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USO DE DIETAS A BASE DE MILHO OU SORGO E CANTAXANTINA SOBRE O DESEMPENHO DE MATRIZES DE CORTE E SUAS PROGÊNIES

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS, Brasil
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USO DE DIETAS A BASE DE MILHO OU SORGO E CANTAXANTINA SOBRE O DESEMPENHO DE MATRIZES DE CORTE E SUAS PROGÊNIES

Carlos Eduardo Bonilla Vivas

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

Orientador: Prof. Alexandre Pires Rosa

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elaborada por
Carlos Eduardo Bonilla Vivas

como requisito parcial para obtenção do grau de
Mestre em Zootecnia

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(Ernesto guevara de la serna.)

RESUMO

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

USO DE DIETAS A BASE DE MILHO OU SORGO E CANTAXANTINA SOBRE O DESEMPENHO DE MATRIZES DE CORTE E SUAS PROGÊNIES

AUTOR: CARLOS EDUARDO BONILLA VIVAS
ORIENTADOR: ALEXANDRE PIRES ROSA
Data e Local da Defesa: Santa Maria, 23 de Julho de 2014.

O experimento foi conduzido no Laboratório de Avicultura – LAVIC da Universidade Federal de Santa Maria – UFSM para avaliar aspectos produtivos e reprodutivos de matrizes de corte alimentadas com dietas a base de milho ou sorgo suplementadas ou não com cantaxantina. Foram utilizadas 440 fêmeas e 60 machos da linhagem Cobb 500, distribuídas em um delineamento inteiramente casualizado em arranjo fatorial (2x2), com dois ingredientes; milho (MI) e sorgo (SO), e dois níveis de cantaxantina, 6mg/kg (CX) e 0mg/kg (NCX), totalizando 4 dietas com 5 repetições de 22 fêmeas e 3 machos cada. O período experimental foi dividido em períodos, o primeiro desde 42ª à 53ª, o segundo da 54ª à 65ª semana de idade e um período total. Para a progênie foram realizadas duas avaliações de 1 a 21 dias com 320 pintos machos de um dia cada, oriundos das matrizes na 54ª e 64ª semana de idade, utilizando o mesmo delineamento na avaliação das matrizes, totalizando 4 tratamentos com 10 repetições de 8 machos cada. A taxa da postura foi afetada significativamente pela CX no 2° período. A gravidade especifica foi melhor nos ovos oriundos de SO+CX e SO+NCX do que MI+CX no 1° período, mas o peso da casca foi melhor para SO+CX do que MI+CX nesse período. Para o 2° período o peso do ovo foi melhor no MI+NCX e SO+CX do que MI+CX. A fertilidade e os parâmetros de incubação, os ovos não foram afetados pelas dietas no período total. A CX nas dietas teve uma maior deposição de carotenóides, além de uma maior coloração na gema do ovo. O teor mais baixo de substâncias que reagem com o Ácido Tiobarbitúrico (TBARS) foi nas gemas SO+CX. Um incremento na força do rompimento da membrana vitelina (VMS) foi registrado nas gemas de SO. MI apresentou a maior concentração de luteína e zeaxantina, mas não de CX na gema do ovo. As concentrações dos ácidos graxos saturados e monoinsaturados foram as maiores nas dietas a base de MI, contudo SO teve a maior concentração de ácidos graxos poliinsaturados na gema do ovo. Na 64ª semana de idade das matrizes, a suplementação de CX resultou um menor percentual de mortalidade na progênie. A suplementação de CX e o uso do SO na avicultura representam uma grande oportunidade para as próximas pesquisas.

Palavras-chaves: carotenóides, taxa de postura, qualidade do ovo, TBARS

ABSTRACT

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

CORN OR SORGHUM DIETS AND CANTHAXANTHIN ON BROILER BREEDERS PERFORMANCE AND YOUR OFFSPRING

AUTHOR: CARLOS EDUARDO BONILLA VIVAS
ADVISER: DR. ALEXANDRE PIRES ROSA
Presentation Place and Date: Santa Maria, 23 July, 2014.

An experiment was carried out at Poultry Science Laboratory – LAVIC at the Federal University of Santa Maria – UFSM. To evaluate the productive and reproductive performance of broiler breeders fed with corn or sorghum diets supplemented or not with canthaxanthin. 440 females and 60 males Cobb 500 were used and distributed in a completely randomized design in a factorial arrangement (2x2), with two ingredients; corn (CO) and sorghum (SO), and two leves of canthaxanthin supplementation, 6mg/kg (CX) and 0mg/kg (NCX), totalizing 4 diets with 5 pens with 22 females and 3 males each one. The experimental period was splitted in periods, the first from 42 to 53, the second from 54 to 65 wks of age and in a total period.The offspring performance was evaluated in 2 avaliations from 1 to 21 days. It was used 320 males chicks of 1 day each one. They came from 54 and 64 wks of age of broiler breeders. The design was the same used in the broiler breeders study, totalizing 4 treatments with 10 pens of 8 male each one. The laying rate was affected by the CX supplementation at the second period. The egg specify gravity was better in egg yolks came from SO+CX and SO+NCX than CO+CX in the first period, but the egg weight was better in SO+CX than CO+CX and the same period. At the second period the egg weight was better in CO+NCX and SO+CX than CO+CX. The fertility and incubation parameters, the eggs were not influenced by the diets in the total period. CX in diets had the highest deposition of carotenoids, therefore, the best pigmentation on the egg yolk. The lowest level of the thiobarbituric acid reactive substances (TBARS) was found in SO+CX. The vitelline membrane strength registered an incremented to break it in egg yolks came from SO. CO had the best lutein and zeaxanthin concentrations, but no to CX in the egg yolk. The concentrations of saturated and monounsaturated fatty acids were the highest in CO diets, but, polyunsaturated fatty acids were the best in SO diets. The supplementation of CX in diets reduced mortality offspring at 64 wks of age. The supplementation of canthaxanthin in diet and the use of sorghum in the poultry feed represents a great opportunity for next researches.

Keywords: carotenoids, laying rate, egg quality, TBARS.

SUMÁRIO

INTRODUÇÃO

A avicultura, nos últimos anos, tem alcançado excelentes resultados na sua produção devido ao incessante trabalho e progresso em todas as áreas: nutrição, genética, manejo, sanidade e ambiência, tornando-se uma atividade altamente competitiva no mercado. Atualmente a produção de aves requer maior atenção para diminuição nos custos de alimentação e proteção do meio ambiente, sem causar alterações na qualidade nutritiva da dieta e, consequentemente, comprometimento do desempenho dos animais. Nesse contexto, a utilização de ingredientes para substituir ao milho e a inclusão de alguns aditivos não alimentares são estratégias importantes para os nutricionistas.

Dentre os grãos de cereais, o milho é o mais empregado na alimentação de aves como ingrediente energético devido ao seu alto teor em amido, além de sua propriedade pigmentante na coloração da gema dos ovos e da carcaça das aves. Entretanto, os custos com ração chegam a mais da metade dos custos totais de produção. O milho contribui cerca de 65% da ração o qual representa aproximadamente 40% dos custos desta (COSTA et al. 2006), no entanto, esse fato decorre da crescente demanda interna de milho para produção de biocombustível, gerando um aumento significativo no preço do milho. Isso tem levado pesquisadores a pensarem em alternativas de substituição do milho, buscando-se matérias primas e ingredientes de valores nutritivos conhecidos (CARVALHO et al. 2005). O sorgo é uma das opções que as indústrias de rações e produtores dispõem para utilizar na alimentação animal por ser considerado, juntamente com o milho, a principal fonte de energia para a nutrição animal (RAMSEY et al. 1990).

A substituição do milho pelo sorgo, além de apresentar um preço inferior, tem maior resistência a seca, o que favorece seu cultivo em várias regiões com baixa pluviosidade (FIALHO; BARBOSA, 1997). Outro fator importante a ser considerado na utilização do sorgo é seu teor de taninos, já que estes componentes são capazes de reduzir a digestibilidade de nutrientes (COSTA et al. 2006). Entretanto, com o melhoramento genético do sorgo e a produção de variedades de baixo tanino destinadas à produção de grãos para alimentação de animais monogástricos, os problemas relacionados à presença de taninos foram minimizados (MORENO et al. 2007). Apesar da maior rusticidade, o sorgo perde por ser menos produtivo e pela deficiência de carotenóides em relação ao milho. Esse efeito deprecia o valor do ovo, sendo necessária à inclusão de fontes adicionais de pigmentantes como os carotenóides, além

de outras funções que estes têm (SILVA et al. 2000). A melhora do perfil nutricional do ovo fértil pode ser realizada pela suplementação da dieta materna com nutrientes e aditivos específicos que tem como objetivo aperfeiçoar a viabilidade, eclodibilidade e o crescimento inicial da progênie. Durante todo o período de incubação e, principalmente próximo à eclosão onde o metabolismo é intenso, existe uma produção acentuada de radicais livres e, os ácidos graxos poliinsaturados presentes no tecido embrionário aumentam a exigência dos pintos por antioxidantes, pois estes são mais sensíveis às rupturas provocadas pelos radicais livres (Reis, 2009).

Os carotenóides são conhecidos como precursores de vitamina A, onde possuem importante papel antioxidante, pigmentantes de pró-vitamina e imunomoduladoras, pois removem radicais livres, absorvem e dissipam o excesso de energia destes e reciclam a vitamina E (BÖHM et al. 1997; WILLIAMS et al. 1998). A cantaxantina está incluída no grupo dos carotenóides e, adicionada à dieta dos galos e galinhas, pode exercer seu papel antioxidante de três formas: 1) no embrião; protegendo os tecidos embrionários na incubação, 2) no ovo; protegendo os nutrientes da gema durante o armazenamento para o embrião em desenvolvimento e, 3) nas matrizes pesadas; auxiliando nos mecanismos antioxidantes do sêmen e oviduto, reduzindo o estresse. Scher et al. (2009a) e Souza et al. (2008); adicionaram 6ppm de cantaxantina na dieta de matrizes e observaram redução do número de ovos inférteis e da mortalidade embrionária e melhora nas taxas de eclosão sobre ovos totais e ovos férteis incubados.

Baseados na importância do sorgo e dos carotenóides como antioxidantes e agentes imuno-estimulantes depois da eclosão, foram conduzidos dois experimentos. Experimento I teve como objetivo avaliar a suplementação da cantaxantina ou não em dietas a base de milho ou sorgo em matrizes de corte Cobb 500 sobre seu desempenho produtivo e reprodutivo. Experimento II foi avaliado os efeitos das dietas utilizadas nas matrizes de corte sobre o desempenho produtivo dos pintos de corte de 1 a 21 dias de idade, partindo da hipótese que o uso de cantaxantina na dieta de matrizes de corte melhora seu desempenho produtivo e reprodutivo com relação às dietas não suplementadas.

CAPITULO I

1. ESTUDO BIBLIOGRÁFICO

1.1. Qualidade do milho na nutrição animal

O Brasil vem se destacando como um dos maiores produtores mundiais de carne, principalmente de origem avícola. Dados da CONAB (2013) apontam que a produção do milho no Brasil para a safra 2012/2013 foi de 81007,2 (mil t). Sabidamente, o milho é um cereal rico em energia, sendo considerado como concentrado energético. Segundo Rostagno et al. (2011) grãos inteiros de milho contêm aproximadamente 87,48% de Matéria Seca, 7,88% proteína bruta, 1,73% de fibra bruta, 72,95% de extrato não nitrogenado e 1,27% de matéria mineral. O milho registrou valores de 3381 kcal/kg de energia metavolizable. Isto se deve principalmente ao elevado conteúdo de amido, onde, é rico em amilopectina sendo essa fração de mais fácil digestão, quando comparada à fração de amilose. O milho apresenta ainda quantidade satisfatória de metionina+cistina (0,47%) e nível muito baixo de lisina (0,36%) (ROSTAGNO et al. 2011). Quando misturado ao farelo de soja, haverá a complementação amionoacídica, pois este alimento é rico em lisina, mas eficiente em metionina+cistina. Contudo, para que o milho esteja com boa qualidade nutricional, é necessário que esteja em bom estado de conservação

Costa et al. (2009) não perceberam variações no conteúdo de proteína bruta entre o milho coletado em diferentes casas agropecuárias. O nível deste princípio nutritivo também não variou ao longo do ano quando foram comparados em seis meses diferentes.

Os carotenoides presentes nos grãos de milho se dividem em dois grandes grupos: carotenos (β-caroteno e α-caroteno) e xantofilas (luteina, cantaxantina e zeaxantina), especialmente amarelo-alaranjados, destacam-se como fonte de carotenóides (OLIVEIRA et al. 2006), sendo que 90% desses compostos, nos grãos, são constituídos por luteína e zeaxantina (GOODWIN, 1980), além dos carotenos (β-caroteno e α-caroteno e a β-zeacaroteno) que conjuntamente somam os 10% restantes (KURILICH; JUVIK, 1999; EGESEL et al. 2003). A concentração total de carotenóides varia de 0,15 a 33,11µg.g-1,

distribuídos essencialmente no endosperma do grão do milho (KURILICH; JUVIK, 1999). A distribuição dos carotenóides no grão seco é 74 a 86% no endosperma vítreo, 9 a 23% no endosperma farináceo, 2 a 4% no germe e 1% no farelo, sendo o endosperma vítreo presente em maior proporção (46-54%) que o farináceo (28 a 36%) (BLESSIN et al. 1963)

1.2. Características do sorgo em grão

O grão de sorgo é o quinto principal cereal depois do trigo, arroz, milho e cevada, é nativo da África Oriental, provavelmente da Etiópia (ECOPORT, 2010). Maunder (2002) considerou o sorgo como uma cultura tradicional em grande parte da África e da Ásia, sendo uma cultura introduzida e hibridizada no hemisfério ocidental. Agora é generalizada entre 50°N (EUA e Rússia) e 40°S, a partir do nível do mar até 1000m de altitude (ECOPORT, 2010). As condições de crescimento ideais para sorgo são 25-30°C em mudas e 30°C durante o crescimento, 400-750 mm de precipitação anual, e solos com pH entre 5,5 e 7,5.O sorgo é uma pequena cariopse com estrutura dura e coberta por glumas (ECOPORT, 2009). Na nutrição animal, o sorgo é usado principalmente como fonte de energia na dieta tanto para aves, suínos e ruminantes (BALOLE et al. 2006).

O sorgo é tolerante à seca, graças ao seu sistema radicular. Ele tem um desempenho melhor do que o milho durante a seca e ocupa áreas impróprias para o milho em regiões semi-áridas. É tolerante a salinidade e de certa forma a água (durante um curto período de tempo). É sensível a geadas, suscetível às ervas daninhas nos primeiros estádios de desenvolvimento (FAO, 2009). No Brasil o cereal é cultivado, principalmente, visando à produção de grãos para suprir a demanda das indústrias da alimentação animal e como forragem, para alimentação de ruminantes (DYKES et al. 2005; TABOSA et al. 1993). A produção de sorgo no Brasil na safra 2011/2012 foi de 2179,5 milhões de toneladas (CONAB, 2012).

1.2.1. Composição nutricional do sorgo

A composição de nutrientes do sorgo foi bem documentada (NRC, 1984; ALETOR, 1999, ETUK, 2008). Grãos inteiros de sorgo contêm aproximadamente 87,90% de Matéria

Seca, 8,97% proteína bruta, 2,3% de fibra bruta, 72,26% de extrato não nitrogenado e 1,41% de matéria mineral (ROSTAGNO et al. 2011).

Em contraponto o sorgo pode substituir o grão de milho em grande medida (SUBRAMANIAN et al. 2000). Ensminger e Olentine (1978) relataram que a energia metabolizável (EM) é de 13,70 a 14,04 MJ/kg. Contudo, Abubakar et al. (2006) relataram um valor ligeiramente inferior, calculado de 12,15 MJ/kg a 12,92 MJ/kg (BARRETT; LARKIN, 1974; WU; BEM, 1980; KUBIEZEK et al. 1984) e Segundo Rostagno et al. (2011) o sorgo de baixo tanino registrou valores de 3189 kcal/kg de energia metavolizable

O sorgo contém baixos níveis de lisina, mas alto teor de triptofano (0,10%) em relação ao milho (PURSEGLOVE, 1972; OLOMU, 1995, ROSTAGNO et al. 2011). McDonald et al. (2000) relataram que tanto o milho como o sorgo apresentam limitaçãoes de aminoacios essenciais como, arginina, lisina, metionina, cistina e triptofano (FAO, 1995; OLOMU, 1995).

O grão de sorgo contém compostos fenólicos, como: ácidos fenólicos, flavonóides e taninos, sendo os dois primeiros inócuos aos animais. Já os taninos estão concentrados no pericarpo da semente e formam complexos com carboidratos e principalmente proteínas, reduzindo assim sua digestibilidade e piorando a palatabilidade, pois confere ao sorgo sabor adstringente (BRUZEGUEZ et at. 2001).

Pesquisas demostraram que o grão de sorgo é deficiente em pigmentos como as xantofilas, importantes para promover a coloração da gema dos ovos e carcaça das aves de corte (ANDRIGUETTO et al. 1990).

1.2.2. Uso do sorgo nas dietas das aves

Considerando-se o valor nutritivo, custo e disponibilidade, o sorgo vem sendo projetado como a próxima alternativa ao milho na alimentação de aves (MAUNDER, 2002). Um estudo realizado por Subramanian e Metta (2000) indica que o sorgo é tão ideal como o milho para aves porque apresenta uma composição nutricional semeliante. Da mesma forma, Augusto et al. (1974) relataram que geralmente o sorgo pode substituir todo o milho em dietas avícolas, sendo necessário o fornecimento de xantofilas para a pigmentação da pele e da gema de ovo. Spiridon et al. (1979) não observaram efeito depressivo do sorgo no crescimento e eficiência alimentar em frangos de corte, mesmo quando 100% do milho foi substituído pelo

sorgo, no entanto, as carcaças de aves alimentadas com dieta a base de sorgo foram mais leves do que as dietas controle.

