune, uma estratégia vantajosa é o aporte de nutrientes via dieta que tenham capacidade imunomodulatória. Entre esses nutrientes destacam-se alguns específicos aminoácidos, ácidos graxos, vitaminas, minerais, ácidos orgânicos, carotenoides, entre outros.

Os nutrientes podem ter ações diferentes conforme o tipo de estímulo imunológico que o animal está sendo submetido. Por isso, muito ainda se discute quanto ao nível de inclusão adequado de cada nutriente para cada tipo de resposta almejada. Sabe-se ainda que quando o assunto é imunomodulação, nem sempre a maior suplementação é a que promove a melhor resposta. O efeito dose-resposta de vitamina E em relação à produção de anticorpos vacinais foi amplamente explorado (Friedman et al., 1998; Leshchinsky & Klasing, 2001; Lin & Chang 2006). O NRC (2005) indica a exigência de 10 UI/kg de dieta. Chegou-se a conclusão que níveis um pouco mais altos (25-50 UI/kg) estimulam a produção de anticorpos, porém níveis ainda mais elevados (>100 UI/kg) são supressivos. Em leitões, a produção de IgG anti-albumina de soro bovino (BSA) foi estimulada com a inclusão de 1% de CLA na dieta, enquanto com 2% o efeito não foi observado (Moraes et al., 2012).

A interação entre nutrientes também é foco de interesse em pesquisas mais recentes com foco em imunomodulação. Existem basicamente dois tipos de interação: o sinergismo, que promove um efeito positivo, e o antagonismo, quando o efeito é negativo. Essas interações podem ocorrer na

absorção dos nutrientes, transporte, armazenamento e até mesmo na atividade biológica. Algumas relações, especialmente entre vitaminas e minerais, já vêm sendo exploradas há mais tempo. O efeito sinérgico da suplementação com vitamina E e selênio talvez seja uma das mais estudadas e os efeitos positivos na produção de anticorpos (Spallholz et al., 1973; Panda & Rao, 1994) e na proliferação de linfócitos (Finch & Turner, 1989; Pollock et al., 1994) já são conhecidos. Já a combinação de vitamina E e vitamina A causa efeito antagônico na atividade fagocítica e produção de anticorpos (Tengerdy & Nockels, 1975; Tengerdy & Brown, 1977). Pesquisas avaliando interações entre outros nutrientes, como por exemplo carotenoides e ácidos graxos, ainda são escassas, porém o que já foi observado mostra que existe um grande potencial de exploração nessa linha.

2. 4. 1 O ácido linoleico conjugado

O ácido linoleico conjugado (CLA, 18:2), apesar do nome semelhante ao ácido linoleico (cis-9, cis-12 18:2), pouco se assemelha ao último nas funções biológicas. O CLA se refere a um conjunto de isômeros geométricos e posicionais do ácido linoleico e este é o motivo pelo qual possui esta nomenclatura (Pariza et al., 2001; Park, 2009). A diferença estrutural entre o CLA e o ácido linoleico é que o primeiro possui duplas ligações conjugadas, ou seja, estão separadas apenas por uma ligação simples entre as insaturações enquanto o ácido linoleico possui 2 ligações simples entre as insaturações e estas sempre serão nos carbonos 9 e 12. No CLA, as duplas ligações conjugadas podem variar dos carbonos 6 até o 12 e para cada isômero posicional são possíveis quatro pares geométricos (cis-trans, trans-cis, cis-cis, trans-trans). Sendo assim, o CLA não é citado por ser um ácido graxo n-6 como o ácido linoleico. Os isômeros de CLA mais estudados são o cis-9, trans-11 e o trans-10, cis-12 por já terem demonstrado benefícios de sua atividade biológica (Pariza et al., 2001; Palmquist et al., 2005; Wallace et al., 2007; Kennedy et al., 2010).

A maior fonte natural de CLA é a gordura de ruminantes, presente na carne ou no leite, embora alguns óleos derivados de plantas também contenham CLA em menor quantidade (Chin et al., 1992; Gnadig, 2003). As preparações comerciais de CLA são obtidas por isomerização alcalina de óleos ricos em ácido linoleico, como o de cártamo ou de girassol e tendem a conter uma mistura homogênea de isômeros cis-9, trans-11 e trans-10, cis-12 (Nagao & Yanagita, 2008), não havendo diferença estrutural entre o CLA natural e o sintético (Kramer et al., 1998; Hur et al., 2007).

2. 4. 1. 1 O ácido linoleico conjugado e o sistema imune

Uma das formas hipotetizadas para o CLA exercer sua atividade imunomodulatória é através da sua incorporação nos fosfolipídeos das membranas celulares. Pela incorporação do CLA nas membranas ser competitiva com a de outros ácidos graxos, ele acaba diminuindo o acúmulo de ácido araquidônico (derivado do ácido linoleico, n-6) e consequentemente, a produção de seus eicosanoides derivados (Pariza et al., 2000; Belury, 2002; He et al., 2007). Os principais são a prostaglandina E_2 (PGE_2) e o leucotrieno B_4 (LTB_4), que são potentes mediadores pró-inflamatórios. Os mecanismos e os

eventos envolvidos na substituição de ácidos graxos nos compartimentos celulares já foram bem estudados em humanos (Vidgren et al., 1997), ratos (Abel et al., 1997), cordeiros (Kim et al., 2007) e frangos (Selvaraj & Cherian, 2004a, Selvaraj & Cherian, 2004b). Esses trabalhos mostram que existe um limite para a substituição de ácidos graxos n-6 e em nenhuma das células do sistema imune estudadas, ácidos graxos n-3 foram capazes de substituir completamente os n-6. O ácido araquidônico chega ao seu nível mínimo após duas semanas de uma dieta rica em ácidos graxos n-3 e o aumento da suplementação com n-3 não é mais capaz de interferir na concentração de ácido araquidônico (Abel et al., 1997).

Além dos efeitos indiretos na diminuição da produção de PGE_2 e LTB_4, o CLA atua como ligante de PPAR diminuindo a expressão de TLR-4 e o reconhecimento de LPS bacterianos (Dasu et al., 2009) e consequentemente também a produção das citocinas pró-inflamatórias IL-1, IL-6, IL-8 e TNF-α (Tizzard, 2009). No trabalho de Reynolds et al. (2009), observou-se que ratos alimentados com CLA tiveram maior expressão de PPARγ e reduzida expressão de TLR-4 nas células dendríticas. Quando inibidor específico de PPARγ foi adicionado, o efeito do CLA na redução de TLR-4 foi revertido, sugerindo que os efeitos anti-inflamatórios do CLA podem ser mediados pela regulação do TLR-4 pelo PPARγ.

Já foi observado também efeito do CLA sobre sistema imune humoral. Alguns estudos mostraram que ele é capaz de aumentar produção de imunoglobulinas totais em humanos (Song et al., 2005) e de imunoglobulinas antígeno específicas em aves (He et al., 2007), em leitões (Corino et al., 2002; Moraes et al., 2012) e em ratos (Sugano et al., 1998; Yamasaki et al., 2003; Ramírez-Santana et al., 2009). O mecanismo pelo qual o CLA interfere na síntese de anticorpos ainda não está claro, mas uma possível explicação seria pelo seu efeito na produção de citocinas. O CLA é capaz de estimular a produção de IL-2 enquanto reduz a de IL-4 (Yang & Cook, 2003). A IL-2, por sua vez, aumenta a síntese de IgG, IgA e IgM (Kawano & Noma, 1996) enquanto a IL-4 aumenta a síntese de IgE (Pène et al., 1988).

2. 4. 2 A luteína

A luteína ($C_{40}H_{56}O_2$) é um dos mais de 600 carotenoides naturalmente encontrados no reino vegetal, porém não pode ser sintetizada pelos animais (Kalaria et al., 2011). A luteína pertence à classe das xantofilas, que são um grupo de carotenoides que contêm oxigênio na sua estrutura e que não possuem ou tem diminuída atividade pró-vitamina A.

As fontes mais ricas em luteína presentes na alimentação humana são os vegetais verde escuros, como o espinafre e couve, mas também são fontes os alimentos de coloração amarela, como milho e gema de ovo (Sommerburg et al., 1998). Para humanos, a luteína é conhecida principalmente por sua importância na saúde ocular por diminuir o risco de catarata e degeneração macular relacionada à idade (Brown et al., 1999; Mares-Perlman et al., 2001). Para frangos de corte e poedeiras, a luteína é incluída na dieta a partir de produtos à base de extratos de flores do gênero *Tagetes* ou da espécie de *Calendula officinalis.* A suplementação é feita com o intuito de atender mercados que têm preferência por pigmentação da carne e de gema do ovo

com tons amarelos-alaranjados.

2. 4. 2. 1 A luteína e o sistema imune

Nos últimos tempos, a luteína vem recebendo mais atenção em pesquisas não apenas pelas suas propriedades pigmentantes, mas também por seu efeito antioxidante (Sies et al., 1992). Este fitonutriente mostrou inibir a produção de mediadores pró-inflamatórios tais como óxido nítrico, interleucinas, TNF-α, COX-2 e NF-κB (Selvaraj et al., 2006, Mengoni et al., 2010, Kim et al., 2008; Hadad & Levy, 2012). Os estudos indicam que a luteína diminui a produção dos radicais superóxido (Kim et al., 2008; Hadad e Levy, 2012) e peróxido (Kim et al., 2008) e então indiretamente reduz a cascata de eventos estimulada pelo NF-κB.

Os efeitos da luteína, assim como de outros carotenoides, sobre o sistema imune humoral não estão bem definidos. O título de anticorpos em resposta à vacinação contra Doença de Newcastle não foi influenciado em frangos provenientes de matrizes suplementadas com luteína, cantaxantina ou β-caroteno (Haq & Bailey,1995; Haq et al.,1996). Entretanto, no trabalho de Rajput et al. (2013), a alimentação de frangos contendo curcumina foi eficiente para elevar os títulos vacinais contra o mesmo agente, enquanto a luteína promoveu níveis intermediários. Bédécarrats & Leeson (2006) relataram que o título vacinal contra o vírus da bronquite infecciosa aumentou quando a luteína foi suplementada em 125 ppm, enquanto o nível mais alto (250 ppm) não causou efeito.

Estudos mais recentes mostram que a luteína atua como ligante de RXR, que juntamente com o PPAR, atua na minimização da intensidade da resposta inflamatória alterando o perfil da transcrição de genes durante esses eventos (Nikawa et al., 1995; Selvaraj & Klasing, 2006; Selvaraj et al., 2010).

2. 4. 3 O ácido linoleico conjugado e a luteína

Tanto os carotenoides quanto os ácidos graxos são componentes da membrana celular e, conhecendo as suas particularidades, é possível que ambos exerçam ações complementares e que esta interação entre eles seja ainda mais importante durante a resposta inflamatória aguda. A intensificação na formação de ROS pelos macrófagos apesar de ter como alvo o patógeno agressor, acaba causando mudanças estruturais nas membranas celulares por atingir os lipídeos. Como o CLA é um ácido graxo poli-insaturado, é mais susceptível a ação das ROS e a ação antioxidante da luteína poderia proteger a sua estrutura.

Uma hipótese alternativa ou complementar à descrita acima para interação entre a luteína e o CLA é através da estimulação da via PPAR-RXR. Sabe-se que o CLA é um ligante de PPAR (O'Shea et al., 2004; Jaudszus et al., 2008), porém a modulação da atividade anti-inflamatória exercida por PPAR requer a presença de um ligante de RXR, como a luteína (Selvaraj et al., 2010). A interação entre a luteína e outro ácido graxo, o eicosapentanoico, foi observada tanto *in vivo*, em frangos, quanto *in vitro* através da ativação da via PPAR-RXR e da diminuição na expressão de iNOS, possivelmente via supressão do NF-κB (Selvaraj & Klasing, 2006). Recentemente, o mesmo grupo de pesquisa testou a luteína em conjunto com óleo de peixe em dietas para

frangos de corte e constatou novamente o efeito positivo de ambos os nutrientes na regulação de PPAR e RXR e diminuição na expressão de IL-1. Foi observado ainda que o efeito da luteína foi dependente do nível de inclusão de gordura na dieta (Selvaraj et al., 2010).

Poucos ainda são os trabalhos que descrevem a relação entre ácidos graxos e luteína no sistema imune e os mecanismos envolvidos ainda não estão claros. Entretanto, a proteção antioxidante da luteína sobre os ácidos graxos de membrana, incluindo o CLA, e a manipulação nutricional da via PPAR-RXR parecem ser estratégias interessantes para diminuir a injúria celular causada pela inflamação.

3 OBJETIVOS E HIPÓTESES

O objetivo deste estudo foi avaliar o efeito da suplementação dietética com CLA e com luteína, bem como suas possíveis interações, no desempenho e na resposta imune de frangos de corte.

As hipóteses testadas foram:

1. A luteína, suplementada na dieta em 50 mg/kg, e o CLA, suplementado em 1 ou 2%, interagem melhorando o desempenho zootécnico de frangos de corte de 1 a 20 dias de idade.

2. A produção de IgY em resposta à inoculação com BSA é aumentada em frangos de corte suplementados com luteína (50 mg/kg) e/ou CLA (1 ou 2%).

3. O desafio com LPS afeta o ganho de peso de frangos de corte e este efeito é evitado ou atenuado quando luteína (50 mg/kg) e/ou CLA (1 ou 2%) são suplementados na dieta.

4. A suplementação de dietas de frangos de corte com luteína (50 mg/kg) e/ou CLA (1 ou 2%) atenua os efeitos da resposta inflamatória aguda consequentes a um desafio com LPS.

5. Podem haver respostas distintas entre os níveis de CLA (1 ou 2%) na interação com luteína (50 mg/kg) tanto para desempenho quanto para resposta imune.

CAPÍTULO II

RUNNING HEAD: CONJUGATED LINOLEIC ACID AND LUTEIN IN CHICKENS

Effects of conjugated linoleic acid and lutein on the growth performance and immune response of broiler chickens

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ABSTRACT The effects of lutein and conjugated linoleic acid (CLA) on the growth performance and immune response of broiler chickens were evaluated in the presence and absence of *Salmonella* lipopolysaccharide (LPS) immune challenge. Cobb chicks (360; 1 to 22 d of age) were used in a 3 x 2 factorial arrangement of CLA (0, 1 and 2%) and lutein (0 and 50 mg/kg) dietary levels. At d 8 and 15, birds were injected with BSA to assess IgY production. At d 20, birds were injected with LPS. Samples of liver, spleen and duodenum were collected at 3 and 16 h post-LPS challenge for RT-qPCR analysis of RXRα, RXRγ, PPARα, PPARγ, TLR-4, IL-1β, IL-2, IL-10, and IL-12 gene expression. CLA decreased BW, BW gain (BWG), and G:F from d 1 to 20, but these effects were reversed when lutein was included in the 1% CLA diet ($P < 0.001$). The production of IgY anti-BSA increased following a 2% CLA supplementation ($P < 0.01$). LPS increased the liver:BW ratio at 3 h post-injection ($P < 0.001$) and decreased BWG at 3, 16 and 40 h ($P < 0.001$), with no influence of dietary treatments. Lutein decreased plasmatic nitric oxide levels ($P < 0.01$). LPS downregulated PPARα mRNA in the duodenum ($P = 0.02$) and liver ($P = 0.04$), and PPARγ ($P = 0.01$) and RXRα ($P = 0.08$) in the spleen; these effects were not reversed by CLA or lutein as initially hypothesized. Although LPS upregulated IL-1β ($P = 0.02$) and IL-12 ($P = 0.07$) expression, lutein downregulated these pro-inflammatory cytokines in the liver ($P = 0.03$ and $P = 0.07$, respectively). Lutein decreased splenic ($P = 0.09$) but increased hepatic ($P = 0.06$) TLR-4 mRNA. A dietary CLA supplementation of 2% increased hepatic RXRα ($P = 0.10$). In conclusion, CLA decreased broiler chicken growth performance, but lutein can prevent this negative effect (depending on CLA dose). Lutein had an anti-inflammatory effect, and a 2% CLA supplementation improved the humoral immune response.

Key words: broiler, CLA, LPS challenge, lutein, performance

INTRODUCTION

The interactions between carotenoids and fatty acids are important because both carotenoids and fatty acids are components of the cell membrane (Nagler et al., 2003). Conjugated linoleic acid (CLA) consists of a group of positional and geometric isomers of linoleic acid with 2 conjugated double bonds at various carbon positions in the fatty acid chain. The most frequently studied CLA isomers for biological activity are the *cis*–9, *trans*–11 and the *trans*–10, *cis*–12 (Pariza et al., 2001). Studies have found that CLA has beneficial immunomodulatory properties (Lai et al., 2005; He et al., 2007) and positively influences body composition (Sirri et al., 2003; Cordero et al., 2010) by integrating to the lipids of CLA-fed animals.

Lutein is the second most abundant carotenoid in nature (Surai and Sparks, 2000) and is the major dietary xanthophyll. Xanthophylls are oxygenated carotenoids with diminished or no provitamin A activity. In addition to pigmentation properties, studies have shown that lutein can enhance humoral and cellular immunity (reviewed by Chew and Park, 2004) and influence the innate response as a result of its antioxidant activity (Krinsky, 1993; Liu et al., 2008). During regular cell metabolism, many reactive oxygen intermediates are formed (Carpentier et al., 2009); this production is intensified during the inflammatory response to kill pathogens (Rosen et al., 2005). However, in addition to protecting the host against the pathogens, reactive oxygen intermediates may cause damage to the cell membranes of the host by targeting polyunsaturated fatty acids, such as CLA, in the phospholipid bilayer. Lutein serves as an antioxidant by decreasing the degradation that is induced by reactive oxygen species on the lipid components of cell membranes (Sujak et al., 1999).

Selvaraj and Klasing (2006) reported that lutein and eicosapentaenoic acid interact

to modify gene expression through the peroxisome proliferator X receptor (PPAR) and retinoic acid X receptor (RXR) pathways in chickens and by altering inflammatory responses to lipopolysaccharides (LPS) in HD11 cell lines. Because fatty acids are ligands for PPARs (Hwang, 2000; O'Shea et al., 2004; Jaudszus et al., 2008) and lutein is a ligand for RXRs (Nikawa et al., 1995; Selvaraj and Klasing, 2006; Selvaraj et al., 2010), an interaction between CLA and lutein can be expected. PPAR/RXR heterodimers attenuate the acute phase response intensity by suppressing NF-κB transcription, downregulating TLR-4 expression and, consequently, downregulating LPS recognition by macrophages (Dasu et al., 2009).

