UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

LICOPENO NA DIETA DE SUÍNOS NA FASE DE TERMINAÇÃO

Autora: Marcelise Regina Fachinello
Orientador: Prof. Dr. Paulo Cesar Pozza
Coorientadora: Profª. Drª. Eliane Gasparino

MARINGÁ
Estado do Paraná
Maio-2017

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TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Produção Animal

APROVADA em 05 de maio de 2017.

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Orientador

"É muito melhor arriscar coisas grandiosas, alcançar triunfos e glórias, mesmo se expondo ao fracasso, do que formar fila com os pobres de espírito, que nem gozam muito, nem sofrem muito, porque vivem nessa penumbra cinzenta que não conhece vitória nem derrota"

Franklin Delano Roosevelt

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BIOGRAFIA DO AUTOR

Marcelise Regina Fachinello, filha de José Fachinello e Judite Buratti Fachinello, nasceu em Xavantina, Estado de Santa Catarina, Brasil, no dia 26 de maio de 1987.

Em julho de 2006, ingressou no curso de graduação em Zootecnia - Ênfase em sistemas orgânicos de produção, pela Universidade Estadual de Santa Catarina – UDESC – Brasil, concluindo-o em dezembro de 2010.

Em março de 2012, iniciou no Programa de Pós-graduação em Zootecnia, em nível de mestrado, área de concentração Produção Animal, na Universidade Estadual de Maringá, realizando estudos na área de "Avaliação de alimentos para suínos" submetendo-se à de defesa de dissertação em fevereiro de 2014.

Em 2014, iniciou o Curso de Pós-Graduação em Zootecnia, em Nível de Doutorado, na Universidade Estadual de Maringá, realizando estudos na área de Nutrição de Suínos. Submeteu-se ao Exame Geral de Qualificação em 21 de fevereiro de 2017 e, em 5 de maio de 2017, submeteu-se à defesa da Tese.

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RESUMO

A carne suína é um alimento ricos em proteínas e lipídios que são altamente susceptíveis à oxidação, o que pode comprometer suas características nutricionais e sensoriais. Os recentes avanços na pesquisa apontam uma alternativa de atenuar estes efeitos indesejáveis com a utilização de antioxidantes dietéticos. Dentre os antioxidantes naturais, o licopeno é conhecido por proteger as células contra danos causados por espécies reativas de oxigênio e impede a peroxidação lipídica, além de exercer ações sobre os componentes lipídicos do sangue e o sistema imune. Assim, o objetivo deste trabalho foi avaliar níveis de suplementação de licopeno para suínos machos castrados e fêmeas, dos 75 aos 100 kg, sobre o desempenho, características quantitativas da carcaça, qualidade da carne, parâmetros bioquímicos no plasma, expressão gênica de enzimas antioxidantes e respostas imunitárias. Foram utlizados 80 suínos, sendo 40 machos castrados e 40 fêmeas, com peso médio inicial de 75,04 ± 1,6 kg, distribuídos em um esquema fatorial 2 x 5, sendo dois sexos (machos e fêmeas) e cinco níveis de licopeno (0; 12,5; 25,0; 37,5 e 50,0 mg/kg de ração). Adicionalmente foram incluídos ao modelo o efeito do período de armazenamento da carne (0, 24, 48 e 72 horas) para as análises do radical DPPH e TBARS do músculo *longissimus lumborum* e, para a determinação de IgG, foram incluídos os períodos de coleta (0, 12 e 24 dias). Os dados foram submetidos à análise de variância a 5% utilizando o programa computacional SAS. Não foram observadas interações (P>0,05) entre o sexo e os níveis de licopeno para as variáveis de desempenho. O sexo influenciou o consumo diário de ração (P=0,001) e a conversão alimentar (P=0,001), sendo inferior para as fêmeas. O desdobramento da interação entre os níveis de licopeno e o sexo, revelou uma redução linear na expressão gênica das enzimas superóxido dismutase (SOD) (P=0,018) e catalase (P=0,001), no fígado, para as fêmeas suínas. As fêmeas apresentaram menor expressão gênica da SOD (P=0,001) com 50,0 mg de suplementação de licopeno e para a catalase (P=0,001) e glutattiona

peroxidase (P=0,001), nos níveis de 0; 12,5 e 50,0 mg de licopeno, em relação aos machos castrados. A suplementação de licopeno na dieta proporcionou melhorias no perfl lipídico do plasma sanguíneo, pois à medida que os níveis de suplementação dietética de licopeno aumentaram, o colesterol total (P=0,001), LDL (P=0,001) e a relação LDL:HDL (P=0,001) reduziram, e aumentaram os níveis de HDL (P=0,001). As fêmeas apresentaram maiores concentrações plasmáticas de ureia (P=0,001) e triglicerídeos (P=0,001) e menores concentrações de HDL (P=0,001), LDL (P=0,001), além de menor relação LDL:HDL (P=0,001) em relação aos machos castrados. Houve diferença quanto ao sexo para as características quantitativas da carcaça, em que os machos castrados apresentaram maior rendimento da carcaça quente (P=0,049), rendimento da carcaça refrigerada (P=0,023), espessura de toucinho (P=0,001) e gordura abdominal (P=0,001) e as fêmeas um maior rendimento de carne magra (P=0,001). A perda de líquido no descongelamento reduziu linearmente (P=0,024) em função dos níveis de licopeno, reduzindo em até 17,46% em relação ao tratamento sem licopeno. Os machos apresentaram maiores intensidades de coloração para as variáveis a* (P=0,001) e b* (P=0,045), tendo tendência ao vermelho e amarelo. Foi observada interação (P=0,006) entre os períodos de armazenamento e os níveis de licopeno na dieta para a oxidação lipídica do músculo *longissimus lumborum*. O desdobramento revelou em uma redução da oxidação lipídica à medida que aumentou-se a suplementação de licopeno na dieta, em todos os períodos avaliados (0, 24, 48 e 72 horas). Obteve-se um aumento linear da oxidação lipídica com o aumento dos dias de armazenamento, para todos os níveis de licopeno avaliados (0; 12,5; 25,0; 37,5 e 50,0 mg/kg de ração). Não foram observadas interações (P>0,05) para a inibição do radical DPPH na carne, no entanto, o radical DPPH foi influenciado pelo período de armazenamento e pelos níveis de suplementação de licopeno na dieta. A inibição do radical DPPH na carne foi reduzida até as 72 h. Com relação ao nível de suplementação de licopeno na dieta, houve um aumento (P=0,001) na captura do radical DPPH por antioxidantes na carne. A oxidação lipídica do fígado foi reduzida pela suplementação de licopeno na dieta de suínos, em que ao nível de 34,47 mg de licopeno/kg de ração houve a menor oxidação. A captura do radical DPPH por antioxidantes no fígado foi aumentada (P=0,001), resultando em um aumento do poder antioxidante exercido pelo licopeno no fígado, devido ao aumento da suplementação dietética de licopeno. As fêmeas apresentaram menor concentração de malonaldeído (P=0,001) e maior captura do radical DPPH (P=0,001) por antioxidantes, em relação aos machos castrados. O aumento da inclusão do licopeno na dieta de suínos aumentou a

albumina no plasma (P=0,012). À medida que os níveis de licopeno aumentaram na dieta, a concentração de linfócitos aumentou (P=0,045) de forma linear. A concentração de neutrófilos e a relação neutrófilos:linfócitos foram influenciadas (P<0,05) pelos níveis de licopeno na dieta, resultando em uma menor concentração de neutrófilos ao nível de 17,49 mg licopeno/kg e 16,46 mg de licopeno para a menor relação neutrófilos:linfócitos. Os eosinófilos também foram influenciados (P=0,050) pela suplementação do licopeno na dieta, em que o nível de 22,69 mg de licopeno/kg de ração resultou em uma maior resposta dos eosinófilos ao licopeno dietético. Houve interação (P=0,011) entre os períodos de coleta e os níveis de licopeno para a produção de IgG anti-BSA. O desdobramento revelou uma maior produção de IgG Anti-BSA até a suplementação de 20,06 mg de licopeno/kg de ração aos 24 dias de coleta e a produção de IgG Anti-BSA aumentou ao longo dos dias de avaliação, em todos os níveis de suplementação de licopeno. A suplementação de licopeno na dieta de suínos machos e fêmeas, dos 75 aos 100 kg, é um potente modulador do perfil lipídico, reduzindo os níveis de colesterol total e lipoproteínas de baixa densidade e aumentando as lipoproteínas de alta densidade, reduzindo também a expressão gênica das enzimas superóxido dismutase e catalase no fígado de fêmeas suínas. Além disso, reduziu a perda de líquido no descongelamento e foi efetivo na proteção contra oxidação do músculo *longissimus lumborum* e fígado, assim como alterou as respostas imunes. Os melhores resultados foram obtidos com a suplementação de 50,0 mg de licopeno/kg de ração para a qualidade da carne, fígado, expressão gênica e perfíl lipídico e 20,06 mg de licopeno/kg de ração apresentou a melhor resposta imune.