Smithhard (2002) relatou o mau desempenho das aves alimentadas com alto teor de tanino de sorgo, mesmo quando foi suplementada com soja. No entanto Cullison (1987) relatou que o sorgo pode substituir 50% do milho, sem nenhum efeito adverso sobre o desempenho dos animais, embora o ganho de peso foi reduzido em 10% ou mais.

Estudos feitos por Kumar et al. (2007) revelaram que a alimentação da dieta a base de sorgo vermelho reconstituído com um teor de tanino de 16g/kg para frangos de corte não exerceu influência apreciável na utilização de nutrientes mesmo em uma subsitituição total do milho. Os resultados obtidos com sorgos nativos na Índia não mostraram nenhum efeito adverso na produção de ovos, tenha sido substituído em um 15% ou com substituição total do milho. Frangos alimentados com sorgo nativo ou melhorado em um nível de substituição de 45% para o milho registraram um desempenho comparável ao milho em todos os parâmetros mensurados (SUBRAMANIAN; METTA, 2000).

Os autores Silva (2003) e Garcia et al. (2005) trabalhando com níveis de 0, 25, 50, 75 e 100% de substituição de milho pelo sorgo com baixo teor de tanino, verificaram que os frangos apresentaram médias similares para consumo de ração, ganho de peso e conversão alimentar nas fases inicial e de crescimento. Pinto et al. (2005), avaliando o desempenho e a qualidade dos ovos de poedeiras alimentadas com dietas contendo sorgo em substituição parcial ou total ao milho, não observaram comprometimento no desempenho das aves, porém, uma diminuição na pigmentação da gema dos ovos com o aumento na substituição do milho pelo sorgo, sendo necessário à inclusão de uma fonte de carotenóides.

O sorgo é de extrema importância na alimentação de aves domésticas nos países onde está disponível e pode ser utilizado totalmente ou parcialmente nas dietas avícolas (Jacquin, 1991). Cultivares de sorgos livres de taninos são os preferidos pelas aves, mas é possível alimenta-las com um sorgo de maior tanino, no entanto, a digestibilidade permanece inferior para o sorgo de baixo tanino (DAGHIR, 2008). Para melhor qualidade e desempenho das aves, é necessário adicionar gordura, pigmentantes e metionina nas dietas a base de sorgo, melhorando assim sua digestibilidade (BLAIR, 2008).

1.3. Participação da nutrição das aves na resposta imune

Diversos autores vêm estudando a participação dos diferentes componentes das dietas na resposta imune das aves (GORE; QURESHI, 1997; KLASING, 1998; VIRDEN et at. 2004), demonstrado que a interação entre nutrição e imunidade está cada vez mais sólida. Considerando o ciclo de reprodução e incubação das aves, observa-se que, durante a vida embrionária, as aves são dependentes dos nutrientes contidos no saco vitelínico. Dessa forma, ao enfatizar que o desenvolvimento e maturação do sistema imune iniciam durante a vida embrionária e prosseguem após o nascimento, fica clara a importância das matrizes (KLASING, 1998). De acordo com Leeson e Summers (2001), os componentes do sistema de defesa das aves necessitam de várias vitaminas, que participam de sistemas enzimáticos, fundamentais para sua síntese, portanto, níveis deficientes podem resultar em menor atividade desse sistema.

Estudos mostram a importância da nutrição na primeira semana de vida para desenvolvimento e maturação do sistema digestório. Segundo Maiorka (2002) o fornecimento de ração aos pintos, logo após a eclosão, minimiza a utilização dos nutrientes oriundos do saco vitelínico para o desenvolvimento intestinal, beneficiando o sistema imunológico dessas aves. Nesse mesmo conceito, Dibner et al. (1998) sugerem que a nutrição nas primeiras 24 horas de vida exerce efeito sobre o peso da bolsa de Fabrícius, responsável pela maturação dos linfócitos B. Conforme Klasing (1998), diversos nutrientes podem participar da modulação do sistema imune por vários mecanismos e alguns, como a vitamina E, exercem efeito regulatório direto sobre esse sistema, além de ter grande importância como agente antioxidante natural (UNDERWOOD; SUTTLE, 1999).

A composição da gema, da clara e da casca é relativamente constante, sendo que moderadas modificações possíveis são dependentes da composição da dieta da reprodutora. A melhora do perfil nutricional do ovo pode otimizar o desenvolvimento corporal do embrião (RICHARDS; STEELE, 1987).

1.4. Oxidação lipídica

Radicais livres (RL) são substâncias químicas que apresentam número ímpar de elétrons, sendo assim muito instáveis e altamente energéticos (ARAÚJO, 2006). Para se tornarem estáveis, os radicais livres transferem a energia acumulada para as substâncias próximas, principalmente aos ácidos graxos poliinsaturados (PUFA). Os lipídeos são suscetíveis ao ataque por RL e sua oxidação pode ser muito prejudicial devido a sua continuidade como uma reação em cadeia. Portanto, os lipídeos contendo PUFA são particularmente propensos ao ataque de radicais livres (PAPAS, 1999) e à deterioração oxidativa, sendo utilizados na determinação da eficácia de antioxidantes naturais (WANASUNDARA; SHAHIDI, 1998).

Em alimentos, a formação de RL ocorre pela ação direta de fontes externas de energia, como luz, calor e radiação. A oxidação dos lipídeos é uma das mais importantes causas da deterioração dos alimentos, devido à formação de sabores e odores indesejáveis (rancidez oxidativa), bem como a formação de substâncias tóxicas potencialmente perigosas quando ingeridas pelas aves e pelo homem. Este aspecto é de grande importância, porque provoca uma queda na qualidade nutricional dos mesmos (NAWAR, 1996). A rancidez oxidativa envolve a reação do oxigênio com ácidos graxos, por mecanismos químicos e enzimáticos como a autoxidação, fotoxidação e lipoxidação (ARAÚJO, 2006).

Na ausência de antioxidantes apropriados, os PUFA formam radicais livres e podem ter um efeito pró-oxidante significativo levando à depleção da vitamina E e dos carotenoides, tendo um aumento dos produtos de oxidação (MEYDANI, 1996; WANDER et al. 1996). Por conseguinte, é um requisito necessário ter uma ingestão aumentada de antioxidantes para acompanhar um consumo elevado de ácidos graxos poliinsaturados para obter as ações benéficas dos mesmos (WISEMAN, 1996).

A oxidação natural de carotenóides depende da sua estrutura, sendo os mais facilmente oxidáveis o β-caroteno, luteína e violaxantina. Estes compostos são facilmente oxidados em função do grande número de duplas ligações conjugadas. No tecido intacto, os pigmentos estão protegidos da oxidação; entretanto, danos físicos ao tecido ou sua extração aumentam sua suscetibilidade à oxidação. Uma antioxidação intensa irá resultar na quebra dos pigmentos e sua descoloração (RIBEIRO; SERAVALLI, 2004; MELÉNDEZ-MARTINEZ et al. 2004b). Ao final da oxidação, ocorre a perda total da cor e da atividade biológica, pois podem ser

formados apocarotenoides; por exemplo, na degradação do β-caroteno são formados β-apo-12´-carotenal, β-apo-10´-carotenal e β-apo-8´-carotenal (RODRIGUEZ-AMAYA, 1999c).

Segundo Silva et al. (1999), é possível distinguir estas três etapas de evolução oxidativa da seguinte forma: a) desaparecimento dos substratos de oxidação–lipídio insaturado (oxigênio); b) aparecimento dos produtos primários de oxidação–peróxidos e hidroperóxidos, cuja estrutura depende da natureza dos ácidos graxos presentes; e c) aparecimento dos produtos secundários de oxidação, obtidos por cisão e rearranjo dos peróxidos. Os produtos secundários são produzidos no decorrer da decomposição dos primários. Dentre esses, um dos principais é o malondialdeído (SHAHIDI; WANASUNDARA, 1992).

O malondialdeído (MDA) é formado durante a oxidação dos PUFA por cisão beta dos PUFA peroxidados, principalmente do ácido araquidônico (LIMA; ABDALLA, 2001). Este aldeído com três átomos de carbono ($C_3H_4O_2$) sendo muito utilizado para avaliar a oxidação lipídica em alimentos e principalmente o estresse oxidativo em amostras biológicas, através do teste de substâncias reativas com o ácido tiobarbitúrico, conhecido como TBARS. O princípio do TBARS baseia-se na reação de uma molécula de MDA com duas de ácido tiobarbitúrico (TBA), em meio ácido e sob altas temperaturas, formando um complexo vermelho, que pode ser determinado por absorção no visível (532 nm) ou por fluorescência.

Em paralelo, acredita-se que o rápido desenvolvimento das genéticas atuais de frango de corte e aumento do estresse poderia conduzir a uma produção acentuada de radicais livres (SURAI, 2000). O aumento de radicais livres tem sido correlacionado com a redução do desempenho zootécnico dos animais (SURAI 2001), além que está envolvido no início e na progressão de várias doenças (UNDERWOOD; SUTTLE, 1999).

1.5. Ácidos graxos

Com base na presença ou não de duplas ligações os ácidos graxos são definidos como saturados (AGS), aqueles que só têm ligações simples; monoinsaturados (AGMI), aqueles que contem uma única dupla ligação e poliinsaturados (AGP), quando estão presentes duas ou mais duplas ligações. Alguns ácidos graxos poliinsaturados não podem ser sintetizados pelo organismo, portanto devem ser ingeridos na alimentação. Estes ácidos são chamados ácidos graxos essenciais (HUNTER; ROBERTS, 2000).

Os ácidos graxos saturados se encontram, predominantemente, em alimentos como carne, ovos, queijo, leite e manteiga, óleos de coco e palma, como também vegetais hidrogenados. O ácido oléico é o mais comum dos ácidos graxos monoinsaturados e se encontra na maioria das gorduras animais, incluindo aves, carne bovina e de cordeiro, bem como em azeitonas, sementes e nozes. Já, os ácidos graxos poliinsaturados se classificam, principalmente, nas séries ômega 6 (ω-6) e ômega 3 (ω-3), os mesmos que se diferenciam na posição da primeira dupla ligação, contando desde o grupo metílico terminal da cadeia do ácido graxo, que apresentam insaturações separadas apenas por um carbono metilênico, com a primeira insaturação no sexto e terceiro carbono. O ácido linoléico é o expoente mais importante da série (ω-6) e está presente de forma abundante nos óleos vegetais como óleo de girassol, cártamo, milho, soja, algodão, etc.

O ácido α-linolênico, representante da família ω-3, é encontrado em quantidades apreciáveis em sementes oleaginosas como canola, soja e linhaça (DZIEZAK, 1988; MARTIN et al. 2006). O ácido linoléico pode ser metabolizado em outros ácidos ω-6, incluindo os ácidos γ-linolênico, dihomo-γ-linolênico e araquidônico. O ácido α-linolênico é metabolizado em outros da série ω-3, entre eles eicosapentaenóico (EPA) e docosahexaenóico (DHA). Este processo metabólico é mediado pelas enzimas chamadas elongases e dessaturases, as quais participam na formação dos PUFA, ω-6 e ω-3, resultando em uma competição metabólica entre os dois grupos (SALEM, 1999). Um excesso de ácido linoléico vai impedir a transformação do α-linolênico em seus derivados EPA e DHA, o mesmo acontecerá no caso contrário, com um menor consumo do ácido linoléico haverá uma diminuição da formação do ácido araquidônico (MADSEM et al. 1999). Isto significa que deve existir uma proporção maior de ácido linoléico que de α-linolênico.

Segundo Cheiran (2008), os ácidos graxos são componentes majoritários dos lipídios da gema de ovo e constituem cerca de 4g de seu peso médio. Os lipídios do ovo estão concentrados na gema e são constituídos por triacilglicerois, fosfolipídios e colesterol. Os fosfolipídios são mais ricos em ácidos graxos insaturados do que os triacilglicerois, sendo que a composição dos ácidos graxos destes lipídeos pode variar em função do alimento ingerido na ave. O conteúdo lipídico do ovo pode ser influenciado pela linhagem, pelo tamanho do ovo e pelos componentes da ração, além do tipo de gordura adicionada na dieta (BARRETO et al. 2006).

1.6. Carotenóides

Carotenóides são moléculas orgânicas com funções antioxidantes, pigmentantes, pró-vitamina e imunomoduladoras. Os animais e o homem não são capazes de sintetizar esses pigmentos, mas são capazes de fazer algumas alterações fundamentais para a estrutura química (WILLIAMS et al. 1998).

Britton et al. (2004) relatou que os carotenóides desempenham papéis importantes na coloração de muitas plantas, invertebrados, peixes, anfíbios, répteis e aves (GOODWIN, 1984). Os carotenóides são polienos isoprenóides, um grupo químico que inclui cantaxantina. Quimicamente, os carotenóides se dividem em dois grupos: carotenóides hidrocarbonados, denominados carotenos, e carotenóides oxigenados, denominados xantofilas (GOODWIN, 1965).

Uma das funções de alguns carotenóides é que agem como provitamina A (SURAI; SPEAKE, 1998), enquanto que outros têm mecanismos de proteção no corpo e ação antioxidante (BURTON, 1989), aumentando assim seu sistema imunológico (BENDICH, 1989; BLANCH, 1999). No entanto, vários estudos têm demonstrado a função da luteína e cantaxantina na pigmentação das gemas de ovos e frangos (HENCKEN, 1992; SAYLOR, 1986). A maioria dos carotenóides naturais está relacionada com a ocorrência da pigmentação das aves de forma livre (HENCKEN, 1992). Estes compostos não são naturalmente sintetizadas pelas galinhas, sendo os mesmos principalmente derivados da dieta (BREITHAUPT, 2007). Junto com a pigmentação em aves também está envolvido o metabolismo de crescimento e fertilidade. Apesar disso, esse sistema também é responsável pela instabilidade e consequente isomerização e oxidação das moléculas de carotenóides durante o processamento e estocagem do alimento que o contém (RODRIGUES; AMAYA, 1999).

1.6.1. Cantaxantina

Cantaxantina é o β, β-caroteno 4,4-Diona, sendo que esta foi isolada pela primeira vez a partir do cogumelo comestível, *Cantharellus cinnabarinus* (HAXO, 1950). Cantaxantina também é indicada por ser produzida em várias algas verdes como carotenóides secundários

(CZYGAN,1968), e assim como em algas azuis-verdes (HERTZBERG; LIAAEN-JENSEN, 1966). Também tem sido encontrada em bactérias (SAPERSTEIN; STARR, 1954), crustáceos (DAVIES et al. 1970; THOMMEN; WACKERNAGEL, 1964) e várias espécies de peixes (KATAYAMA et al. 1971; 1973; CZECZUGA, 1973). A cantaxantina foi sintetizada pela primeira vez a partir do β-caroteno (PETRACEK; ZECHMEISTER, 1956), seguido pela síntese completa feita por Isler et al. (1956) e Isler e Schudel (1963) sendo produzida através da síntese química a partir de 1962.

De acordo com a Comissão Européia (2002), a cantaxantina é absorvida no intestino delgado e transportada pelo sangue ao fígado, onde parte é transformada em substâncias intermediárias precursoras de vitamina A, como 4-oxoretinol e o restante permanece íntegro, transportado pelas lipoproteínas aos depósitos alvos. Beardsworth e Henández (2003) relataram que a atividade pró-vitamina A da cantaxantina tem sido reconhecida e que esta pode ser transformada em vitamina A nas aves quando está em níveis limitados na dieta. Menos de 40% de cantaxantina na dieta é depositada na gema do ovo e menos do que 10% nos tecidos (SCHIEDT, 1987; HOPPE; KRENNRICH, 1995). A distribuição da cantaxantina nos diferentes tecidos e órgãos após administrada (8mg/kg na dieta) na galinha é a seguinte: ovários (68-69%), fígado (5,2-6,3%), músculo (3,2-7,5%), gordura (1,0-1,2%) e pele (1,1-1,1%) (SCHIEDT, 1987).

A deposição de cantaxantina na gema de ovo é diretamente proporcional ao nível de proteína (BORNSTEIN; BARTOV, 1965; BRAUNLICH, 1974; TYCZKOWSKI; HAMILTON, 1986; GRASHORN et al. 2000). Nas aves, há um efeito predominante na concentração de cantaxantina na gema de ovo, sendo que para uma concentração de 1mg/kg de cantaxantina na ração, sua concentração na gema do ovo atinge cerca de 2-3,1mg/kg (SCHIEDT, 1987; HENCKEN, 1992; GRASHORN et al. 2000; SIDIBE, 2001). Braunlich (1974) relataram uma taxa de deposição de cantaxantina de 2-3mg/kg de gema para cada 1mg/kg de cantaxantina na dieta. Outros estudos realizados utilizando cantaxantina permitiram o isolamento de metabólitos a partir do fígado de poedeiras e frangos de corte (SCHIEDT, 1990), bem como a partir de gema de ovo, baço, rim e tecido adiposo perineal (SCHIEDT, 1987). Cantaxantina representavam 40% do total de resíduos no fígado, sendo o 4-oxoretinol o metabólito principal (30%) (TYCZKOWSKI et al. 1988).

A cantaxantina pode melhorar significativamente a escala de pigmentação em frangos de corte, quando usado em dietas contendo carotenóides amarelos (MARUSICH; BAUERNFEIND, 1981).

1.6.2. Propriedades antioxidantes dos carotenóides

Uma substância antioxidante pode ser definida como: 1) composto ou substância química que inibe a oxidação ou, 2) qualquer substância que, quando presente em baixa concentração comparada a do substrato oxidável, diminui ou inibe significativamente a oxidação do mesmo. Do ponto de vista biológica, se pode definir um antioxidante como compostos que protegem sistemas biológicos contra os efeitos potencialmente danosos de processos ou reações que promovem a oxidação de macromoléculas ou estruturas celulares (ABDALA, 1993).