It was hypothesized that dietary CLA and lutein interact to attenuate the magnitude of acute inflammatory response by combating oxidative stress and modifying PPAR and RXR isomers to influence the expression of inflammatory cytokines and TLR-4. This study also investigated the effects of CLA and lutein on the humoral immune response and growth performance of broiler chickens.

MATERIAL AND METHODS

The experiment was approved by the UC Davis Institutional Animal Care and Use Committee to ensure adherence to animal care guidelines. For 22 d after hatching, 360 broiler chickens (Cobb of mixed sex) were reared under comfortable temperatures in brooder battery cages with woven wire floors (Petersime Inc., Gettysburg, OH, USA). Each cage with 6 birds represented an experimental unit.

Dietary Treatments

The study consisted of a 3 x 2 factorial design in which CLA (0, 1 or 2%; Lutalin,

BASF, Florham Park, NJ) and lutein (0 or 50 mg/kg; ORO GLO, Kemin Industries, Inc., Des Moines, IA) replaced soybean oil and cellulose in the diet, respectively. The diets were provided *ad libitum* throughout the experimental period and were formulated according to the nutritional levels recommended by NRC (1994), differing only in the amounts of the evaluated ingredients and their substitutes. All diets were isocaloric and isonitrogenous and were formulated to contain 0, 1, or 2% of CLA and 0 or 50 mg/kg lutein (Table 1). There were ten replicates with six chicks each per treatment.

LPS Immune Challenge

When chicks were 20 d of age, 3 birds/pen were injected intraperitoneally with 1.5 mg/kg *Salmonella enterica* serotype Typhimurium LPS (L7261, Sigma Chemicals, Saint Louis, MO) to challenge the immune system. The remaining 3 chicks in each pen were not injected. Lipopolysaccharides are key structural components of the membranes of Gram-negative bacteria and cause an inflammatory response. Because saline injections cause mild tissue trauma and inflammation, no injection was used as a control because our study was designed to examine the effects of inflammation vs. no inflammation.

Performance

BW, feed intake, body weight gain (BWG), and G:F were evaluated for 20 d. Before the LPS immune challenge and 3, 16, and 40 h after the immune challenge, all birds were individually weighted to evaluate BWG.

Anti-BSA IgY Production

On d 8 and 15 after hatching, all chickens were inoculated intramuscularly with 0.2 mg/kg of a solution of BSA (A3912 Sigma–Aldrich, Saint Louis, MO) diluted in 0.2 mL PBS at pH 7.4 (P3813, Sigma–Life Science) and 0.2 mL adjuvant (adapted from Takanashi et al., 2002). Complete Freund's Adjuvant (F5881, Sigma–Aldrich) was used in the first inoculation, and Incomplete Freund's Adjuvant (F5881, Sigma–Aldrich) was used in the second inoculation. Blood samples (5 mL) from one bird per experimental unit were collected on d 8, 15, and 21 via the brachial vein using a syringe. The blood samples were centrifuged at 2,000 × *g* for 15 min at 25°C. ELISA plates in a 96-well format (12565500, Fisher Scientific, Fair Lawn, NJ) were coated with BSA (100 µL/well) at a concentration of 500 mg/mL in sodium carbonate buffer (0.2 M, pH 9.2). The plates were then incubated at 4°C for 16 h. Serum samples were tested in duplicate (100 µL/well, 1:2,000) and incubated for 1 h. The plates were incubated for 30 min with 100 µL of rabbit anti-chicken/turkey IgY (IgG) conjugated with peroxidase (1:8,000, A-9046, Sigma–Aldrich). Then, 100 µL of ortho-phenylenediamine solution (P8936, Sigma–Aldrich Fast) was added and the plates were incubated in the dark for 15 min at room temperature. The reaction was terminated with sulfuric acid (2 M, 50 µL/well). The anti-BSA IgY production was quantified by optical density measurements performed with an ELISA plate reader at 495 nm. Between each step listed above, the plates were washed 3 times with PBS Tween–20 (P1319, Sigma–Aldrich), and all incubations were performed in a moist chamber at 37°C unless otherwise noted. To establish serum titer assays, a series of dilutions were tested and the lowest dilution with an optical density of 2 SD above the baseline was chosen.

Acute Phase Response

Nitric Oxide Production. Sixteen hours after the LPS immune challenge, blood samples from two birds per experimental unit (i.e., an LPS-challenged and an unchallenged bird) were collected for nitric oxide (NO) quantification. The blood samples were centrifuged at 2,000 × *g* for 15 min at 25°C. NO in the serum was measured indirectly by quantifying nitrite (a stable end-product of NO metabolism) using the Griess Reagent System (G2930, Promega, Madison, WI). For each blood sample, 100 μL of serum was mixed with 100 μL of Griess reagent (1% sulfanilamide in phosphoric acid, 0.1% N-1-naphthylethylenediamine dihydrochloride in water) in 96-well microplates (12565500, Fisher Scientific) that were then incubated at room temperature for 15 min in the dark. Absorbance at 540 nm was measured with an ELISA plate reader using the serum with PBS (instead of Griess reagent) as a blank. All samples were analyzed in duplicate. A $NaNO_2$ standard curve (0 to 20 μ*M*) was generated in triplicate on each plate containing samples. A linear fit line was generated for all triplicate standards ($R^2 > 0.99$) and used to calculate the concentration of NO_2^- in each sample. The results are expressed as the mean nitrite concentration in the supernatants of duplicate wells.

Liver:BW Ratio. At 3 and 16 h after the LPS immune challenge, two birds per experimental unit (i.e., an LPS-challenged and an unchallenged bird) were euthanized. Their livers were weighted and related to their respective BW.

Inflammatory Cytokines and Receptor mRNA Expression. From the 2 birds per experimental unit that were sacrificed for the liver:BW ratio examination (above), livers, spleens and duodenums were removed and stored in RNA later solution (R0901, Sigma–Aldrich) at -20°C for future RNA analysis. Total RNA from these tissues was

isolated using TRIzol reagent (15596-018, Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. A Turbo-DNAse kit (AM1907, Applied Biosystems, Foster City, CA) was used to treat the samples. RNA concentrations were quantified with a NanoDrop Spectrophotometer (ND1000, Thermo Scientific, Bonn, Germany), and integrity was determined with an Experion Automated Electrophoresis System (700-7000, Bio-Rad, Hercules, CA). RNA samples were reverse transcribed and RT-qPCR analyses were performed with a MyiQ System (170-9740, Bio-Rad). One microgram of RNA was converted to a 20 μL reaction volume of cDNA using the iScript™ Reverse Transcription Supermix kit (170-8841, Bio-Rad) at 25°C for one h, 42°C for 30 min, and then 85°C for 5 min.

The genes analyzed by RT-qPCR were as follows: PPARα (5'-caatgcactggaactggatg-3' and 5'-cgtcaggatggttggtttg-3'), PPARγ (5'-gggcgatcttgacaggaa-3' and 5'-gcctccacagagcgaaac-3'), RXRα (5'-gatgcgagacatgcagatg-3' and 5'-gtcggggtatttgtgcttg-3'), RXRγ (5'-caaacacatctgtgccatctg-3' and 5'-gatgaggcagtccttgttgtc-3'), TLR-4 (5'-agtctgaaattgctgagctcaaat-3' and 5'-gcgacgttaagccatggaag-3'), IL-2 (5'-tctgggaccactgtatgctct-3' and 5'-acaccagtgggaaacagtatca-3'), IL-10 (5'-cgggagctgagggtgaa-3' and 5'-gtgaagaagcggtgacagc-3'), IL-1β (5'-gcatcaagggctacaagctc-3' and 5'-caggcggtagaagatgaagc-3'), IL-12 (5'-agactccaatgggcaaatga-3' and 5'-ctcttcggcaaatggacagt-3') and GAPDH (5'-ggtggtgctaagcgtgttat-3' and 5'-acctctgtcatctctccaca-3'). The final 20 μL of the PCR reaction contained 2 μL of the reverse transcription product, 2 μL of the forward and reverse genes, and 10 μL of iTAq® Universal SYBR Green Supermix (172-5122, Bio-Rad). The PCR cycle conditions of all primer pairs included an initial 60 s denaturation step at 95°C followed by 40 cycles of denaturing (15 s at 95°C), annealing and extension (30 s at 60°C). The

melting profile of each sample was analyzed after every PCR run to confirm PCR product specificity. The melting profile was determined by heating samples at 65°C for 30 s and then increasing the temperature at a linear rate of 20°C/s to 95°C while continuously monitoring fluorescence. Sample PCR amplification efficiencies were determined in the log-linear phase using the LinRegPCR program (Ramakers et al., 2003). The delta–delta equation subtracts sample and reference Ct values from an endogenous control; however, the endogenous control (GAPDH) Ct was affected by treatment in our study ($P < 0.05$) and was therefore removed from the equation. All data were normalized to the mRNA level of the reference group (i.e., the group fed 0 mg lutein and 0% CLA) and reported as the fold-change from the reference. Fold-change from the reference was calculated as $E_S^{(40\text{-Ct Sample})}/E_R^{(40\text{-Ct Reference})}$, where E_S and E_R are the sample and reference PCR amplification efficiencies, respectively (Humphrey et al., 2004).

Statistical Analyses

Data were analyzed by ANOVA and least square (LS) means using the General Linear Model (GLM) procedure (SAS Inst. Inc., Cary, NC) appropriate for a factorial arrangement of treatments in a completely randomized design. The statistical models of growth performance and anti-BSA IgY production included the effects of CLA (0, 1 or 2%) and lutein (0 or 50 mg/kg) supplementation levels and their interactions. For the acute phase response, LPS challenge was also included in the model as a source of variation. Interactions were removed when the observed *P*-value was above 0.1. Pooled SEM was calculated by averaging the SEMs calculated with the GLM procedure of SAS for the variable of interest. A $P \leq 0.1$ was used to indicate statistical significance

for gene expression analyses, and a $P \leq 0.05$ was used to indicate statistical significance for the other variables evaluated.

RESULTS AND DISCUSSION

Lutein and CLA interacted to affect growth performance responses from 1 to 20 d. Contrary to the initial hypothesis, no interaction between the dietary treatments or with the LPS challenge was observed for the immunological response variables.

Performance

Growth data indicated a strong interaction between CLA and lutein. At 1 or 2% of CLA, BW, BWG and G:F decreased between 1 and 20 d; these effects were reversed when lutein was included in the 1% CLA diet ($P < 0.001$; Figure 1). Significant effects or tendencies were also observed in the partial measurement periods (1 to 8, 8 to 15 and 15 to 20 d; Table 2). Feed intake was not affected by treatments. To the best of our knowledge, the effects of the interaction between CLA and lutein on performance have not previously been reported. Working with a different xanthophyll, Hu et al. (2012) observed a BWG decrease as a result of the combination of fucoxanthin + CLA in the diets of rats, but fucoxanthin alone elicited no effect. However, as no CLA control treatment was included, it cannot be determined whether CLA or a synergistic interaction of CLA and fucoxanthin caused this negative effect on BWG.

Most previous studies on dietary carotenoids have focused on improving the pigmentation of animals and animal products. Studies on growth performance and immunomodulatory function have used different carotenoid mixtures, diet inclusions, and measurements, making comparisons with our study difficult. Pérez-Vendrell et al.

(2001) evaluated lutein, zeaxanthin and canthaxanthin at different proportions in broiler diets and found no effect of any carotenoids on performance compared to the control group. Similar results were reported by Ouart et al. (1988) for different xanthophyll sources. In addition, chick performance did not differ when breeders were fed diets containing lutein, canthaxanthin or β-carotene (Haq and Bailey, 1995).

The reported effects of CLA on performance range from positive to negative and neutral. These discrepancies can be attributed to factors such as differences in animal species, dietary concentration or type of CLA isomer used. Feeding CLA at 0.5 to 1% improved growth, feed efficiency, fat-to-lean tissue repartitioning or a combination thereof in rats (Chin et al., 1994), mice (West et al., 1998) and pigs (Dugan et al., 1997; Ostrowska et al., 1999). In chickens, Jiang et al. (2014) observed no significant effects on growth performance with 1% CLA supplementation. Simon et al. (2000), Du and Ahn (2002), Sirri et al. (2003), and Zhang et al. (2008) reported similar results with up to 2.4% CLA supplementation in chicken diets. In contrast, Szymczyk et al. (2001) and Suksombat et al. (2007) tested CLA inclusions at 0, 0.5, 1, and 1.5% for chickens and observed linear reductions in growth performance until 42 d of age, particularly at higher levels of CLA. In our study, CLA exhibited a negative effect on performance that could be observed not only at 2% CLA inclusion but also at 1% CLA inclusion, the lowest level tested. These results are consistent with our recent findings: feeding broiler chickens 0.5 or 1% CLA decreased the growth rate between 1 and 21 d old (Cardinal et al., 2014). West et al. (1998) attributed the decreased growth performance of mice that were fed CLA to an increase on energy expenditure, and suggested that this effect was related to the loss of body fat. The mechanisms by which CLA could affect energy metabolism are still not clear, but was suggested CLA inhibits fat storage in adipocytes

coupled with both elevated β-oxidation in skeletal muscle and an increase in skeletal mass (Park et al., 1999). Park et al. (1997) reported enhaced lipolysis and decreased lipoprotein lipase activity as a result of adding CLA to the culture medium of 3T3-L1 adipocytes. The lipoprotein lipase is an enzyme which hydrolyses free fatty acids from circulating triacylclyceride; the fatty acids are then taken up by the adipocytes and re-esterified. Additionally, the same authors observed an increase on carnitine palmitoyltransferase activity, which is rate-limiting for fatty acid β-oxidation, in skeletal muscle of mice. Intriguingly, in the study of Cardinal et al. (2014), CLA decreased the growth rate between 1 and 21 d old, but did not affect performance between 21 and 42 d. In the present study, CLA also decreased performance in the starter period (1 to 20 d). If we consider the effect of CLA on chicken performance to be primary related to changes in the adipose tissue, we would expect CLA to exhibit a stronger effect in the growing phase (when fat deposition is greater), rather than in the starting phase (when deposition is mainly protein). This lack of agreement with previous studies suggests that the mechanisms by which CLA affects chicken performance are complex and require further clarification.

Anti-BSA IgY Production

The production of anti-BSA IgY increased ($P \leq 0.01$) in the group that was fed 2% CLA, indicating a positive stimulation of the humoral response (Table 3). Similar to our findings, dietary CLA was considered to increase antigen-specific immunoglobulin in pigs (Lai et al., 2005; Moraes et al., 2012) and birds (He et al., 2007). Dietary CLA was also reported to increase total immunoglobulin production in humans (Song et al., 2005) and rats (Ramirez-Santana et al., 2009). In contrast, Cook et al. (1993) observed no

effect of 0.5% CLA supplementation on antibody levels of SRBC in chicks or BSA in rats. The mechanism by which CLA affects immunoglobulin synthesis is unclear, but a possible explanation is that dietary CLA influences IL production. It was reported that IL–2 increased IgG, IgA, and IgM synthesis (Kawano and Noma, 1996). Although this effect was not observed in our study, Yang and Cook (2003) reported that CLA could stimulate IL–2. Thus, it is possible that CLA can indirectly promote IgG, IgA, and IgM synthesis.

Lutein did not affect the production of anti-BSA antibodies. Similarly, Haq and Bailey (1995) and Haq et al. (1996) found no differences in the antibody titers for Newcastle Disease in chicks hatched from breeders fed diets supplemented with lutein, canthaxanthin, β-carotene or vitamin E. In contrast, Rajput et al. (2013) observed that antibody titers that were measured following vaccination for Newcastle Disease were greater in LPS-induced chickens fed curcumin; those fed lutein exhibited an intermediate level compared to the control group. Bédécarrats and Leeson (2006) also observed a positive effect of lutein on humoral immunity; they observed an enhanced amplitude of the secondary antibody response to a live infectious bronchitis virus vaccine in hens fed 125 ppm lutein. Interestingly, a higher dose of lutein (250 ppm) failed to significantly boost the antibody response. However, it is not always the greatest concentration of nutraceutical supplementation that promotes the strongest immune response. Contradictory reports regarding the effect of carotenoids on chick antibody titers can be attributed to the use of different antigens, vaccination methods, and assays to measure antibodies in the different studies (Haq et al., 1995).

Acute Phase Response

Body Weight Gain. An acute effect of LPS was identified in the BWG results ($P < 0.001$, Figure 2). At 3 h post-LPS injection, BWG was 88.60% lower in the challenged group than in the control group. From 3 to 16 and 16 to 40 h post-injection, challenged birds had similar BWG; however, they did not compensate for their lower BWG in the first 3 h post-injection. Decreased BWG is expected to be a consequence of an acute inflammatory response due to a general catabolic state and reduction in feed intake (Klasing and Johnstone, 1991; Johnson, 1997). Jiang et al. (2010) detected a BWG decrease of 22.5% in LPS-challenged broilers compared to unchallenged broilers fed *ad libitum*. The results from a third group of unchallenged broilers that were pair fed with the LPS-challenged group suggested that 59% of the BWG decrease was due to reduced feed intake and 41% was attributable to other factors, likely associated with immune-induced metabolic inefficiency.

One hypothesis for the anti-inflammatory effects of CLA and lutein is that they are able to modulate receptors and cytokines to attenuate the events associated with the acute phase response and, consequently, prevent the expected decrease in performance. However, neither CLA nor lutein was able to reverse or attenuate the deleterious effect of LPS challenge on BWG. Contrary to performance results from 1 to 20 d, no interaction of CLA and lutein was observed for BWG after the LPS challenge (data not shown). Cook et al. (1993) reported that a 0.5% CLA supplementation at 1 mg/kg body weight prevented LPS-induced growth depression in chicks; this dose was somewhat lower than that used in our study (1.5 mg/kg). Interestingly, Takahashi et al. (2002) reported that CLA prevented the reduction in BWG and G:F after 3 consecutive LPS challenges every other day at a much higher dose (200 mg/kg).

Liver:BW Ratio. LPS increased the liver:BW ratio at 3 h post-injection ($P < 0.001$) but not at 16 h, illustrating the acute effect of this antigen (Table 3). Increased liver weight is expected during an acute inflammatory response because the liver is the site of acute phase protein synthesis (Xie et al., 2000). These proteins act to increase humoral immunity, preventing tissue pathology induced by local inflammation and aiding in the reparative process (Klasing and Austic 1984). Neither lutein nor CLA influenced this response. Meriwether et al. (2010) described an LPS challenge and dietary lutein interaction in which chicks that were not fed lutein exhibited LPS-induced increases in liver weight, but chicks that were fed 40 mg/kg dietary lutein did not.