Palavras chaves: antioxidante, carne suína, oxidação, radicais livres, imunidade

ABSTRACT

Meat pork is a food rich in proteins and lipids that are highly susceptible to oxidation, which can compromise your nutritional and sensory characteristics. The recent advances in research point to an alternative to attenuate these undesirable effects with the use of dietary antioxidants. Among the natural antioxidants, lycopene is known to protect cells against damage caused by reactive oxygen species and prevents lipid peroxidation, in addition to exerting actions on the lipid components of the blood and the immune system. The objective of this work was to evaluate the levels of lycopene supplementation for barrows and gilts pigs, from 75 to 100 kg, on performance, carcass quantitative characteristics, meat quality, plasma biochemical parameters, gene expression of antioxidant enzymes and Immune responses. Eighty pigs were used, 40 barrows and 40 gilts, with a means initial weight of 75.04 ± 1.6 kg. In a 2 x 5 factorial scheme, two sexes (male and female) and five levels of lycopene (0, 12.5, 25.0, 37.5 and 50.0 mg/kg of diet) were additionally included in the model, the effect of the meat storage period (0, 24, 48 and 72 hours) for analyzes of the radical DPPH and TBARS of the *longissimus lumborum* muscle and, for the determination of IgG, the collection periods (0, 12 and 24 days). Data were submitted to analysis of variance at 5%, using the SAS computer program. No interactions (P>0.05) were observed between sex and levels lycopene to the performance variables. Sex influenced daily feed intake (P=0.001) and feed:gain ratio (P=0.001), being lower for gilts. The unfolding of the interaction between levels of lycopene and sex revealed a linear reduction in the gene expression of superoxide dismutase (SOD) enzymes (P=0.018) and catalase (P=0.001) in the liver for the gilts pigs. Gilts showed lower SOD gene expression (P=0.001) with 50.0 mg of lycopene supplementation and for catalase (P=0.001) and glutathione peroxidase (P=0.001) at levels of 0; 12.5 and 50.0mg of lycopene, in relation to barrows. Lipoprotein supplementation in the diet

provided improvements in the lipid profile of the blood plasma, as the levels of dietary supplementation of lycopene increased, total cholesterol (P=0.001), LDL (P=0.001) and LDL:HDL ratio (P=0.001) reduced, and increased levels of HDL (P=0.001). Gilts presented higher plasma concentrations of urea (P=0.001) and triglycerides (P=0.001) and lower concentrations of HDL (P=0.001), LDL (P=0.001) and besides lower LDL:HDL ratio (P=0.001) in to relation barrows. There was a difference in sex for the quantitative characteristics of the carcass, in which the barrows had higher hot carcass yield (P=0.049), cold carcass yield (P=0.023), backfat thickness (P=0.001) and abdominal fat (P=0.001) higher Lean meat yield (P=0.001). Thawing loss reduced linearly (P=0.024) as a function levels of lycopene, reducing by up to 17.46% in relation to treatment without lycopene. The barrows had higher staining intensities for the variables a* (P=0.001) and b* (P=0.045), having a tendency to red and yellow. Interaction (P=0.006) was observed between storage periods and levels of lycopene in the diet for the *longissimus lumborum* muscle. Interaction (P=0.006) was observed between storage periods and levels of lycopene in the diet for lipid oxidation of the *longissimus lumborum* muscle. The unfolding revealed in a reduction of lipid oxidation as the dietary lycopene supplementation was increased in all evaluated periods (0, 24, 48 and 72 hours). Linear increase of the lipid oxidation was obtained with the increase the days of storage days for all lycopene levels evaluated (0, 12.5, 25.0, 37.5 and 50.0 mg/kg of diet). No interactions (P>0.05) were observed for the inhibition of the DPPH radical in the meat, however, the DPPH radical was influenced by the storage period and levels of lycopene supplementation in the diet. Inhibition of the DPPH radical in meat was reduced up until at 72h. Regarding the level of lycopene supplementation in the diet, there was an increase (P=0.001) in the capture of the DPPH radical by antioxidants in the meat. The lipid oxidation of the liver was reduced by the supplementation of lycopene in the diet of pigs, where at the level of 34.47 mg of lycopene/kg of diet, there was the lowest oxidation. The capture of the DPPH radical by antioxidants in the liver was increased (P=0.001), resulting in an increase of the antioxidant power exerted by the lycopene in the liver, due to the increase of the dietary supplementation of lycopene. The gilts showed lower concentration of malonaldehyde (P=0.001) and higher DPPH (P=0.001) radical capture by antioxidants, compared to barrows. The increase in the inclusion of lycopene in the diet of pigs increased (P = 0.012) the albumin in the plasma. As levels of lycopene increased in the diet, the lymphocyte concentration increased (P=0.045) in linear fashion. The neutrophil concentration and the neutrophil:lymphocyte ratio were influenced

(P<0.05) by the levels of lycopene in the diet, resulting in a lower concentration of neutrophils of level of lycopene 17.49 mg/kg and 16.46 mg of lycopene/kg of diet to the lowest the neutrophil:lymphocyte ratio. The eosinophils were also influenced (P=0.050) by the supplementation of lycopene in the diet, in which level of 22.69 mg of lycopene/kg of diet resulted in a greater response of eusinophils to dietary lycopene. There was interaction (P=0.011) between the collection period and the lycopene levels for the anti-BSA IgG production. The unfolding showed a higher production of anti-BSA IgG until the supplementation of 20.06 mg of lycopene/kg of diet at 24 days of collection and the production of Anti-BSA IgG increased throughout the evaluation days at all levels of lycopene supplementation. Supplementation of lycopene in barrows and gilts pigs, from 75 to 100 kg, is a potent lipid profile modulator, reducing levels of total cholesterol and low density lipoprotein and increasing high density lipoprotein, reducing also the gene expression of the superoxide dismutase and catalase enzymes in the liver of pig gilts. In addition, it reduced the thawing loss and was effective in the protection against oxidation of the *longissimus lumborum* muscle and liver, as well as altered the immune responses. The best results were obtained with the supplementation of 50.0 mg of lycopene/kg of diet for meat quality, liver, gene expression and lipid profile, and 20.06 mg of lycopene/kg of diet presented the best immune response.

Keywords: antioxidant, pork meat, oxidation, free radicals, immunity

I- INTRODUÇÃO

A carne suína tem importante participação na economia mundial, pois em 2015 foram produzidas aproximadamente 110,321 milhões de toneladas (United States Department of Agriculture - USDA, 2016). Para que a cadeia produtiva seja mais eficiente, alguns aspectos produtivos devem ser melhorados para garantir produtos com melhor qualidade, prevenindo o estresse oxidativo dos animais, melhorando as características da carcaça, os valores de qualitativos da carne e, consequentemente, aumentar o tempo de prateleira dos produtos. A qualidade e a segurança dos alimentos representam uma das características mais marcantes exigidas pelo mercado consumidor. Portanto, estes conceitos estão bem sedimentados na cadeia da carne suína, sendo foco da produção de animais precoces, eficientes e que apresentem carcaças com elevada relação carne:gordura, associada a um ótimo padrão de qualidade (Silva et al., 2013).

A qualidade da carne pode ser influenciada pelo estresse oxidativo, o qual é caracterizado pelo desequilíbrio entre a formação de radicais livres e a capacidade de defesa celular contra estes radicais (Cadenas e Davies, 2000). Em consequência da produção excessiva destes radicais, ocorre a redução da vida de prateleira dos produtos alimentícios industrializados e também da carne *in natura*, pois está intimamente relacionada aos processos de oxidação de substâncias orgânicas. Nestes produtos, as principais reações de oxidação observadas são o escurecimento enzimático e a oxidação de lipídeos (Degáspari e Waszczynskyj, 2004).

Uma alternativa é o uso de antioxidantes em rações, com o intuito de proteção da deterioração oxidativa e melhora do status antioxidante dos tecidos e, consequentemente, a prevenção de processos oxidantes na carne (Haak et al., 2008). Um equilíbrio entre oxidantes e antioxidantes sustenta condições fisiológicas ótimas no corpo, pois a superprodução de oxidantes pode provocar um desequilíbrio que leva ao estresse oxidativo, o que pode resultar em danos celulares de constituintes como as proteínas, lipídos e DNA (Ahn, 2002).

Estes compostos podem atuar de forma direta, participando na neutralização das ações dos radicais livres, ou indireta, através de sistemas enzimáticos presentes tanto no organismo, localizados dentro das células, ou na circulação sanguínea (Montero, 1996). Desta maneira, os antioxidantes retardam os danos oxidativos causados pela peroxidação lipídica, que afetam principalmente os atributos de qualidade da carne, como cor, sabor, textura e seus valores nutricionais (Halliwell e Gutteridge, 2015), o que é de fundamental importância para a indústria suinícola, devido à influência direta na qualidade e no tempo de prateleira da carne *in natura* e processada (Rodrigues et al., 2003).

Dentre os componentes não enzimáticos da defesa antioxidante podem ser destacados o β-caroteno, o licopeno e a luteína (Papas, 1999), que exercem funções antioxidantes em fases lipídicas, bloqueando os radicais livres que danificam as membranas lipoproteicas (Sies e Stahl, 1995). O licopeno, por sua vez, caracteriza-se por ser um excelente pigmentante, é lipossolúvel, composto por onze ligações conjugadas e duas ligações duplas não conjugadas, tido como o carotenoide que possui a maior capacidade sequestrante do oxigênio singlete, possivelmente devido à presença das duas ligações duplas não conjugadas, o que lhe oferece maior reatividade, além de ser um carotenoide sem a atividade pró-vitamina A (Shami e Moreira, 2004). Atua sequestrando o oxigênio singlete e removendo os radicais peróxidos, modulando o metabolismo carcinogênico, inibindo a proliferação celular, estimulando a comunicação entre células e elevando a resposta imune (Olson, 2008). Sua proteção também recai sobre lipídeos, lipoproteínas de baixa densidade (LDL), proteínas e DNA (Rao e Agarwal, 1998).