O sistema de defesa antioxidante do organismo compreende uma gama variada de substâncias que atuam em diferentes níveis. O sistema primário constitui-se em uma primeira linha de defesa formada por substâncias que impedem a geração de espécies reativas ou através da retirada das mesmas de forma impedir sua interação com alvos celulares, ou seja, bloqueiam a etapa de iniciação da cadeia radicular. Nesse grupo encontram-se: a) enzimas antioxidantes, b) quelantes e proteínas como a transferrina e a ceruloplasmina que transportam ferro e cobre impedindo que sejam liberados e, catalisam a formação de espécies oxidantes e c) substâncias não-enzimáticas como o ascorbato, albumina e carotenóides que sequestram radicais superóxido e hidroxila, ou suprimem oxigênio singlete (ABDALA, 1993).

Os carotenóides protegem as células de danos oxidativos provocados por radicais livres e por espécies reativas de oxigênio (EROs) (constituem moléculas não radiculares derivadas do oxigênio, como peróxido de hidrogênio (H_2O_2)) que podem ser gerados no citoplasma, nas mitocôndrias ou na membrana, atacando lipídeos, proteínas, carboidratos e DNA (SHAMI; MOREIRA, 2004).

A proteção antioxidante é fornecida pelos carotenóides acíclicos, que possuem nove ou mais duplas ligações conjugadas, por exemplo, o licopeno é mais eficaz que o β-caroteno, pois o licopeno possui onze duplas ligações conjugadas de cadeia acíclica, enquanto o β-caroteno possui nove duplas ligações conjugadas de cadeia cíclica nas extremidades (DI MASCIO et al. 1989; MCBRIDE, 1996). Esses carotenóides são capazes de sequestrar espécies reativas de oxigênio, como o radical peroxil (ROO) e o oxigênio singleto ($^1O2$) (FOOTE et al. 1970), estabilizando o elétron desemparelhado do radical. Os carotenóides são, por conseguinte, capazes de retirar do meio, espécies altamente reativas (BURTON; INGOLD, 1984). A ordem crescente da capacidade de sequestrar o oxigenio singlento por parte dos carotenos e xantofilas é: licopeno, astaxantina ou cantaxantina, β-caroteno ou

bixina, luteína e crocina (FONTANA et al. 2000). Por serem apolares, os carotenóides ficam mergulhados nas membranas sequestrando radicais gerados nestes ambientes (TRUSCOTT, 1996). Os carotenóides ao combaterem as espécies reativas de oxigênio, podem interagir de três maneiras diferentes: transferência de elétrons, remoção de íons de hidrogênio ou adição de espécies radiculares.

Tem sido demonstrado que o aumento da vitamina E (SURAI, 1999) ou concentrações de carotenóides (SURAI, 2002) na gema de ovo estão associadas com um aumento da resistência à peroxidação lipídica. No entanto, os ácidos graxos poliinsaturados de cadeia longa na dieta podem mudar substancialmente o perfil dos ácidos graxos da gema de ovo e ter efeitos prejudiciais sobre as defesa contra os antioxidantes. É interessante notar uma correlação positiva entre carotenóides e vitamina E na concentração na gema de ovo das aves silvestres (HARGITAI et al. 2006). Porém, está é uma forte correlação entre vários carotenóides em níveis intra e interespecíficos nas aves (COHEN; MCGRAW, 2009).

1.6.3. Importância da transferência dos carotenóides ao embrião

O sucesso do processo de incubação e do desenvolvimento corporal logo após a eclosão é dependente de inúmeros fatores relacionados com o ovo. O tamanho do ovo e o formato da casca podem ser importantes (PAPPAS et al. 2006). Além disso, a limitação na disponibilidade de nutrientes seja pela quantidade ou pela forma em que se apresentam levam ao insucesso do processo de incubação (VIEIRA, 2007), uma diminuição da eclodibilidade, que também esta correlacionada com um aumento na mortalidade da primeira semana (PAPPAS et al. 2006).

Durante a embriogênese, os carotenóides são transferidos a partir da gema para os tecidos em desenvolvimento (PLACK, 1963). Desses, a vitamina E, os minerais e os carotenóides são transferidos do alimento das matrizes para o ovo e, subsequentemente, para os tecidos embrionários, enquanto que os outros são sintetizados no organismo do animal (SURAI, 2000). O embrião das aves desenvolve-se dentro de um sistema fechado, o ovo, que contém todos os nutrientes necessários para os 21 dias do processo de desenvolvimento embrionário (SPEAKE et al. 1998). A gema é rica em lipídeos, vitaminas lipossolúveis A e E, e também uma gama de carotenóides (GRIFFIN et al. 1984).

Após a absorção, os carotenóides são rapidamente depositados nos tecidos adiposos do frango, no peito, pernil e na pele. O tecido embrionário necessita de efetiva proteção antioxidativa, pois contêm grandes quantidades de ácidos graxos poliinsaturados (SPEAKE et al. 1998). Vários aspectos da ação do carotenóide podem ser considerados benéficos para o desenvolvimento do embrião. Os carotenóides podem aumentar a capacidade antioxidante total disponível para o embrião, protegendo assim os tecidos em desenvolvimento dos efeitos prejudiciais de espécies reativas de oxigênio e radicais livres (SIES; STAHL, 2003).

Durante os desenvolvimentos embrionários outros compostos antioxidantes são sintetizados em vários tecidos. Estes incluem a glutationa, o ácido ascórbico e ácido úrico, bem como enzimas antioxidantes (peroxidase de glutationa (GSH), catalase (CA) e superóxido dismutase (SOD)) (SURAI, 2006). Existe especificidade de tecido associada com a síntese de tais antioxidantes e em combinação com a vitamina E mais carotenóides, que contribuem como um sistema antioxidante no embrião em desenvolvimento para seu uso após eclosão (SURAI, 2006). O papel dos antioxidantes naturais dentro da dieta materna na manutenção da qualidade do ovo e da galinha não deve ser subestimado (ROCHA et al. 2010).

1.6.4. Utilização de carotenóides na dieta das matrizes de corte

Para observar os efeitos da suplementação de cantaxantina (Carophyll Red®) na dieta de matrizes com 30 semanas de idade sobre o sistema antioxidante do embrião e pinto, SURAI et al. (2003) utilizaram cinco dietas: controle, com menos de 2mg de cantaxantina/kg na dieta e sem cantaxantina, e 3, 6, 12 e 24mg de cantaxantina/kg na dieta. Os níveis de cantaxantina nos pintos de um dia, no fígado dos pintos de sete dias e na gema do ovo, no fígado e no saco vitelino dos embriões com 16 dias aumentaram ($P<0,001$) de maneira crescente de acordo com o aumento na suplementação das dietas nas matrizes de corte com CarophyllRed®. No grupo controle, a cantaxantina não foi identificada em nenhuma das amostras acima citadas. A concentração da vitamina A na gema do ovo e nos tecidos embrionários e dos pintos não foi afetada pelas diferentes concentrações de cantaxantina. Isso porque a cantaxantina não é considerada um carotenóide precursor desta vitamina, a menos que a ave esteja com hipovitaminose A. A cantaxantina demonstrou efeito positivo sobre a concentração da vitamina E de três formas: 1) aumentou a assimilação de gamatocoferol da

dieta e sua transferência para gema do ovo e consequentemente aumentou as concentrações desta substância no fígado do embrião e do pinto após a eclosão; 2) aumentou as concentrações de alfa-tocoferol nos tecidos e no plasma dos pintos com um dia de idade, pois a cantaxantina agiu como antioxidante durante o desenvolvimento embrionário sequestrando RL e deixando menos espécies reativas disponíveis para reagir com a vitamina E. Dessa forma, a vitamina E foi "economizada" e seus níveis aumentados; e 3) possivelmente auxiliou na regeneração da vitamina E, através da transferência do elétron dos carotenóides para o radical alfatocoferoxil. Os autores concluíram que os carotenóides podem modular o sistema antioxidante do embrião e pinto, ajudando a manter sua eficiência.

Surai e Sparks (2001) compararam ovos, embriões e pintos produzidos por matrizes alimentadas com duas dietas: a base de milho, rica em carotenóides, especialmente luteína e zeaxantina – 11,8mg de carotenóides/kg, e a base de trigo – 5,6mg de carotenóides/kg. Os ovos provenientes de galinhas alimentadas com dieta a base de milho apresentaram maiores concentrações ($P<0,01$) de beta+gama-tocoferol, carotenóides totais, luteína e zeaxantina, assim como os tecidos dos pintos nascidos destes ovos. Os autores concluíram que a dieta da matriz tem importante função na formação do sistema antioxidante durante o desenvolvimento embrionário e que a dieta a base de milho aumenta o potencial antioxidante da gema do ovo e dos tecidos embrionários quando comparada à dieta a base de trigo.

Scher et al. (2009a) avaliaram os efeitos de 25-(OH)D3 (69ppb de principio ativo), cantaxantina (60ppm de principio ativo) e 25-(OH)D3 mais cantaxantina, nas dietas de matrizes de corte da linhagem Cobb 500, durante 20 incubações (uma por semana). E verificaram que as melhores taxas de eclosão, eclodibilidade e fertilidade, foram obtidas por matrizes alimentadas com dietas contendo 25-(OH)D3 e cantaxantina, além disso, os aditivos contribuíram para uma redução do percentual de mortalidade embrionária durante o período avaliado.

Koutsos et al. (2003) verificaram que a dieta materna rica em carotenóides influenciou a concentração desses nos tecidos dos frangos até 28 dias. Segundo Karadas et al. (2005) a dieta da matriz enriquecida com carotenóides é o principal fator que influencia a concentração de carotenóides no fígado dos pintos na primeira semana de vida e, que a partir desta idade a alimentação do pinto passa a influenciar estes níveis. Baseados no importante papel dos carotenóides como antioxidante imediatamente após a eclosão, estes autores concluíram que o consumo de carotenóides pela matriz, além de influenciar a incorporação desses pelos tecidos da progênie, pode ser necessário para aumentar a viabilidade da mesma.

Em experimentos recentes, Souza et al. (2008) adicionaram 6ppm de cantaxantina (60g de Carophyll Red®/ton) na dieta das matrizes, observaram redução do número de ovos inférteis, mortalidade embrionária e melhora nas taxas de eclosão sobre ovos totais e ovos férteis incubados.

Na verdade, os efeitos maternos são mensurados pela qualidade dos ovos, sendo as mesmas fontes de descendência, variação fenotípica podendo influenciar no curso dos processos evolutivos. A mãe aloca uma gama diversificada de antioxidantes para o ovo, com a função de proteger ao embrião do estresse oxidativa. Além, as propriedades imunomoduladores dos carotenóides podem ser de importância para a progênie (CONSTANTINI; MOLLER, 2008).

Koutsos et al. (2003), mostraram o efeito materno da concentração dos carotenóides em pintos de 4 semanas de idade, sugerindo que alguns antioxidantes, incluindo os carotenóides, poderiam afetar a expressão dos genes durante seu desenvolvimento. Isto pode resultar em melhores defesas por parte dos antioxidantes relacionadas com uma maior eclodibilidade e melhor viabilidade pós-eclosão. O autor mostrou que as concentrações de carotenóides na gema de ovo tiveram um efeito profundo sobre o desenvolvimento do sistema imune pós-eclosão. Eles sugeriram que a depleção dos carotenóides na gema do ovo pode melhorar o sistema imune da ave (KOUTSOS et al. 2007). Embora esta hipótese precisa de mais esclarecimentos, é claro que esse efeito da dieta materna é visto além de pintos recém-nascidos, e poderia ser um exemplo de programação nutricional materna responsável por várias mudanças na vida pós-natal. O ambiente nutricional que prevaleceu durante o desenvolvimento fetal foi duradouro sobre a fisiologia e metabolismo do pinto (LANGLEY-EVANS, 2009).

Borba (2011) para observar os efeitos da suplementação de cantaxantina (Carophyll Red®) e / ou 25-(OH)D_3 sobre o desepenho de matrizes de corte, Cobb 500 desde a 25 até 52 semanas de idade foram avaliadas 4 dietas experimentais a base de milho e soja; sendo a primeira sem adição dos aditivos, a segunda com adição de 25-(OH)D_3 adicionado ao produto HY-D®, a terceira com adição de cantaxantina e a quarta com adição de 25-(OH)D_3 + cantaxantina. Avaliou os parâmetros zootécnicos e reprodutivos das reprodutoras pesadas, sendo que os diferentes tratamentos utilizados não afetaram a taxa de postura (P=0,5148). A gravidade específica, pesos de gema, albúmen e casca de ovo também não foram afetados pelos tratamentos (P>0,05). Matrizes pesadas que receberam suplementação de cantaxantina na dieta, apresentaram maior deposição de carotenóides na dieta.

2. HIPÓTESIS E OBJETIVOS

2.1. Hipótese

Uma dieta que apresenta baixo teor de carotenóides produz um efeito negativo nos aspetos reprodutivos das matrizes de corte.

Matrizes de corte alimentadas com dietas suplementadas com cantaxantina apresentarão melhor desempenho zootécnico e parâmetros qualitativos de ovos do que as aves não suplementadas.

Existe um efeito positivo no desempenho da progênie das matrizes de corte alimentadas com dietas suplementadas com cantaxantina.

O sorgo pode ser uma alternativa na substituição total do milho na dieta de matrizes de corte sem produzir efeitos negativos no desempenho das matrizes de corte.

2.2. Objetivos

2.2.1. Geral

Avaliar a eficácia das dietas a base de milho ou sorgo com ou sem suplementação de cantaxantina sobre o desempenho reprodutivo e qualitativo de ovos de matrizes de corte da linhagem Cobb da 42ª a 65ª semanas de idade e suas progênies.

2.2.2. Específicos

- Avaliar a eficácia das dietas em relação à produção de ovos e parâmetros de incubação das matrizes de corte.
- Avaliar a qualidade dos ovos durante o período reprodutivo de estudo.

- Determinar o poder antioxidante da cantaxantina sobre a gema do ovo.
- Avaliar a composição dos carotenóides presentes na gema do ovo.
- Determinar o desempenho da progênie das matrizes de corte alimentadas com dietas a base de milho ou sorgo com ou sem suplementação de cantaxantina.

CAPÍTULO II

Corn or sorghum diets supplemented or not with canthaxanthin on productive and reproductive performance of broiler breeder

Este capítulo é apresentado de acordo com as normas para publicação no **Periódico Poultry Science.**

Corn or sorghum diets supplemented or not with canthaxanthin on productive and reproductive performance of broiler breeder

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ABSTRACT The effects of the corn or sorghum diets supplemented or not with canthaxanthin on productive and reproductive performance of broiler breeder were studied. 440 females and 60 roosters were placed in an open-sided house with 20 pens of 4.615m². The experimental period was of 24 wks and splitted in 2 periods. First period was from 42 to 53 and second period was from 54 to 65 wks of age of broiler breeders. At the 42 weeks of age started the trial and females and roosters were distributed in a completetly randomized desing in a factorial arrangement (2x2) with 4 diets of 2 ingredients; corn (CO) or sorghum (SO) and 2 levels of canthaxanthin; 6mg/kg (CX) and 0mg/kg (NCX), with similar body weight (BW) and uniformity. BW was measured every 28 days and the mortality in the end. The egg production (%), egg specify gravity (g/m³), egg weight (g), percentage of yolk weight (%), albumen weight (%), eggshell weight (%) and yolk colorimetric score were calculated weekly. Thirteen incubations were recorded in all experimental period to evaluate fertility (%) and incubation parameters (%) (Hatching, hatchability of fertile eggs, contaminated, pipped, second quality chick and chick weight (g)). BW, mortality, yolk and albumen weight, fertility and incubation parameters were not affected by diets. An increase in egg production at second period was observed (P=0.0066) in CX breeder's diets. Egg specify gravity and eggshell weight were improved at first period by SO+CX (P=0.0138, P=0.0209), occurring the same in egg weight but at the second period (P=0.0251). CX in diets improved egg yolk pigmentation in both periods (P<0.001). CX in breeder's diets improved egg production at the critical period and egg yolk coloration, as well as SO improved some egg quality parameters in this study, being an excellent opportunity to keep researching your effect breeders.

Key words: carotenoid, egg production, fertility, egg quality.

INTRODUCTION

Throughout the animal nutrition history some raw materials stand out for their quality as a source of nutrients or by the amount of inclusion in the diet, such as corn and soybean meal (Casartelli et al., 2005). In the search of alternative raws, the sorghum is an important cereal grain for human and animal consumption throughout the world. Total sorghum production is forecasted at 61 million tonnes in 2012 in the world, which makes it the fifth important grain (Hassan, 2013) and fourth in Brazil (Faostat, 2007; Ibge, 2010), which your production in this country was more than two million tons and field productivity was 2.580kg/ha-¹ at the period of 2010/2011 (Companhia Nacional de Abastecimento, 2011). The sorghum and corn grain are comparable in nutritional composition, sorghum shows protein levels and energy content slightly higher than corn, but the corn has more oil, quantity of lysine, methionine, carotenoids and vitamin A than sorghum, but the tryptophan is similar in both grains (Butolo, 2002; Healy et al., 1994; Sauvant et al., 2004).