Nitric Oxide Production. Lutein had an anti-inflammatory effect, decreasing plasma NO ($P < 0.01$) independently of the LPS challenge 16 h prior to measurement (Table 3). Although NO plays important roles in host defense (Baran et al., 1996), its increased release may promote inflammation and induce extensive cellular damage associated with oxidative stress (Tidball, 2005; Barnham et al., 2004). Thus, reducing NO production substantially impairs the inflammatory process. Hadad and Levy (2012) tested the role of LPS-induced macrophages on the release of some pro-inflammatory mediators. The authors suggested that LPS activates NOX2 NADPH oxidase and results in the transference of electrons from NADPH to molecular oxygen, thus generating superoxide and activating the NF-κB signaling pathway. As consequence, TNF-α production is increased and subsequently upregulates COX-2, iNOS and NOX2 NADPH oxidase, which promote the cellular release of PGE_2, NO and additional superoxide, respectively. Lutein acts to inhibit the production of superoxide and, thus, the subsequent cascade of events. Kim et al. (2008) concluded that lutein scavenges not

only superoxide but also H_2O_2, which also increases the expression of NF-κB-regulated genes (TNF-α, COX-2, iNOS and IL-1β) in LPS-stimulated macrophages. However, in our study, neither the LPS x lutein interaction nor the LPS main effect influenced the plasmatic NO production 16 h after LPS injection. LPS promoted an acute inflammatory response, and we observed differences in the liver:BW ratio at 3 h post-challenge but not at 16 h. Possibly, if we had quantified plasmatic NO sooner post-challenge, differences between the LPS-challenged and the unchallenged birds could have been noted.

Conflicting effects of CLA on NO production have been reported. In our study, CLA did not alter NO concentration in chicken plasma. CLA either decreased (Eder et al., 2003; Jenko and Vanderhoek, 2008) or increased (Coen et al., 2004) NO production during *in vitro* trials with endothelial cells. NO production was also increased in white blood cell cultures isolated from pigs that were fed 1% CLA, but lower levels of CLA inclusion (0.12, 0.25 and 0.5%) did not influence this response (Wiegand et al., 2011).

Inflammatory Cytokines and Receptor mRNA Expression. No interaction was observed between dietary treatment and LPS challenge for gene expression. Therefore, the time of sample collection (3 or 16 h post-LPS challenge) was not considered as a main effect of dietary treatment. No differences among treatments were found for RXRγ, IL-2 and IL-10 in any of the tissues sampled (data not shown).

LPS downregulated PPARα mRNA in the duodenum ($P = 0.02$) and liver ($P = 0.04$) and PPARγ ($P = 0.01$) and RXRα ($P = 0.08$) in the spleen, but these expected effects were not reversed by CLA or lutein as initially hypothesized (Figure 3). Conjugated linoleic acid is a ligand for PPAR (O'Shea et al., 2004); however, PPARs are only

active as heterodimers with RXR (Selvaraj et al., 2006). Previous studies have shown that RXR can be activated by lutein (Selvaraj and Klasing, 2006; Selvaraj et al., 2010), and we hypothesized that feeding CLA and lutein to LPS-challenged birds would create an anti-inflammatory response through the PPAR-RXR pathway. Despite the lack of an interaction of the two dietary treatments, chickens fed 2% CLA exhibited increased hepatic RXRα mRNA ($P = 0.1$), but there was no effect of CLA on PPAR in any of the tissues sampled (Figure 4). The capacity to specifically bind and activate RXRα has been attributed to other unsaturated fatty acids (Lengqvist et al., 2004; Steineger et al., 2005). A mix of PUFA (from fish and sunflower oil) increased RXRα expression in the spleen and the liver of chickens (Sevaraj et al., 2010), as did eicosapentaenoic acid in chickens and HD11 macrophage cultures (Selvaraj and Klasing, 2006). Docosahexaenoic acid, arachidonic acid, and oleic acid had similar effects on cell cultures (Lengqvist et al., 2004). These findings suggest that CLA acts in a similar manner to other fatty acids and has the potential to exert important effects on RXR-mediated gene transcription.

LPS-challenged chickens exhibited higher IL-1β ($P = 0.02$) and IL-12 ($P = 0.07$) mRNA expression levels. IL-1β synthase is induced by transcription factor NF-κB after innate cells are exposed to LPS, which binds to TLR-4 and initiates the inflammatory response cascade. IL-1β plays a central role in the inflammation response, activating many different cell types and inducing a range of pro-inflammatory activities, such as the induction of IL-12 in dendritic cells (Wesa and Galy, 2001). In the present study, LPS did not increase TLR-4. These results agree with other trials conducted in our lab in which, despite a lack of difference in TLR-4 expression, IL-1β and IL-12 increased and IL-10 decreased at 3 h after the LPS-challenge (Santin et al., unpublished data).

Lutein decreased hepatic IL-1β ($P = 0.03$) and IL-12 ($P = 0.07$), the same cytokines that were upregulated by LPS challenge in the liver (Figure 5). Lutein also decreased splenic ($P \leq 0.09$) but increased hepatic ($P = 0.06$) TLR-4 mRNA. These contradictory TLR-4 results are difficult to interpret. In general, RT-qPCR results indicated that lutein may act as an anti-inflammatory nutrient, despite its acute phase response stimulation. These results agree with the antioxidant effects of lutein on plasmatic NO production. Lutein acts as antioxidant by preventing NF-κB signaling pathway stimulation by superoxide and, consequently, NO, IL-1β, and IL-12 production. These reductions in inflammatory markers reduce tissue injury (Feingold et al., 2004; Li et al., 2005) and the anorectic consequences of an inflammatory response (Klasing, 1998).

Carotenoids are not as well absorbed as vitamins C and E (Halliwell et al., 2000), and lutein may therefore play an important role as an intestinal antioxidant. The intestinal mucosa not only absorbs these nutrients but also continuously acts in response to dietary and microbial antigenic components by producing pro-inflammatory mediators (Witting and Zeitz, 2003; Burkey et al., 2009). Few studies have tested the effect of carotenoids on chicken intestines, although a dietary mix of 40% lutein and 60% zeaxanthin at 20 or 30 mg/kg decreased pro-inflammatory cytokines in the duodenum (LITAF), jejunum (IL-1β, IFN-γ), and ileum (IFN-γ) of birds (Gao et al., 2012). However, the same study reported that the liver was the site where a large number of effects were observed in response to carotenoid inclusion in the diet, including the downregulation of the pro-inflammatory IL-6, IL-1β, IFN-γ, LITAF and the upregulation of the anti-inflammatory IL-10. In the present study, lutein influenced TLR-4, IL-1β and IL-12 in the liver and TLR-4 in the spleen, but no difference was observed in the duodenum. These findings suggest that the main action site for

carotenoids may be in the liver, at least compared to the intestine and spleen.

In conclusion, dietary CLA and lutein can be used to beneficially modulate broiler chicken immune response, regardless of LPS stimulation. Our findings suggest that lutein has an anti-inflammatory effect, and a 2% CLA supplementation promotes a beneficial humoral immune system response.

Lutein and CLA interact to modify broiler chicken growth performance. Conjugated linoleic acid at 1 or 2% decreased the BWG and G:F of chickens from 1 to 20 d of age, but this effect was reversed when lutein was included in the 1% CLA diet. The profound synergistic relationship between lutein and CLA should be clarified in future studies.

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Table 1. Composition of experimental diets, as-fed basis

Item	Diet
Ingredient, %	
Soybean meal	44.36
Corn	41.54
Vegetable oil[1]	7.88
Calcium phosphate	1.97
Limestone	1.41
Cellulose[2]	1.35
Salt	0.5
DL-Methionine	0.32
Vitamin premix[3]	0.25
Mineral premix[4]	0.25
L-Lysine HCl 95%	0.1
Choline chloride	0.07
Chemical composition, %	
ME, kcal/kg	3,200
CP	25
Fat	9.9
Ca	1.1
Available P	0.5
Total lysine	1.5
Total methionine	0.69
Total methionine + cystine	1.08

[1]Diets contained either 0, 1 or 2% CLA, as follows: in the 0% CLA diets, all vegetable oil was soybean oil; in the 1% CLA diets, 6.21% soybean oil and 1.67% CLA-rich oil were used; in the 2% CLA diets, 4.54% soybean oil and 3.34% CLA-rich oil were used.

[2]Lutein was included in substitution to cellulose at 50 mg/kg diet.

[3]Provided per kilogram of diet: vitamin A, 5,406 IU; vitamin D3, 450 IU; vitamin E, 53.06 IU; vitamin K, 1.50 mg; biotin, 0.67 mg; choline, 2000 mg; folic acid, 6.74 mg; niacin, 69.72 mg; pantothenic acid, 23.31 mg; pyridoxine, 12.87 mg; riboflavin, 16.7 mg; thiamin, 17.87 mg; and vitamin B12, 0.02 mg.

[4]Provided per kilogram of diet: Fe, 46.3 mg; Mn, 111.3 mg; Cu, 12.16 mg; Se, 0.148 mg; Zn, 73.91 mg; and I, 4.879 mg.

Table 2. Effects of dietary supplementation with CLA and lutein on the growth performance of broiler chickens[1]

	CLA, %				Lutein, mg/kg			*P*-value		
Item[2]	0	1	2	SEM[3]	0	50	SEM	CLA	Lutein	Lutein x CLA
BW, g										
d 1	45.5	45.6	45.6	0.0	45.6	45.6	0.0	0.535	0.821	0.987
d 8	142.7	142.4	138.2	1.5	141.5	140.7	1.2	0.078	0.623	0.019
d 15	417.6[a]	404.2[b]	396.9[b]	4.7	406.1	406.5	3.9	0.011	0.950	0.002
d 20	688.4[a]	671.1[b]	660.5[b]	5.2	672.9	673.9	4.2	0.001	0.859	0.001
FI, g										
1 - 8 d	116.3	116.8	114.0	1.2	116.5	114.9	1.0	0.215	0.228	0.028
8 - 15 d	323.6	320.7	315.5	3.5	320.7	319.2	2.9	0.269	0.721	0.091
15 - 20 d	357.5	350.6	355.0	2.7	354.3	354.5	2.2	0.202	0.954	0.491
Overall	797.2	789.7	784.5	5.2	791.3	789.6	4.3	0.235	0.785	0.278
BWG, g										
1 - 8 d	97.2	96.8	92.6	1.5	96.0	95.1	1.2	0.073	0.618	0.019
8 - 15 d	274.9[a]	261.8[b]	258.7[b]	3.8	264.6	265.8	3.1	0.009	0.783	0.004
15 - 20 d	270.8	266.9	263.6	4.6	266.7	267.5	3.8	0.551	0.891	0.023
Overall	642.7[a]	628.7 [b]	614.9[c]	5.1	627.3	628.4	4.1	0.001	0.577	0.001
G:F										
1 - 8 d	0.835	0.829	0.812	0.007	0.823	0.828	0.006	0.092	0.601	0.001
8 - 15 d	0.848[a]	0.815[b]	0.820[b]	0.016	0.824	0.832	0.013	0.035	0.46	0.055
15 - 20 d	0.757	0.761	0.742	0.011	0.752	0.754	0.009	0.470	0.905	0.014
Overall	0.806[a]	0.797[ab]	0.784[b]	0.005	0.793	0.798	0.004	0.012	0.344	0.001

[1]There were 10 replicates/treatment, each with 6 broiler chickens.
[2]BW = body weight; FI = feed intake; BWG = body weight gain; G:F = gain to feed ratio.
[3]Pooled SEM.
[a, b]Means in the same row that lack a common superscript differ significantly ($P < 0.05$).

Table 3. Effect of *Salmonella* lipopolysaccharide (LPS) challenge and dietary supplementation with CLA and lutein on plasmatic anti-BSA IgY, nitric oxide production, and liver:BW ratio at 3, 16, and 40 h after LPS challenge[1]

	CLA, %				Lutein, mg/kg			LPS[2]			*P* - value[4]		
Item	0	1	2	SEM[3]	0	50	SEM	+	-	SEM	CLA	Lutein	LPS
IgY[5]													
8 d	0.04	0.06	0.07	0.02	0.06	0.06	0.01	-	-	-	0.54	0.9	-
15 d	0.47	0.29	0.49	0.1	0.43	0.4	0.08	-	-	-	0.34	0.82	-
21 d	1.19[b]	1.24[b]	1.58[a]	0.1	1.35	1.31	0.08	-	-	-	0.01	0.73	-
Nitric oxide, μ*M*													
16 h after LPS	615	616	587	34	660	552	28	581	631	28	0.79	0.01	0.2
Liver:BW, %[6]													
3 h after LPS	2.91	3.08	3.08	0.06	3.03	3.02	0.05	3.18	2.87	0.05	0.06	0.99	0.001
16 h after LPS	3.74	3.73	3.78	0.09	3.81	3.69	0.07	3.66	3.84	0.07	0.89	0.22	0.08

[1]There were 10 replicates/treatment, each with 6 broiler chickens.

[2]*Salmonella* LPS challenge was performed on d 20.

[3]Pooled SEM.

[4]*P*-values above 0.2 for interactions were removed from the analyses.

[5]Values represent the mean optical density of anti-BSA IgY in blood samples. Inoculations with BSA occurred on d 8 and 15.

[6]BW = body weight.

[a, b]Means in the same row that lack a common superscript differ significantly ($P \leq 0.01$).

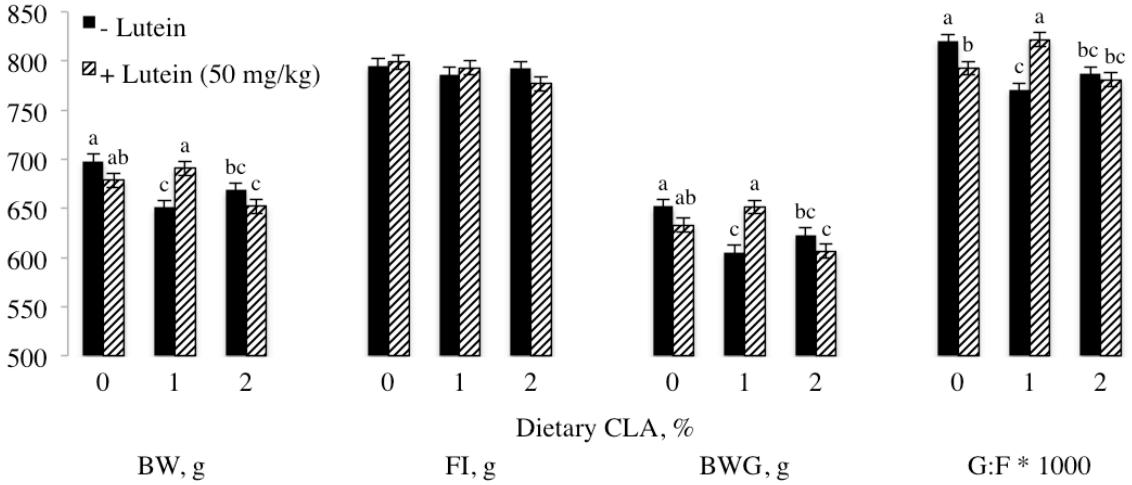

Figure 1. Interaction effect of dietary supplementation with CLA and lutein on broiler chickens from 1 to 20 d of age on growth performance. Values are means ± SE. Means that lack a common superscript differ significantly ($P \leq 0.05$).

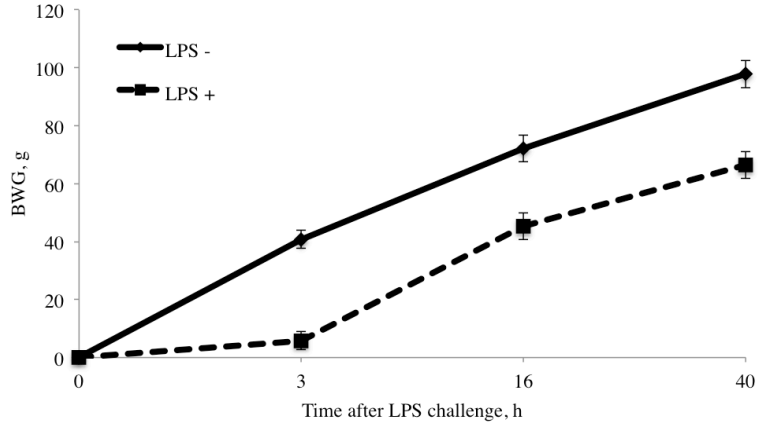

Figure 2. Effect of *Salmonella* lipopolysaccharide (LPS) challenge on chicken BWG at 3, 16 and 40 h after LPS injection. $P < 0.001$. Error bars indicate SEM.

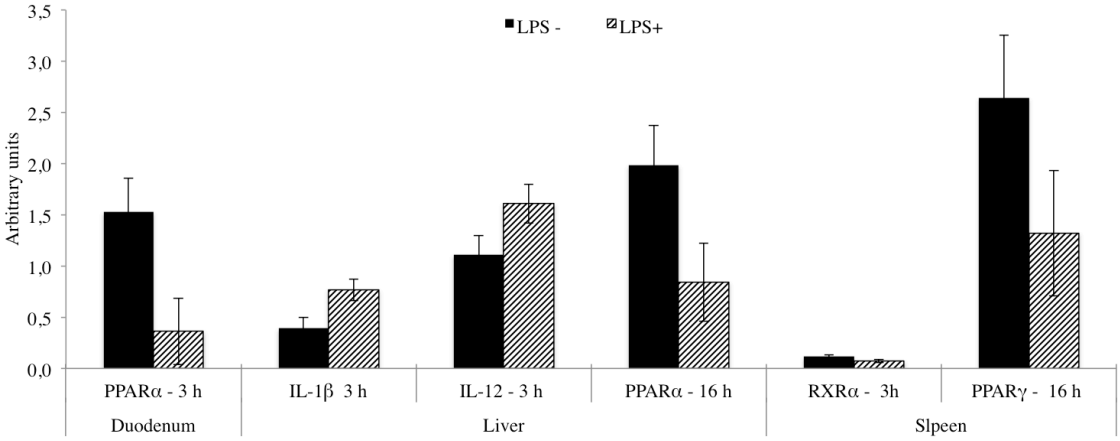

Figure 3. Gene expression in chicken duodenum, spleen and liver at 3 or 16 h post-LPS challenge. Values are means ± SE. *P*-values from ANOVA: duodenal PPARα, $P = 0.02$; hepatic IL-1β, $P = 0.02$; IL-12, $P = 0.07$; PPARα, $P = 0.04$; splenic RXRα, $P = 0.08$; PPARγ, $P = 0.01$.