Esta mólecula pode doar elétrons para suprir e neutralizar os radicais livres, considerada fonte de envelhecimento e a causa de várias doenças degenerativas antes que possa prejudicar a célula (Agarwal e Rao, 2000; Livny et al., 2002). Ainda, pode aumentar os níveis de HDL (Blum et al., 2006), proteção contra os danos celulares induzidos por oxigênio (Markovic et al., 2006) e modulação da massa magra com redução da gordura corporal (Sukardi, 2006).

Também apresenta efeito na produção animal, atuando sobre o desempenho e qualidade de carne de codornas japonesas (Botsoglou et al., 2004) e frangos de corte (Leal et al., 1999), na qualidade de ovos de codornas (Sahin et al., 2006) e de galinhas poedeiras (Olson et al., 2008). Em suínos, a suplementação do licopeno resultou na redução efetiva do estresse oxidativo, reduzindo as concentrações de malondialdeído no corpo e atuou protegendo as células mononucleares do sangue contra o aumento dos danos ao DNA (Pajk et al., 2006).

Desta forma, o licopeno apresenta-se como um potente antioxidante que oferece proteção contra o dano celular causado por espécies reativas de oxigênio e vem sendo utilizado na alimentação humana. No entanto, sua função como antioxidante para suínos ainda é pouco estudada. São escassos os trabalhos que referenciam o potencial antioxidante do licopeno sobre a qualidade da carne suína e, sua ação sobre a redução do estresse oxidativo e manutenção da qualidade da carne, por meio do retardo ou prevenção da oxidação lipídica, assim como sua ação sobre o sistema imune.

1. Produção e valor nutricional da carne suína

A demanda da carne suína tem aumentado nas últimas décadas como resultado de mudanças nos padrões de consumo, à medida que os rendimentos aumentaram nos países em desenvolvimento e também o rápido crescimento das economias (Food and Agriculture Organization of the United Nations – FAO, 2016). Atualmente, a carne suína é a proteína animal mais produzida e consumida no mundo. Somente em 2016, foram produzidas cerca de 110,321 milhões de toneladas (USDA, 2016) representando 42,9% do consumo mundial de carnes, sendo a China o principal produtor e consumidor mundial de carne suína, e o Brasil como o quarto maior produtor e o quinto maior consumidor de carne suína (USDA, 2016).

A carne suína é muito apreciada pelo seu reconhecido valor nutricional e sensorial, sendo a espécie mais consumida e a mais utilizada para a fabricação de derivados. A carne suína magra é composta por cerca de 75.1% de água, 22,8% de proteína, 1,2% de gordura e 1% de matéria mineral, porém na carcaça suína a percentagem de gordura aumenta consideravelmente, sendo 47,0% de gordura, água 41,1%, proteínas 11,2% e matéria mineral 0,6% (FAO, 2017). O perfil lipídico do músculo *longuissimus lumborum* do suíno é composto por cerca 38,90% de ácidos graxos saturados, 60,65% de ácidos graxos insaturados, destes 43,8% são ácidos graxos monoinsaturados e 16,85% de poli-insaturados (Janiszewski et al., 2016), o que torna a carne suína altamente susceptível à oxidação lipídica devido à predominância de ácidos graxos insaturados (Juntachote et al., 2006), pois quanto maior o grau de instauração dos ácidos graxos, maior é a susceptibilidade da carne fresca à oxidação (Boselli et al., 2008). A cor e a estabilidade lipídica são os principais fatores que limitam a qualidade e a aceitabilidade da carne e dos produtos cárneos (Gatellier et al., 2001). E os processos oxidativos podem afetar

diretamente os principais atributos de qualidade da carne como cor, sabor, textura e seus valores nutricionais (Halliwell e Gutteridge, 2015).

2. Mecanismos de oxidação

Os danos oxidativos aos lipídeos ocorrem ainda no animal vivo, devido a um desequilíbrio entre a produção de espécies reativas de oxigênio e os mecanismos de defesa do animal. Em particular, no período imediato pós-abate e durante a manipulação, processamento, armazenamento e cozimento da carne, os danos aos lipídeos podem ser acentuados (Morrissey et al., 1998).

Nos animais vivos, as células podem continuamente sofrer ação de agentes estressores oriundos de fontes internas e externas, que podem atuar como aceleradores das reações de peroxidação, com formação de radicais livres (Bergman et al., 2001). Porém, nesta fase, os animais têm disponíveis fatores intrínsecos que podem controlar as reações de oxidação, tais como as enzimas antioxidantes superóxido dismutase, catalase e glutationa peroxidase (Halliwell e Gutteridge, 2015), proteínas transportadoras e seus mecanismos (Chan et al., 1994), e os antioxidante dietéticos, tais como o cobre, manganês, zinco, selênio e ferro e vitaminas como o ácido ascórbico, vitamina E e carotenoides como o β-caroteno, licopeno e luteína; bioflavonoides como genisteína, quercetina e taninos como catequinas (Papas, 1999).

A carne no pós-morte é diferente do tecido vivo, uma vez que o metabolismo muda gradualmente quando os músculos não são mais continuamente alimentados com oxigênio. Desta forma, é altamente improvável que os mecanismos de defesa disponíveis para a célula no animal vivo ainda funcionem no período pós-abate, devido às alterações quantitativas em vários metabólitos e propriedades físicas (Carlsen et al., 2005). Logo após o abate, as alterações bioquímicas que acompanham a conversão do músculo em carne dão origem a condições para o processo de peroxidação autocatalítica, pois o sistema antioxidante natural não é mais controlado de forma rigorosa e o equilíbrio entre os fatores prooxidativos e a capacidade antioxidante favorece a oxidação.

Dentre os fatores no pós-abate que contribuem para a oxidação da carne, está o cessamento da circulação sanguínea, consequentemente o cessamento rápido da circulação de nutrientes; a instalação de um metabolismo anaeróbico que promove a produção de ácido lático, o que leva a uma queda no pH de 7,4 para aproximadamente 6,0-5,5; comprometimento do sistema de enzimas antioxidantes; inativação das proteínas

sequestrantes de ferro e a iniciação da oxidação lipídica das membranas (Morrissey et al., 1998). Além disso, ocorre a desnaturação das proteínas e a proteólise, e os potenciais reagentes são misturados a reações não fisiológicas (Carlsen et al., 2005). Nesta fase, o grau de extensão da oxidação é influenciado pelos eventos ocorridos durante a vida do animal, tais como sua alimentação, o estresse e sua saúde.

Os processos de moagem, corte, emulsificação e cozimento também aceleram a oxidação lipídica. A principal razão é que com o processamento ocorre o rompimento da integridade das membranas musculares e, alteram os compartimentos celulares, com a liberação do ferro cataliticamente ativo da mioglobina e de outras proteínas (Gray e Pearson, 1987). A interação deste e de outros fatores pró-oxidantes com os ácidos graxos insaturados resulta na geração de radicais livres e na propagação das reações oxidativas, afetando diretamente a cor, sabor, textura, valor nutritivo e a segurança dos alimentos.

Os atributos de qualidade dos produtos cárneos deterioram-se devido à oxidação dos lipídeos durante o processamento e armazenamento. A oxidação lipídica é responsável pelo desenvolvimento de produtos de oxidação primária e secundária, resultando na redução da qualidade nutricional e alterações de sabor (Maqsood e Benjakul 2011). A deterioração oxidativa pode ocorrer tanto em moléculas de lipídeos como em proteínas da carne, e é umas das principais causas de deterioração funcionais, sensoriais e nutricionais da qualidade da carne e dos seus produtos (Ventanas et al., 2006; Liu et al., 2015), que podem causar riscos à saúde e perdas econômicas em função da inferioridade na qualidade (Naveena et al., 2008).

As alterações associadas à oxidação dos lipídeos incluem o desenvolvimento de odores e sabores desagradáveis, deteriorações da cor, propriedades reológicas, bem como formação potencial de compostos tóxicos. Por outro lado, a oxidação de proteínas está relacionada à deterioração da cor e textura, além da perda de nutrientes como aminoácidos essenciais e a diminuições na digestibilidade das proteínas (Ventanas et al., 2006) .

A oxidação lipídica ocorre principalmente por um mecanismo auto-catalítico induzido por radicais livres, e é dependente da presença de alguns fatores iniciadores, tais como luz, calor, sensibilizadores, metais e/ou espécies reativas de oxigênio ou nitrogênio (Mariutti e Bragagnolo, 2017). E estes processos de oxidação ocorrem basicamente em três fases, conhecidas como iniciação, propagação e terminação (Kumar et al., 2015).