In addition to its value for bird feeding, sorghum also has several advantages in regard to the environment. It is more drought-resistant than corn (Farre and Faci, 2006) and can be more easily grown without irrigation (Hassan, 2013). The sorghum cost is between 70 and 80% of the corn price, as well as it is economically attractive, due by the recent increases in corn prices (Rocha et al., 2008). As for nutritional, sorghum dietary inclusion was associated with worse feed conversion efficiency in broilers, due by the presence of tannins (Robertson & Perez-Maldonado, 2006). But, through the genetic improvement of sorghum, the problems related with the presence of tannins were reduced (Jacob et al., 1996). Therefore, the dietary inclusion of new sorghum cultivars did not affect the broiler performance (Garcia et al., 2005a; Rocha et al., 2008) and technically it may replace up to 100% in corn diets of broilers (Rocha et al., 2008) and laying hens (Assuena et al., 2008) without negative effect on the birds performance (Metta and Subramanian, 2000;. Zanzad et al, 2000). When it is used on

hen's diets, it is recommended the inclusion of pigments like carotenoids (Silva et al., 2000). Although, it is known that the xanthophylls are yellow or red carotenoid pigments are present in some plant. The corn contains about 20mg kg^{-1} (Cheeke, 1999) consequently, one of the problems is that sorghum is poor in natural pigments, its use in the diet of laying hens causes decreases in egg yolk color (Subramanian and Metta, 2000; Zanzad et al., 2000).

Carotenoids belong to a group of more 500 pigments. In avian species; the primary carotenoids of economical and ecological interest are xanthophylls, including lutein, its isomer zeaxanthin, and canthaxanthin (Goodwin, 1984). Broilers use these compounds for skin pigmentation and for growth and fertility maintenance (Schiedt, 1998) In addition to their importance in signaling secondary sexual characteristics in animals (Griffith et al., 2006), these pigments are also powerful immunostimulants (Lozano, 1994, 2001; Fenoglio et al., 2002). Canthaxanthin is an important carotenoid that could be efficiently deposited in egg yolk and further distributed in the chick embryonic tissues (Surai and Speake, 1998). In the egg, canthaxanthin is transferred from the egg yolk to the developing embryo and distributed in many organs and tissues (Llaurado et al., 1997; Surai et al., 2003; Karadas et al., 2005), which it might help protect the developing bird against oxidative damage, particularly during the sensitive periods of hatching and early posthatch life (Robert et al., 2007). The effects of supplementation of canthaxanthin in corn or sorghum broiler breeder's diets on the reproductive performance (e.g., fertility and hatchability) and on the productive performance (e.g., egg production, egg quality and egg yolk coloration) were assessed as a means to improve your performance.

MATERIALS AND METHODS

The present study was carried out in the Poultry Science Laboratory-LAVIC of the Federal University of Santa Maria (UFSM). 660 broiler breeder hens and 90 roosters Cobb

500 (22 wks old) were acquired from a commercial poultry company to be used in the trial. They were place in an open-sided house with a wood shaving floor. Birds were reared following the Cobb 500 broiler breeder guidelines (Cobb, 2008; Rostango et al., 2011). The trial was initiated from 42 until 65 wks old. The total experimental period was of 24 wks. It was splitted in two experimental periods. The first period was from 42 to 53 wks of age and the second period was from 54 to 65 wks of age. At 42 wks of age, the breeder hens and roosters were weighed, and 440 hens and 60 roosters were selected according to the body weight (BW) in order to compose the experimental groups.

The selected birds were placed in 20 pens; each pen had 4.615m² (3.24 x 1.42m) and, each pen was equipped with an automatic drinker, one tube feeders to the breeder hens, and a trough-type feeder for the roosters. The corn or sorghum and soybean meal were used as base to elaborate the experimental diets, which all of them were bought at the same place. After of this, a sample was collected, and sent at Laboratório de Bromatologia e Nutrição de Ruminantes (LABRUMEN-UFSM), Santa Maria (RS), Brazil and CBO laboratories in Campinas (SP), Brazil to make bromatological analysis and carotenoids concentrations at DSM Nutritional Products Ltd – Switzerland. After corn, sorghum and soybean meal was done (Table 1), the experimental diets were formulated according of the nutritional needs of Cobb 500 broiler breeders and subsequently, a sample of the diets was collected and sent to the same laboratories (Table 2).

Birds were fed with corn or sorghum-soybean-based mash diets supplemented or not with canthaxanthin. The supply of the feed was strictly controlled, in accordance with the recommendations of the breeder company. Water was *ad libitum*, and a photoperiod of 13 hours light/day was used during the first weeks (22 wks), gradually it was increasing until they received 16 hours and 30 minutes of light/day at 54 wks until at the end of experiment.

Treatments

The birds were distributed in 4 experimental groups with similar BW and uniformity (average of 3.71kg and uniformity of 90%). Each one of the 4 experimental treatments was randomly assigned with 5 replicates, each one with 22 hens and 3 roosters by pen. The birds were fed with the same basal diet (corn and soybean) from 22 until 41 wks old. From 42 to 65 wks of age, 4 groups were fed with the different experimental diets in this period. First group received corn diet with 0mg/kg of canthaxanthin (Carophyll Red; DSM Nutritional Products Ltd., Basel, Switzerland), second group received corn diet with 6mg/kg of canthaxanthin; third group received sorghum diet with 0mg/kg of canthaxanthin and fourth group received sorghum diet with 6mg/kg of canthaxanthin. Hens and roosters received the same diets in all periods (Table 2).

Experimental responses measured

Body weight (BW) was evaluated every 28 days and accumulated mortality (total number of dead birds/initial number of birds x 100%) was calculated in the end of the experiment. Eggs were collected and recorded 6 times per day. The egg production by hen housed was calculated in each experimental period used. Egg weight, percentage of yolk weight, albumen weight, eggshell weight and yolk colorimetric score were determinated in a specify day of each experimental period using 15 eggs/treatment (5 replicates of 3 eggs each one). Egg weight, yolk weight and albumen weight were determinated through a precision scale (0.001g). The egg specify gravity was determined throughout the immersion of the eggs in saline solutions with densities of 1070; 1075; 1080; 1085; 1090; 1095 and 1100g/cm^3 using Archimede's principle as described by Peebles and McDaniel (2004). The eggshell was weighed after being dried in ambient temperature by 3 days (Rodriguez-Navarro et al., 2002).

Egg yolk colorimetric score was conducted using DSM Yolk Color Fan in a scale from 1 until 15 by the technique of Vuilleumier, (1969).

Incubation parameters

For each treatment, fertility, hatchability of fertile eggs, hatchability of total eggs set and healthy chicks were recorded in 13 incubations throughout 24 experimental weeks (Total period). Eggs were collected 6 times daily and classified by pen for hatching process. Only clean and without visible abnormalities eggs were considered suitable for incubation. After of this, they were stored in a period of 7 days in a room with controlled temperature (18-20°C and 75-80% Relative Humidity). The incubation was carried out in a commercial multi-stage incubator (Casp, Amparo, SP, Brazil) at 37.5°C and 60% Relative Humidity. On day 18, the eggs were transferred to the hatcher with 36.5°C and 65% Relative Humidity to complete incubation. At 21 day, chicks were taken out of hatcher, weighed and classified into first and second-quality. Chicks were considered second quality, when they possessed bad umbilical scarring, beak abnormalities, leg weakness or excessively wet downy feathers, therefore, the chicks without these characteristics were considered as first quality. The hatching rate was determinated in relation to the total incubated eggs. To evaluate the hatching rate of fertile eggs, fertility and embryonic mortality, the nonhatched eggs were submitted to embryo diagnostics. They were classified throughout of a macroscopic visual examination as infertile, embryonic mortality at the first 48 hs of incubation (EM1); embryonic mortality occurring between 72 hs and 147 hs of incubation (EM2); embryonic mortality occurring between 192 hs and 336 hs of incubation (EM3), embryonic mortality occurring between 360 and 504 hs of incubation (EM4). The egg pipped was classified as dead-pipped embryos or pipped eggs that have broken the shell but nor emerged at the time to removal of chicks from the hatcher and

the embryo is still alive. Eggs with abnormal contents with green or black coloration and emitting rotten smell or eggs that blow up on opening were considered contaminated.

Experimental design and statistical analysis

The experimental design was a completely randomized design in a factorial arrangement with two ingredients (corn and sorghum) and two leves of canthaxanthin (0mg/kg and 6mg/kg), totalizing four treatments with five replicates pens of 22 females and 3 males of Cobb 500 broiler breeders each one. The total embryonic mortality was corrected by adjust of normality with square root of X. All the data were subject to Analysis of Variance. When was observed significant differences at 5% in the variance average was applied Tukey test for comparison among the treatments. Statistical procedures were performed by the use of SAS software (2009).

RESULTS AND DISCUSSION

Sorghum has not been used in poultry feeding for many years, because in old varieties, the seeds contained condensed tannins, mainly flavonoids (Bate-Smith, 1969; Watterson and Butler, 1983) therefore, it reduces the diet's digestibility and the growth of the birds (Elkin et al., 1996; Nyachoti et al., 1997). Genetic selection programs were devoted to develop tannin-free varieties (Conan et al., 1992) and now sorghum is commonly used in poultry feeding (Gualtieri and Rapaccini, 1990; Selle et al., 2010) but, few details refer in broiler breeders. In this study the BW and mortality during the experimental period were not affected by using the sorghum diets (Table 3).

The egg production was not affected by CX supplementation in corn or sorghum diets at the first period (Table 4). Nonetheless, at the second period, the supplementation of CX in diets had a positive influence on the egg production (P=0.0066). The result found is very

important, because it may indicate the functional significant positive effect of this xanthophyll in the critical period of the egg production, accepting the hypothesis proposed that potential increase in the antioxidant status in broiler breeder supplemented with canthaxanthin might result in a better egg production than birds not supplemented.

In others studies found higher egg production in sorghum diet than diets without this grain (Ebadi et al., 2000). The result of this study at the second period was disagree with the previous studies (Sell et al., 1983 and Gualtirir & Rappaccini, 1990) which showed that replacement corn by sorghum grain decrease the egg production. At the experiment of Zanzad et al. (2000) only the percentage of egg production was slightly lower when sorghum was replaced in 100% of corn, although, it was different for the experiment realized.

Rosa et al. (2012) three hundred and sixty females and 36 roosters were placed in an open-sided house allocated into 12 pens. At 42 weeks of age, the breeder hens and roosters were distributed into two experimental groups with similar body weight and uniformity. From 46 to 66 weeks of age, one group received 6ppm of CX supplemented in corn diet, and the other group received the diet without addition of CX (control corn diet). Those fed with CX in diet had significantly better egg production during certain periods than those without CX in diet, but overall there was not difference between treatments groups.

Previous studies also have reported that different forms of xanthophylls have different deposition rate in eggs (Bowen et al., 2002). Many studies have suggested that some carotenoids, such as B-carotene is beneficial for the productivity of laying females (Damron et al., 1984; Meng and Shan, 2002; Liang et al., 2004). Nevertheless, the important function of carotenoids as antioxidants has been established (Krinsky, 2001; El-Agamey et al., 2004) and a potential benefit exists for the production of laying hens, when undergoing a certain degree of stress. Despite these researches, at the present study when the canthaxanthin was supplemented in broiler breeder diets, it showed a potential benefits on the egg production.

At the present study was found a positive effect on the egg specify gravity at the first period (Table 5), whereas sorghum diets (supplemented or not) were better than corn diet supplemented with CX (P=0.0138). In the same context, but to the second period, an improvement on egg weight was found in sorghum diet supplemented with CX and corn diet without supplementation (P=0.0251). When the supplementation was in sorghum diet the eggshell weight was better than corn diet at the first period (P=0.0209). It is clearly observable how the breeder's diets can influence on the egg quality, specifically on these parameters. However, at the second period demonstrated that the dietary raw material and CX had not effects on some egg quality characteristics such as egg specific gravity, eggshell, yolk and albumen weight. These data corroborate the results obtained with no feed consumption of CX (Khaton et al., 1999; Hasin et al., 2006) on egg gravity (Saha et al., 1999; Hasin et al., 2006) in hens fed with a diet enriched with CX.

Pigmentation of egg yolks are influenced mostly by layer diet (Colin et al., 2004), but it does not only depend on the total amount of pigments, although also on the proportion of yellow and red carotenoids ingested. Low levels of red pigments added to diets with higher levels of yellow pigments result in a very intense yolk color (De Groote, 1970), whereas supplementation of a weakly colored yellow basis diet with a high level of canthaxanthin gives egg off-colors. The effects of current levels of canthaxanthin use in laying hens on coloration of fresh and boiled eggs have been investigated (Grashorn et al., 2000). Canthaxanthin can be efficiently deposited and distributed in the ovary, egg yolk and embryos in both wild and domestic birds (Hencken, 1992; Nys, 2000; Surai et al., 2001ª, 2003; Blount et al., 2002). Because, the canthaxanthin mainly is responsible by orange and red color in plants and animals. Therefore, at the present study was demonstrated that supplementation of canthaxanthin in broiler breeder's diets increased the egg yolk color from yellow (6.00 and 6.00) to orange-red (14.00 and 15.00) (Table 5).

In both period of the current study, the corn and sorghum diets supplemented with CX had the biggest deposition on pigments in the egg yolk (P<0.0001). The diets without canthaxanthin had lower pigmentation on egg yolk than diets supplemented with canthaxanthin. However, the corn diet without CX had better pigmentation than sorghum diet without CX (P<0.0001). This happened, because the deposition of canthaxanthin in egg yolk is directly proportional of the dietary level (Bornstein and Bartov, 1965; Braunlich, 1974; Tyczkowski and Hamilton, 1986; Grashorn et al., 2000). Therefore, when reaching high levels, this results in a lower deposition rate (Marusich et al., 1974; Belyavin and Marangos, 1987; Puchal, 1988). In general, pigment supplementation had not been associated with changes in production (Angeles and Scheideler, 1998; Garcia et al., 2002; Soto-Salanova, 2003), nevertheless, in this experiment was observed the contrary of this affirmation, whereas broiler breeder fed with CX in diets had a better egg production than diets without this carotenoids.

Proportional reductions in yolk pigmentation, which the substitution of corn by sorghum were also found in others authors (Zanzad et al., 2000; Garcia et al., 2002) and that these effects are due by the low content of xanthophylls (Subramanian and Metta, 2000). In this study was found that egg yolk color was enhanced by a diet containing CX, which the intact yolk color was enhanced quickly by a diet containing CX in breeder's diets, when it was previously fed with corn-soybean meal without additional pigments.

Rosa et al. (2012) two weeks after the start of the supplementation, the pigmentation was measured with the color fan, which it was 9.33 in the control group and 14.67 in the CX group (P<0.0001) and this difference in yolk coloration remained stable throughout the experiment. These results are in agreement with previous observations (Surai et al., 2003; Zhang et al., 2011) and for this study.

Canthaxanthin is absorbed in the small intestine and transported via the blood to the liver. Thus, a part of the absorption of canthaxanthin undergoes metabolic change and it is transformed into 4'-hydroxyechinenone and isozeaxanthin, but also 4-oxoretinol, a vitamin A precursor in laying hens and broilers (Tyczkowski and Hamilton, 1986; Schiedt, 1998). The remaining unchanged canthaxanthin is transported by lipoproteins via blood to the target deposition sites. Less than 40% of the dietary canthaxanthin is deposited in egg yolk, and lower than 10% on the body tissues (Schiedt, 1987; Hoppe and Krennrich, 1995). Further studies carried out using radiolabeled canthaxanthin has allowed the isolation of metabolites from the liver of both laying hens and broiler chicks (Schiedt, 1990) as well as from egg yolk, spleen, kidney and perineal fat of layers (Schiedt, 1987). Natural xanthophyll is well-absorbed by hen intestinal cells (Gouveia et al., 1996) and is transferred to the yolk (Donald & William, 2002) after being released into the circulatory system (Salma et al., 2007). However, in natural products, xanthophylls are unstable and effective levels may decline as a result of oxidation during prolonged storage.

During egg incubation, vitamin E and carotenoids are effectively transferred from the egg yolk to embryo development and maximum concentrations of these compounds in the liver of newly hatched chicks are considered to be an adaptive mechanism to protect tissues from the oxidative stress of hatching (Surai, 2002; Surai et al., 1996). Despite those researches and affirmations, at the present study, it was not proven, because the supplementation of CX in corn or sorghum diets, it did not enhance the hatching and hatchability of fertile eggs of broiler breeder throughout the experiment (Table 6). This observation is disagree with Liaurado et al. (1997), who reported an improvement of hatchability by adding 6mg/kg of canthaxanthin in a commercial broiler breeder diet. Despite of this, Meijerhof (1992) reported that the reduction in hatchability as a function of storage, due to the reduction in embryo viability is caused by changes occurring in the embryo or egg.

At the present study, canthaxanthin supplementation in corn or sorghum diet during the 24 wks did not improve broiler breeder fertility (Table 6). In a research with lutein and xanthophylls carotenoids on broiler breeder diets, Pizzey and Bédécarrats (2007) did not observe significant differences in fertility, embryonic survival, or hatchability, when evaluating dietary supplementation of lutein at 30 or 12mg/kg.

In another research, Surai and Sparks (2001) compared eggs, embryos and chicks of broiler breeder hens fed by two diets: corn-based, rich in carotenoids, especially lutein and zeaxanthin - carotenoids with 11.8mg/kg, and wheat-based with 5.6mg carotenoids/kg. Eggs came from hens fed with corn diet had higher ($P \leq 0.01$) concentrations of beta + gamma-tocopherol, carotenoids, lutein and zeaxanthin, as well as the tissues of chicks hatched than eggs came from hens fed with wheat diet. The authors concluded that the mother diet has an important role in the formation of the antioxidant system during embryonic development and that the corn-based diet increases the antioxidant potential of egg yolk and embryonic tissues when compared to wheat based diet.

In the analysis of nonhatched eggs, like a contaminated or pipped did not have a reduction of the percentage of these variables in broiler breeder hens fed with supplementation of canthaxanthin in corn or sorghum (Table 6).