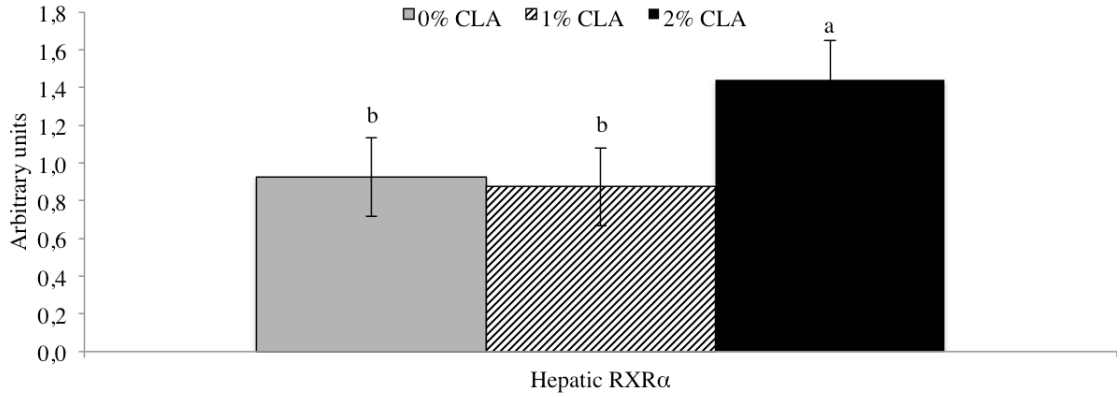

Figure 4. RXRα mRNA expression in the liver of chickens fed different levels of CLA. Values are means ± SEM. Means lacking a common superscript differ ($P < 0.10$).

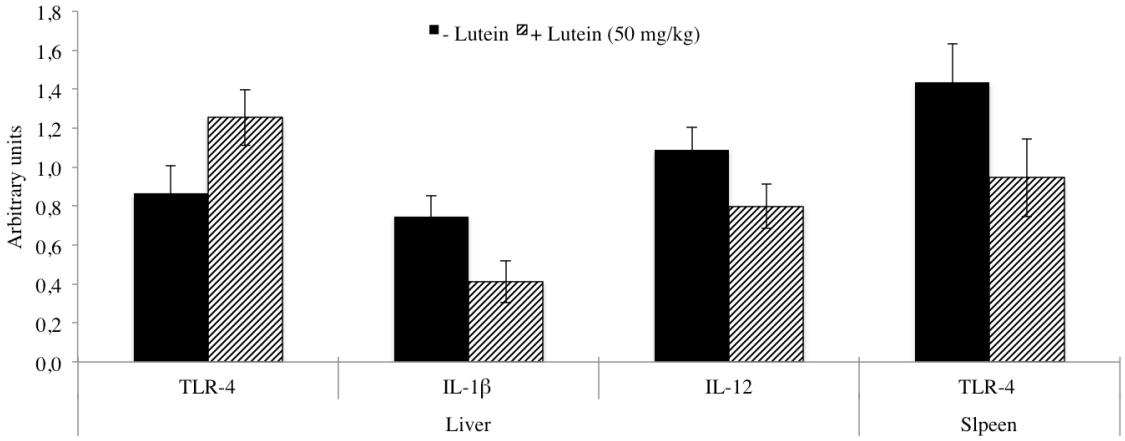

Figure 5. Gene expression in the spleen and the liver of chickens fed different levels of lutein. Values are means ± SE. *P*-values from ANOVA: hepatic TLR-4, $P = 0.06$; IL-1β, $P = 0.03$; IL-12, $P = 0.07$; splenic TLR-4, $P = 0.09$.

CAPÍTULO III

CONSIDERAÇÕES FINAIS

O CLA e a luteína suplementados na dieta de frangos de corte de 1 a 22 dias promoveram efeitos imunomodulatórios positivos, porém, não foi observada interação entre ambos os nutrientes para as avaliações relacionadas com a resposta imune. O CLA, adicionado em 2% na dieta, foi benéfico para o sistema imune humoral elevando a produção de IgY em resposta ao estímulo com BSA e também foi capaz de aumentar a expressão de RXRα no fígado. A luteína, de uma forma geral, se mostrou como um agente anti-inflamatório, diminuindo o óxido nítrico plasmático e também a expressão de TLR-4 no baço e de IL-1β e IL-12 no fígado apesar ter de aumentado a expressão de TLR-4 hepática. Os efeitos observados para CLA e para luteína foram independentes do desafio com LPS.

Já para os resultados de desempenho, uma forte interação entre CLA e luteína foi observada, de forma que a suplementação com 1 ou 2% de CLA pioraram o peso corporal, o ganho de peso e a eficiência alimentar de 1 a 20 dias de idade, mas estes efeitos foram revertidos quando a luteína foi incluída na dieta contendo 1% de CLA. Esta observação não foi relatada previamente em outras pesquisas e abre um leque de possibilidades para novos estudos que explorem essa relação. Podemos pensar inclusive, que caso opte-se pela inclusão de CLA na dieta de frangos de corte almejando seus benefícios imunomodulatórios ou como melhorador da carcaça, a inclusão conjunta de luteína pode ser uma boa estratégia para evitar possíveis quedas de desempenho. Entretanto, devemos ter consciência de que os efeitos individuais da luteína e do CLA, assim como seus efeitos relacionados, são dependentes dos seus níveis de inclusão na dieta e é possível que haja um nível mais adequado para cada tipo de resposta. Nesse caso, deve-se decidir qual resposta é mais desejada de uma forma geral ou ainda trabalhar de acordo com cada situação.

O desafio com LPS atendeu o objetivo proposto de provocar uma resposta inflamatória aguda, comprovado pelos sinais clínicos de apatia e sonolência dos frangos, pela queda no ganho de peso até 40 horas após sua inoculação, pelo aumento na relação fígado:peso corporal, e também pela

mudança na expressão de genes envolvidos na resposta inflamatória. O fato de não ter sido observada interação entre o LPS nem com o CLA e nem com a luteína mostra que os nutrientes não foram eficientes para reverter ou amenizar o estresse causado pelo modelo de desafio adotado. Entretanto, pesquisas prévias relatam resultados conflitantes que podem ser atribuídos em parte às diferenças metodológicas como por exemplo espécie animal utilizada, idade, nível de inclusão dos nutrientes na dieta, modelo de desafio imunológico adotado e respostas avaliadas, ressaltando nessas, a importância nos intervalos de tempo para as coletas de amostras quando se emprega um desafio agudo. Essas diferenças metodológicas prejudicam a percepção global do estado da arte no que diz respeito a avaliação da interação dos nutrientes com o sistema imune. Estudos com o objetivo de esclarecer as metodologias mais adequadas para serem empregadas neste tipo de avaliação ainda são escassos e trariam um grande avanço científico para a área.

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APÊNDICE 1. Observações experimentais: desempenho zootécnico

Tratamento			Variável Resposta (g)				
CLA (%)	Luteína (mg/kg)	Rep	PC 1 d	PC 8 d	CR 1-8 d	GP 1-8 d	GP:CR 1-8 d
0	0	1	45,2	143,2	117,7	98,0	0,83
0	0	2	45,5	146,5	119,0	101,0	0,85
0	0	3	45,3	148,7	119,0	103,3	0,87
0	0	4	45,7	137,2	108,3	91,5	0,84
0	0	5	45,7	157,7	123,2	112,0	0,91
0	0	6	45,5	132,8	105,8	87,3	0,83
0	0	7	45,8	140,0	113,7	94,2	0,83
0	0	8	45,5	139,8	109,8	94,3	0,86
0	0	9	45,7	137,8	112,5	92,2	0,82
0	0	10	45,3	144,7	121,2	99,3	0,82
1	0	1	45,5	134,3	113,0	88,8	0,79
1	0	2	45,7	145,5	120,2	99,8	0,83
1	0	3	45,3	130,8	117,5	85,5	0,73
1	0	4	45,5	137,8	114,2	92,3	0,81
1	0	5	45,7	151,5	123,3	105,8	0,86
1	0	6	45,7	161,2	132,2	115,5	0,87
1	0	7	45,5	131,7	109,3	86,2	0,79
1	0	8	45,5	140,3	117,3	94,8	0,81
1	0	9	45,7	128,2	113,2	82,5	0,73
1	0	10	45,7	137,2	112,8	91,5	0,81
2	0	1	45,7	134,2	108,7	88,5	0,81
2	0	2	45,3	139,5	112,5	94,2	0,84
2	0	3	45,7	150,2	124,5	104,5	0,84
2	0	4	45,8	134,7	110,8	88,8	0,80
2	0	5	45,7	150,3	123,7	104,7	0,85
2	0	6	45,5	141,8	115,7	96,3	0,83
2	0	7	45,5	137,3	114,8	91,8	0,80
2	0	8	45,5	147,5	123,5	102,0	0,83
2	0	9	45,7	137,3	119,5	91,7	0,77
2	0	10	45,3	146,5	119,0	101,2	0,85

Rep=repetição; PC=peso corporal, CR=consumo de ração; GP=ganho de peso

APÊNDICE 1 - CONTINUAÇÃO. Observações experimentais: desempenho zootécnico

Tratamento			Variável Resposta (g)				
CLA (%)	Luteína (mg/kg)	Rep	PC 1 d	PC 8 d	CR 1-8 d	GP 1-8 d	GP:CR 1-8 d
0	50	1	45,5	140,0	120,8	94,5	0,78
0	50	2	45,5	136,3	110,8	90,8	0,82
0	50	3	45,5	138,0	116,0	92,5	0,80
0	50	4	45,5	149,0	120,5	103,5	0,86
0	50	5	45,2	140,7	115,2	95,5	0,83
0	50	6	45,8	152,2	122,8	106,3	0,87
0	50	7	45,5	142,3	113,2	96,8	0,86
0	50	8	45,7	144,3	124,5	98,7	0,79
0	50	9	45,7	141,8	115,8	96,2	0,83
0	50	10	45,3	140,8	117,0	95,5	0,82
1	50	1	45,5	151,7	119,3	106,2	0,89
1	50	2	45,5	142,3	111,0	96,8	0,87
1	50	3	45,7	142,3	112,0	96,7	0,86
1	50	4	45,5	135,3	115,2	89,8	0,78
1	50	5	45,5	157,7	124,0	112,2	0,90
1	50	6	45,5	147,2	117,3	101,7	0,87
1	50	7	45,7	141,5	112,2	95,8	0,85
1	50	8	45,7	147,2	117,5	101,5	0,86
1	50	9	45,7	144,3	120,3	98,7	0,82
1	50	10	45,7	140,2	113,2	94,5	0,84
2	50	1	45,2	131,5	106,7	86,3	0,81
2	50	2	45,7	139,5	112,0	93,8	0,84
2	50	3	45,5	124,3	104,7	78,8	0,75
2	50	4	45,5	141,7	119,2	96,2	0,81
2	50	5	45,5	133,5	117,2	88,0	0,75
2	50	6	45,5	134,8	108,0	89,3	0,83
2	50	7	45,3	137,7	112,3	92,3	0,82
2	50	8	46,3	132,7	107,5	86,3	0,80
2	50	9	45,7	132,2	107,3	86,5	0,81
2	50	10	45,7	137,0	112,2	91,3	0,81

Rep=repetição; PC=peso corporal, CR=consumo de ração; GP=ganho de peso

APÊNDICE 1 - CONTINUAÇÃO. Observações experimentais: desempenho zootécnico

Tratamento			Variável Resposta (g)			
CLA	Luteína		PC	CR	GP	GP:CR
(%)	(mg/kg)	Rep	15 d	8-15 d	8-15 d	8-15 d
0	0	1	411,0	314,3	267,8	0,85
0	0	2	430,0		283,5	
0	0	3	433,2	336,5	284,5	0,85
0	0	4	405,0	303,8	267,8	0,88
0	0	5	448,8	343,0	291,2	0,85
0	0	6	404,8	322,5	272,0	0,84
0	0	7	399,8	330,5	259,8	0,79
0	0	8	411,0	311,0	271,2	0,87
0	0	9	399,2	306,2	261,3	0,85
0	0	10	430,7	331,5	286,0	0,86
1	0	1	371,7	291,0	237,3	0,82
1	0	2	415,3	336,7	269,8	0,80
1	0	3	362,8	320,3	232,0	0,72
1	0	4	381,7	302,2	243,8	0,81
1	0	5	421,5	335,0	270,0	0,81
1	0	6	431,3	339,3	270,2	0,80
1	0	7	364,8	298,2	233,2	0,78
1	0	8	405,0	333,8	264,7	0,79
1	0	9	375,5	312,7	247,3	0,79
1	0	10	386,8	304,0	249,7	0,82
2	0	1	404,2	310,0	270,0	0,87
2	0	2	409,5	332,8	270,0	0,81
2	0	3	434,8	337,0	284,7	0,84
2	0	4	387,7	301,3	253,0	0,84
2	0	5	421,5	313,2	271,2	0,87
2	0	6	410,5	315,3	268,7	0,85
2	0	7	412,0	349,3	274,7	0,79
2	0	8	408,3	327,8	260,8	0,80
2	0	9	387,5	315,2	250,2	0,79
2	0	10	417,8	323,8	271,3	0,84

Rep=repetição; PC=peso corporal, CR=consumo de ração; GP=ganho de peso

APÊNDICE 1 - CONTINUAÇÃO. Observações experimentais: desempenho zootécnico

Tratamento			Variável Resposta (g)			
CLA	Luteína		PC	CR	GP	GP:CR
(%)	(mg/kg)	Rep	15 d	8-15 d	8-15 d	8-15 d
0	50	1	415,8	328,3	275,8	0,84
0	50	2	392,7	290,2	256,3	0,88
0	50	3	432,3	341,8	294,3	0,86
0	50	4	408,3	327,0	259,3	0,79
0	50	5	429,8	328,0	289,2	0,88
0	50	6	427,7	323,7	275,5	0,85
0	50	7	412,3	322,0	270,0	0,84
0	50	8	425,0	334,0	280,7	0,84
0	50	9	414,5	325,2	272,7	0,84
0	50	10	421,7	331,0	280,8	0,85
1	50	1	457,7	342,5	306,0	0,89
1	50	2	417,7	307,0	275,3	0,90
1	50	3	429,0	324,0	286,7	0,88
1	50	4	420,7	324,5	285,3	0,88
1	50	5	462,0	344,5	304,3	0,88
1	50	6	418,3	313,5	271,2	0,86
1	50	7	354,7	299,3	213,2	0,71
1	50	8	437,0	331,9	289,8	0,87
1	50	9	369,2	322,0	224,8	0,70
1	50	10	402,7	331,5	262,5	0,79
2	50	1	380,0	283,8	248,5	0,88
2	50	2	395,2	318,5	255,7	0,80
2	50	3	362,2	286,5	237,8	0,83
2	50	4	417,5	343,0	275,8	0,80
2	50	5	374,0	308,8	240,5	0,78
2	50	6	387,0	315,7	252,2	0,80
2	50	7	402,8	322,0	265,2	0,82
2	50	8	378,2	299,3	245,5	0,82
2	50	9	376,2	291,2	244,0	0,84
2	50	10	372,0	315,8	235,0	0,74

Rep=repetição; PC=peso corporal, CR=consumo de ração; GP=ganho de peso

APÊNDICE 1 - CONTINUAÇÃO. Observações experimentais: desempenho zootécnico

Tratamento			Variável Resposta (g)			
CLA	Luteína		PC	CR	GP	GP:CR
(%)	(mg/kg)	Rep	20 d	15-20 d	15-20 d	15-20 d
0	0	1	738,8	372,0	327,8	0,88
0	0	2	702,5	361,2	272,5	0,75
0	0	3	690,7	352,0	257,5	0,73
0	0	4	707,2	358,2	302,2	0,84
0	0	5	713,8	356,3	265,0	0,74
0	0	6	679,5	358,3	274,7	0,77
0	0	7	677,5	353,3	277,7	0,79
0	0	8	695,8	365,7	284,8	0,78
0	0	9	682,3	349,8	283,2	0,81
0	0	10	694,8	362,3	264,2	0,73
1	0	1	637,5	345,5	265,8	0,77
1	0	2	659,5	350,0	244,2	0,70
1	0	3	638,2	354,2	275,3	0,78
1	0	4	657,0	365,2	275,3	0,75
1	0	5	675,8	356,7	254,3	0,71
1	0	6	679,8	349,2	248,5	0,71
1	0	7	626,3	349,2	261,5	0,75
1	0	8	657,5	354,3	252,5	0,71
1	0	9	621,0	347,5	245,5	0,71
1	0	10	660,7	345,5	273,8	0,79
2	0	1	686,7	355,3	282,5	0,80
2	0	2	663,3	345,2	253,8	0,74
2	0	3	669,2	348,8	234,3	0,67
2	0	4	638,5	342,0	250,8	0,73
2	0	5	691,5	366,5	270,0	0,74
2	0	6	691,3	365,2	280,8	0,77
2	0	7	649,7	347,0	237,7	0,68
2	0	8	657,0	350,0	248,7	0,71
2	0	9	652,5	341,8	265,0	0,78
2	0	10	689,8	361,0	272,0	0,75

Rep=repetição; PC=peso corporal, CR=consumo de ração; GP=ganho de peso

APÊNDICE 1 - CONTINUAÇÃO. Observações experimentais: desempenho zootécnico

Tratamento			Variável Resposta (g)			
CLA	Luteína		PC	CR	GP	GP:CR
(%)	(mg/kg)	Rep	20 d	15-20 d	15-20 d	15-20 d
0	50	1	703,0	373,0	287,2	0,77
0	50	2	668,3	355,3	275,7	0,78
0	50	3	691,7	365,2	259,3	0,71
0	50	4	691,7	364,5	283,3	0,78
0	50	5	684,2	368,5	254,3	0,69
0	50	6	677,3	344,8	249,7	0,72
0	50	7	659,7	339,3	247,3	0,73
0	50	8	657,7	344,5	232,7	0,68
0	50	9	655,0	351,0	240,5	0,69
0	50	10	698,0	355,5	276,3	0,78
1	50	1	717,3	369,5	259,7	0,70
1	50	2	675,5	344,7	257,8	0,75
1	50	3	699,0	356,0	270,0	0,76
1	50	4	680,5	339,5	259,8	0,77
1	50	5	698,3	347,8	236,3	0,68
1	50	6	684,2	344,5	265,8	0,77
1	50	7	635,0		280,3	
1	50	8	763,0	381,0	326,0	0,86
1	50	9	675,3	322,8	306,2	0,95
1	50	10	681,3	340,3	278,7	0,82
2	50	1	714,4	399,6	334,4	0,84
2	50	2	651,7	341,8	256,5	0,75
2	50	3	620,8	344,8	258,7	0,75
2	50	4	670,0	367,3	252,5	0,69
2	50	5	625,8	348,2	251,8	0,72
2	50	6	635,0	348,0	248,0	0,71
2	50	7	659,5	363,3	256,7	0,71
2	50	8	644,0	358,7	265,8	0,74
2	50	9	642,3	351,0	266,2	0,76
2	50	10	658,3	354,3	286,3	0,81