Na fase de iniciação, a presença de pro-oxidantes ou espécies reativas de oxigênio (ROS), ou qualquer condição favorável à oxidação, resulta na perda de um radical de hidrogênio a partir de ácidos graxos insaturados, gerando um radical alquilo de ácido

graxo no átomo de carbono, que requer menos energia (Mariutti e Bragagnolo, 2017). Na fase de propagação, o oxigênio molecular reage com o radical alquilo de um ácido graxo e resulta na formação do radical peróxido e, estes reagem com outros ácidos graxos formando hidroperóxidos e devido à sua alta instabilidade, eles se decompõem gerando novos radicais (Kumar et al., 2015). A decomposição de hidroperóxidos é acelerada na presença de metais de transição e, no caso da carne e dos produtos à base de carne, o ferro é o principal responsável (Erickson, 2008).

As duas primeiras fases conduzem à formação de radicais, que são rapidamente transformados em compostos não radicais tais como dienos conjugados e hidroperóxidos, que são ambos considerados produtos primários da oxidação lipídica. Estes compostos se decompõem ainda mais e dão origem a compostos de carbonilo, cetonas, álcoois e aldeídos, que são considerados produtos secundários da oxidação lipídica (Kumar et al., 2015; Guyon et al., 2016).

Na última etapa, uma grande quantidade de radicais peroxilo é acumulada e estes radicais reagirão entre si gerando compostos não radicais, e sob pressão atmosférica, a combinação entre radicais peroxilo ocorrerá formando compostos intermediários instáveis que se decompõem em compostos estáveis (Masuda et al., 2010). Durante esta etapa, o consumo de oxigênio e o teor de hidroperóxido tendem a diminuir, as mudanças sensoriais aumentam e as alterações físicas começam a ocorrer (Mariutti e Bragagnolo, 2017).

A taxa de oxidação lipídica é diretamente proporcional à insaturação de ácidos graxos, o que finalmente determina a cor e a estabilidade oxidativa dos produtos cárneos (Hallenstvedt et al., 2012). E a cor, principalmente relacionada à atratividade da carne pelo consumidor, tem esta característica influenciada pela oxidação lipídica, que acelera a taxa de formação da metmioglobina, que é a forma oxidada da mioglobina, tornando a carne com coloração mais marrom (Faustman et al., 2010).

A oxidação das proteínas pode ocorrer de forma semelhante no músculo animal. Falowo et al. (2014) observaram que os radicais peroxilo formados durante a oxidação lipídica são absorvidos por átomos de hidrogênio em proteínas para formar radicais proteicos, que são convertidos em peróxidos de alquilo e contribuem para a produção de radicais alcoxilo e derivados hidroxilo. Dos produtos secundários de oxidação lipídica, os aldeídos são compostos chave porque reagem prontamente com as proteínas, levando a modificações de suas propriedades sensoriais e nutricionais (Guyon et al., 2016).

A estabilidade dos lipídeos na carne e nos produtos à base de carne é influenciada por muitos fatores, incluindo a espécie, tipo de músculo, quantidade e tipo de gordura na dieta, estado nutricional do animal no abate, presença ou ausência de doença ou infecção, além do tipo de transformação a que a carne é submetida (Morrissey et al., 1998).

3. Espécies reativas de oxigênio (ROS)

As ROS são produzidas a partir de oxigênio molecular no metabolismo celular normal e podem ser divididas em radicais livres e em não radicais. São caracterizadas em radicais livres quando as moléculas contêm um ou mais elétrons desemparelhados e são, assim, a reatividade para a molécula; e em não radicais, quando dois radicais livres compartilham seus elétrons desemparelhados (Birben, 2012). O elétron não pareado de um radical livre é representado como um ponto em negrito (**.**) e estes radicais livres, quando relacionados ao oxigênio, são chamados de ROS (Gutteridge e Mitchell, 1999). A geração dos radicais livres pode ocorrer no citoplasma, mitocôndrias ou na membrana celular (Akbarian et al., 2016).

Os radicais livres, em baixas a moderadas concentrações, funcionam em processos celulares fisiológicos, mas em concentrações elevadas, produzem modificações adversas aos componentes celulares, tais como lipídeos, proteínas e DNA (Valko et al., 2006), além de estarem envolvidos com o desenvolvimento de várias doenças (Speisky e Jiménez, 2000).

As três principais ROS produzidas com significado fisiológico são o radical superóxido (O_2^-), o radical hidroxila (•OH), e o peróxido de hidrogênio (H_2O_2) (Birben, 2012). Essas substâncias são capazes de oxidar macromoléculas biológicas, como proteínas e lipídeos. Estudos relacionados à oxidação de proteínas apontam que o O_2 pode oxidar seletivamente resíduos de aminoácidos ou agregados proteicos, podendo levar à destruição dos mesmos. Os peróxidos proteicos ainda podem reagir com outras biomoléculas, como o DNA, gerando um dano adicional (Ronsein et al., 2006).

A formação de um superóxido ocorre quando um elétron é adicionado a uma molécula de oxigênio e, a partir desta espécie reativa, as demais são sintetizadas (Miller et al., 1990). O superóxido é produzido em diversos processos biológicos, entre eles, encontra-se a cadeia transportadora de elétrons das mitocôndrias. Os elétrons normalmente são transferidos através da cadeia de transporte de elétrons mitocondrial

para a redução de oxigênio à água, mas cerca de 1 a 3% de todos os elétrons que saem do sistema produzem superóxido (Birben, 2012).

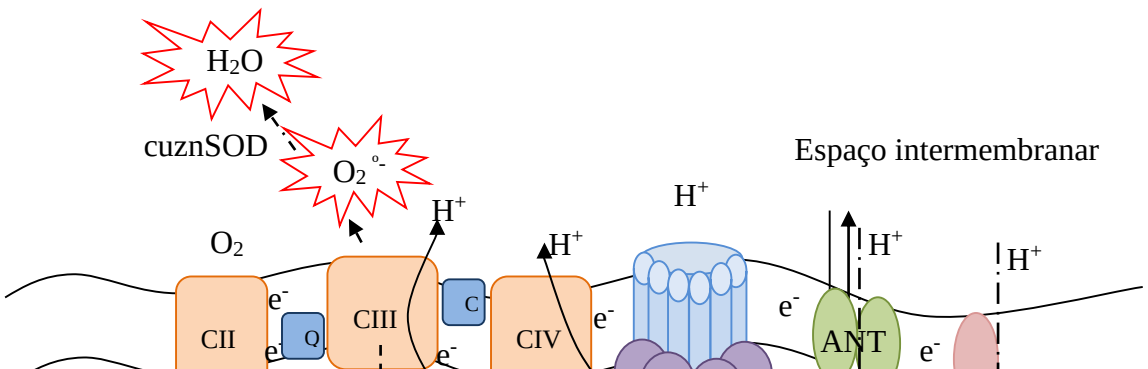
As espécies reativas de oxigênio são produzidas normalmente nos processos biológicos celulares. Entretanto, mudanças no equilíbrio entre oxidante/antioxidante em favor dos oxidantes são chamadas de "estresse oxidativo". Nesta condição, grandes quantidades de ROS estão presentes e isto se deve não apenas à superprodução destes, mas também à deficiência nos sistemas de defesas antioxidantes (Halliwell e Gutteridge, 2015).

4. Sistemas antioxidantes

Os antioxidantes atuam em diferentes níveis, na proteção do organismo. O primeiro mecanismo de defesa contra os radicais livres é impedir a sua formação, principalmente, pela inibição das reações em cadeia com o ferro e o cobre. Além disso, os antioxidantes são capazes de interceptar os radicais livres gerados pelo metabolismo celular, ou por fontes exógenas, impedindo o ataque sobre os lipídeos, os aminoácidos das proteínas, a dupla ligação dos ácidos graxos poli-insaturados e as bases do DNA, evitando a formação de lesões e a perda da integridade celular (Bianchi e Antunes, 1999). São amplamente definidos como substâncias facilmente oxidadas pelas ROS, diminuindo a velocidade pela qual as ROS podem reagir sobre componentes celulares cruciais, como as membranas lipídicas, DNA ou proteínas (Pajk et al., 2006).

4.1. Antioxidantes enzimáticos

Os sistemas antioxidantes enzimáticos são os primeiros a agir, evitando o acúmulo do radical ânion superóxido e hidrogênio. Este sistema é representado principalmente pelas enzimas superóxido dismutase e catalase, e pelo sistema de defesa da glutationa (Figura 1) (Akbarian et al., 2016). Estas enzimas atuam de forma cooperativa ou sinérgica para garantir a proteção celular global (Day, 2009).