Rocha et al. (2008) considered the embryo mortality from 15 days of incubation, because in this period of incubation starts the embryo increased metabolism. This increased metabolism means higher oxygen consumption (Wilson et al., 1992) and increased oxidation of yolk lipids to meet the energy requirements of the embryo (Latour et al., 2000). Those associated with the high concentration of polyunsaturated fatty acids (PUFA) of embryonic tissues (Surai et al., 1997), factors make the embryo more susceptible to peroxidation. Thus, the addition of canthaxanthin in diet increased the number of arrays in the egg yolk of antioxidants that protect the embryonic tissues, resulting in lower mortality in this period,

when compared to embryos that did not receive canthaxanthin. But, the results to embryo mortality for this experiment were not significant affected during the different phases and periods studied (Table 7). Therefore, the canthaxanthin supplementation or not in corn or sorghum diets did not have effect about these variables. The same result was observed using lutein supplementation, which Pizzery and Bédécarrats (2007) observed that embryonic mortality throughout the incubation period did not differ significantly between treatments. Despite of this result, previous studies have found that dietary supplementation of hens with antioxidant, such as vitamin E, improved the activity of the embryo antioxidant system, thus decreasing the susceptibility of tissues to lipid peroxidation (Surai et al., 2000; Surai and Sparks, 2001). Regarding the chicks produced during the period evaluated, there were not statistical differences in chick BW or chick quality.

A research about the supplementation of canthaxanthin in broiler breeder diets was done by Rosa et al. (2012), which observed the positive effect of CX supplementation of the maternal diet on fertility, hatchability and embryonic mortality. In fact, CX in comparison to the control improved the fertility (92.1 vs 91.0%, $P<0.02$) and hatchability (93.7 vs 91.3%, $P=0.0003$) and reduced embryonic mortality (3.7 vs 5.5%, $P<0.0003$). As a result, hatching rate was significantly (86.2 vs 83.0%, $P=0.0001$) improved. To the differences stages of embryonic mortality, CX was most efficient for its prevention into the first 48 hours (1.04 vs 1.8%, $P=0.008$) as well as between day 15 and 21 of incubation (1.44 vs 2.07, $P=0.017$). It can be postulate that CX supplementation improved breeder fertility ($P=0.0171$) by improving survival and storage of spermatozoa within the reproductive tract of hen. The same result were found by Souza et al. (2008) and Scher et al. (2009) which added 6ppm of canthaxanthin (60g Carophyll Red/ton of feed) to the diet of broiler breeder hens, and they observed a reduction in the number of infertile egg and embryo mortality and improvement in overall rates of eggs hatching and hatching eggs hatched.

In this research, the results showed an important effect of canthaxanthin supplementation in diets on the egg production and how a raw alternative materials low in carotenoids and pigments, as sorghum resulted in a positive influence on the broiler breeder performance and egg quality, such as egg specific gravity, egg weight and the percentage of eggshell weight when this was supplemented with canthaxanthin. The potential positive effects of supplementation of canthaxanthin on the sorghum and others material raw with low level of carotenoids certainly need further study.

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Table 1. Nutritional composition of corn or sorghum grain and soybean meal.

Nutritional composition			
Ingredient [1]	Corn Grain	Sorghum Grain	Soybean meal
Humidity (%)	11.37	13.14	12.32
Dry matter (%)	88.63	86.86	87.68
Crude Protein (%)	7.71	7.48	45.36
Gross Energy (kcal/kg)	3855	3895	4030
Crude fibre (%)	1.40	1.80	5.07
Ether extract (%)	4.40	1.72	2.02
Mineral matter (%)	1.21	1.45	6.23
Calcium (%)	0.01	0.14	0.30
Available P (%)	0.26	0.23	0.52
Alpha-Tocopherol (mg/kg)	9.39	4.97	1.06
Lutein (mg/kg)	3.80	0.49	-
Zeaxanthin (mg/kg)	4.25	0.22	-
Canthaxanthin (mg/kg)	5.45	5.54	-

[1] Determined by analysis at LABRUMEN/UFSM (Humidity (%),Dry matter (%), Crude protein (%), Gross Energy (kcal/kg), Crude fibre (%), Ether Extract (%), Mineral Matter (%),Calcium (%) and Available P (%)) and at CBO analyses laboratoriais (Alpha-Tocopherol (mg/kg), Lutein (mg/kg), Zeaxanthin (mg/kg) and Canthaxanthin (mg/kg)).

Table 2. Composition nutritional of corn or sorghum diets supplemented or not with canthaxanthin in broiler breeders.

Item		
Ingredient	Corn Diet (%)	Sorghum Diet (%)
Corn	69.06	-
Sorghum	-	67.58
Soybean meal (46% of CP)	21.15	20.68
Dicalcium phosphate	1.60	1.61
Limestone, 38% Ca	6.99	7.08
Salt	0.40	0.40
Vitamin-mineral premix[1]	0.50	0.50
DL-Methionine	0.08	0.10
Vegetable oil	0.22	2.05
Treaments		
Control	0.000	0.000
Canthaxanthin (mg/kg)[2]	6.00	6.00
Estimated composition		
Metabolizable Energy (kcal/kg)	2800	2800
Crude Protein (%)	15.50	15.50
Arginine total (%)	0.93	0.94
Lysine total (%)	0.79	0.73
Methionine total (%)	0.35	0.35
Methionine + cysteine total (%)	0.63	0.59
Threonine total (%)	0.57	0.57
Tryptophan total (%)	0.17	0.20
Isoleucine total (%)	0.61	0.64
Leucine total (%)	1.43	1.49
Valine total (%)	0.80	0.82
Phenylalanine total (%)	0.80	0.82
Calcium (%)	3.20	3.20
Available phosphorus (%)	0.39	0.39
Sodium (%)	0.19	0.18
Potassium (%)	0.60	0.61
Nutritional composition analyzed[3]		
Crude Protein (%)	14.80	14.26
Gross Energy (kcal/kg)	3583	3680
Ether Extract (%)	1.18	2.01
Mineral Matter (%)	9.09	10.28
Calcium (%)	3.27	3.28
Total phosphorus (%)	0.56	0.58
Vitamin A (UI/kg)	9620[4] (12900)[5]	5810[4] (9790)[5]
Vitamin D3 (UI/kg)	2380[4] (3360)[5]	575[4] (1600)[5]
Alpha-Tocopherol (mg/kg)	36.8[4] (37.4)[5]	34.5[4] (32.2)[5]
Lutein (mg/kg)	3.84[4] (4.05)[5]	0.95[4] (0.99)[5]
Zeaxanthin (mg/kg)	4.41[4] (5.98)[5]	0.28[4] (0.33)[5]
Canthaxanthin (mg/kg)	< detection limit[4] (5.88)[5]	< detection limit[4] (6.58)[5]

[1]Mineral and Vitamin Premix: Levels per kg of product: Vit. A 2,090,000 IU, Vit. E 7.600 mg, Vit. D3 332.500 IU, Vit. K3 950 mg; Nicotinic Acid 8.500 mg, Vit. B1 475mg, Vit. B12 3.800mcg, Vit B2 1.900 mg, Vit B6 950 mg, Folic Acid 237.5 mg, Biotin 38mg, Choline 72.000mg; 3.800mg Pantothenic Acid, Copper 12.400mg; 12.000mg Iron, Iodine 160mg, Manganese 14.000 mg, Selenium 108 mg and Zinc 14.000 mg. [2]Canthaxanthin 10% (Carophyll Red; DSM Nutritional Products Ltd., Basel, Switzerland). [3]Determined by analysis at LABRUMEN/UFSM (Crude protein (%), Gross Energy (kcal/kg), Ether Extract (%), Mineral Matter (%),Calcium (%) and Total P (%)) and at CBO analyses laboratoriais (Vitamin A (UI/kg), Vitamin D3 (UI/kg), Alpha-Tocopherol (mg/kg), Lutein (mg/kg), Zeaxanthin (mg/kg) and Canthaxanthin (mg/kg)). [4]Content in basal diet. [5]Content in the basal diet supplemented with 6 mg/kg of canthaxanthin.

Table 3. Effect of broiler breeder hens fed with corn or sorghum diets supplemented or not with canthaxanthin on the body weight (kg)¹ and mortality (%)¹.

Factor	Body weight (kg)							Mortality (%)
	42 wk	45 wk	49 wk	53 wk	57 wk	61 wk	65 wk	42-65 wk
Ingredient								
Corn	3.70	3.76	3.85	3.92	3.91	3.98	4.00	0.9091
Sorghum	3.71	3.78	3.85	3.92	3.94	3.99	4.01	0.0000
Canthaxanthin (mg/kg)								
0	3.70	3.77	3.85	3.89	3.92	3.95	3.97	0.4545
6	3.71	3.76	3.85	3.95	3.93	4.01	4.04	0.4545
Interaction								
Corn x 0 Canthaxanthin	3.68	3.75	3.84	3.85	3.92	3.94	3.97	0.9091
Corn x 6 Canthaxanthin	3.73	3.77	3.87	3.99	3,90	4.01	4.04	0.9091
Sorghum x 0 Canthaxanthin	3.72	3.79	3.87	3.93	3.92	3.96	3.98	0.0000
Sorghum x 6 Canthaxanthin	3.70	3.76	3.83	3.92	3.96	4.02	4.04	0.0000
Source of Variation	,,,P Value,,							
Ingredients	0.9359	0.7283	0.9666	0.9502	0.5369	0.8707	0.8792	0.1765
Canthaxanthin	0.8705	0.8527	0.9443	0.2881	0.8294	0.3881	0.1964	1.000
Interaction	0.5693	0.6413	0.5017	0.2099	0.5397	0.8847	0.9720	1.000
Average	3.71	3.77	3.85	3.92	3.93	3.98	4.01	0.45
SEM	0.14	0.11	0.12	0.14	0.09	0.12	0.11	1.44
CV %	3.65	2.84	3.16	3.46	2.37	3.11	2.71	1.44

[1] Data represent means from 5 replicates (i.e., pens) per treatment.

Table 4. Effect of canthaxanthin supplementation or not in corn or sorghum diets on the egg production[1] (%) of broiler breeder hens.

Factor	Egg Production (%)	
	I Period	II Period
Ingredient		
Corn	71.66	55.21
Sorghum	71.38	56.35
Canthaxanthin (mg/kg)		
0	71.72	54.35 [b]
6	71.32	57.21 [a]
Interaction		
Corn x 0 Canthaxanthin	72.08	54.21
Corn x 6 Canthaxanthin	71.24	56.22
Sorghum x 0 Canthaxanthin	71.36	54.49
Sorghum x 6 Canthaxanthin	71.39	58.21
Source of Variation	,,,P Value,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
Ingredients	0.7677	0.2328
Canthaxanthin	0.6750	0.0066
Interaction	0.6549	0.3646
Average	71.52	55.78
SEM	2.11	2.05
CV %	2.95	3.67

[a – b] Means within a row with different superscripts differ significantly ($P < 0.05$).

[1] Data represent means from 5 replicates (i.e., pens) per treatment.

Table 5. Performance of broiler breeder hens fed with corn or sorghum diets supplemented or not with canthaxanthin[1].

Factor	Egg specify gravity (g/m^3)		Egg weight (g)		Egg yolk (%)		Egg albumen (%)		Egg shell (%)		Egg yolk coloration score (1-15)	
	I Period	II Period	I Period	II Period	I Period	II Period	I Period	II Period	I Period	II Period	I Period	II Period
Ingredient												
Corn	1083.83[b]	1084.59	70.86	74.52	29.76	30.58	60.87	59.98	9.28[b]	9.45	12.00[a]	12.00[a]
Sorghum	1085.18[a]	1084.81	71.59	75.03	29.88	30.66	60.60	59.74	9.50[a]	9.60	9.00[b]	9.00[b]
Canthaxanthin (mg/kg)												
0	1084.71	1084.62	71.52	74.99	29.68	30.55	60.88	60.00	9.40	9.46	6.00[b]	6.00[b]
6	1084.30	1084.88	70.93	74.56	29.96	30.69	60.59	59.73	9.38	9.58	14.00[a]	15.00[a]
Interaction												
Corn x 0 Canthaxanthin	1084.59[ab]	1084.57	71.39	75.16[a]	29.57	30.33	61.01	60.27	9.38[ab]	9.40	9.00[b]	10.00[b]
Corn x 6 Canthaxanthin	1083.08[b]	1084.61	70.32	73.88[b]	29.94	30.82	60.74	59.69	9.19[b]	9.49	14.00[a]	15.00[a]
Sorghum x 0 Canthaxanthin	1084.83[a]	1084.47	71.64	74.81[ab]	29.78	30.76	60.76	59.72	9.42[ab]	9.52	3.00[c]	2.00[c]
Sorghum x 6 Canthaxanthin	1085.53[a]	1085.15	71.54	75.24[a]	29.97	30.56	60.43	59.77	9.58[a]	9.68	14.00[a]	15.00[a]
Source of Variation						P value						
Ingredients	0.0040	0.5543	0.1299	0.1635	0.5846	0.7291	0.2555	0.3252	0.0055	0.0602	<.0001	<.0001
Canthaxanthin	0.3184	0.3404	0.2218	0.2402	0.2028	0.5622	0.2210	0.2607	0.8182	0.1062	<.0001	<.0001
Interaction	0.0138	0.3845	0.3074	0.0251	0.6745	0.1704	0.9064	0.1915	0.0209	0.6763	<.0001	<.0001
Average	1084.51	1084.70	71.23	74.78	29.82	30.62	60.74	59.86	9.39	9.52	10.00	11.00
SEM	0.89	0.82	1.03	0.77	0.47	0.54	0.52	0.52	0.15	0.16	0.41	0.30
CV %	0.08	0.07	1.45	1.03	1.58	1.77	0.86	0.86	1.59	1.74	4.11	2.79

[a – b - c] Means within a row with different superscripts differ significantly ($P < 0.05$).

[1] Data represent means from 5 replicates (i.e., pens) per treatment.

Table 6. Reproductive responses evaluated in broiler breeder hens fed with corn or sorghum diets supplemented or not with canthaxanthin[1].

Factor	Hatching (%)		Hatchability of fertile eggs (%)		Fertility (%)		Chick weight (g)		Contaminated (%)		Pipped (%)		Second quality chick (%)	
	I Period	II Period	I Period	II Period	I Period	II Period	I Period	II Period	I Period	II Period	I Period	II Period	I Period	II Period
Ingredient														
Corn	83.12	79.37	87.19	87.61	95.25	90.99	51.11	52.30	0.43	0.68	3.22	3.73	6.13	6.64
Sorghum	85.53	78.63	88.06	87.12	97.10	92.28	50.76	52.80	0.34	0.55	3.01	4.01	6.69	7.71
Canthaxanthin (mg/kg)														
0	83.68	77.37	87.47	86.81	95.57	90.72	51.11	52.89	0.32	0.73	2.89	4.34	6.75	7.90
6	84.97	80.63	87.79	87.93	96.78	92.55	50.76	52.20	0.45	0.49	3.34	3.41	6.08	6.45
Interaction														
Corn x 0 Canthaxanthin	81.27	79.06	85.90	87.38	94.52	91.22	51.47	52.93	0.42	0.54	3.56	4.30	7.04	6.88
Corn x 6 Canthaxanthin	84.96	79.68	88.48	87.84	95.98	90.77	50.76	51.66	0.44	0.81	2.89	3.16	5.23	6.40
Sorghum x 0 Canthaxanthin	86.08	75.68	89.04	86.24	96.62	90.22	50.76	52.85	0.23	0.92	2.22	4.37	6.46	8.91
Sorghum x 6 Canthaxanthin	84.97	81.58	87.09	88.01	97.58	94.33	50.75	52.74	0.46	0.18	3.79	3.65	6.92	6.51
Source of Variation	P Value													
Ingredients	0.3411	0.8695	0.5274	0.8016	0.3792	0.6729	0.4629	0.2164	0.5920	0.7104	0.6982	0.7018	0.5822	0.4682
Canthaxanthin	0.6063	0.4731	0.8170	0.5657	0.5627	0.5485	0.4618	0.0944	0.4133	0.4957	0.4312	0.2238	0.5089	0.3322
Interaction	0.3431	0.5595	0.1116	0.7342	0.9058	0.4561	0.4806	0.1559	0.4890	0.1565	0.0604	0.7795	0.2708	0.5147
Average	84.32	79.00	87.63	87.27	96.17	91.63	50.93	52.55	0.38	0.61	3.12	3.87	6.41	7.17
SEM	5.49	9.92	3.01	4.27	4.68	6.67	1.06	0.86	0.34	0.76	1.23	1.64	2.23	3.23
CV %	6.51	12.56	3.43	4.88	4.76	7.28	2.09	1.65	87.98	124.65	39.69	42.47	34.75	45.02

[1] Data represent means from 5 replicates (i.e., pens) per treatment.

Table 7. Total embryonic mortality[1,2] (%) in broiler breeder hens fed with corn or sorghum diets supplemented or not with canthaxanthin.

Factor	Total embryonic mortality (%)		EM1[3] (%)		EM2[4] (%)		EM3[5] (%)		EM4[6] (%)	
	42-53 wk	54-65 wk	42-53 wk	54-65 wk	42-53 wk	54-65 wk	42-53 wk	54-65 wk	42-53 wk	54-65 wk
Ingredient										
Corn	8.67	8.96	4.02[a]	3.93	1.25	0.98	1.97	2.10	1.43	1.95
Sorghum	7.44	9.73	2.75[b]	4.01	1.15	1.49	1.67	2.23	1.87	2.00
Canthaxanthin (mg/kg)										
0	8.58	9.72	3.50	4.74	1.35	1.03	1.78	2.18	1.94	1.76
6	7.53	8.98	3.27	3.20	1.05	1.44	1.86	2.15	1.35	2.18
Interaction										
Corn x 0 Canthaxanthin	9.82	9.16	4.12	3.90	1.68	0.92	2.15	2.40	1.87	1.95
Corn x 6 Canthaxanthin	7.52	8.76	3.92	3.97	0.81	1.04	1.79	1.81	0.99	1.95
Sorghum x 0 Canthaxanthin	7.33	10.27	2.88	5.59	1.02	1.13	1.41	1.97	2.02	1.57
Sorghum x 6 Canthaxanthin	7.55	9.19	2.62	2.43	1.29	1.84	1.93	2.49	1.72	2.42
Source of Variation					P value					
Ingredients	0.1974	0.6516	0.0487	0.9596	0.7288	0.1186	0.3572	0.8011	0.2788	0.8820
Canthaxanthin	0.2737	0.6633	0.7048	0.3070	0.2767	0.2001	0.8043	0.9511	0.1495	0.2222
Interaction	0.1891	0.8400	0.9751	0.2873	0.0512	0.3582	0.1931	0.2932	0.4667	0.2195
Average	8.06	9.35	3.39	3.97	1.20	1.23	1.82	2.17	1.65	1.97
SEM	2.05	3.73	1.33	3.28	0.60	0.69	0.72	1.13	0.86	0.74
CV %	25.49	39.92	39.38	82.53	50.16	56.33	39.40	52.33	52.75	37.75

[a – b] Means within a row with different superscripts differ significantly ($P < 0.05$).