Rep=repetição; PC=peso corporal, CR=consumo de ração; GP=ganho de peso

APÊNDICE 1 - CONTINUAÇÃO. Observações experimentais: desempenho zootécnico

Tratamento			Variável Resposta (g)		
CLA	Luteína		CR	GP	GP:CR
(%)	(mg/kg)	Rep	1-20 d	1-20 d	1-20 d
0	0	1	803,9	693,6	0,86
0	0	2			
0	0	3	807,5	645,3	0,80
0	0	4	770,3	661,5	0,86
0	0	5	822,5	668,2	0,81
0	0	6	786,7	634,0	0,81
0	0	7	797,5	631,7	0,79
0	0	8	786,5	650,3	0,83
0	0	9	768,5	636,7	0,83
0	0	10	815,0	649,5	0,80
1	0	1	749,5	592,0	0,79
1	0	2	806,8	613,8	0,76
1	0	3	792,0	592,8	0,75
1	0	4	781,5	611,5	0,78
1	0	5	815,0	630,2	0,77
1	0	6	820,7	634,2	0,77
1	0	7	756,7	580,8	0,77
1	0	8	805,5	612,0	0,76
1	0	9	773,3	575,3	0,74
1	0	10	762,3	615,0	0,81
2	0	1	774,0	641,0	0,83
2	0	2	790,5	618,0	0,78
2	0	3	810,3	623,5	0,77
2	0	4	754,2	592,7	0,79
2	0	5	803,3	645,8	0,80
2	0	6	796,2	645,8	0,81
2	0	7	811,2	604,2	0,74
2	0	8	801,3	611,5	0,76
2	0	9	776,5	606,8	0,78
2	0	10	803,8	644,5	0,80

Rep=repetição; PC=peso corporal, CR=consumo de ração; GP=ganho de peso

APÊNDICE 1 - CONTINUAÇÃO. Observações experimentais: desempenho zootécnico

Tratamento			Variável Resposta (g)		
CLA	Luteína		CR	GP	GP:CR
(%)	(mg/kg)	Rep	1-20 d	1-20 d	1-20 d
0	50	1	822,2	657,5	0,80
0	50	2	756,3	622,8	0,82
0	50	3	823,0	646,2	0,79
0	50	4	812,0	646,2	0,80
0	50	5	811,7	639,0	0,79
0	50	6	791,3	631,5	0,80
0	50	7	774,5	614,2	0,79
0	50	8	803,0	612,0	0,76
0	50	9	792,0	609,3	0,77
0	50	10	803,5	652,7	0,81
1	50	1	831,3	671,8	0,81
1	50	2	762,7	630,0	0,83
1	50	3	792,0	653,3	0,82
1	50	4	779,2	635,0	0,81
1	50	5	816,3	652,8	0,80
1	50	6	775,3	638,7	0,82
1	50	7			
1	50	8	830,4	717,3	0,86
1	50	9	765,2	629,7	0,82
1	50	10	785,0	635,7	0,81
2	50	1	790,1	669,2	0,85
2	50	2	772,3	606,0	0,78
2	50	3	736,0	575,3	0,78
2	50	4	829,5	624,5	0,75
2	50	5	774,2	580,3	0,75
2	50	6	771,7	589,5	0,76
2	50	7	797,7	614,2	0,77
2	50	8	765,5	597,7	0,78
2	50	9	749,5	596,7	0,80
2	50	10	782,3	612,7	0,78

Rep=repetição; PC=peso corporal, CR=consumo de ração; GP=ganho de peso

APÊNDICE 2. Observações experimentais: IgY anti-BSA aos 15 dias

Tratamento				Tratamento			
CLA (%)	Luteína (mg/kg)	Rep	IgY[1] 15 d	CLA (%)	Luteína (mg/kg)	Rep	IgY[1] 15 d
0	0	1	0,245	0	50	1	0,318
0	0	2	1,981	0	50	2	0,511
0	0	3		0	50	3	0,098
0	0	4		0	50	4	0,248
0	0	5	0,216	0	50	5	0,890
0	0	6		0	50	6	0,531
0	0	7	0,628	0	50	7	0,252
0	0	8	0,445	0	50	8	
0	0	9	0,514	0	50	9	
0	0	10	0,088	0	50	10	0,110
1	0	1	0,094	1	50	1	
1	0	2	0,784	1	50	2	0,068
1	0	3		1	50	3	
1	0	4		1	50	4	0,224
1	0	5	0,110	1	50	5	
1	0	6	0,071	1	50	6	
1	0	7		1	50	7	
1	0	8	0,076	1	50	8	0,426
1	0	9	0,478	1	50	9	0,093
1	0	10	0,381	1	50	10	0,664
2	0	1	0,721	2	50	1	0,190
2	0	2	0,173	2	50	2	
2	0	3	1,062	2	50	3	0,649
2	0	4	0,163	2	50	4	0,928
2	0	5	0,083	2	50	5	0,918
2	0	6	0,175	2	50	6	0,742
2	0	7	0,303	2	50	7	0,088
2	0	8	0,490	2	50	8	0,834
2	0	9	0,112	2	50	9	0,091

Rep=repetição.

[1]Valores de densidade óptica de IgY anti-BSA obtidos pelo teste de ELISA indireto.

APÊNDICE 3. Observações experimentais: IgY anti-BSA aos 21 dias

Tratamento					Tratamento				
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	IgY[1] 21d	CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	IgY[1] 21d
0	0	-	1	1,29	0	0	+	1	1,29
0	0	-	2	1,99	0	0	+	2	1,71
0	0	-	3	1,62	0	0	+	3	1,68
0	0	-	4	2,27	0	0	+	4	1,97
0	0	-	5	0,19	0	0	+	5	1,70
0	0	-	6		0	0	+	6	
0	0	-	7		0	0	+	7	0,55
0	0	-	8	0,56	0	0	+	8	1,20
0	0	-	9	0,27	0	0	+	9	1,68
0	0	-	10	1,05	0	0	+	10	0,72
1	0	-	1	0,69	1	0	+	1	0,72
1	0	-	2	2,17	1	0	+	2	2,21
1	0	-	3	1,04	1	0	+	3	1,23
1	0	-	4	1,91	1	0	+	4	0,95
1	0	-	5	1,39	1	0	+	5	0,63
1	0	-	6	0,59	1	0	+	6	0,70
1	0	-	7	0,43	1	0	+	7	0,57
1	0	-	8	2,23	1	0	+	8	2,62
1	0	-	9		1	0	+	9	1,89
1	0	-	10	0,91	1	0	+	10	0,70
2	0	-	1	1,71	2	0	+	1	1,94
2	0	-	2	0,49	2	0	+	2	1,43
2	0	-	3	1,85	2	0	+	3	1,59
2	0	-	4	1,93	2	0	+	4	2,05
2	0	-	5	0,50	2	0	+	5	2,46
2	0	-	6	1,06	2	0	+	6	1,25
2	0	-	7	2,18	2	0	+	7	1,85
2	0	-	8	0,91	2	0	+	8	1,30
2	0	-	9	1,59	2	0	+	9	1,41
2	0	-	10	2,42	2	0	+	10	1,19

Rep=repetição.

[1]Valores de densidade óptica de IgY anti-BSA obtidos pelo teste de ELISA indireto.

APÊNDICE 3 - CONTINUAÇÃO. Observações experimentais: IgY anti-BSA aos 21 dias

Tratamento					Tratamento				
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	IgY[1] 21d	CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	IgY[1] 21d
0	50	-	1	1,07	0	50	+	1	0,88
0	50	-	2	0,79	0	50	+	2	0,59
0	50	-	3	0,96	0	50	+	3	0,70
0	50	-	4	1,27	0	50	+	4	1,14
0	50	-	5	0,91	0	50	+	5	0,55
0	50	-	6	1,68	0	50	+	6	1,92
0	50	-	7	1,72	0	50	+	7	0,88
0	50	-	8	1,15	0	50	+	8	0,85
0	50	-	9	0,64	0	50	+	9	1,54
0	50	-	10		0	50	+	10	1,70
1	50	-	1	0,80	1	50	+	1	
1	50	-	2	0,30	1	50	+	2	1,00
1	50	-	3	1,81	1	50	+	3	0,51
1	50	-	4	2,27	1	50	+	4	1,22
1	50	-	5		1	50	+	5	2,29
1	50	-	6	0,92	1	50	+	6	1,09
1	50	-	7		1	50	+	7	1,16
1	50	-	8	0,96	1	50	+	8	1,57
1	50	-	9	1,78	1	50	+	9	1,96
1	50	-	10	1,05	1	50	+	10	0,41
2	50	-	1		2	50	+	1	0,78
2	50	-	2	0,85	2	50	+	2	1,71
2	50	-	3	1,18	2	50	+	3	2,16
2	50	-	4	0,44	2	50	+	4	0,95
2	50	-	5	1,69	2	50	+	5	2,69
2	50	-	6	2,64	2	50	+	6	2,67
2	50	-	7	2,21	2	50	+	7	2,44
2	50	-	8	1,88	2	50	+	8	1,76
2	50	-	9	2,26	2	50	+	9	0,65
2	50	-	10	0,43	2	50	+	10	1,06

Rep=repetição.

[1]Valores de densidade óptica de IgY anti-BSA obtidos pelo teste de ELISA indireto.

APÊNDICE 4. Observações experimentais: ganho de peso após o desafio com LPS

Tratamento				Variável Resposta (g)		
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	GP 0a3 h	GP 0a16 h	GP 0a40 h
0	0	-	1	15	47	
0	0	-	2	55	66	74
0	0	-	3	38	61	82
0	0	-	4	32	75	92
0	0	-	5	49	70	126
0	0	-	6	37	65	100
0	0	-	7	48	97	112
0	0	-	8	66	70	116
0	0	-	9	25	97	76
0	0	-	10	67	67	117
1	0	-	1	-8	23	76
1	0	-	2	74	265	126
1	0	-	3	32	56	187
1	0	-	4	34	65	85
1	0	-	5	63	76	116
1	0	-	6	42	60	103
1	0	-	7	53	64	70
1	0	-	8	54	65	113
1	0	-	9		68	63
1	0	-	10	32	58	72
2	0	-	1	32	68	89
2	0	-	2	22	71	109
2	0	-	3	63	64	154
2	0	-	4	28	63	118
2	0	-	5	56	98	20
2	0	-	6	47	91	73
2	0	-	7	58	86	83
2	0	-	8	69	69	154
2	0	-	9	-24	88	87
2	0	-	10	55	95	85

Rep=repetição; GP=ganho de peso.

APÊNDICE 4 - CONTINUAÇÃO. Observações experimentais: ganho de peso após o desafio com LPS

Tratamento				Variável Resposta (g)		
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	GP 0a3 h	GP 0a16 h	GP 0a40 h
0	50	-	1	45	52	112
0	50	-	2	36	73	94
0	50	-	3	26	55	97
0	50	-	4	65	93	105
0	50	-	5	58	87	119
0	50	-	6	48	92	121
0	50	-	7	33	66	109
0	50	-	8	56	89	144
0	50	-	9	41	-9	105
0	50	-	10	38		-109
1	50	-	1	30	91	172
1	50	-	2	42	65	111
1	50	-	3	15	36	90
1	50	-	4	42	72	84
1	50	-	5	45	104	136
1	50	-	6	58	36	111
1	50	-	7	26	62	67
1	50	-	8		48	
1	50	-	9	20	42	110
1	50	-	10	51	83	103
2	50	-	1	-5		90
2	50	-	2	42	56	72
2	50	-	3	26	68	78
2	50	-	4	56	75	117
2	50	-	5	40	66	61
2	50	-	6	53	83	85
2	50	-	7	66	62	103
2	50	-	8	37	103	103
2	50	-	9	30	42	93
2	50	-	10	37	95	106

Rep=repetição; GP=ganho de peso.

APÊNDICE 4 - CONTINUAÇÃO. Observações experimentais: ganho de peso após o desafio com LPS

Tratamento				Variável Resposta (g)		
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	GP 0a3 h	GP 0a16 h	GP 0a40 h
0	0	+	1	-31	-15	51
0	0	+	1	21	48	66
0	0	+	3	26	77	90
0	0	+	4	37	33	80
0	0	+	5	8	52	76
0	0	+	6	22	75	56
0	0	+	7	-12	47	95
0	0	+	8	-26	32	74
0	0	+	9	-24	18	58
0	0	+	10	-8	42	87
1	0	+	1	-9	73	36
1	0	+	2	25	73	62
1	0	+	3	9	47	215
1	0	+	4	-10	26	59
1	0	+	5	31	46	90
1	0	+	6	14	24	55
1	0	+	7	25	43	67
1	0	+	8	13	31	84
1	0	+	9	-21	207	41
1	0	+	10	4	47	32
2	0	+	1	9	40	47
2	0	+	2	-8	45	60
2	0	+	3	37	62	77
2	0	+	4	11	5	74
2	0	+	5	19	55	72
2	0	+	6	9	38	83
2	0	+	7	33	45	88
2	0	+	8	-19	20	62
2	0	+	9	-38	88	69
2	0	+	10	5	17	14

Rep=repetição; GP=ganho de peso.

APÊNDICE 4 - CONTINUAÇÃO. Observações experimentais: ganho de peso após o desafio com LPS

Tratamento				Variável Resposta (g)		
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	GP 0a3 h	GP 0a16 h	GP 0a40 h
0	50	+	1	7	31	51
0	50	+	2	-11	14	31
0	50	+	3	-20	51	20
0	50	+	4	1	27	73
0	50	+	5	18	47	-9
0	50	+	6	0	72	75
0	50	+	7	74	66	131
0	50	+	8	34	93	28
0	50	+	9	19	16	70
0	50	+	10	2	164	73
1	50	+	1	-14	48	80
1	50	+	2	-13	31	98
1	50	+	3	-18	108	90
1	50	+	4	0	41	65
1	50	+	5	70	91	118
1	50	+	6	11	46	91
1	50	+	7	5	-10	
1	50	+	8	-29	15	19
1	50	+	9	-25	-22	44
1	50	+	10	-23	26	61
2	50	+	1	-12	23	43
2	50	+	2	-15	22	51
2	50	+	3	-4	30	40
2	50	+	4	99	20	62
2	50	+	5	65	84	90
2	50	+	6	-6	44	77
2	50	+	7	-7	29	77
2	50	+	8	8	18	58
2	50	+	9	-36	23	61
2	50	+	10	16	30	27

Rep=repetição; GP=ganho de peso.

APÊNDICE 5. Observações experimentais: relação fígado:peso corporal 3 e 16 horas após o desafio com LPS

Tratamento				Variável Resposta	
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	Fígado:PC (%) 3 h	Fígado:PC (%) 16 h
0	0	-	1	2,84	3,91
0	0	-	2	2,75	2,91
0	0	-	3	2,52	3,42
0	0	-	4	3,01	5,17
0	0	-	5	2,89	3,50
0	0	-	6	2,80	5,02
0	0	-	7	2,29	4,19
0	0	-	8	2,85	3,53
0	0	-	9	2,86	4,06
0	0	-	10	2,93	3,69
1	0	-	1	3,26	4,86
1	0	-	2	2,84	5,25
1	0	-	3	3,09	4,73
1	0	-	4	2,68	3,36
1	0	-	5	3,12	4,01
1	0	-	6	3,13	3,96
1	0	-	7	2,95	4,10
1	0	-	8	2,97	3,50
1	0	-	9	2,47	3,44
1	0	-	10	3,42	3,13
2	0	-	1	2,61	3,30
2	0	-	2	3,25	3,66
2	0	-	3	2,40	5,53
2	0	-	4	2,78	3,48
2	0	-	5	2,86	4,93
2	0	-	6	3,02	3,05
2	0	-	7	2,76	3,50
2	0	-	8	2,45	3,03
2	0	-	9	3,67	4,18
2	0	-	10	2,36	3,31

Rep=repetição; PC=peso corporal.

APÊNDICE 5 - CONTINUAÇÃO. Observações experimentais: relação fígado:peso corporal 3 e 16 horas após o desafio com LPS

Tratamento				Variável Resposta	
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	Fígado:PC (%) 3 h	Fígado:PC (%) 16 h
0	50	-	1	2,90	3,72
0	50	-	2	3,34	4,05
0	50	-	3	2,54	3,24
0	50	-	4	2,86	4,25
0	50	-	5	3,02	3,14
0	50	-	6	3,22	4,46
0	50	-	7	3,14	3,70
0	50	-	8	2,61	3,43
0	50	-	9	2,65	4,77
0	50	-	10	2,59	
1	50	-	1	2,68	3,59
1	50	-	2	2,76	3,35
1	50	-	3	2,58	3,36
1	50	-	4	2,73	3,23
1	50	-	5	2,67	4,10
1	50	-	6	3,05	3,91
1	50	-	7	2,68	3,83
1	50	-	8		3,62
1	50	-	9	2,73	3,14
1	50	-	10	2,53	4,59
2	50	-	1	3,61	
2	50	-	2	2,97	3,20
2	50	-	3	3,50	3,10
2	50	-	4	2,92	4,19
2	50	-	5	2,87	3,42
2	50	-	6	2,77	4,11
2	50	-	7	3,02	4,28
2	50	-	8	2,50	3,72
2	50	-	9	2,89	3,73
2	50	-	10	3,01	3,75

Rep=repetição; PC=peso corporal.