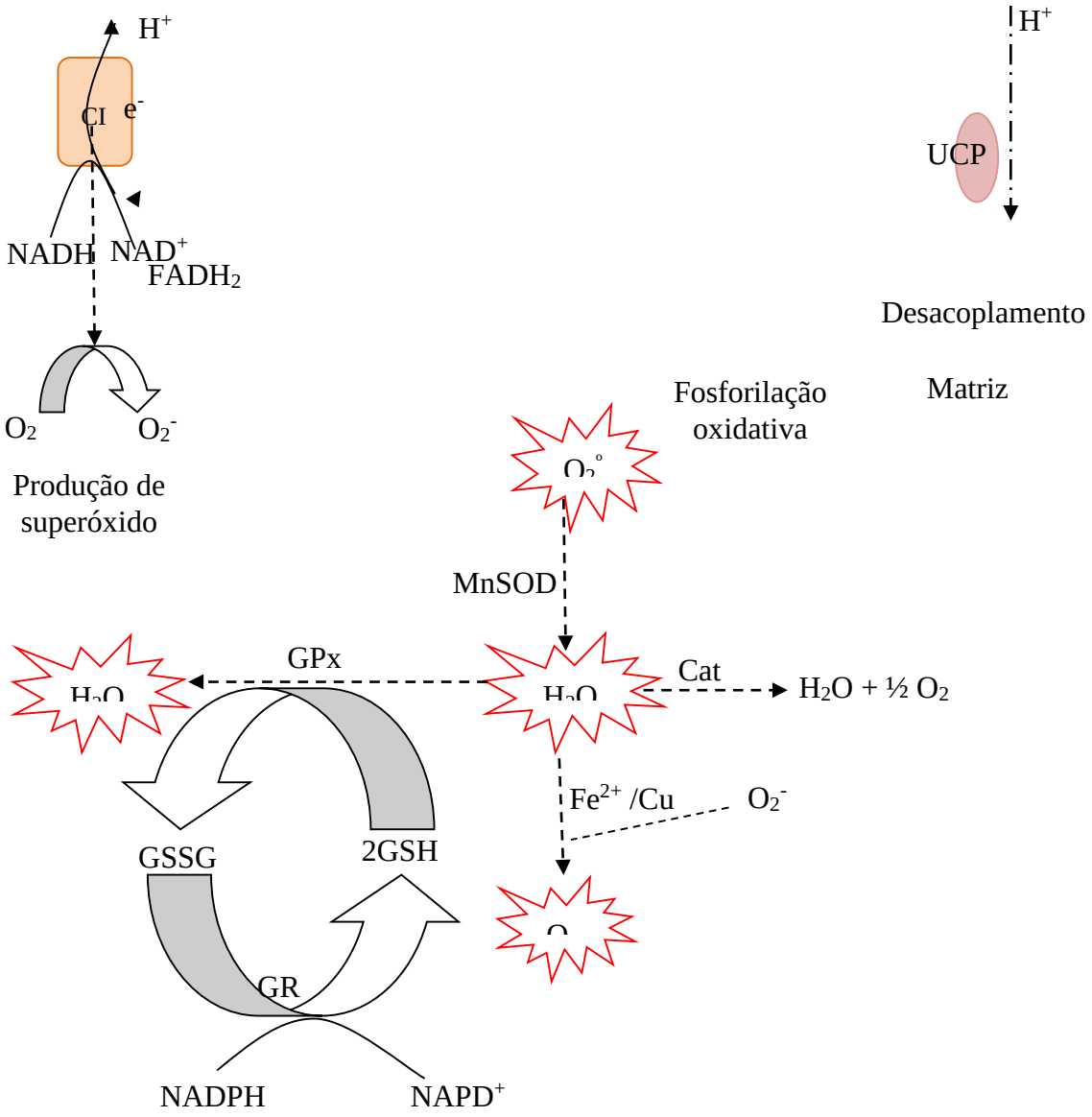


Figura 1 - Produção e combate às espécies reativas de oxigênio. Fonte: Adaptado de Akbarian et al. (2016).

A SOD desempenha um papel essencial na proteção do organismo contra danos causados pelo radical ânion superóxido e é a enzima mais importante para catalisar eficientemente a dismutação de superóxido em O_2 e H_2O_2, que são desintoxicados à H_2O e O_2 pela CAT ou para H_2O por GPx (Michiels et al., 1994). Atualmente, três isoformas distintas de SOD foram identificadas em mamíferos (Covarrubias et al., 2008). Estes são superóxido dismutase de cobre, zinco (CuZnSOD, SOD1), superóxido-dismutase de manganês (MnSOD, SOD2) (Takada et al., 2009) e superóxido-dismutase extracelular (EC-SOD, SOD3). A CuZnSOD encontra-se quase que exclusivamente em espaços citoplasmáticos intracelulares e compreende 90% de SOD total (Noor et al., 2002).

A catalase é dependente do NADPH e tem a capacidade de remover o peróxido de hidrogênio do interior das células, formado pela ação da SOD, em que dismuta o peróxido de hidrogênio a oxigênio e água, por meio da reação $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$ (Agarwal et al., 2011).

A glutationa está envolvida em uma variedade de ações biológicas, sendo que a principal é a defesa contra os radicais livres (Morand et al., 1997). O sistema da glutationa é composto, além da GSH, pelas enzimas glutationa oxidase (GO), glutationa peroxidase (GPx) e pela glutationa redutase (GR). Portanto, a defesa depende da atividade de todo um conjunto. Para reduzir as espécies oxidantes, a glutationa se oxida à glutationa dissulfeto (GSSG), pela ação das enzimas GO e GPx. Na forma de GSSG, a glutationa está incapacitada para nova reação. A enzima glutationa redutase é a responsável por regenerar a GSH, a partir de GSSG, permitindo assim que a mesma molécula seja usada mais de uma vez no combate às espécies reativas de oxigênio (Figura 1) (Huber et al., 2008). A família das enzimas glutationa peroxidases pode ser dividida em duas classes (Arthur, 2000): enzimas dependentes de selênio e enzimas selênio independentes, estas chamadas de glutationa S-tranferases e com atividade relacionada à hidroperóxidos orgânicos.

4.2. Antioxidantes não enzimáticos

Segundo o Codex Alimentarius, antioxidante é um aditivo alimentar que prolonga a vida de prateleira de alimentos por meio da proteção contra a deterioração causada pela oxidação. É definido quimicamente como um composto aromático, podendo ser natural ou sintético (Araújo, 2011). Do ponto de vista qualitativo, e de segurança alimentar, o uso de antioxidantes através da suplementação das rações animais corresponde a uma das ferramentas para atingir esses objetivos, minimizando a oxidação lipídica, que é uma das principais causas de deterioração da carne e de seus subprodutos (Soares, 2009).

Em rações para animais com ingredientes que apresentam elevada quantidade de ácidos graxos insaturados, como óleos de origem vegetal, a utilização de antioxidantes sintéticos ou naturais visa proteger seus constituintes dos efeitos da oxidação lipídica (Mariutti e Bragagnolo, 2009). Os mecanismos antioxidantes dos compostos usados para o controle da oxidação incluem o controle de radicais livres, onde agentes sequestrantes de radicais livres reagem mais rapidamente com estes radicais em comparação com os ácidos graxos insaturados; o controle de pró-oxidantes, pela ação de agentes quelantes ou

complexantes de metais de transição; e pela ação de carotenoides sobre o oxigênio singlete (Damodaran et al, 2010).

Os antioxidantes exógenos, especialmente fornecidos por alimentos, são essenciais para evitar o estresse oxidativo (Laguerre et al., 2007). Os antioxidantes agem impedindo a peroxidação lipídica, por meio da eliminação dos radicais iniciadores, impedindo o início da reação em cadeia ao agirem como catalisadores de ligações, como íons metálicos para evitar a iniciação da geração de radicais e decomposição de peróxidos, assim eles não podem ser reconvertidos em radicais iniciadores e realizando a quebra da cadeia, para evitar a captação contínua de hidrogênio por radicais ativos (Dorman et al., 2003). Entre os grupos de antioxidantes, estão presentes os carotenoides, que reagem com os radicais livres, notavelmente com os radicais peróxidos e com o oxigênio molecular, sendo a base de sua ação antioxidante.

Os carotenoides como o β-caroteno, licopeno, zeaxantina e luteína exercem funções antioxidantes em fases lipídicas, bloqueando os radicais livres que danificam as membranas lipoproteicas (Sies e Stahl, 1995). Devido à sua estrutura polieno, o licopeno possibilita um sistema rico em elétrons, sendo um alvo elegível para os reagentes eletrofílicos. Assim, executa uma alta reatividade contra radicais livres e oxigênio (Krinsky, 1998).

5. O licopeno e seu modo de ação como antioxidante

O licopeno é um carotenoide e está presente em um número limitado de alimentos de cor vermelha, como tomates e seus produtos, goiaba, melancia, mamão e pitanga (Arab e Steck, 2000). Os tomates e derivados aparecem como as maiores fontes de licopeno (Djuric e Powell, 2001). O tomate cru apresenta, em média, 30 mg de licopeno/kg do fruto (Stahl e Sies, 2003).

O licopeno é um representante dos carotenoides de hidrocarbonetos, com uma estrutura de cadeia aberta, acíclica, formado por quarenta átomos de carbono, com a fórmula $C_{40}H_{56}$ e constituído de 13 ligações duplas, das quais onze são ligações duplas conjugadas (Figura 2), construindo assim um cromatóforo (Shi, 2000). Esta estrutura de polieno conjugado apresenta a cor rubi e confere as propriedades antioxidantes do licopeno, por isso tal reatividade se deve à sua estrutura química (Vitale et al., 2010). Pela forma, o licopeno é provavelmente um antioxidante eficaz como agente quimiopreventivo, considerado um carotenoide de grande reatividade, tanto para o

oxigênio, como para os radicais livres. Devido à sua estrutura acíclica e à ausência de um anel β-ionona, nenhuma atividade pro-vitamina A pode ser conferida ao licopeno, diferenciando-o em relação ao α- e β- caroteno (Breemen e Pajkovic, 2008).