[1] Data represent means from 5 replicates (i.e., pens) per treatment.

[2] Total embryonic mortality was analyzed statistically after being normalized by $\sqrt{(x + 1.5)}$.

[3] EM1 = embryonic mortality at the first 48 hs of incubation.

[4] EM2 = embryonic mortality occurring between 72 hs and 168 hs of incubation.

[5] EM3 = embryonic mortality occurring between 192 hs and 336 hs of incubation.

[6] EM4 = embryonic mortality occurring between 360 hs and 504 hs of incubation.

CAPÍTULO III

Corn or sorghum diets and canthaxanthin effect on lipid peroxidation, fatty acid composition of fertile eggs and offspring's performance of broiler breeder

Este capítulo é apresentado de acordo com as normas para publicação no **Periódico Poultry Science.**

Corn or sorghum diets and canthaxanthin effect on lipid peroxidation, fatty acid composition of fertile eggs and offspring's performance of broiler breeder

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ABSTRACT The effects of the corn or sorghum diets supplemented or not with canthaxanthin on lipid peroxidation, fatty acids profile of fertile eggs and offspring's performance of broiler breeder were studied. Forty four hundred and sixty females and roosters were placed in an open-sided house with 20 pens, each pen with 4.615m² from 45 to 65 weeks of age. At the 45 weeks of age, the breeder hens and roosters were distributed in four experimental diets with two ingredients; corn (CO) or sorghum (SO) and two levels of canthaxanthin; 6mg/kg (CX) and 0mg/kg (NCX), with similar body weight (BW) and uniformity. BW was measured every 28 days and your mortality in the end. Two incubations were recorded to evaluate incubation parameters and your offspring's performance. Thiobarbituric Acid Reactive Substances (TBARS), vitelline membrane strength (VMS), carotenoids concentration, fatty acids profile in egg yolk and offspring's performance were determinated. BW and mortality were not affected by the inclusion or not of CX in CO or SO breeder's diets. The lowest TBARS was found in SO+CX. The VMS was light in egg yolks produced by hens fed with CO+NCX. The CO diets had the highest level of lutein (mg/kg) and zeaxanthin (mg/kg) in egg yolk. It showed as a rich fount of these carotenoids, but CX results shows an interesenting level concentration in SO+CX. The Profile of Saturated and Monounsaturated Fatty Acids shown the highest concentration in CO, but sorghum in breeder's diets improved the Profile Polyunsaturated Fatty Acids shown the highest concentration in egg yolk, therefore, the supplementation of CX in diets showed a low level of TBARS and offspring´s mortality and improved the viability of the offspring. It is a great opportunity to keep going the positive effect of this carotenoid in material raw with low level of carotenids in breeders' diets.

Key words: carotenoids, thiobarbituric reactive substances, vitelline membrane strength.

INTRODUCTION

Breeding programmes are designed to produce a broiler with high potential for growth, yield and feed efficiency. Where, the broiler production is the quickest means of achieving high-quality animal protein in shortest possible time. In raising poultry, feed expenditure accounts are about 70% - 75% of the total cost. These traits may compromise your performance, because they are designed to maximise this genetic potential, since there is a trade-off between growth and health related traits with responses to husbandry programs, age, sex and genetic line (Siegel et al., 2001; Esonu et al., 2006; Sharif et al., 2012).

Sorghum is the fifth most cultivated cereal in the world, being surpassed only by wheat, rice, corn and barley. Sorghum has high dry matter production and characteristics of tolerance to drought and heat (Fornasieri Filho & Fornasieri 2009). Sorghum presents excellent nutritional value which it allows the partially or totally replace of corn in poultry diets (Assuena et al., 2008). However, it has low carotenoids content if compared with the corn, resulting in egg yolks with a low pigmentation level. This problem may be solved by the inclusion of pigments in layer diets (Assuena et al., 2008).

The fundamental importance of carotenoids in poultry nutrition is based on the fact that these substances are the principal's compouds of egg yolk color and skin chicken color, and they may be controlled by inclusion in diet (Bornstein and Bartov, 1966). Moreover, the deposition of carotenoids into the yolk is subject to regulation by a range of factors including diet, laying order, reproductive output, maternal infection and paternal ornamentation (Royle et al., 1999; Saino et al., 2002; Bortolotti et al., 2003). It has been shown that increase of vitamin E (Surai, 1999) or carotenoid concentrations (Surai, 2002) in the egg yolk are associated with increased resistance in the lipid peroxidation. Such protection is likely to be important, because several tissues of the avian embryo are rich in highly polyunsaturated lipids, which are very susceptible to peroxidative attack (Speake et al., 1998).

Canthaxanthin in the egg is trasferred from the yolk developing embryo and distributed in many organs and tissues (Llaurado et al., 1997; Surai et al., 2003; Karadas et al., 2005) therefore, it might help protect the developing bird against oxidative damage, particularly during the sensitive periods of hatching and early posthatch life (Robert et al., 2007). Postnatal development of the chicken is a crucial time for the maturation of major physiological systems, including the immune system, as well as, the time of high risk of peroxidation (Surai, 2002). Thefore, an increased of supplementation in maternal diet with carotenoids, in particular CX, it could help to maintain the antioxidant system efficiently and increase the chick viability. There are several studies in literatures of sorghum use with the inclusion of artificial and natural pigments in poultry feeds, and report that them do not interfere in poultry performance, meat and egg quality (Sklan et al., 1989; Harder et al., 2010; Moura et al., 2011). The enhancement of the antioxidant effect on the fertile egg and offspring's performance of broiler breeder as a result of supplementing of canthaxanthin in corn or sorghum in maternal diets will be evaluated in this study.

MATERIALS AND METHODS

The present study was carried out in the Poultry Science Laboratory-LAVIC at the Federal University of Santa Maria (UFSM). The study was dived in two experiments: **Experiment I**: 660 broiler breeder hens and 90 roosters Cobb 500 (22 wks old) were acquired from a commercial poultry company to be used in the trial. They were place in an open-sided house with a wood shaving floor. Birds were reared following broiler breeder guidelines (Cobb, 2008; Rostango et al., 2005). The trial was initiated from 45 to 65 wks of age. At 45 wks of age, the breeder hens and roosters were weighed, and 440 hens and 60 roosters were selected according to the body weight (BW) in order to compose the experimental groups.

The selected birds were placed in 20 pens; each pen had 4.615m² (3.24 x 1.42m) and, each pen was equipped with an automatic drinker, one tube feeders to the breeder hens, and a trough-type feeder for the roosters. The corn or sorghum and soybean meal were used as base to elaborate the experimental diets, which all of them were bought at the same place. After of this, a sample was collected, and sent at Laboratório de Bromatologia e Nutrição de Ruminantes – (LABRUMEN-UFSM) Santa Maria (RS), Brazil and CBO laboratories in Campinas (SP), Brazil to make bromatological analysis and carotenoids concentrations at DSM Nutritional Products Ltd – Switzerland. After corn, sorghum and soybean meal was done (Table 1), the experimental diets were formulated according of the nutritional needs of Cobb 500 broiler breeders and subsequently, a sample of the diets was collected and sent to the same laboratories (Table 2).

Birds were fed with corn or sorghum-soybean-based mash diets supplemented or not with canthaxanthin. The supply of the feed was strictly controlled, in accordance with the recommendations of the breeder company. Water was *ad libitum*, and a photoperiod of 13 hours light/day was used during the first week (22 wks), gradually, it was increasing until they received 16 hours and 30 minutes of light/day at 54 wks until at the end of experiment.

Experiment II: To determinate the offspring's performance at 54 and 64 wks of age of broiler breeder of the experiment I, 320 chicks hatched males (1 day old) were used in each one, they were sexed and after of this weighted. The experiment was from 1 to 21 days of age,. The selected birds were housed in battery with 5 floors of 20 compartments in 40 pens. Each pen was 0.5m² and equipped with one feeder type channel and two nipple drinkers. Birds were reared following the Cobb 500 broiler guidelines, with environment controlled. Water and feed were provided *ad libitum* for 21 days being that birds received the same basal diet (Table 2).

Treatments

Experiment I: The birds were distributed in 4 experimental groups with similar BW and uniformity (average of 3.77kg and uniformity of 90%). Each one of the 4 experimental treatments was randomly assigned with 5 replicates, each one with 22 hens and 3 roosters by pen. The birds were fed with the same basal diet (corn and soybean) from 22 until 42 wks old. From 45 to 65 wks of age, the 4 groups were fed with the different experimental diets. First group received corn diet with 0mg/kg of canthaxanthin (Carophyll Red; DSM Nutritional Products Ltd., Basel, Switzerland), second group received corn diet with 6mg/kg of canthaxanthin; third group received sorghum diet with 0mg/kg of canthaxanthin and fourth group received sorghum diet with 6mg/kg of canthaxanthin. Hens and roosters received the same diets in all periods (Table 2).

Experiment II: The offspring's were distributed into 4 experimental groups with similar BW and uniformity. The 4 experimental groups were randomly assigned in 10 replicates, each one, with 8 chicks by pen. The 4 groups were divided in chicks coming from broiler breeders fed with four different diets like demonstrated at the experiment I.

Experimental responses measured

Experiment I: To evaluate BW, hens and roosters were weighted every 28 days and the accumulated mortality (total number of dead birds/initial number of birds x 100%) was calculated in the end of the experiment. Eggs were collected and recorded 6 times per day. The laying rate per hen housed was calculated weekly.

Lipid oxidation was assessed on the basis of Malondialdehit (MDA) formed during refrigerated storage time. MDA was a compound used as an index of lipid peroxidation (Botsoglou et al., 2005). The antioxidant potential of canthaxanthin deposited in the egg was evaluated through the Thiobarbituric Acid Reactive Substances (TBARS) method as

described by Jentzsch et al. (1996). To evaluate the antioxidant effect of canthaxanthin at 50, 54, 58 and 62 wks of age in the total experimental period, 15 egg yolks/treatment (5 replicates of 3 egg yolk each) were colleted and recorde by pen and stored in a temperature-controlled room (18°C) by 7 days. After of this was done a pool of each replicate, totaling 20 samples (3 pooled egg yolks each one).

To determinate the vitelline membrane strength (VMS) in fresh egg yolks was used 15 egg yolks/treatment (5 replicates of 3 eggs each). The samples were analyzed according the method of Lyon et al. (1972), taking care not to be near the germinal disc and chalaza. This test was evaluated 6 times during the experiment (50, 52, 54, 56, 58 and 60 wks of age in the total experimental period) using TA.XT Plus Texture Analyzer 123 with capacity of 50 pounds of force. The test was done with a needle with a rounded tip of 2mm in diameter, test speed of 3.20mm/s, post-test speed of 10.00mm/s, distance of 36mm and auto force of 0.1g applied on the egg yolk.

To determinate the content of xanthophylls; canthaxanthin (mg/kg), lutein (mg/kg) and zeaxanthin (mg/kg) were collected and recorded with 15 egg yolks/treatment (5 replicates of 3 eggs each) at 54 and 64 wks of age in the total experimental period. After of this was done a pool of each replicate, totaling 20 samples (3 pooled egg yolks each one) and sent to Mycological Research Laboratory (LAPEMI/UFSM) Santa Maria (RS), Brazil to be lyophilized. When the lyophilization of samples was finished, they were sent to DSM Nutritional Products Ltd – Switzerland to be analysis.

The fatty acids profile was determinate using 15 egg yolks/treatment (5 replicates of 3 eggs each) at 54 wks of age. They were collected, recorded and sent to analysis at CBO laboratories in Campinas (SP), Brazil.

For each treatment, fertility, hatchability of fertile eggs, hatchability of total eggs set and healthy chicks were recorded in 2 incubations throughout the experiment (54 and 64 wks

of age). Eggs were collected 6 times daily and classified by pen for hatching process. Only clean and without visible abnormalities eggs were considered suitable for incubation, thereafter, they were stored in a period of 7 days in a room with controlled temperature (18-20°C and 75-80% Relative Humidity). The incubation was carried out in a commercial multi-stage incubator (Casp, Amparo, SP, Brazil) at 37.5°C and 60% Relative Humidity. On day 18, the eggs were transferred to the hatcher with 36.5°C and 65% Relative Humidity to complete the incubation. At 21 days, chicks were taken out of hatcher, weighed and classified into first and second-quality. Chicks were considered second quality, when they possessed bad umbilical scarring, beak abnormalities, leg weakness or excessively wet downy feathers, therefore, the chicks without these charateristics were considered as first quality. The hatching rate was determinated in relation of the total incubated eggs. To evaluate the hatching rate of fertile eggs, fertility and embryonic mortality, the nonhatched eggs were submitted to embryo diagnostics. They were classified throughout of a macroscopic visual examination as infertile, embryonic mortality at the first 48 hs of incubation (EM1); embryonic mortality occurring between 72 hs and 147 hs of incubation (EM2); embryonic mortality occurring between 192 hs and 336 hs of incubation (EM3), embryonic mortality occurring between 360 and 504 hs of incubation (EM4). The egg pipped was classified as dead-pipped embryos or pipped eggs that have broken the shell but nor emerged at the time to removal of chicks from the hatcher and the embryo is still alive. Eggs with abnormal contents with green or black coloration and emitting rotten smell or eggs that blow up on opening were considered contaminated.

Experiment II: To determinate the offspring's performance, the birds were weighed at 1 day of age by a replicate basis. Body weight (g), body weight gain (g), feed intake (g), feed conversion (g/g) and cumulative mortality (%) were measured at 7, 14 and 21 days of age. The viability (%), average daily gain (%) and production efficiency index (%) were measured at 21 days of age.

Experimental design and statistical analysis

The experimental design was a completely randomized design in a factorial arrangement with two ingredients (corn and sorghum) and two leves of canthaxanthin (0 mg/kg and 6 mg/kg), totalizing four treaments with five replicates pens of 22 females and 3 roosters of Cobb 500 broiler breeders each one. All the data were subject to Analysis of Variance. The chicks coming from broiler breeders had the same statistical design of the broilers breeders, totalizing four treaments with 10 replicates pens of 8 chicks each one. When it was observed significant differences at 5% in the variance average was applied Tukey test for comparison among the treatments. Statistical procedures were performed by the use of SAS software (2009). The variables of the profile fatty acids and mortality of the offspring were corrected by the ajust of normality with square root of X.

RESULTS AND DISCUSSION

The body weight and mortality were not affected by corn or sorghum breeder's diets per 20 wk supplemented or not with 6ppm of canthaxanthin (Data not shown). Rosa et al. (2012) showed that broiler breeders fed with 0 or 6ppm of canthaxanthin from 46 to 66 wks of age had the same BW.

MDA is widely used as a marker to evaluate the degree of lipid oxidation in foods and oxidative stress in biological samples through the testing of TBARS (Rocha et al., 2010). Various antioxidants, such as carotenoids, selenium and vitamin E can provide protective effects against lipid peroxidation in embryonic tissues and resistance to lipid peroxidation on the egg yolk (Surai and Speake, 1998; Surai et al., 1999; Surai, 2000; Surai, 2002). However, carotenoid-fed females produced eggs with reduced susceptibility to lipid peroxidation in vitro, suggesting that carotenoid availability can limit the egg quality (Blount et al., 2002). The idea of exploiting the beneficial effects of dietary CX supplementation in breeder diets

was further developed by Zhang et al. (2011) which, reported that egg yolk enrichment with CX was associated with a significant improvement of the antioxidant status on the egg yolk ($P<0.05$), therefore, these important improvements in the antioxidant status of egg yolk could be due that CX transfer or improvements of vitamin E status (Surai et al., 2003). In the present study, the CX supplementation in breeder's diets had a significantly effect on the antioxidants status on the egg yolks ($P=<0.0001$) in the total experimental period. As well as the sorghum diets with supplementation of canthaxanthin showed the lowest TBARS ($P=0.0002$) in the egg yolks. But, the egg yolks of broiler breeder fed without supplementation of canthaxanthin had the worst TBARS (Table 3) in the total period. Despite the sorgum had showed low level of lutein and zeaxanthin in grain (Table 1) and diet (Table 2), the supplementation of 6ppm of canthaxanthin in diet allowed it has better antioxidant status than the others egg yolks. But, despite the corn had had an important level of lutein and zeaxanthin in grain and diet, when the canthaxanthin was supplemented, it was not showed better TBARS than sorghum.

In a field trial, Robert et al. (2007) studied the effect of canthaxanthin in Ross breeders on the antioxidant status of their progeny. They observed that the antioxidant status of sera of 1-day old, chicks were significantly higher and the TBARS level significantly lower with 6 ppm canthaxanthin than those not supplemented in the breeder feed. According to these authors, the results indicated that the maternal supplementation with canthaxanthin (6ppm) enhanced antioxidant capability and depressed oxidative stress in chicks. Rosa et al. (2012) in an experiment with broiler breeders fed with 6ppm of canthaxanthin or not from 46 to 66 wks of age observed a reduction of TBARS in yolks from stored hatching eggs produced by breeders fed CX. The reduction in TBARS was observed in eggs submitted for analysis on the same day that they were produced ($P=0.0214$) and in eggs stored for four ($P<0.0002$), eight ($P<0.0003$) and twelve days ($P<0.0001$).