APÊNDICE 5 - CONTINUAÇÃO. Observações experimentais: relação fígado:peso corporal 3 e 16 horas após o desafio com LPS

Tratamento				Variável Resposta	
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	Fígado:PC (%) 3 h	Fígado:PC (%) 16 h
0	0	+	1	3,10	3,46
0	0	+	1	2,77	3,53
0	0	+	3	3,21	3,22
0	0	+	4	2,86	3,16
0	0	+	5	2,47	4,60
0	0	+	6	2,93	4,41
0	0	+	7	3,29	3,79
0	0	+	8	2,96	2,76
0	0	+	9	3,36	3,71
0	0	+	10	2,99	3,61
1	0	+	1	3,73	3,14
1	0	+	2	3,21	4,44
1	0	+	3	3,37	4,20
1	0	+	4	3,34	3,16
1	0	+	5	2,71	4,39
1	0	+	6	3,42	3,76
1	0	+	7	3,42	3,84
1	0	+	8	2,91	4,15
1	0	+	9	3,49	3,01
1	0	+	10	4,65	3,48
2	0	+	1	3,11	3,43
2	0	+	2	3,41	3,63
2	0	+	3	3,08	3,52
2	0	+	4	2,76	3,41
2	0	+	5	3,01	3,62
2	0	+	6	3,35	3,36
2	0	+	7	3,20	3,56
2	0	+	8	2,99	3,64
2	0	+	9	3,40	4,43
2	0	+	10	3,20	4,53

Rep=repetição; PC=peso corporal.

APÊNDICE 5 - CONTINUAÇÃO. Observações experimentais: relação fígado:peso corporal 3 e 16 horas após o desafio com LPS

Tratamento				Variável Resposta	
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	Fígado:PC (%) 3 h	Fígado:PC (%) 16 h
0	50	+	1	2,46	3,61
0	50	+	2	2,92	3,66
0	50	+	3	3,42	3,49
0	50	+	4	2,95	3,53
0	50	+	5	3,23	3,94
0	50	+	6	2,95	3,39
0	50	+	7	2,73	3,28
0	50	+	8	3,04	3,13
0	50	+	9	3,39	4,02
0	50	+	10	2,99	3,22
1	50	+	1	4,11	2,88
1	50	+	2	3,33	3,83
1	50	+	3	2,78	3,86
1	50	+	4	6,12	2,79
1	50	+	5	3,31	3,01
1	50	+	6	2,99	3,58
1	50	+	7	2,80	3,41
1	50	+	8	3,29	4,04
1	50	+	9	2,88	3,83
1	50	+	10	2,74	3,27
2	50	+	1	3,13	3,67
2	50	+	2	3,39	3,27
2	50	+	3	3,60	3,40
2	50	+	4	2,77	4,05
2	50	+	5	2,64	4,10
2	50	+	6	3,48	3,87
2	50	+	7	4,29	4,24
2	50	+	8	3,13	4,16
2	50	+	9	3,60	4,34
2	50	+	10	3,65	3,83

Rep=repetição; PC=peso corporal.

APÊNDICE 6. Observações experimentais: óxido nítrico (NO) plasmático 16 h após o desafio com LPS

Tratamento				
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	NO (µM)
0	0	-	1	
0	0	-	2	838,8
0	0	-	3	546,2
0	0	-	4	
0	0	-	5	807,9
0	0	-	6	826,5
0	0	-	7	701,7
0	0	-	8	471,2
0	0	-	9	787,2
0	0	-	10	
1	0	-	1	811,6
1	0	-	2	
1	0	-	3	
1	0	-	4	505,3
1	0	-	5	941,1
1	0	-	6	780,5
1	0	-	7	559,8
1	0	-	8	716,8
1	0	-	9	531,6
1	0	-	10	447,2
2	0	-	1	
2	0	-	2	837,4
2	0	-	3	705,4
2	0	-	4	741,2
2	0	-	5	
2	0	-	6	876,4
2	0	-	7	676,9
2	0	-	8	431,1
2	0	-	9	760,6
2	0	-	10	

Rep=repetição.

APÊNDICE 6 - CONTINUAÇÃO. Observações experimentais: óxido nítrico (NO) plasmático 16 h após o desafio com LPS

Tratamento				
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	NO (µM)
0	50	-	1	
0	50	-	2	614,6
0	50	-	3	748,3
0	50	-	4	645,8
0	50	-	5	771,5
0	50	-	6	
0	50	-	7	480,4
0	50	-	8	
0	50	-	9	508,8
0	50	-	10	
1	50	-	1	
1	50	-	2	302,0
1	50	-	3	745,8
1	50	-	4	664,2
1	50	-	5	718,3
1	50	-	6	
1	50	-	7	461,3
1	50	-	8	
1	50	-	9	555,6
1	50	-	10	
2	50	-	1	
2	50	-	2	593,4
2	50	-	3	572,1
2	50	-	4	417,3
2	50	-	5	621,7
2	50	-	6	
2	50	-	7	399,9
2	50	-	8	568,1
2	50	-	9	370,3
2	50	-	10	532,1

Rep=repetição.

APÊNDICE 6 - CONTINUAÇÃO. Observações experimentais: óxido nítrico (NO) plasmático 16 h após o desafio com LPS

Tratamento				
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	NO (µM)
0	0	+	1	627,1
0	0	+	2	
0	0	+	3	
0	0	+	4	606,4
0	0	+	5	339,5
0	0	+	6	761,0
0	0	+	7	465,3
0	0	+	8	710,5
0	0	+	9	732,3
0	0	+	10	
1	0	+	1	860,0
1	0	+	2	
1	0	+	3	949,5
1	0	+	4	359,3
1	0	+	5	846,1
1	0	+	6	
1	0	+	7	416,6
1	0	+	8	650,3
1	0	+	9	448,0
1	0	+	10	635,8
2	0	+	1	
2	0	+	2	275,3
2	0	+	3	793,1
2	0	+	4	
2	0	+	5	960,6
2	0	+	6	1098,8
2	0	+	7	440,3
2	0	+	8	312,8
2	0	+	9	474,3
2	0	+	10	

Rep=repetição.

APÊNDICE 6 - CONTINUAÇÃO. Observações experimentais: óxido nítrico (NO) plasmático 16 h após o desafio com LPS

Tratamento				
CLA (%)	Luteína (mg/kg)	Desafio LPS	Rep	NO (µM)
0	50	+	1	949,0
0	50	+	2	
0	50	+	3	245,9
0	50	+	4	386,0
0	50	+	5	746,5
0	50	+	6	
0	50	+	7	368,6
0	50	+	8	439,6
0	50	+	9	
0	50	+	10	510,0
1	50	+	1	
1	50	+	2	527,9
1	50	+	3	
1	50	+	4	883,8
1	50	+	5	541,9
1	50	+	6	
1	50	+	8	309,2
1	50	+	8	710,4
1	50	+	9	576,7
1	50	+	10	
2	50	+	1	699,2
2	50	+	2	423,8
2	50	+	3	
2	50	+	4	440,0
2	50	+	5	359,4
2	50	+	6	540,5
2	50	+	7	
2	50	+	8	522,4
2	50	+	9	373,2
2	50	+	10	680,9

Rep=repetição.

APÊNDICE 7. Observações experimentais: expressão gênica de receptores e citocinas no fígado 3 h após o desafio com LPS

Tratamento				Variável Resposta					
CLA	Luteína	Desafio		Expressão Gênica					
(%)	(mg/kg)	LPS	Rep	RXRα	RXRγ	PPARγ	IL-1β	IL-10	IL-12
0	0	-	1	1,304	1,364	0,272	0,778	1,030	1,010
0	0	-	2				1,005	0,822	0,176
0	0	-	3	0,808	0,639	1,159	0,387	0,590	1,584
0	0	-	4	0,572	0,259		0,577	0,875	
0	0	-	5	0,471	0,213	0,209		2,286	0,803
1	0	-	2	0,438	2,193	1,856	0,079	0,418	1,128
1	0	-	3	2,170			0,830	3,899	0,687
1	0	-	4	0,239	0,294		0,055	0,239	0,311
1	0	-	5		0,229	0,636	1,471	2,986	
2	0	-	1	2,133	4,717	1,781	0,254	0,820	0,939
2	0	-	2				0,654	1,291	
2	0	-	3	0,961	1,093	1,955	0,103	0,948	1,226
2	0	-	4	2,082			0,327	1,063	0,455
2	0	-	5		0,643		0,076	3,211	2,988
0	50	-	2	1,398				0,704	1,038
0	50	-	3	1,163	1,059	1,744	0,255	1,070	0,412
0	50	-	4	1,703	0,367	0,123	0,073	1,208	0,322
0	50	-	5				0,175	3,766	
1	50	-	2	0,894	1,313		0,290	0,955	0,562
1	50	-	3	1,327	4,107		0,212	0,610	2,006
1	50	-	4	1,056	0,354		0,175	1,857	0,737
1	50	-	5		0,067		0,037	1,383	2,435
2	50	-	1				1,216	2,408	1,826
2	50	-	2	2,511			0,310	0,741	1,675
2	50	-	3	0,728	1,143	2,262	0,081	0,894	0,820
2	50	-	4	0,636		1,605	0,369	0,590	1,833
2	50	-	5		0,060		0,306	0,849	0,721

Rep=repetição.

APÊNDICE 7 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no fígado 3 h após o desafio com LPS

Tratamento				Variável Resposta					
CLA	Luteína	Desafio		Expressão Gênica					
(%)	(mg/kg)	LPS	Rep	RXRα	RXRγ	PPARγ	IL-1β	IL-10	IL-12
0	0	+	1	0,320	3,418		0,415	0,846	2,836
0	0	+	2	0,588	0,237	1,775	1,066	0,699	0,812
0	0	+	3	2,224				2,375	
1	0	+	2	0,463		5,342	1,534	1,100	2,539
1	0	+	3			0,208			0,568
1	0	+	4	0,417	0,696	0,212	0,424	1,081	2,574
1	0	+	5					0,929	
2	0	+	1	1,427		0,054	2,341	1,074	3,180
2	0	+	2	1,514		2,441		1,020	1,722
2	0	+	4	1,151	4,750	4,798	0,550	0,746	2,768
2	0	+	5	0,716	0,407	0,140		1,159	1,235
0	50	+	2	0,692	0,539	0,410	0,656	0,869	0,787
0	50	+	3			0,241			0,972
0	50	+	4	0,572			0,115	0,749	0,982
0	50	+	5	0,264	0,110		0,160	1,155	0,576
1	50	+	1					3,324	2,062
1	50	+	2	0,235	2,608		0,698	0,958	
1	50	+	3	1,883	0,272		0,565	0,897	0,413
1	50	+	4	0,659	3,600		0,421	1,070	2,967
1	50	+	5			5,380	1,254	0,652	3,326
2	50	+	1	1,213	1,781	0,794	1,142	0,906	0,385
2	50	+	2	0,243	1,331		0,218	0,932	
2	50	+	3	0,392	1,331		0,091	1,977	0,187
2	50	+	4	2,442	1,016			0,089	2,120
2	50	+	5	3,189	0,372			0,820	0,891

Rep=repetição.

APÊNDICE 8. Observações experimentais: expressão gênica de receptores e citocinas no fígado 16 h após o desafio com LPS

Tratamento				Variável Resposta		
CLA	Luteína	Desafio		Expressão Gênica		
(%)	(mg/kg)	LPS	Rep	RXRα	RXRγ	PPARα
0	0	-	1	1,393	1,935	3,83
0	0	-	2	1,656		5,766
0	0	-	3	0,699	0,887	4,163
0	0	-	4		0,393	0,011
0	0	-	5	0,066	0,369	
1	0	-	1	1,498		0,828
1	0	-	2	0,251	0,048	
1	0	-	4	0,08	0,275	
1	0	-	5	0,388	0,063	
2	0	-	1	0,602		6,669
2	0	-	2	1,179	2,652	4,716
2	0	-	3	1,733	1,111	0,746
2	0	-	4	1,806	2,024	
2	0	-	5	0,411	0,244	0,002
0	50	-	1		0,254	0,161
0	50	-	2	0,114	0,034	0,015
0	50	-	3	0,666	0,113	0,004
0	50	-	4	0,137	1,644	0,565
0	50	-	5	0,069	0,091	
1	50	-	1	1,388		6,716
1	50	-	2	0,1	0,315	0,134
1	50	-	3	0,61	1,062	1,191
1	50	-	4	1,045	1,507	4,206
1	50	-	5	0,247	0,058	0,005
2	50	-	1	0,564	0,08	0,004
2	50	-	2			0,087
2	50	-	3	0,343	1,131	2,317
2	50	-	4	0,178		0,006
2	50	-	5	0,58	0,3	

Rep=repetição.

APÊNDICE 8 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no fígado 16 h após o desafio com LPS

Tratamento				Variável Resposta		
CLA	Luteína	Desafio		Expressão Gênica		
(%)	(mg/kg)	LPS	Rep	RXRα	RXRγ	PPARα
0	0	+	1	1,045	2,483	0,243
0	0	+	2	0,197	1,451	2,17
0	0	+	3	0,416	0,424	0,682
0	0	+	4	0,414	0,884	1,085
0	0	+	5	0,202	0,171	
1	0	+	1		0,414	0,573
1	0	+	2	0,162	0,046	0,009
1	0	+	3			0,647
1	0	+	4	2,681	2,745	0,596
1	0	+	5	0,357	0,066	0,001
2	0	+	1	2,039	2,652	
2	0	+	2	0,317	0,577	2,562
2	0	+	3	0,794	0,998	
2	0	+	4	0,189	0,526	0,144
2	0	+	5	0,324	0,045	0,003
0	50	+	1	0,477	0,308	1,72
0	50	+	2	0,251	0,931	3,1
0	50	+	3	0,285	0,045	0,003
0	50	+	4			
0	50	+	5	0,231	0,011	0,004
1	50	+	1		1,005	1,962
1	50	+	2	0,533	0,279	0,236
1	50	+	3			0,687
1	50	+	4	1,838	1,793	1,793
1	50	+	5	0,133	0,061	
2	50	+	1	0,217	0,161	1,229
2	50	+	2		0,19	0,248
2	50	+	3		3,91	
2	50	+	4		3,176	0,682
2	50	+	5	1,407	0,281	0,023

Rep=repetição.

APÊNDICE 8 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no fígado 16 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	IL-1β	IL-10	IL-12	TLR-4
0	0	-	1	0,835	0,643	1,408	0,521
0	0	-	2	1,275	1,3	0,65	
0	0	-	3	0,488	0,359	1,093	0,621
0	0	-	4	3,605			0,438
0	0	-	5	0,534	0,657		1,417
1	0	-	1	2,532	0,572	1,108	
1	0	-	2	0,318	0,435	1,03	0,217
1	0	-	4	0,333	0,214	0,547	0,552
1	0	-	5	0,541	0,317		0,675
2	0	-	1	1,778	0,65	1,346	
2	0	-	2	0,892	0,942	1,923	
2	0	-	3	0,471	0,221	0,68	1,234
2	0	-	4		0,063	1,551	0,775
2	0	-	5	0,611	1,433	1,155	0,568
0	50	-	1	0,796	0,526	0,311	0,524
0	50	-	2	0,219	0,215	0,634	
0	50	-	3	0,707	0,23	0,38	0,961
0	50	-	4	0,588	0,09	0,259	
0	50	-	5	0,516	0,493	1,045	0,849
1	50	-	1	0,109	0,481	0,825	
1	50	-	2	0,545	0,377	1,739	1,002
1	50	-	3	0,838	0,137	0,444	2,924
1	50	-	4	1,5	0,259	0,455	1,634
1	50	-	5	2,838	1,152	0,68	
2	50	-	1	0,766	0,562	1,369	0,566
2	50	-	2	0,173	0,158	0,326	
2	50	-	3	0,176	0,169	0,754	
2	50	-	4	0,724	2,013		
2	50	-	5	0,664	0,586	0,28	1,977

Rep=repetição.

APÊNDICE 8 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no fígado 16 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	IL-1β	IL-10	IL-12	TLR-4
0	0	+	1	2,346	0,855	0,891	0,781
0	0	+	2	1,121	0,683	2,054	1,291
0	0	+	3	0,832	0,29	0,701	0,372
0	0	+	4	0,859	0,275	0,339	1,048
0	0	+	5	0,766	0,219	0,76	0,792
1	0	+	1	0,151	0,832	1,056	1,07
1	0	+	2	1,061	0,276	0,474	1,183
1	0	+	3		0,858	0,396	
1	0	+	4		1,193	0,729	1,929
1	0	+	5	1,185	0,325	3,223	0,652
2	0	+	1		0,63		0,797
2	0	+	2	0,309	0,586	0,891	0,528
2	0	+	3	1,905	0,357	0,806	2,45
2	0	+	4	0,352	0,084	1,023	0,372
2	0	+	5	0,568	0,515	1,697	0,499
0	50	+	1	0,635	0,536		
0	50	+	2	0,428	0,352	1,332	2,155
0	50	+	3	0,247	0,435	0,637	0,393
0	50	+	4		1,031	1,557	
0	50	+	5	1,58	0,861	0,864	1,163
1	50	+	1	0,225	0,376	1,02	0,183
1	50	+	2	0,294	0,259	0,444	0,854
1	50	+	3	2,219	1,897	0,729	1,295
1	50	+	4	2,166	0,43	0,643	2,216
1	50	+	5	3,531	0,639	0,519	
2	50	+	1	0,32	0,353	0,296	1,651
2	50	+	2	0,247	0,133	1,038	
2	50	+	3	2,462	1,675	2,232	
2	50	+	5	1,094	0,798	0,543	1,078

Rep=repetição.

APÊNDICE 9. Observações experimentais: expressão gênica de receptores e citocinas no baço 3 h após o desafio com LPS

Tratamento				Variável Resposta		
CLA	Luteína	Desafio		Expressão Gênica		
(%)	(mg/kg)	LPS	Rep	RXRα	RXRγ	PPARα
0	0	-	1	0,059	0,507	1,017
0	0	-	2	0,15	1,752	0,345
0	0	-	3	0,138	0,792	
0	0	-	4	0,238		
0	0	-	5		0,175	0,639
1	0	-	1	0,022	0,274	
1	0	-	2	0,031	0,536	0,186
1	0	-	3	0,284	1,356	0,375
1	0	-	4	0,331	4,466	
2	0	-	1	0,036	0,51	0,303
2	0	-	2	0,036	0,763	
2	0	-	3	0,196	0,91	0,675
2	0	-	4		0,23	
2	0	-	5	0,124	0,916	0,949
0	50	-	1	0,11	0,333	1,579
0	50	-	2	0,027	0,254	0,075
0	50	-	3	0,067	1,042	0,457
0	50	-	4	0,245	2,988	2,6
0	50	-	5	0,007	0,598	1,478
1	50	-	1	0,116	1,23	
1	50	-	2	0,036		1,082
1	50	-	3	0,302	3,061	
1	50	-	4	0,039	0,423	0,125
2	50	-	1	0,066	0,515	
2	50	-	2			0,549
2	50	-	3	0,264	2,988	0,35
2	50	-	4	0,007	0,466	0,254
2	50	-	5	0,019	0,344	0,122

Rep=repetição.