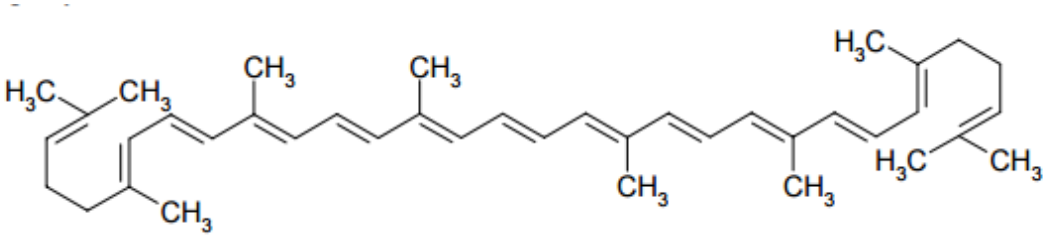


Figura 2- Estrutura do licopeno.

O licopeno é conhecido por ser o reagente de eliminação do oxigênio mais potente entre os carotenoides e, além disso, fornece a capacidade de intervir em reações iniciadas por radicais livres, como a de OH^-, ou radicais peróxido (Krinsky, 1998; Di Marcio et al., 1989). Possivelmente, isso se deva à presença das duas ligações duplas não conjugadas, o que lhe oferece maior reatividade (Shami e Moreira, 2004).

A capacidade de eliminar o oxigênio aumenta com a abertura do anel β-ionona na estrutura da molécula, como encontrado por comparação direta do licopeno, γ-caroteno e β-caroteno. Este pode ser o resultado da diminuição do nível de energia, que fornece a capacidade de se aproximar de um estado tripleto de energia. A partir desse momento, a transferência de energia do estado excitado do oxigênio pode ser facilmente adaptada (Di Marcio et al., 1989).

O licopeno atua como antioxidante através de vários mecanismos. Espécies altamente reativas de oxigênio, também chamadas de oxigênio singlet ($^1O_2$), são capazes de oxidar os ácidos nucleicos, ácidos graxos insaturados ou aminoácidos, e podem ser extintas por carotenoides/licopeno, por meio da reação indicada a seguir (Krinsky, 1998):

$$^1O_2 + \text{Licopeno} \rightarrow {}^3O_2 + {}^3\text{Licopeno}$$

$$3\text{Licopeno} \rightarrow \text{Licopeno} + \text{calor}$$

A quantidade superior de energia da molécula de licopeno, adquirida nesta reação, faz atingir o estado tripleto, e é dispensado através de interações vibracionais, resultando na liberação de energia térmica, sendo a estrutura do polieno conjugado responsável por esta reação. Como a molécula imediatamente restabelece seu estado fundamental, um outro ciclo de eliminação de $^1O_2$ pode ser ativado, proporcionando assim a possibilidade

de cada molécula do carotenoide eliminar cerca de 1000 moléculas de $^1O_2$ (Krinsky, 1998).

O licopeno, e outros carotenoides, também são conhecidos por suas atividades antioxidantes no sentido de impedir reações de radicais livres. Radicais de peroxil são formados no organismo durante o processo de peroxidação lipídica, o que pode levar à destruição de seções lipofílicas. A inativação destas espécies reativas resulta no desenvolvimento de produtos de adição de radicais. Os produtos da oxidação dos carotenoides incluem a formação de epóxidos localizados no anel β-ionona, bem como situados na ligação dupla central da cadeia polieno conjugada. Outros produtos desta reação são a formação de cetonas e aldeídos no anel β-ionona (Sies e Stahl, 1995). A inibição destas reações radicais pelo licopeno pode abrigar as membranas a partir de peroxidação lipídica (Stahl e Sies, 2003).

A maior atividade biológica do licopeno tem sido atribuída à sua capacidade antioxidante e vários estudos têm avaliado o papel do licopeno como um potencial antioxidante *in vivo*. Estudos usando tomates, ou produtos do tomate, demonstraram uma redução nos danos ao DNA (Bowen et al., 2002), diminuição do estresse oxidativo em linfócitos (Porrin et al., 2000) e diminuição da oxidação do LDL ou peroxidação lipídica (Agarwal e Rao., 2000). Lian e Wang (2008) observaram um possível efeito indireto do ácido apo-10'licopenóico, produto da clivagem do licopeno, após 24 h de tratamento com ácido apo-10'-licopenoico. Foi observada uma diminuição dependente da dose na produção endógena de ROS e na inibição da liberação de lactato desidrogenase.

Como o licopeno exibe o maior potencial de eliminação do oxigênio entre todos os carotenoides, suas propriedades antioxidantes também variam de acordo com os isômeros. O licopeno 5-*cis* foi detectado como o mais potente, seguido do cis-9. As propriedades antioxidantes mais fracas foram relatadas para o isomero *all-trans* (Chasse et al., 2001).

Além de suas reações com radicais, também tem sido demonstrado que o licopeno regula positivamente elementos de resposta antioxidante, a partir de enzimas de fase II, ou seja, enzimas antioxidantes e detoxificantes (Breemen e Pajkovic, 2008).

5.1. Absorção e metabolismo do licopeno

O licopeno ingerido na sua forma natural *(trans*-licopeno) é pouco absorvido, no entanto, o processamento mediante o calor rompe as paredes celulares e possibilita

transformar as formas isoméricas *trans* do licopeno em *cis* (5-*cis*, 9-*cis*, 13-*cis* e 15-*cis*) aumentando a sua biodisponibilidade (Perdomo et al., 2012). Portanto, a biodisponibilidade do licopeno está relacionada com as formas isoméricas apresentadas. Clinton et al. (1996) demonstraram que 79 a 91% do licopeno presente nos tomates e seus produtos encontram-se sob a forma do isômero *trans* (*trans*-licopeno), em contraste com os níveis de licopeno sérico e tissulares, que se encontram em mais de 50% na forma de isômero *cis*-licopeno. Por isso, espera-se que existam mecanismos de isomerização *in vivo* (Clinton et al., 1996).

O β-caroteno é o carotenoide mais estudado quanto à absorção, embora mais estudos sejam necessários sobre a absorção dos isomeros *cis* e os fatores que influenciam a absorção do β-caroteno, pois de acordo com Wang (2012) pode afetar de forma semelhante a absorção do licopeno devido à similaridade de sua estrutura.

Uma vez no estômago, os carotenoides são liberados da matriz e disolvem-se na emulsão do conteúdo gástrico, passando ao duodeno para serem absorvidos na mucosa intestinal (Figura 3). Como os carotenoides são substâncias lipossolúveis, o processo de absorção e transporte ocorre similarmente ao das gorduras. Desta forma, precisam ser incorporadas às micelas e, em seguida, os carotenoides são absorvidos por difusão passiva (Borel at al., 2005). Entretanto, existem estudos sobre o envolvimento de um processo ativo na absorção de carotenoides, através da proteína transortadora *Scavenger Receptor class B type I* (SR- B1) que é encontrada no intestino delgado, bem como no fígado, glândulas suprarenais, ovários, placenta, rins, próstata e cérebro (Lobo et al., 2010). Portanto, SR-B1 pode ser parcialmente responsável pelo transporte de carotenoides, a partir de lipoproteína para os tecidos e dos tecidos para lipoproteínas (Von Linting et al., 2008).

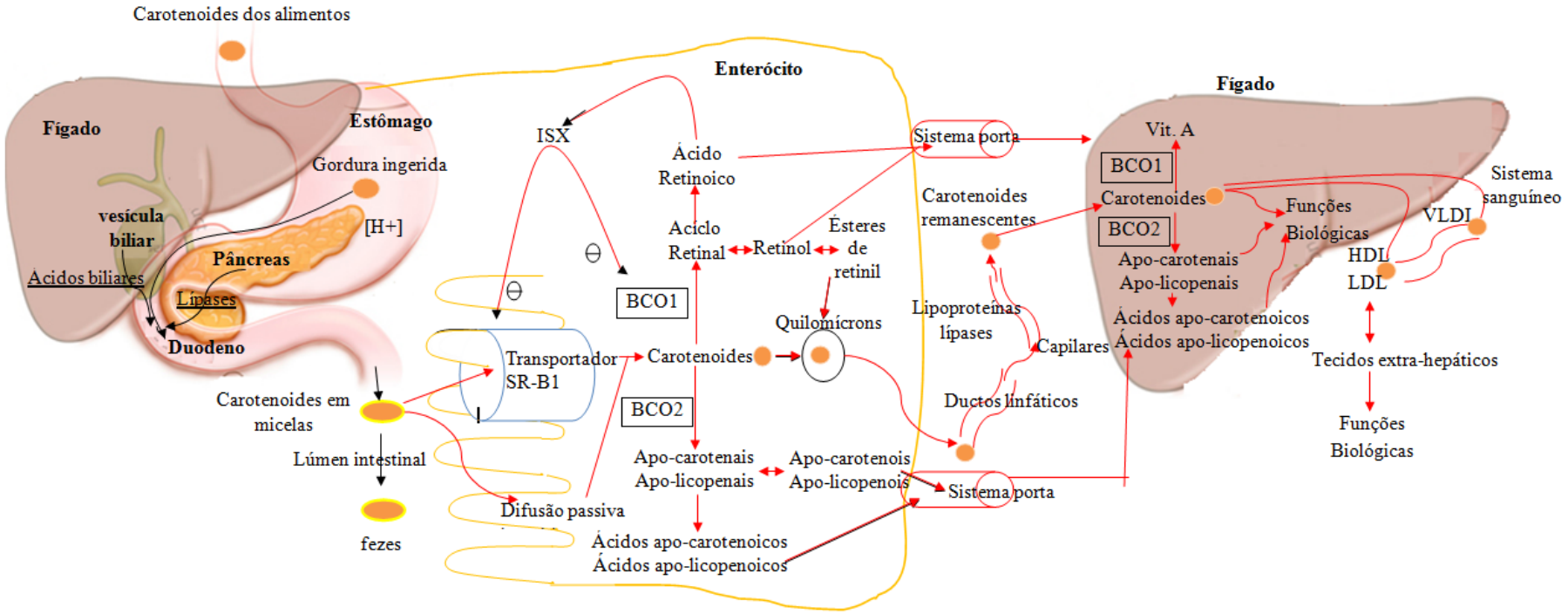


Figura 3 - Ilustração esquemática simplificada de absorção, metabolismo e transporte de carotenoides. Fonte: adaptado e expandido de Borel et al., (2005) e Wang (2012).