The vitelline membrane of the egg separates the yolk from albumen. It is also the last barrier to microorganisms invading the yolk. Structural integrity of the vitelline membrane is important to prevent microorganisms from entering nutrient-rich yolk (Tan et al., 1992).

The VMS evaluated in the different grains demonstrated that VMS was lighter in eggs produced by hens fed with corn diets than sorghum diets (P=0.0235), and when added the canthaxanthin this parameter had the same effect (P=0.0145) (Table 3). Yolk vitelline membrane has an essential role in embryogenesis as well; the sperm has to penetrate the membrane for fertilization to occur (Sim et al., 2000). Many factors can affect the quality and strength of the vitelline membrane, with storage duration and temperature being the most important (Feeney et al., 1956; and Kato et al., 1979). In anothers researchs about the inclusion of antioxidants in diets Scheideler et al. (2010) when evaluated diets in laying hens for 12 wks during summer (34 to 35°C high temperatures daily), to investigate the effects of feeding higher levels of the dietary antioxidants DL-α-tocopherol and selenium (inorganic or organic) on egg production and egg quality. High basal levels of selenium were present in the corn-soybean meal diets (0.25ppm), resulting in selenium treatment levels of 0.55 or 0.75ppm. The supplemented α-tocopherol treatments were 50, 100, or 150 (IU/kg) of the diet. When the hens were supplemented with vitamin E, the fresh eggs and egg stored per 2 wks the yolk vitelline membrane was improved. In summary, vitamin E and selenium can be supplemented to a laying hen ration to improve the vitelline membrane strength of fresh and aged eggs, while also increasing the levels of these nutrients in the egg yolk.

The deposition of canthaxanthin in egg yolk is directly proportional their level on diet (Bornstein and Bartov, 1965; Braunlich, 1974; Tyczkowski and Hamilton, 1986; Grashorn et al., 2000). Canthaxanthin did not affect vitamin A concentration in the egg yolk, embryonic or postembryonic chick tissues. In general, canthaxanthin is considered to be a non-provitamin A carotenoid. However, in circumstances of vitamin A deficiency, canthaxanthin probably could

also be converted to vitamin A (Hudon, 1994) and this carotenoid may in fact be a substrate for 15,15¹-carotene-dioxygenase (EC 1.13.11.21; Canfield et al., 1992). At the present study, the analysis of canthaxanthin was not according as the expected, because it was higher in sorghum grain (Table 1) and sorghum diet supplemented with CX (Table 2) than corn grain and diet. Nevertheless, to lutein and zeaxanthin concentrations were the highest in corn. The deposition of CX in the egg yolk was the higest in sorghum diet supplemented with CX ($P<0.0001$), however the sorghum diet without supplementation of CX had the lowest deposition of CX in egg yolk (Table 4). The corn diets showed the highest lutein ($P<0.0001$) and zeaxanthin ($P<0.0001$) concentrations in egg yolk. It shows as corn is a rich source of carotenoids to the egg yolk (Table 4). To address some features of CX activity in breeder chicken, a study was conducted at the Scottish Agricultural College (SAC) (Surai et al., 2003) using 320 female's broiler breeder. Four treatments were compared, a control diet containing <2mg/kg total xanthophylls, and then test diets supplemented with either 3, 6, 12 and 24mg/kg of a comercial CX product (CAROPHYLL® Red 10% (Roche Vitamins (UK) Ltd, Heanor, UK). The trial data showed that inclusion of CX into the maternal diet caused a significant dose-dependent response in terms of its accumulation in the egg yolk. The most important finding of this study was a positive effect of CX on vitamin E in the developing chicks.

Researches have shown that over 90% of the total energy required by the embryo is provided by the β-oxidation of lipids derived from the yolk (Noble and Cocchi, 1990; Romanoff, 1960; Cherian and Sim, 1993; Farkas et al., 1996a; Speake et al., 1996, 1998). During the incubation occur changes in the fatty acid composition (Noble and Cocchi, 1990). Thus, the changes in the composition of fatty acids are important and occur in several times during their development embrionary. A significant effect in the concentration of saturated fatty acids on the egg yolk was found (Table 5), pointing out that fertile eggs are rich sources

of saturated fatty acids, especially those egg yolks coming from broiler breeder fed with corn diets (P<0.0001). When analyzing differents fatty acids in the egg yolk, the lauric acid (C12) coming from broiler breeder fed with corn diet supplemented with CX had better concentration than sorghum diet supplemented with this carotenoid (P=0.0362). The broiler breeder fed with corn diet had higher myristic (C14), palmitic (C16), arachidic (C20) and lignoceric (C24) acids than broiler breeder fed with sorghum diet (P=0.0429, P=0.0003, P=0.0167, P=<0.0001, respectively). Meantime, the same effect of the corn diet observed in saturated fatty acids was shown in monounsaturated fatty, palmitoleic (C16:1) and oleic (C18:1n9c) acids (Table 6) (P=0.0003, P=0.0391, P=0.0019, respectively).

In the Polyunsaturated Fatty Acids, the sorghum diets showed a better concentration than corn diets (Table 7). To linolenic acid (C18:3n3), egg yolks of broiler breeder fed with sorghum diets had a higher concentration than corn diets (P<0.0001). Linolenic acid is specifically required during the embryonic development and early post-hatch growth, because, significant concentrations of some long chain fatty acid are derived from the linolenic lipids and, they are found in the retina and brain (Budowski and Crawford, 1986; Anderson et al., 1989). Linoleic acid can affect the fluidity, permeability, receptor activity and enzymatic function of biomembranes by the changing fatty acid composition (Murphy, 1990). When supplementation of this essential fatty acid is deficient in bird diets, your performance is also affected, such as, reduction in the egg production; mortality increased during incubation and reduced growth (Hertad et al., 2000). In this research, the sorghum diets showed the highest concentrations of linoleic acid (C18:2n6c) (P=0.0002). Concerning to arachidonic acid (C20:4n6) of egg yolks coming from broiler breeder fed with corn diets showed a higher level concentration than sorghum diets (P=0.0005). In relation to the results of docosahexaenoic acid (DHA) (C22:6n3), the sorghum diets showed higher concentration than corn diets (P=0.0001). Maldjian et al. (1995) mentioned that arachidonic acid is

relatively resistant to β-oxidation during embryogenesis and exits activities conversion of linoleic acid to arachidonic acid in the yolk sac. Cherian et al. (1997) also suggest that the concentration of arachidonic acid and DHA are different during the incubation, because these acids show a transfer standard from the yolk to the embryo different of the others. Chick embryo tissues contain a high proportion of polyunsaturated fatty acids in the lipid fraction and therefore need antioxidant defence. Tissues of newly hatched chicks express a range of antioxidant defences including natural antioxidants and antioxidant enzymes as well as antioxidant enzyme cofactors (Se, Zn, Mn and Fe) (Surai, 2002). The relationship of polyunsaturated fatty acids and saturated fatty acids was not significative, but to the relationship of n6/n3, the corn diets had a better relationship than sorghum diets (P=<0.0001) (Data not shown).

The parameters of hatching (%), hatchability of fertile eggs (%), fertility, chick weigh, contaminated, pipped and total embryonic mortality (%), EM1, EM2, EM3, EM4 were not affected by the supplementation or not of CX in corn or sorghum diet in their interactions (P>0.05) (data not shown). The carotenoid pigmentation in poultry is also involved in growth metabolism and fertility (Scheldt, 1998), whereas others have protective mechanisms in the body and act as physiological antioxidants (Burton, 1989), thus enhancing the immune system (Bendich, 1989; Blanch, 1999). Leeson and Summers (1997) reported that the protein, amino acids and linoleic acid are the most important nutritional factors that affect the egg weight.

According to Breque et al. (2003), long term storage of spermatozoa is supported by a complex antioxidant defence system present within the oviduct that protects spermatozoa against lipid peroxidation. CX may play an important role in that system. As for the positive effect of CX on hatchability, this can be explained by participation in antioxidant defences during embryonic development (Surai, 2002). The positive effect of CX on hatchability was not showed in this experiment. To Rosa et al. (2012), the most important finding of your

study was the positive effect of CX supplementation on the maternal diet, specifically on fertility, hatchability and embryonic mortality.

Besides of chicks own emerging immune capacity, which is relatively weak at the time of hatching, the chick has access of maternally derived immunoglobulins that are deposited in the yolk during vitellogenesis (Apanius, 1998). In the finding that maternal supplementation not only enhances the carotenoid provision during embryonic life, but also continues influencing the chick's carotenoid status throughout the first week after hatching, suggests that this maybe an effective way to boost the antioxidant defences of the offspring (Surai, 2002). Since CX possesses antioxidant properties in vitro and in vivo (Surai, 2002), and it is effectively transferred from the diet to the egg and, further to the developing embryo, it can be considered as an effective modulator of the antioxidant system of the egg and the embryo. Despite of these reserches and affirmations, some studies of domestic hens have shown that supplementation of the maternal diet with carotenoids (β-carotene (Haq et al., 1996)), combination of lutein, citranaxanthin, canthaxanthin and β-apo-80- carotenoic acid (Surai., et al 1998), the results in carotenoid-enrichment of egg yolk and chick tissues reduces tissue susceptibility to lipid peroxidation (Surai, et al 1998) and oxidative stress in vitro (Lawlor et al., 1995). In this research, the offspring of broiler breeder supplemented or not with canthaxanthin in corn or sorghum diets did not show a significantly difference in the BW in the experimental period (Table 8). Taking into account the results presented by Koutsos et al. (2003), which showed that maternal effect of carotenoids on their concentrations in 4 weeks-old chickens, it is possible suggests that various antioxidants, including carotenoids, could affect gene expression during embryonic development. This could result in a better antioxidant defences related to higher hatchability and improved chick viability post-hatch. Zhang et al. (2011) evaluating the addition of canthaxanthin to the BW of 1-day old newly hatched chicks, weight gain in the first 21 days posthatching and feed conversion ratio (FCR)

were not significantly different between the 2 groups (supplemented and not supplemented), and this supplementation in breeder diet reduced the mortality of chicks in the early 21 days posthatching (0 and 4% for CX and control groups). This positive effect was observed in this research, which at the 64 wks of age of broiler breeder, the offspring coming from broiler breeders fed with canthaxanthin in diet had a lesser percentage mortality than offspring coming from broiler breeder fed without canthaxanthin (P=0.0408). It has been shown that the inclusion of canthaxanthin in broiler breeder´s diet, produce a lower 7-day mortality for their progeny (Sarabia, 2010), thanks in part, because it had a better antioxidant capacity (Robert et al., 2007; Zhang et al., 2011). In a field trial, Robert et al. (2008) studied the effect of CX in ROSS breeders on the antioxidant status of their progeny. According to these authors, the results indicated that maternal supplementation with CX (6ppm) enhances antioxidant capability and depresses oxidative stress in chicks. Due to the results found in this research, the viability was better in offspring coming from broiler breeder fed with cathaxanthin than offspring coming from broiler breeder fed without canthaxanthin at 64 wks of age (P=0.0408). According with Koutsos et al. (2003) and Karadas et al. (2005), highlighted that consumption of carotenoids by broiler breeders, besides influencing the incorporation of the tissue's offspring, may also help to increase the viability thereof. However, Surai et al. (2003) concluded that carotenoids can modulate the antioxidant system of the chicken embryo, thereby helping to maintain its efficiency. Thus, the alternative substitution of corn by sorghum in broiler breeder diet did not change the performance of progeny, adding canthaxanthin had not effect on performance (Table 9). In the present study, the levels of stress, bacterial and viral infections were low, and the benefits of an enhanced antioxidant status attributable to canthaxanthin supplementation may not have been observed in your total expresion.

In conclusion in the present study, the sorghum diet supplemented with canthaxanthin showed the best antioxidant status of the egg yolk (TBARS) and, canthaxanthin supplementation in diets reduced the mortality and improved the viability of the offspring. The corn diets showed better saturated and monounsaturated profile acids than sorghum, but it showed better polyunsaturated acids profile in egg yolks and VMS than corn diets. Even more researches to clarify the positive effect of canthaxanthin supplementation in material raw with low level of carotenoids and how acts to improve the reproductive characteristic of broiler breeder and offspring's performance requires further studies.

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Table 1. Nutritional composition of corn, sorghum and soybean grain.

Nutritional composition			
Ingredient [1]	Corn Grain	Sorghum Grain	Soybean meal
Humidity (%)	11.37	13.14	12.32
Dry matter (%)	88.63	86.86	87.68
Crude Protein (%)	7.71	7.48	45.36
Gross Energy (kcal/kg)	3855	3895	4030
Crude fibre (%)	1.40	1.80	5.07
Ether extract (%)	4.40	1.72	2.02
Mineral matter (%)	1.21	1.45	6.23
Calcium (%)	0.01	0.14	0.30
Available P (%)	0.26	0.23	0.52
Alpha-Tocopherol (mg/kg)	9.39	4.97	1.06
Lutein (mg/kg)	3.80	0.49	-
Zeaxanthin (mg/kg)	4.25	0.22	-
Canthaxanthin (mg/kg)	5.45	5.54	-

[1] Determined by analysis at LABRUMEN/UFSM (Humidity (%),Dry matter (%), Crude protein (%), Gross Energy (kcal/kg), Crude fibre (%), Ether Extract (%), Mineral Matter (%),Calcium (%) and Available P (%)) and at CBO analyses laboratoriais (Alpha-Tocopherol (mg/kg), Lutein (mg/kg), Zeaxanthin (mg/kg) and Canthaxanthin (mg/kg)).

Table 2. Composition nutritional of corn or sorghum diets supplemented or not with canthaxanthin in broiler breeders and offspring diet.

Item			
Ingredient (%)	Corn Diet (%)	Sorghum Diet (%)	Offspring Diet (%)
Corn	69.06	-	55.15
Sorghum	-	67.58	-
Soybean meal (46% of CP)	21.15	20.68	37.27
Dicalcium phosphate	1.60	1.61	1.81
Limestone, 38% Ca	6.99	7.08	1.04
Salt	0.40	0.40	0.40
Vitamin-mineral premix[1]	0.50	0.50	0.50[6]
DL-Methionine	0.08	0.10	0.11
L-Lysine 98%	-	-	0.09
Vegetable oil	0.22	2.05	4.03
Treaments			
Control	0.000	0.000	-
Canthaxanthin (mg/kg)[2]	6.00	6.00	-
Estimated composition			
Metabolizable Energy (kcal/kg)	2800	2800	3050
Crude Protein (%)	15.50	15.50	22.00
Arginine total (%)	0.93	0.94	-
Lysine total (%)	0.79	0.73	1.30
Methionine total (%)	0.35	0.35	0.57
Methionine + cysteine total (%)	0.63	0.59	0.92
Threonine total (%)	0.57	0.57	0.84
Tryptophan total (%)	0.17	0.20	0.24
Isoleucine total (%)	0.61	0.64	-
Leucine total (%)	1.43	1.49	-
Valine total (%)	0.80	0.82	-
Phenylalanine total (%)	0.80	0.82	-
Calcium (%)	3.20	3.20	1.00
Available phosphorus (%)	0.39	0.39	0.45
Sodium (%)	0.19	0.18	0.20
Potassium (%)	0.60	0.61	-
Nutritionalc omposition analyzed[3]			
Crude Protein (%)	14.80	14.26	-
Gross Energy (kcal/kg)	3583	3680	-
Ether Extract (%)	1.18	2.01	-
Mineral Matter (%)	9.09	10.28	-
Calcium (%)	3.27	3.28	-
Total phosphorus (%)	0.56	0.58	-
Vitamin A (UI/kg)	9620[4] (12900)[5]	5810[4] (9790)[5]	-
Vitamin D3 (UI/kg)	2380[4] (3360)[5]	575[4] (1600)[5]	-
Alpha-Tocopherol (mg/kg)	36.8[4] (37.4)[5]	34.5[4] (32.2)[5]	-
Lutein (mg/kg)	3.84[4] (4.05)[5]	0.95[4] (0.99)[5]	-
Zeaxanthin (mg/kg)	4.41[4] (5.98)[5]	0.28[4] (0.33)[5]	-
Canthaxanthin (mg/kg)	< detection limit [4] (5.88)[5]	< detection limit [4] (6.58)[5]	-

[1]Mineral and Vitamin Premix: Levels per kg of product: Vit. A 2,090,000 IU, Vit. E 7.600 mg, Vit. D3 332.500 IU, Vit. K3 950 mg; Nicotinic Acid 8.500 mg, Vit. B1 475mg, Vit. B12 3.800mcg, Vit B2 1.900 mg, Vit B6 950 mg, Folic Acid 237.5 mg, Biotin 38mg, Choline 72.000mg; 3.800mg Pantothenic Acid, Copper 12.400mg; 12.000mg Iron, Iodine 160mg, Manganese 14.000 mg, Selenium 108 mg and Zinc 14.000 mg. [2]Canthaxanthin 10% (Carophyll Red; DSM Nutritional Products Ltd., Basel, Switzerland). [3]Determined by analysis at LABRUMEN/UFSM (Crude protein (%), Gross Energy (kcal/kg), Ether Extract (%), Mineral Matter (%),Calcium (%) and Total P (%)) and at CBO analyses laboratoriais (Vitamin A (UI/kg), Vitamin D3 (UI/kg), Alpha-Tocopherol (mg/kg), Lutein (mg/kg), Zeaxanthin (mg/kg) and Canthaxanthin (mg/kg)). [4]Content in basal diet. [5]Content in the basal diet supplemented with 6 mg/kg of canthaxanthin.