APÊNDICE 9 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no baço 3 h após o desafio com LPS

Tratamento				Variável Resposta		
CLA	Luteína	Desafio		Expressão Gênica		
(%)	(mg/kg)	LPS	Rep	RXRα	RXRγ	PPARα
0	0	+	1	0,025	2,157	
0	0	+	2	0,014		0,169
0	0	+	3	0,162		
0	0	+	4	0,024	3,834	2,777
0	0	+	5	0,028	3,072	0,613
1	0	+	1	0,066	0,423	0,178
1	0	+	2	0,048	0,858	0,235
1	0	+	3	0,057	3,315	0,175
1	0	+	4	0,009	0,238	0,082
1	0	+	5	0,019	0,582	0,07
2	0	+	1	0,081	1,017	1,201
2	0	+	2			0,062
2	0	+	3	0,109	0,43	
2	0	+	5	0,134	1,992	
0	50	+	1	0,049	0,523	0,195
0	50	+	2	0,067	0,752	0,303
0	50	+	3	0,137		0,194
0	50	+	4	0,151		0,541
0	50	+	5	0,007	1,148	0,052
1	50	+	1	0,014	0,339	0,406
1	50	+	2	0,081	4,284	0,904
1	50	+	3	0,173	0,952	0,383
1	50	+	4	0,045	0,371	0,396
1	50	+	5	0,007	0,165	0,056
2	50	+	1	0,157	0,697	
2	50	+	2	0,065	0,417	0,11
2	50	+	3	0,301		1,897
2	50	+	4	0,008	1,045	
2	50	+	5	0,007	0,204	0,082

Rep=repetição.

APÊNDICE 9 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no baço 3 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	IL-1β	IL-2	IL-12	TLR-4
0	0	-	1	0,388	0,404	0,372	0,465
0	0	-	2	3,444	0,571	0,545	0,136
0	0	-	3	2,461		0,901	1,516
0	0	-	4	1,252		2	3,227
0	0	-	5	0,243	1,673	2,732	3,238
1	0	-	1			0,41	2,667
1	0	-	2	2,936	0,89	3,458	0,147
1	0	-	3	4,299			0,901
1	0	-	4	0,514	0,188	1,439	0,766
2	0	-	1	3,769			0,088
2	0	-	2	0,473	0,214	1,925	0,681
2	0	-	3	1,261		3,422	3,411
2	0	-	4	0,383		5,736	3,084
2	0	-	5	0,975	0,884	2,437	1,046
0	50	-	1	0,554			0,31
0	50	-	2	1,261		1,297	0,296
0	50	-	3	3,408	0,223		1,371
0	50	-	4	1,71		3,095	1,177
0	50	-	5	0,613		4,423	1,31
1	50	-	1	0,692		1,993	3,34
1	50	-	2	0,923	0,286	0,85	
1	50	-	3	2,027		1,202	0,7
1	50	-	4	0,955	0,328	4,79	0,832
2	50	-	1				1,847
2	50	-	2	0,724	0,142		0,276
2	50	-	3	5,025	0,094	0,75	
2	50	-	4	0,298			0,796
2	50	-	5	0,243	1,286	1,083	0,646

Rep=repetição.

APÊNDICE 9 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no baço 3 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	IL-1β	IL-2	IL-12	TLR-4
0	0	+	1		0,352	0,779	2,713
0	0	+	2	2,062	0,596	1,227	1,324
0	0	+	3	4,938	0,12	1,608	2,751
0	0	+	4	5,025	0,854	2,713	
0	0	+	5		0,634		2,412
1	0	+	1	0,755	0,604	1,4	2,242
1	0	+	2	1,698		0,613	0,856
1	0	+	3	2,083		0,976	0,176
1	0	+	4	0,632	0,471	1,098	0,209
1	0	+	5	0,185	1,322	1,919	0,398
2	0	+	1			1,939	1,01
2	0	+	2	1,02	0,647		0,782
2	0	+	3	2,807		0,983	
2	0	+	5		0,592	3,891	
0	50	+	1	0,528		2,136	3,127
0	50	+	2	4,82	0,239		0,163
0	50	+	3	2,946		1,765	0,597
0	50	+	4			4,112	0,187
0	50	+	5	0,666		0,705	0,293
1	50	+	1			0,471	0,644
1	50	+	2		1,135	1,227	1,202
1	50	+	3	1,871	0,31	2,918	0,901
1	50	+	4	2,817	0,199	3,138	0,859
1	50	+	5	0,458	0,519	3,837	0,66
2	50	+	1		0,282	0,557	0,693
2	50	+	2	1,489		1,38	0,236
2	50	+	3			3,399	0,793
2	50	+	4			5,598	2,063
2	50	+	5	0,268	0,418	2,63	0,318

Rep=repetição.

APÊNDICE 10. Observações experimentais: expressão gênica de receptores e citocinas no baço 16 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	RXRα	PPARα	PPARγ	IL-1β
0	0	NOLPS	1	1,897	0,643		0,605
0	0	NOLPS	2	0,216	0,285	0,773	1,549
0	0	NOLPS	3	0,787	0,829	4,784	0,914
0	0	NOLPS	4	0,469	1,579	0,463	0,74
0	0	NOLPS	5			2,796	1,576
1	0	NOLPS	1	0,422			0,557
1	0	NOLPS	2	0,309			0,369
1	0	NOLPS	3	1,669	1,865	2,012	2,763
1	0	NOLPS	4	1,795	2,264	2,403	0,26
1	0	NOLPS	5	2,142	0,515	2,601	
2	0	NOLPS	1	0,358	1,305		1,94
2	0	NOLPS	2	0,17	0,198		
2	0	NOLPS	3	0,537	1,574		1,13
2	0	NOLPS	4	0,716	0,289	4,784	0,653
2	0	NOLPS	5		0,125		
0	50	NOLPS	1	0,171			0,81
0	50	NOLPS	2	1,595	0,486		1,339
0	50	NOLPS	3	0,234	2,435		1,381
0	50	NOLPS	4	1,082	0,975	2,564	0,761
0	50	NOLPS	5	2,344			
1	50	NOLPS	1	0,873	0,253		0,509
1	50	NOLPS	2	0,197	0,179	0,752	2,668
1	50	NOLPS	3	1,053	1,808	3,9	0,833
1	50	NOLPS	4	0,474	0,088		0,71
1	50	NOLPS	5	2,574	1,172	1,374	
2	50	NOLPS	3			3,478	
2	50	NOLPS	4	1,16	0,107	2,876	0,416
2	50	NOLPS	5	1,3			

Rep=repetição.

APÊNDICE 10 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no baço 16 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	RXRα	PPARα	PPARγ	IL-1β
0	0	LPS	1	1,006	0,747	1,668	1,96
0	0	LPS	2	0,35	0,402		0,229
0	0	LPS	3	1,356			2,323
0	0	LPS	4	1,097			0,612
0	0	LPS	5	1,71	0,671	2,502	1,94
1	0	LPS	1	0,471	0,109	0,441	1,88
1	0	LPS	2	0,942	1,156	1,295	1,785
1	0	LPS	3	1,045	0,724		1,04
1	0	LPS	4	0,982	0,17	0,8	
1	0	LPS	5	2,936	0,168	0,327	
2	0	LPS	1	0,972	1,075	2,757	0,428
2	0	LPS	2	2,249			1,66
2	0	LPS	3	1,595	1,197	1,163	1,153
2	0	LPS	4	0,76	0,226	0,639	0,485
2	0	LPS	5	2,619	0,191	1,273	2,43
0	50	LPS	1	1,884	0,737	0,368	1,232
0	50	LPS	2	1,563	2,41		0,957
0	50	LPS	3	0,276	1,858	0,731	1,44
0	50	LPS	4	0,923	0,142	0,843	1,271
0	50	LPS	5	1,964	0,23		
1	50	LPS	1	0,148	0,913	3,06	0,947
1	50	LPS	2	0,409	0,635		0,61
1	50	LPS	3	0,637	1,346		0,899
1	50	LPS	4	0,503			0,743
1	50	LPS	5	2,988	0,226		
2	50	LPS	1	0,901	1,287	3,478	0,256
2	50	LPS	2	0,249	0,643	0,876	0,573
2	50	LPS	3	1,18	1,379		0,81
2	50	LPS	4	0,855			0,804
2	50	LPS	5	0,904		1,364	

Rep=repetição.

APÊNDICE 10 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no baço 16 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	IL-2	IL-10	IL-12	TLR-4
0	0	NOLPS	1	0,657	1,525	3,92	
0	0	NOLPS	2		2,048	2,763	0,338
0	0	NOLPS	3		1,003	0,397	0,53
0	0	NOLPS	4	1,521	0,57	0,115	1,057
0	0	NOLPS	5		0,56	2,029	1,223
1	0	NOLPS	1		0,734		1,227
1	0	NOLPS	2		2,048	4,457	0,402
1	0	NOLPS	3	0,755	0,752	0,893	0,796
1	0	NOLPS	4	0,273	0,558	0,725	0,338
1	0	NOLPS	5		0,202	1,724	
2	0	NOLPS	1	0,892	1,536	6,004	0,37
2	0	NOLPS	2	0,347			0,862
2	0	NOLPS	3	0,886	0,861	0,143	1,4
2	0	NOLPS	4	0,911	1,082	0,281	0,847
2	0	NOLPS	5		0,216	1,475	0,346
0	50	NOLPS	1	1,075	1,082	4,365	0,644
0	50	NOLPS	2		0,826	3,065	1,828
0	50	NOLPS	3	0,111	0,732	0,585	0,862
0	50	NOLPS	4			0,307	0,547
0	50	NOLPS	5		0,413	0,651	
1	50	NOLPS	1	0,622	0,724	1,43	0,862
1	50	NOLPS	2	0,173	1,734	4,38	
1	50	NOLPS	3	2,979	0,837	0,37	0,538
1	50	NOLPS	4	0,943	0,773	0,523	0,519
1	50	NOLPS	5		1,269	1,372	0,281
2	50	NOLPS	3	0,412	2,113	0,421	
2	50	NOLPS	4	0,639			0,57
2	50	NOLPS	5			0,296	0,747

Rep=repetição.

APÊNDICE 10 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no baço 16 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	IL-2	IL-10	IL-12	TLR-4
0	0	LPS	1		0,528	3,044	
0	0	LPS	2	0,493	0,486	3,184	0,322
0	0	LPS	3	1,083	1,152	0,44	1,429
0	0	LPS	4	0,613	0,461	0,343	1,145
0	0	LPS	5		0,175	1,146	0,637
1	0	LPS	1	0,586	1,12	1,289	0,422
1	0	LPS	2		2,113	2,83	0,349
1	0	LPS	3	0,242	0,721	0,839	0,545
1	0	LPS	4	0,276	0,51	0,973	0,815
1	0	LPS	5	0,886	0,554		
2	0	LPS	1		0,763	0,799	0,09
2	0	LPS	2		1,049	2,43	2,676
2	0	LPS	3	0,745	1,06	0,743	0,735
2	0	LPS	4	1,032	0,449	0,253	0,69
2	0	LPS	5		0,212	0,597	1,133
0	50	LPS	1			0,788	0,428
0	50	LPS	2		1,184	1,533	0,648
0	50	LPS	3	0,534	0,837	0,116	0,859
0	50	LPS	4		1,128	0,166	1,102
0	50	LPS	5		0,292	2,108	0,547
1	50	LPS	1	0,291	0,558	3,774	1,014
1	50	LPS	2	0,549	1,086	1,823	0,798
1	50	LPS	3	0,793	1,433	0,416	0,523
1	50	LPS	4		0,826	0,166	0,582
1	50	LPS	5	0,29	0,789	0,29	0,676
2	50	LPS	1		0,335	4,777	0,815
2	50	LPS	2		2,12	0,644	1,729
2	50	LPS	3	0,296	0,392	0,205	0,2
2	50	LPS	4	0,695		0,144	0,688
2	50	LPS	5			0,735	

Rep=repetição.

APÊNDICE 11. Observações experimentais: expressão gênica de receptores e citocinas no duodeno 3 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	RXRα	RXRγ	PPARα	PPARγ
0	0	-	1	0,369	0,2	0,34	0,245
0	0	-	2	4,234	0,156	0,258	0,046
0	0	-	3	0,212	1,845	0,134	0,231
0	0	-	4	0,872	2,565	4,181	7,803
0	0	-	5	3,463	6,769		
1	0	-	1	0,074	0,099	0,033	
1	0	-	2	0,041	1,365	0,017	
1	0	-	3	2,588		4,853	
1	0	-	5		1,646	6,676	
2	0	-	1	0,828			
2	0	-	2	0,244	0,264	0,232	0,537
2	0	-	4	0,686	1,52		
2	0	-	5	0,425	1,704	3,59	1,536
0	50	-	1	0,12	0,879	4,167	
0	50	-	2	0,836		0,019	
0	50	-	3	0,263	1,839	3,444	3,653
0	50	-	4	1,084	1,013	0,949	1,752
0	50	-	5	0,369	0,624	0,325	0,086
1	50	-	1	0,133	0,699	0,027	0,182
1	50	-	2	0,434	2,264		
1	50	-	3		0,837	2,098	1,365
1	50	-	4	0,492	0,666	1,453	0,041
1	50	-	5	0,184	0,962	0,412	1,209
2	50	-	1	0,454	0,187	0,032	
2	50	-	2	0,291	0,088	0,108	0,119
2	50	-	3	0,348	3,456	1,433	2,027
2	50	-	4	1,667	2,74	0,135	0,158
2	50	-	5	0,884	0,449	1,269	0,344

Rep=repetição.

APÊNDICE 11 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no duodeno 3 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	RXRα	RXRγ	PPARα	PPARγ
0	0	+	1	0,357	0,191	0,036	
0	0	+	2	0,227	0,367	0,122	0,16
0	0	+	3	0,552	0,972	0,287	0,391
0	0	+	5	1,588			
1	0	+	1	0,189	0,226	0,012	0,104
1	0	+	2	0,27	0,955	1,601	
1	0	+	3	0,153	0,789	0,56	0,479
1	0	+	5	0,291	0,566	0,013	
2	0	+	2	0,254	1,332	0,432	0,755
2	0	+	4	0,677	0,812	0,062	0,071
2	0	+	5	0,675	2,187		
0	50	+	1	0,142	0,126	0,534	0,123
0	50	+	4	1,856	2,856	1,06	2,149
0	50	+	5	0,273	0,885	0,737	0,666
1	50	+	2	0,772	3,48		
1	50	+	3	0,071	0,879	0,184	0,197
1	50	+	4	0,583	1,74	0,069	0,025
1	50	+	5	0,148	0,787	0,019	
2	50	+	1	0,445	1,536	0,864	2,264
2	50	+	2	0,338	0,51	0,213	0,952
2	50	+	3		0,678	0,079	
2	50	+	5	0,325	1,105	0,016	

Rep=repetição.

APÊNDICE 11 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no duodeno 3 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	IL-2	IL-10	IL-12	TLR-4
0	0	-	1	0,281	0,307		0,459
0	0	-	2	2,951	1,134	0,543	0,307
0	0	-	3	1,66	0,513	0,562	1,455
0	0	-	4	0,397	3,067		2,725
0	0	-	5	1,829	1,824	0,327	1,791
1	0	-	1	0,748	2,285	0,837	0,408
1	0	-	2	0,953	2,423	0,213	
1	0	-	3	1,637	2,457	0,489	3,285
1	0	-	5		0,984	0,464	
2	0	-	1	0,973	2,597	0,414	2,569
2	0	-	2		0,878	0,9	
2	0	-	4	0,397			
2	0	-	5	1,533	0,436	0,417	0,601
0	50	-	1	0,283	0,456	0,778	0,73
0	50	-	2			0,812	0,383
0	50	-	3	0,522	1,25		2,971
0	50	-	4	1,893	1,799		2,772
0	50	-	5		0,369	0,25	0,973
1	50	-	1	0,638	0,643	0,42	2,821
1	50	-	2		0,808	0,443	0,756
1	50	-	3	0,813	2,398	0,885	1,92
1	50	-	4			3,029	
1	50	-	5	0,973	0,748	1,056	1,501
2	50	-	1	6,13	4,601	0,876	
2	50	-	2	1,32	0,467	0,435	0,735
2	50	-	3	1,47	2,11	0,168	1,981
2	50	-	4	0,406	1,335	2,956	
2	50	-	5		0,461	1,172	1,178

Rep=repetição.

APÊNDICE 11 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no duodeno 3 h após o desafio com LPS

Tratamento				Variável Resposta			
CLA	Luteína	Desafio		Expressão Gênica			
(%)	(mg/kg)	LPS	Rep	IL-2	IL-10	IL-12	TLR-4
0	0	+	1		0,367		
0	0	+	2		1,793	1,998	0,425
0	0	+	3	1,543	0,786	0,452	
0	0	+	5	1,232	2,823	0,091	1,666
1	0	+	1	1,435	1,588		0,229
1	0	+	2	1,367	1,134	0,077	0,353
1	0	+	3	0,579	0,64	0,784	4,426
1	0	+	5	0,254	0,647	0,155	3,497
2	0	+	2		0,872	0,099	0,507
2	0	+	4	2,283	8,616	2,529	4,231
2	0	+	5		1,055	0,191	2,791
0	50	+	1		0,484	0,099	0,383
0	50	+	4	0,816	0,921	2,777	3,044
0	50	+	5		0,977	1,18	2,372
1	50	+	1	0,587	2,059	0,219	0,553
1	50	+	2	0,264	1,725	0,624	0,908
1	50	+	3	0,693	0,462	0,376	2,743
1	50	+	4	0,667	1,799		4,38
1	50	+	5	1,57	1,066	0,223	4,76
2	50	+	1	0,629	5,359	0,172	
2	50	+	2		0,934	0,097	
2	50	+	3	0,412	0,703	0,574	1,186
2	50	+	5	1,592	0,988	0,789	1,598

Rep=repetição.