A regulação da absorção intestinal dos carotenoides parece ser mediada por um mecanismo de *feedback* negativo, envolvendo o fator de transcrição *intestine-specific homeobox* (ISX) (Lobo et al., 2010).

O ISX reprime a expressão da β-caroteno 15',15'-oxigenase (BCO1) e SR-B1 no intestino, facilitando a absorção de lipídeos e carotenoides da dieta (During e Harrison et al., 2004). Devido ao ISX estar sob o controle dos mecanismos dependentes do ácido retinoico e do receptor do ácido retinoico (RAR), durante a insuficiência de vitamina A tanto a expressão da β-caroteno 15',15'-oxigenase (BCO1) como da SR-B1 são induzidas para aumentar a absorção e conversão de β-caroteno em vitamina A (Figura 3).

No entanto, os carotenoides não-provitamina A, como o licopeno, podem ser metabolizados em aciclo-retinal pela BCO1 (Dela Seña et al., 2013). Além disso, acredita-se que a β-caroteno 9',10'- oxigenase (BCO2) é a principal enzima envolvida no metabolismo excêntrico do licopeno (Hu et al., 206), pois catalisa preferencialmente a clivagem dos carotenoides não-provitamina A. Ainda, a BCO2 cliva preferencialmente os isômeros de licopeno 5-*cis* e 13-*cis* em apo-10'-licopenal mas não *all-trans*-licopeno. Uma explicação possível é que a estrutura química dos isómeros *cis* do licopeno pode imitar a estrutura do anel do β-caroteno e encaixar-se mais facilmente à ligação enzima-substrato (Wang, 2012).

Ambas as enzimas BCO1 e BCO2 são altamente expressas no fígado e no intestino (Raghuvanshi et al., 2015), mas também podem ser encontradas no rim, pulmão, coração, baço, próstata, testículo, estômago, cólon e cérebro (Kiefer et al., 2001). No fígado ao nível subcelular, a BCO1 é encontrada no citosol, enquanto BCO2 é encontrada na mitocôndria (Raghuvanshi et al., 2015).

Das glicoproteínas presentes na superfície do duodeno e jejuno, a CD36 está envolvida na captação de ácidos graxos de cadeia longa e das LDL oxidadas. Além disso, pode desempenhar um papel no movimento de carotenoides nas células. Moussa et al. (2011) mostraram que a CD36 está envolvida na absorção de licopeno e luteína pelo tecido adiposo. Carotenoides não-provitamina A, tais como luteína, licopeno e xantofilas podem ser reduzidas pela clivagem da β-caroteno a partir da inibição competitiva da BCO1 (Darroch, 2001).

Depois de serem absorvidos pela mucosa do intestino delgado, os carotenoides são clivados pela BCO1 e ou BCO2 formando a vitamina A e outros metabólitos, ou farão parte dos quilomícrons, a seguir, transportados pela linfa até a circulação central, que carregam para o músculo e tecido adiposo. Nos capilares destes tecidos há a presença das

enzimas extracelulares lipases lipoproteicas, que hidrolisam grande parte do triacilglicerol em ácidos graxos livres e glicerol, que podem ser absorvidos pelas células nos tecidos alvos ou resultando em quilomícrons remanescentes que interagem com receptores nos hepatócitos e são captados por endocitose (Bojórquez et al., 2013).

Uma vez no fígado, os carotenoides podem ser convertidos a retinoides e outros metabólitos, exercendo funções biológicas no próprio fígado ou então incorporados em lipoproteínas, sendo os remanescentes transportados aos tecidos extra-hepáticos (Figura 2). Eles são empacotados juntamente com os triglicerídeos em VLDL e transportados para o sangue, e do sangue para o tecido adiposo os triglicerídeos são removidos e a gordura armazenada em gotículas de gordura dentro do adipócito, no caso do licopeno, a proteína CD36 faz sua absorção no tecido. A extração dos lipídeos das VLDL é acompanhada de perda de parte das apolipoproteinas, convertendo gradativamente as VLDL em LDL que transporta o colesterol para os tecidos extra hepáticos ou de volta ao fígado como HDL.

Cerca de trinta minutos após a ingestão, o licopeno é incorporado às micelas e é absorvido na mucosa intestinal, depois é incorporado aos quilomícrons e transportado através do sistema linfático (Bojórquez et al., 2013). Alguns metabólitos podem ser transportados diretamente para o fígado pelo sangue portal (Wang et al., 1992; Borel et al., 2005; Wang, 2012). Os fatores específicos que regulam a absorção pelos tecidos, a reciclagem de carotenoides de volta para o fígado, e a excreção não são ainda compreendidos (Canene-Adams e Erdman, 2009). Experimentos *in vitro* sugerem a absorção preferencial é do isômero *cis* do licopeno, em relação à forma *trans*, devido à sua estrutura ligeiramente modificada, tornando a molécula mais curta e mais adequada para se difundir nas micelas, sendo então preferencialmente incorporado aos quilomícrons (Boileau e Erdman, 2002).

O licopeno é o carotenoide predominante no soro humano, com níveis plasmáticos que variam de 0,22 a 1,06 nmol/mL, e é encontrado ainda em vários tecidos do corpo, como fígado, rins, glândulas renais, testículos, ovários e próstata (Lu et al., 2011). Sua concentração é proporcional à sua ingestão alimentar, mas é pouco influenciada pelas variações diárias, devido ao fato da meia vida do licopeno no plasma ser de 12 a 33 dias (Cohen, 2002). As principais formas encontradas no sangue são apo-licopenais, incluindo a apo-10’licopenal, (Kopec et al., 2010), e a apo-10'-licopenal resulta tanto em ácido apo-10'-licopenoico e apo-10'-licopenol, dependendo da presença de NAD + ou NADH (Figura 4) (Hu et al., 2006). Lembrando que a suplementação da forma *all-trans* licopeno

resulta em um aumento significativo na concentração de *cis*-licopeno nos tecidos (Liu et al., 2006).

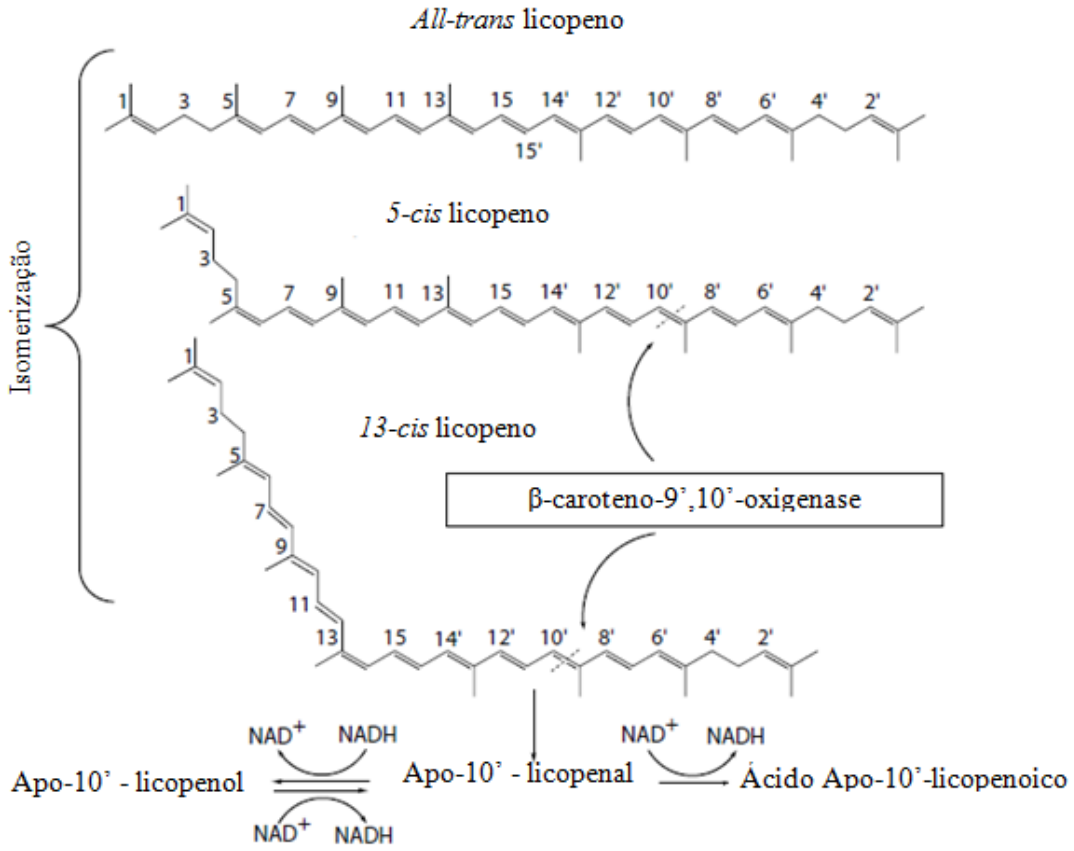


Figura 4 - Ilustração esquemática da via metabólica do licopeno pela β-caroteno-9',10'-oxigenase. Fonte: adaptado Hu et al. (2006).