[6] Mineral and Vitamin Premix: Levels per kg of product: Vit. A 2,090,000 IU, Vit. E 7.600 mg, Vit. D3 332.500 IU, Vit. K3 950 mg; Nicotinic Acid 8.500 mg, Vit. B1 475mg, Vit. B12 3.800mcg, Vit B2 1.900 mg, Vit B6 950 mg, Folic Acid 237.5 mg, Biotin 38mg, Choline 72.000mg; 3.800mg Pantothenic Acid, Copper 12.400mg; 12.000mg Iron, Iodine 160mg, Manganese 14.000 mg, Selenium 108 mg and Zinc 14.000 mg.

Table 3. Effect of canthaxanthin supplementation or not in corn or sorghum diets on TBARS[1] (MDA μg/g) and Vitelline membrane strength[2] (g) of egg yolks of broiler breeder.

Factor	TBARS	Vitelline Membrane Strength
	(μgMDA/g)	(g)
Ingredient		
Corn	20.78	7.76 [b]
Sorghum	20.47	8.09 [a]
Canthaxanthin (mg/kg)		
0	22.67 [a]	7.98
6	18.57 [b]	7.87
Interaction		
Corn x 0 Canthaxanthin	21.80 [a]	7.65 [b]
Corn x 6 Canthaxanthin	19.75 [b]	7.88 [ab]
Sorghum x 0 Canthaxanthin	23.54 [a]	8.32 [a]
Sorghum x 6 Canthaxanthin	17.39 [c]	7.85 [ab]
Source of Variation	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,P Value,,,,,,,,,,,,,,,,,,,	
Ingredients	0.4847	0.0235
Canthaxanthin	<.0001	0.3784
Interaction	0.0002	0.0145
Average	20.62	7,93
SEM	0.96	0.28
CV %	4.68	3.64

[a – b] Means within a row with different superscripts differ significantly ($P < 0.05$).

[1] Data represent means from 5 repetitions of 3 pooled yolks (i.e., pens) per treatment.

[2] Data represent means from 5 repetitions of 3 egg yolks (i.e., pens) per treatment.

Table 4. Effect of canthaxanthin supplementation or not in corn or sorghum diets on the carotenoids levels[1] (mg/kg) of egg yolks of broiler breeder.

Factor	Canthaxanthin (mg/kg)	Lutein (mg/kg)	Zeaxanthin (mg/kg)
Ingredient			
Corn	14.37	14.03 [a]	19.08 [a]
Sorghum	15.42	3.33 [b]	1.58 [b]
Canthaxanthin (mg/kg)			
0	1.92 [b]	8.80	10.60
6	27.85 [a]	8.56	10.06
Interaction			
Corn x 0 Canthaxanthin	3.36 [c]	14.25	19.40
Corn x 6 Canthaxanthin	25.39 [b]	13.81	18.77
Sorghum x 0 Canthaxanthin	0.49 [d]	3.35	1.80
Sorghum x 6 Canthaxanthin	30.36 [a]	3.32	1.36
Source of Variation	,,,P Value,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		
Ingredients	0.1358	<0.0001	<0.0001
Canthaxanthin	<.0001	0.5759	0.4105
Interaction	<.0001	0.6390	0.8775
Average	14.90	8.64	10.33
SEM	1.48	0.93	1.40
CV %	9.99	10.79	13.60

[a – b – c-d] Means within a row with different superscripts differ significantly ($P < 0.05$).

[1] Data represent means from 5 repetitions of 3 pooled yolks (i.e., pens) per treatment.

Table 5. Saturated Fatty Acid Profile [1,2] (%) of egg yolks of broiler breeders fed with corn or sorghum diets supplemented or not with canthaxanthin.

Factor	Saturated Fatty Acid Profile (%)									
	Ac. Lauric C12	Ac. Myristic C14:0	Ac. Pentadecanoic C15:0	Ac. Palmitic C16:0	Ac. Margaric C17:0	Ac. Stearic C18:0	Ac. Arachidic C20:0	Ac. Behenic C22:0	Ac. Lignoceric C24:0	Saturated Fatty Acids
Ingredient										
Corn	0.013	0.256 [a]	0.039	16.87 [a]	0.142 [b]	5.83	0.02 [a]	0.003	0.18 [a]	23.36 [a]
Sorghum	0.006	0.226 [b]	0.040	15.55 [b]	0.172 [a]	5.39	0.01 [b]	0.002	0.02 [b]	21.44 [b]
Canthaxanthin (mg/kg)										
0	0.009	0.235	0.042	16.15	0.153	5.62	0.018	0.002	0.11	22.36
6	0.010	0.247	0.037	16.28	0.159	5.60	0.016	0.003	0.10	22.45
Interaction										
Corn x 0 Canthaxanthin	0.008 [ab]	0.238	0.038	16.78	0.132	5.83	0.020	0.002	0.17	23.22
Corn x 6 Canthaxanthin	0.018 [a]	0.274	0.040	16.97	0.148	5.83	0.020	0.004	0.20	23.50
Sorghum x 0 Canthaxanthin	0.010 [ab]	0.232	0.046	15.51	0.174	5.42	0.010	0.002	0.05	21.49
Sorghum x 6 Canthaxanthin	0.002 [b]	0.220	0.034	15.59	0.170	5.36	0.008	0.002	0.00	21.39
Source of Variation	,,P Value,,,									
Ingredients	0.0944	0.0429	0.8786	0.0003	0.0218	0.0610	0.0167	0.6437	<.0001	<.0001
Canthaxanthin	0.8027	0.3919	0.4490	0.6494	0.6401	0.8991	0.6003	0.6437	0.7181	0.7982
Interaction	0.0362	0.0975	0.2933	0.8521	0.4386	0.9063	0.1284	0.6437	0.1897	0.5886
Average	0.0095	0.241	0.0395	16.21	0.156	5.61	0.017	0.0025	0.1055	22.40
SEM	0.003	0.01	0.01	0.07	0.01	0.09	0.003	0.00	0.03	0.07
CV %	0.29	0.87	0.46	1.77	0.85	3.46	0.27	0.15	2.05	1.59

[a – b] Means within a row with different superscripts differ significantly (*P* < 0.05).

[1] Data represent means from 5 repetitions of 3 pooled yolks (i.e., pens) per treatment.

[2] Saturated Fatty Acid Profile data were analyzed statistically after being normalized by $\sqrt{(x + 1.5)}$.

Table 6. Monounsaturated Fatty Acid Profile [1,2] (%) of egg yolks of broiler breeders fed with corn or sorghum diets supplemented or not with canthaxanthin.

Factor	Monounsaturated Fatty Acid Profile (%)									
	Ac. Myristoleic C14:1	Ac. Palmitoleic C16:1		Ac. Oleic C18:1n9c		Ac. Cis-Eicosenoic C20:1	Ac. Erucic C22:1n9	Ac. Nervonic C24:1	Monounsaturated Fatty Acid	
Ingredient										
Corn	0.049	1.92	a	25.04	a	0.12	0.002	0.004	27.17	a
Sorghum	0.056	1.66	b	22.92	b	0.11	0.003	0.002	24.96	b
Canthaxanthin (mg/kg)										
0	0.046	1.77		23.80		0.11	0.003	0.003	25.92	
6	0.059	1.82		24.17		0.12	0.002	0.003	26.21	
Interaction										
Corn x 0 Canthaxanthin	0.040	1.92		24.73		0.11	0.002	0.004	27.03	
Corn x 6 Canthaxanthin	0.058	1.92		25.36		0.12	0.002	0.004	27.30	
Sorghum x 0 Canthaxanthin	0.052	1.61		22.86		0.10	0.004	0.002	24.81	
Sorghum x 6 Canthaxanthin	0.060	1.72		22.97		0.11	0.002	0.002	25.10	
Source of Variation	,,P Value,,									
Ingredients	0.4982	0.0391		0.0019		0.2184	0.6437	0.3844	0.0003	
Canthaxanthin	0.2163	0.6517		0.5314		0.2184	0.6437	1.000	0.5608	
Interaction	0.6273	0.6517		0.6549		0.7529	0.6437	1.000	0.9836	
Average	0.0525	1.79		23.98		0.11	0.0025	0.003	26.06	
SEM	0.01	0.06		0.13		0.01	0.00	0.00	0.10	
CV %	0.72	3.84		2.51		0.43	0.15	0.16	1.93	

[a – b] Means within a row with different superscripts differ significantly (*P* < 0.05).

[1] Data represent means from 5 repetitions of 3 pooled yolks (i.e., pens) per treatment.

[2] Monounsaturated Fatty Acid Profile data were analyzed statistically after being normalized by √(x + 1.5).

Table 7. Profile Polyunsaturated Fatty Acids [1,2] (%), Unsaturated and Trans Fats (%)² of egg yolks of broiler breeder fed with corn or sorghum diets supplemented or not with canthaxanthin.

Factor	Profile Polyunsaturated Fatty Acids, Unsaturated and Trans Fats (%)											
	Ac. Linoleic C18:2n6c	Ac. Gamma Linolenic C18:3n6	Ac. Linolenic C18:3n3	Ac. Cis-Eicosadienoic C20:2	Ac. Cis-Eicosatrienoic C20:3n6	Ac. Arachidonic C20:4n6	Ac. Cis-Eicosapentaenoic C20:5n3	Ac. Cis-Docosahexaenoic C22:6n3	Polyunsaturated Fatty Acids	Ac. Elaidic C18:1n9t	Trans Fats	Unsaturated Fatty Acids
Ingredient												
Corn	9.71 [b]	0.13	0.25 [b]	0.08 [b]	0.14	1.93 [a]	0.01	0.74 [b]	12.88 [b]	0.09	0.09	40.05
Sorghum	11.62 [a]	0.12	0.59 [a]	0.11 [a]	0.15	1.66 [b]	0.01	1.16 [a]	15.29 [a]	0.07	0.07	40.26
Canthaxanthin (mg/kg)												
0	10.61	0.11	0.43	0.09	0.13	1.85	0.01	0.95	14.06	0.08	0.08	39.99
6	10.73	0.14	0.42	0.10	0.16	1.75	0.01	0.96	14.12	0.09	0.09	40.32
Interaction												
Corn x 0 Canthaxanthin	9.85	0.12	0.26	0.08	0.13	1.91	0.01	0.71	12.96	0.09	0.09	39.99
Corn x 6 Canthaxanthin	9.58	0.15	0.24	0.08	0.15	1.95	0.01	0.78	12.81	0.09	0.09	40.12
Sorghum x 0 Canthaxanthin	11.37	0.11	0.58	0.11	0.13	1.78	0.01	1.18	15.16	0.07	0.07	39.98
Sorghum x 6 Canthaxanthin	11.87	0.12	0.60	0.12	0.16	1.54	0.01	1.13	15.42	0.08	0.08	40.53
Source of Variation							P Value					
Ingredients	0.0002	0.1348	<.0001	0.0072	0.7034	0.0005	0.8300	0.0001	0.0001	0.1092	0.1092	0.7353
Canthaxanthin	0.7579	0.1009	0.9807	0.4047	0.1852	0.1375	0.8300	0.8754	0.9164	0.4335	0.4335	0.5751
Interaction	0.3409	0.8068	0.7179	0.7365	0.8701	0.0377	0.8300	0.4801	0.6768	0.6613	0.6613	0.7253
Average	10.67	0.12	0.42	0.10	0.14	1.80	0.01	0.95	14.09	0.08	0.08	40.15
SEM	0.12	0.01	0.03	0.01	0.01	0.04	0.00	0.05	0.13	0.01	0.01	0.10
CV %	3.56	0.82	2.29	0.81	1.22	2.12	0.33	3.75	3.42	0.79	0.79	1.57

[a – b] Means within a row with different superscripts differ significantly ($P < 0.05$).

[1] Data represent means from 5 repetitions of 3 pooled yolks (i.e., pens) per treatment.

²The Profile Polyunsaturated Fatty Acids and Unsaturated and Trans Fats data were analyzed statistically after being normalized by $\sqrt{(x + 1.5)}$.

Table 8. Body weight[1] (g) of offspring coming from broiler breeders fed with corn or sorghum diets supplemented or not with canthaxanthin.

Factor	Body Weight 54 wk (g)				Body Weight 64 wk (g)			
	1d	7d	14d	21d	1d	7d	14d	21d
Ingredient								
Corn	51.59	162.94	471.11	987.43	52.53	180.81	487.76	988.74
Sorghum	51.61	165.90	477.59	994.07	52.57	185.36	496.56	997.59
Canthaxanthin (mg/kg)								
0	51.62	166.53	478.18	1004.11	52.63	182.54	492.61	993.89
6	51.58	162.31	470.53	977.39	52.48	183.63	491.71	992.44
Interaction								
Corn x 0 Canthaxanthin	51.61	164.40	472.93	998.23	52.60	178.05	485.40	986.86
Corn x 6 Canthaxanthin	51.58	161.48	469.30	976.63	52.46	183.58	490.13	990.63
Sorghum x 0 Canthaxanthin	51.63	168.65	483.43	1009.99	52.65	187.04	499.83	1000.93
Sorghum x 6 Canthaxanthin	51.59	163.15	471.75	978.15	52.49	183.69	493.29	994.25
Source of Variation	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,P Value,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,							
Ingredients	0.7844	0.3569	0.4490	0.7513	0.7765	0.2054	0.4424	0.6833
Canthaxanthin	0.4138	0.1929	0.3718	0.2069	0.2602	0.7597	0.9366	0.9464
Interaction	1.000	0.6875	0.6371	0.8070	0.9246	0.2166	0.6220	0.8095
Average	51.60	164.42	474.35	990.75	52.55	183.08	492.15	993.17
SEM	0.14	10.04	26.75	53.02	0.41	11.16	35.80	68.05
CV %	0.27	6.11	5.64	6.64	0.79	6.09	7.27	6.85

[1] Data represent means from 10 replicates (i.e., pens) per treatment.

Table 9. Offspring performance[1] coming from broiler breeder fed with corn or sorghum diets supplemented or not with canthaxanthin.

Factor	Body weight gain 1-21days		Feed consumption/bird 1-21days		Feed conversion 1-21days		Mortality 1-21days		Viability		Average daily gain		Production efficiency index	
	54 wk	64 wk	54 wk	64 wk	54 wk	64 wk	54 wk	64 wk	54 wk	64 wk	54 wk	64 wk	54 wk	64 wk
	(g)		(g)		(g/g)		(%)		(%)		(%)		(%)	
Ingredient														
Corn	935.84	936.88	1305.15	1332.37	1.40	1.42	1.61	1.25	98.39	98.75	44.56	44.61	315.97	309.89
Sorghum	942.47	945.02	1319.39	1374.59	1.40	1.46	1.34	1.25	99.20	98.75	44.88	45.00	319.02	306.14
Canthaxanthin (mg/kg)														
0	952.49	941.94	1319.67	1368.50	1.39	1.45	2.32	2.50 ^{a}	98.21	97.50 ^{b}	45.36	44.85	323.00	300.23
6	925.81	939.96	1304.88	1338.46	1.41	1.43	0.63	0.00 ^{b}	99.38	100.00 ^{a}	44.09	44.76	311.99	315.80
Interaction														
Corn x 0 Canthaxanthin	946.62	935.59	1309.46	1341.67	1.40	1.43	1.96	2.50	98.04	97.50	45.08	44.55	321.40	301.84
Corn x 6 Canthaxanthin	925.05	938.16	1300.84	1323.08	1.41	1.41	1.25	0.00	98.75	100.00	44.05	44.67	310.53	317.94
Sorghum x 0 Canthaxanthin	958.37	948.28	1329.88	1395.32	1.39	1.47	2.68	2.50	98.39	97.50	45.64	45.16	324.60	298.61
Sorghum x 6 Canthaxanthin	926.56	941.76	1308.91	1353.85	1.41	1.44	0.00	0.00	100.00	100.00	44.12	44.85	313.45	313.66
Source of Variation	,,P Value,,,													
Ingredients	0.7517	0.7061	0.5971	0.1996	0.95.04	0.2450	0.8711	1.000	0.5477	1.000	0.7517	0.7061	0.8227	0.6683
Canthaxanthin	0.2075	0.9271	0.5833	0.3587	0.6687	0.3023	0.3077	0.0408	0.3865	0.0408	0.2075	0.9271	0.4211	0.0813
Interaction	0.8070	0.8332	0.8186	0.7254	0.9293	0.7945	0.5529	1.000	0.7379	1.000	0.8070	0.8332	0.9917	0.9520
Average	939.15	940.95	1312.27	1353.48	1.40	1.44	1.47	1.25	98.79	98.75	44.72	44.81	317.50	308.01
SEM	65.75	67.75	84.48	102.15	0.13	0.08	0.89	0.75	4.18	3.72	3.13	3.22	42.79	27.46
CV %	7.00	7.20	6.43	7.54	9.22	5.70	59.92	50.82	4.24	3.77	7.00	7.20	13.47	8.91

$^{a-b}$ Means within a row with different superscripts differ significantly ($P < 0.05$).

[1] Data represent means from 10 replicates (i.e., pens) per treatment.

CONCLUSÕES

Peso corporal das aves e os parâmetros de incubação não foram influenciados pelas diferentes dietas usadas.

O presente estudo demonstrou que suplementação de cantaxantina na dieta das matrizes de corte melhorou significativamente a taxa de postura, TBARS e coloração da gema do ovo.

O sorgo por ser uma matéria prima baixa em carotenóides quando foi suplementada com a cantaxantina melhorou significativamente a gravidade especifica do ovo, peso do ovo, peso da casca do ovo e ácidos graxos poliinsaturados presentes na gema do ovo.

As gemas de ovos provenientes de dietas a base de milho se mostraram como uma fonte rica em xantofilas, ácidos graxos saturados e monoinsatarudados.

A progênie oriunda de matrizes de corte alimentadas com dietas suplementadas com 6ppm de cantaxantina apresentou uma baixa mortalidade e a melhor viabilidade no período de 1 a 21 dias de idade.

Matrizes de corte alimentadas com dietas a base de sorgo não apresentaram efeitos negativos nos parâmetros evaluados.

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