APÊNDICE 12. Observações experimentais: expressão gênica de receptores e citocinas no duodeno 16 h após o desafio com LPS

Tratamento				Variável Resposta				
CLA	Luteína	Desafio		Expressão Gênica				
(%)	(mg/kg)	LPS	Rep	RXRγ	IL-1β	IL-2	IL-10	IL-12
0	0	-	1	0,716	1,924	0,710	1,520	1,220
0	0	-	2	0,356	0,521	2,242	1,663	1,335
0	0	-	3		1,319	0,588	1,078	0,372
0	0	-	4	0,103	0,707	0,357	0,955	0,951
0	0	-	5	1,482	1,884	2,990	0,384	1,737
1	0	-	1	0,131	0,969	1,414	0,776	1,392
1	0	-	2	0,241	0,416	1,153	0,798	0,627
1	0	-	3	1,255	1,305	0,189	1,042	0,988
1	0	-	5	0,172		1,161		2,491
2	0	-	1	0,783	1,399	0,547	1,176	1,001
2	0	-	2	0,250		0,949	1,413	0,808
2	0	-	3	0,149	2,013	1,094	0,806	0,304
2	0	-	4	0,133	1,209		1,252	0,691
2	0	-	5	0,143		2,838	1,530	2,832
0	50	-	1	2,700	2,027		1,003	1,005
0	50	-	2	0,152	1,128	0,261	0,622	0,691
0	50	-	3	0,716	0,982	0,568	0,704	0,363
0	50	-	4	0,185	0,716	0,838	1,117	0,964
0	50	-	5	0,075	1,222	3,171	2,393	2,784
1	50	-	1	0,185	0,474		0,992	0,546
1	50	-	2	1,112	0,683		1,144	0,652
1	50	-	4		1,574	0,753	1,071	0,411
1	50	-	5		1,820	3,784	0,901	2,932
2	50	-	1	1,251	1,256	1,315		0,954
2	50	-	3	1,151	1,230	1,400	0,826	0,589
2	50	-	5	0,828		1,828	2,062	5,510

Rep=repetição.

APÊNDICE 12 - CONTINUAÇÃO. Observações experimentais: expressão gênica de receptores e citocinas no duodeno 16 h após o desafio com LPS

Tratamento				Variável Resposta				
CLA	Luteína	Desafio		Expressão Gênica				
(%)	(mg/kg)	LPS	Rep	RXRγ	IL-1β	IL-2	IL-10	IL-12
0	0	+	1	1,870	1,160	1,266	1,042	0,283
0	0	+	2					
0	0	+	3	0,087	0,765	0,437	0,755	0,715
0	0	+	4	0,326	0,792	0,284	0,737	0,357
0	0	+	5	0,059	0,989	0,997	2,069	2,803
1	0	+	1	0,332	0,704	0,549	0,781	0,715
1	0	+	2	0,680	0,879		0,969	1,022
1	0	+	3	0,159	0,673	0,886	1,265	0,428
1	0	+	4	0,332	0,582	0,618	0,864	0,559
2	0	+	1	0,325	0,613	0,396	1,663	0,619
2	0	+	2	1,970	1,473	0,402	1,438	0,342
2	0	+	3	0,393	2,418	2,497		0,254
2	0	+	5	0,044	1,300	2,173		4,076
0	50	+	1	0,701	1,337	0,370	0,789	0,187
0	50	+	2		1,845	1,597	1,546	0,364
0	50	+	3		0,673	0,695	0,949	0,402
0	50	+	5	0,148	1,413	1,390	1,808	2,553
1	50	+	1	0,293	1,770	0,609	0,582	1,055
1	50	+	3	0,127		2,144		0,738
1	50	+	5	0,238	0,678	1,979	1,845	1,299
2	50	+	1	0,701			1,035	0,616
2	50	+	2	0,109	0,843	2,667	0,626	0,964
2	50	+	3	0,187	1,010		0,611	0,698
2	50	+	4			1,010	0,304	0,322

Rep=repetição.

APÊNDICE 13. Efeito da interação entre o CLA e a luteína no desempenho de frangos de corte

	PESO MÉDIO (PM)			CONSUMO DE RAÇÃO (CR)			GANHO DE PESO (GP)			GP:CR		
				CR 1 a 8 d, g			GP 1 a 8 d, g			GP:CR 1 a 8 d		
CLA / Luteína	0%	1%	2%	0%	1%	2%	0%	1%	2%	0%	1%	2%
0 mg/kg				115,0 ab	117,3 a	117,3 a	97,3 a	94,3 ab	96,4 a	0,845 ab	0,802 c	0,821 bc
50 mg/kg				117,7 a	116,2 a	110,7 b	97,0 a	99,4 a	88,9 b	0,825 bc	0,855 a	0,803 c
SEM					1,7			2,2			0,011	
Valor de P					0,03			0,02			0,001	
	PM 8 d, g			CR 8 a 15 d, g			GP 8 a 15 d, g			GP:CR 8 a 15 d		
CLA / Luteína	0%	1%	2%	0%	1%	2%	0%	1%	2%	0%	1%	2%
0 mg/kg	142,8 a	139,9 ab	141,9 a	322,1 ab	317,3 ab	322,6 a	274,5 a	251,8 b	267,5 a	0,850 a	0,794 c	0,830 abc
50 mg/kg	142,6 a	145 a	134,5 b	325,1 a	324,1 a	308,5 b	275,5 a	271,9 a	250 b	0,848 ab	0,838 ab	0,812 bc
SEM		2,2			5,0			5,4			0,013	
Valor de P		0,02			0,08			0,004			0,055	
	PM 15 d, g			CR 15 a 20 d, g			GP 15 a 20 d, g			GP:CR 15 a 20 d		
CLA / Luteína	0%	1%	2%	0%	1%	2%	0%	1%	2%	0%	1%	2%
0 mg/kg	417,4 a	391,7 b	409,4 a				280,9 a	259,7 b	259,6 b	0,782 a	0,738 ab	0,737 b
50 mg/kg	418,0 a	416,9 a	384,5 b				260,6 b	274,1 ab	267,7 ab	0,731 b	0,783 a	0,747 ab
SEM		6,7						6,5			0,016	
Valor de P		0,002			0,490			0,02			0,01	
	PM 20 d, g			CR 1 a 20 d, g			GP 1 a 20, g			GP:CR 1 a 20		
CLA / Luteína	0%	1%	2%	0%	1%	2%	0%	1%	2%	0%	1%	2%
0 mg/kg	698,3 a	651,3 c	669 bc				652,3 a	605,8c	623,4 bc	0,820 a	0,771 c	0,787 bc
50 mg/kg	678,7 ab	691 a	652,2 c				633,1 ab	651,6 a	606,6 c	0,793 b	0,822 a	0,781 bc
SEM		7,4						7,2			0,007	
Valor de P		0,001			0,280			0,0001			0,0001	

Médias seguidas por diferentes letras diferem significativamente ($P \leq 0.05$).

APÊNDICE 14. Normas para a publicação de artigos na revista Poultry Science

POULTRY SCIENCE INSTRUCTIONS TO AUTHORS[1]

Editorial Policies and Procedures

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

Contact Information for Journal Staff

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

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

Care and Use of Animals

Authors must make it clear that experiments were conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and laboratory techniques. Experiments shall be conducted in accordance with the principles and specific guidelines presented in *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 3rd edition, 2010 (Association Headquarters, Champaign, IL 61820); and, if applicable, *Guide for the Care and Use of Laboratory Animals* (United States Department of Human Health and Services, National Institutes of Health, Publication Number ISBN 0-309-05377-3, 1996); or *Guide to the Care and Use of Experimental Animals*, 2nd ed. Volume 1, 1993 (Canadian Council on Animal Care). Methods of killing experimental animals must be described in the text. In describing surgical procedures, the type and dosage of the anesthetic agent must be specified. Intra-abdominal and intrathoracic invasive surgery requires anesthesia. This includes caponization. The editor-in-chief of *Poultry Science* may refuse to publish manuscripts that are not compatible with these guides. If rejected solely on that basis, however, the paper may be resubmitted for reconsideration when accompanied by a written verification that a committee on animal care in research has approved the experimental design and procedures involved.

Types of Articles

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

Research Notes. Research Notes are short notes giving the results of complete experiments but are less comprehensive than full-length articles. Preliminary or progress reports will not be accepted. The running head shall be "RESEARCH NOTE." Research Notes will be published as a subsection of the scientific section in which they were reviewed. Research Notes are limited to five printed pages including tables and figures. Manuscripts should be prepared according to the guidelines for full-length articles.

Symposium Papers. The symposium organizer or chair must present the proposal and tentative budget to the Board of Directors at the summer meeting one full year before the symposium is to be scheduled. The symposium chair must then develop detailed symposium plans, including a formal outline of the talks approved

[1]Updated 2014.

and full budgetary expectations, which must be brought to the Board of Directors at the January meeting prior to the meeting at which the symposium is scheduled. The symposium chair must decide whether or not the symposium is to be published and will inform the editor-in-chief of this decision at the January meeting. If the decision is not to publish the symposium, the individual authors retain the right to submit their papers for consideration for the journal as ordinary manuscripts. If publication is decided upon, all manuscript style and form guidelines of the journal shall be followed. Manuscripts must be prepared electronically, including figures and tables, and then uploaded onto the *Poultry Science* Manuscript Central site within 2 weeks after the annual meeting. The symposium chair will review the papers and, if necessary, return them to the authors for revision. The symposium chair then forwards the revised manuscript to the editor-in-chief for final review. Final revisions by the author and recommendations for acceptance or rejection by the chair must be completed by December 31 of the year in which the symposium was presented. Manuscripts not meeting this deadline will not be included in the published symposium proceedings. Symposium papers must be prepared in accordance with the guidelines for full-length articles and are subject to review. Offprints and costs of pages are the responsibility of the author.

Invited Papers. Invited papers, such as the World's Poultry Science Association lecture, should be submitted online; the editorial office will then make these papers available to the editor-in-chief. These papers are subject to review, and all manuscript style and form guidelines of the journal shall be followed. Invited papers are exempt from page charges but not offprint charges.

Review Papers. Review papers are accepted only if they provide new knowledge or a high-caliber synthesis of important knowledge. Reviews are not exempt from pages charges. All *Poultry Science* guidelines for style and form apply.

Invited Reviews. Invited Reviews will be approximately 10 published pages and in review format. The editor-in-chief will send invitations to the authors and then review these contributions when they are submitted. Nominations or suggestions for potential timely reviews are welcomed and should be sent directly to the editor-in-chief.

Contemporary Issues. Contemporary Issues in *Poultry Science* will address critical issues facing poultry scientists and the poultry industry. As such, submissions to this section should be of interest to any poultry scientist, to the industry, to instructors and faculty teaching contemporary issues classes, and to undergraduate and graduate students. The section will consist of short papers (approximately 2 published pages) written in essay format and will include an abstract, appropriate subheadings, and references.

Rapid Communications. We aim for receipt-to-decision times of a month or less, and accepted papers will have priority for publication in the next available issue of *Poultry Science*. These papers will present informative and significant new findings, such as tissue-specific gene expression profile data with full-length cDNA and genomic gene structure characterization. These papers will be short (2 to 4 published pages), adhere to journal format, and include references and an abstract. Rapid Communications should **not** be preliminary reports or incomplete studies. Authors will select Rapid Communications as the paper type when submitting the paper.

Book Reviews. *Poultry Science* publishes reviews of books considered to be of interest to the readers. The editor-in-chief ordinarily solicits reviews. Unsolicited reviews must be sent directly to the editor-in-chief for approval. Book reviews shall be prepared in accordance to the style and form requirements of the journal, and they are subject to editorial revision. No page charges will be assessed.

Letters to the Editor. The purpose of letters will be to discuss, critique, or expand on scientific points made in articles recently published in *Poultry Science*. Introduction of unpublished data will not be allowed, nor will material based on conjecture or speculation. Letters must be received within 6 months of an article's publication. Letters will be limited to 400 words and 5 references (approximately 3 double-spaced, typed pages including references). Letters shall have a title. Author name(s) and affiliation(s) shall be placed between the end of the text and list of references. Letters will be sent electronically directly to the editor-in-chief for consideration. The author(s) of the original paper(s) will be provided a copy of the letter and offered the opportunity to submit for consideration a reply within 30 days. Replies will have the same page restrictions and format as letters, and the titles shall end with "—Reply." Letters and replies will be published together. Acceptability of letters will be decided by the editor-in-chief. Letters and replies shall follow appropriate *Poultry Science* format and may be edited by the editor-in-chief and a technical editor. If multiple letters on the same topic are received, a representative letter concerning a specific article will be published. All letters may not be published. Letters and replies will be published as space permits.

SUBMISSION OF ELECTRONIC MANUSCRIPTS

Authors should submit their papers electronically (http://mc.manuscriptcentral.com/ps). Detailed instructions for submitting electronically are provided online at that site. Authors who are unable to submit electronically should contact the editorial office (nes.diaz@oup.com) for assistance.

Copyright Agreement

Authors shall complete the Manuscript Submission and Copyright Transfer form for each new manuscript submission; faxed copies are acceptable. The form is

to complete an offprint order requesting the number of offprints desired and the name of the institution, agency, or individual responsible for publication charges.

Color Charges. The cost to publish in color in the print journal is $600 per color image; a surcharge for offprints will also be assessed. At the time of submission on ScholarOne Manuscripts, authors will be asked to approve color charges for figures that they wish to have published in color in the print journal. Color versions of figures will be included in the online PDF and full-text article at no charge.

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 New International Dictionary,* or the *Oxford American English Dictionary.* Authors should follow the style and form recommended in *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers.* 2006. 7th ed. Style Manual Committee, Council of Science Editors, Reston, VA.

Authors should prepare their manuscripts with Microsoft Word and upload them using the fewest files possible to facilitate the review and editing process.

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

Preparing the Manuscript File

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

Headings

Major Headings. Major headings are centered (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 not have abbreviations.

Under the title, names of authors should be typed (first name or initial, middle initial, last name). 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., [1]Corresponding 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). 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. Papers dealing with the effects of feed additives or graded levels of a specific nutrient must give analyzed values for the relevant additive or nutrient in the diet(s). If products were used that contain different potentially active compounds, then analyzed values for these coupounds must be given for the diet(s). Exceptions can only be made if appropriate methods are not available. 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 d-α-tocopherol

In the instance of vitamin D_3, cholecalciferol is the acceptable term on the basis that 1 IU of vitamin D_3 = 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 time-sequence analysis.

Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. For 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 (σ^2)], which defines or describes a population, is estimated by a statistic (x, s^2). 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. Significant digits in data reported should be restricted to 3 beyond the decimal point, unless warranted by the use of specific methods.

Results and Discussion

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

must have GenBank accession numbers prior to submitting. One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experiments are adequately replicated. The results of experiments must be replicated, either by replicating treatments within experiments or by repeating experiments.

Acknowledgments

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

Appendix

A technical Appendix, if desired, shall follow the 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. 50:354–365. doi:10.1637/7498-010306R.1

Book:

Metcalfe, J., M. K. Stock, and R. L. Ingermann. 1984. The effects of oxygen on growth and development of the chick embryo. Pages 205-219 in Respiration and Metabolism of Embryonic Vertebrates. R. S. Seymour, ed. Dr. W. Junk, Dordrecht, the Netherlands.

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

Federal Register:

Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. 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. Accessed Feb. 2006. http://www.emedicine.com/neuro/topic20.htm.

El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, 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 $600; a surcharge for color reprints ordered will be assessed. Authors must agree in writing to bear the costs of color production after acceptance and prior to publication of the paper.

Miscellaneous Usage Notes

Abbreviations. Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD). A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the 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 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
AME_n	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	immunoglobulin
IL	interleukin
IU	international units
kb	kilobase pairs
kDa	kilodalton
L	liter*
L:D	hours light:hours darkness in a photoperiod (e.g., 23L:1D)
m	meter
μ	micro
M	molar
MAS	marker-assisted selection
ME	metabolizable energy
ME_n	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
r^2	coefficient of determination, simple
R^2	coefficient of determination, multiple
RFLP	restriction fragment length polymorphism
RH	relative humidity
RIA	radioimmunoassay
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
SD	standard deviation
SDS	sodium dodecyl sulfate
SE	standard error
SEM	standard error of the mean
SRBC	sheep red blood cells
SNP	single nucleotide polymorphism
T	thymine
TBA	thiobarbituric acid
T cell	thymic-derived cell
TME	true metabolizable energy
TME_n	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 camera-ready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow *Poultry Science* guidelines.

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

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

Use the slant line only when it means "per" with 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

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://www.oxfordjournals.org/our_journals/ps/for_authors/index.html

SI Units. The following site (National Institute of Standards and Technology) provides a comprehensive guide to SI units and usage: http://physics.nist.gov/Pubs/SP811/contents.html

Figure Preparation Guidelines. Current detailed information on figure preparation can be found at http://www.oxfordjournals.org/for_authors/figures.html

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VITA

Mariana Lemos de Moraes, filha de Uraci Terezinha Lemos de Moraes e José Luiz de Moraes, nasceu em 12 de abril de 1985 em Porto Alegre. Cursou ensino fundamental e médio no Colégio Marista Nossa Senhora do Rosário em Porto Alegre.

Ingressou no Curso de Medicina Veterinária da Universidade Federal do Rio Grande do Sul (UFRGS) no segundo semestre de 2003 e obteve o título da graduação em dezembro de 2008. No período entre março de 2006 e dezembro de 2008, foi bolsista de iniciação científica no Laboratório de Ensino Zootécnico, onde acompanhou vários projetos de pesquisa em nutrição de aves e suínos e apresentou trabalho em três edições do Salão de Iniciação Científica da UFRGS. Durante o período de graduação, também estagiou na Nutron Alimentos Ltda, no setor de Nutrição do Hospital de Clínicas Veterinárias da UFRGS e no Hospital do Jockey Club de Porto Alegre.

Em março de 2009, ingressou no Programa de Pós-Graduação em Zootecnia da UFRGS, área de concentração "Produção Animal", linha de pesquisa em "Nutrição e Alimentação de Não-Ruminantes". Obteve o título de Mestre em Zootecnia em fevereiro de 2011, com a defesa da dissertação intitulada "Efeito do ácido linoleico conjugado no desempenho e na resposta imune de leitões recém-desmamados" com orientação da Prof[a] Andréa Machado Leal Ribeiro.

Entre agosto de 2009 e março de 2012, cursou MBA em Gestão Ambiental da Fundação Getúlio Vargas, tendo como trabalho de conclusão o tema "A gestão ambiental na suinocultura".

Em abril de 2011, ingressou no Doutorado no Programa de Pós-Graduação em Zootecnia da UFRGS, área de concentração "Produção Animal", linha de pesquisa em "Nutrição e Alimentação de Não-Ruminantes" com orientação da Prof[a] Andréa Machado Leal Ribeiro. De agosto de 2012 a setembro de 2013, foi bolsista do programa de Doutorado Sanduiche no grupo de pesquisa liderado pelo Prof. Kirk Klasing na Universidade da California, Davis.

Durante a vida acadêmica, participou de projetos de pesquisa em nutrição de aves e suínos, apresentou trabalhos em congressos nacionais e internacionais, ministrou aulas para graduação, publicou artigos, resumos e capítulo de livro como autora e coautora.