6. Licopeno na alimentação animal

No Brasil, o licopeno é controlado pela Agência Nacional de Vigilância Sanitária (ANVISA) e é classificado como um alimento com alegações de propriedades funcionais e ou de saúde. Segundo a ANVISA, o produto, para ser comercializado, deve apresentar o processo detalhado de obtenção e padronização da substância, incluindo solventes e outros compostos utilizados, além de apresentar laudo com o teor de resíduos dos solventes utilizados e grau de pureza (Agência Nacional de Vigilância Sanitária - ANVISA, 2009).

O licopeno é muito estudado em humanos, e resultou em inúmeras evidências que comprovam seu efeito preventivo sobre doenças associadas ao estresse oxidativo, como o câncer e doenças cardiovasculares (Rao e Shen, 2002). Em animais, tem aumentado cada vez mais o interesse em se estudar este composto antioxidante, com o intuito de avaliar possíveis benefícios sobre a produtividade, saúde animal, bem estar e qualidade dos produtos.

Diversos trabalhos utilizando o extrato do licopeno ou fontes de licopeno, como os produtos derivados do tomate, foram realizados nas mais diversas espécies de animais, avaliando seus possíveis benefícios. Em peixes, o licopeno foi testado (800 mg/kg na ração), com os animais submetidos ao estresse por contenção, e observou-se que o licopeno não alterou os parâmetros de desempenho (ganho de peso e conversão alimentar). Porém, a inclusão do licopeno na dieta aboliu os efeitos negativos do estresse de contenção, evitando a elevação dos níveis de cortisol, conteúdo de TBARs do filé e atividade das enzimas catalase, glutationa redutase e lactato desidrogenase no músculo branco e vermelho. Em vista disso, os autores concluíram que o licopeno reduz os efeitos negativos do estresse, sem alterar o desempenho de juvenis de tilápias do Nilo (Girao et al., 2012).

Estudos desenvolvidos com aves apontaram que o licopeno pode atenuar os efeitos negativos quando estes animais são expostos a desafios. Leal et al. (1999) observaram que frangos suplementados com licopeno e expostos às micotoxinas mostraram redução no peso corporal, consumo de ração e conversão alimentar, mas essas reduções foram, em parte, atenuadas pela suplementação de licopeno.

Em codornas, Sahin et al. (2006) estudaram a suplementação dietética de licopeno (50, 100 e 200 mg/kg de ração) associado a diferentes temperaturas, de estresse (32°C) e termoneutra (22°C), e observaram que a suplementação de licopeno aumentou linearmente o consumo de ração, o ganho de peso e a eficiência alimentar, sob condições de estresse pelo calor (34°C), mas o desempenho de aves criadas a uma temperatura termoneutra não foi influenciado pela suplementação de licopeno. Os mesmos autores também observaram efeitos positivos do licopeno sobre os níveis de VLDL e HDL, que aumentaram e as concentrações de LDL foram reduzidas, principalmente a uma concentração dietética de 200 mg de licopeno/kg de ração.

Em experimentos desenvolvidos com frangos de corte por Sevcikova et al. (2008), os autores observaram efeitos positivos do licopeno sobre o perfil lipídico do plasma

sanguíneo. Este comportamento do perfil lipídico do plasma, quando os animais são suplementados com licopeno, se repete em efeitos simililares em humanos, em que pessoas alimentadas com uma dieta rica em tomate mostraram um aumento do HDL em 15,2% (Blum et al., 2006) e redução do colesterol total com suplementação do licopeno (Rao e Shen, 2002).

Estudos realizados com poedeiras demonstraram que ovos provenientes de poedeiras comerciais, alimentadas com 65, 257 e 650 mg de licopeno/kg de ração, apresentaram maior intensidade de coloração da gema em relação aos ovos de aves alimentadas com rações sem licopeno, de forma que a coloração ocorreu por meio da adição de 420 mg de licopeno/kg de ração (Olson et al., 2008).

Utilizando o suíno como modelo para seres humanos, Parjk et al. (2006) avaliaram o potencial do licopeno sobre a redução do estresse oxidativo, quando foi incluido 615 g/dia de tomate na ração de suínos, e constataram que o licopeno atua na redução efetiva do estresse oxidativo por reduzir as concentrações de malonaldeído no corpo e por atuar na proteção de células mononucleares do sangue contra o aumento de danos ao DNA. Chung et al. (2014) avaliaram diferentes inclusões de subprodutos do processamento do tomate, sobre a qualidade da carne suína, mas não observaram efeitos sobre a cor e pH.

O licopeno proporciona resultados positivos não somente para a saúde do animal e seu bem estar, pois também pode melhorar a qualidade dos produtos, como carne e ovos, o que resulta em alimentos com maior qualidade ao consumidor. Desta forma, pode ser uma alternativa interessante para ser usado na alimentação animal.

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II- OBJETIVOS GERAIS

Avaliar níveis de suplementação de licopeno para suínos machos castrados e fêmeas, dos 75 aos 100 kg.

Objetivos específicos:

- ✓ Avaliar o efeito de diferentes níveis de licopeno sobre o desempenho;
- ✓ Avaliar o efeito de diferentes níveis de licopeno sobre as características da carcaça, qualidade da carne e a oxidação lipídica do músculo *longissimus lumborum* e fígado;
- ✓ Avaliar o efeito de diferentes níveis de licopeno suplementar sobre a expressão gênica de enzimas no fígado de suínos;
- ✓ Estudar a suplementação de licopeno sobre os parâmetros imunológicos e bioquímicos do plasma de suínos.

III- EFFECTIVENESS OF LYCOPENE IN THE DIET REGARDING THE EXPRESSION OF FREE RADICAL-FIGHTING ENZYMES AND MODULATION OF THE BLOOD LIPID PROFILE OF PIGS

ABSTRACT

The objective of this work was to evaluate the effect of lycopene addition in the diet of barrows and gilts on the performance, lipid profile of the blood and the gene expression of antioxidant enzymes. Eighty pigs were used, 40 barrows and 40 gilts, with a mean initial weight of 75.04 ± 1.6 kg. They were distributed in a 2 x 5 factorial arrangement, consisting of both sexes (male and female) and five levels of lycopene (0, 12.5, 25.0, 37.5 and 50.0 mg/kg of diet). The variables were submitted to the statistical analysis adopting the level of significance of 5%. No interactions ($P > 0.05$) were observed between sex and levels of lycopene for the performance of the animals. Sex influenced daily feed intake ($P = 0.001$) and feed:gain ratio ($P = 0.001$), being lower for gilts. The interaction between lycopene levels and sex resulted in a linear reduction in the gene expression of the enzymes superoxide dismutase (SOD) ($P = 0.018$) and catalase ($P = 0.001$) in the liver for the gilts. Gilts presented lower SOD gene expression ($P= 0.001$) with 50.0 mg of lycopene supplementation and catalase ($P = 0.001$) at the levels of 0; 12.5 and 50.0 mg of lycopene and also of that of glutathione peroxidise ($P= 0.001$), in relation to barrows. No interactions ($P > 0.050$) were observed between the factors and the biochemical parameters of the blood. Total cholesterol decreased ($P = 0.001$) as dietary levels of lycopene supplementation increased, as did low-density lipoprotein (LDL) ($P = 0.001$). Lycopene supplementation in the diet provided improvements in the lipid profile of the blood plasma, since it increased the levels of high density lipoprotein (HLD) ($P = 0.001$), and the LDL:HDL ratio decreased ($P = 0.001$) with the inclusion of lycopene in the diet. Gilts had higher plasma concentrations of urea ($P = 0.001$) and triglycerides ($P = 0.001$) and lower concentrations of HDL ($P = 0.001$), LDL ($P = 0.001$) and the LDL:HDL ratio ($P = 0.004$). Dietary lycopene does not influence the performance of pigs, being a potent modulator of the lipid profile, reducing the levels of total cholesterol and low density lipoproteins and increasing the high density lipoproteins, as well as reducing the gene expression of superoxide dismutase enzymes and catalase in the liver of gilts.

Keywords: antioxidant, carotenoids, enzymes, blood

1. **Introduction**

Reactive oxygen species are normally produced in cellular biological processes. However, changes in the oxidant/antioxidant balance, in favor of oxidants, is called "oxidative stress." In this condition, large quantities of reactive oxygen species (ROS) are present, not only due to overproduction but also due to a deficiency in antioxidant defense systems (Halliwell and Gutteridge, 2015). Excess free radicals react with membrane lipids and proteins and can induce cellular and tissue damage (Valko et al., 2006). The defense against these free radicals in the animal body begins to occur naturally through the action of antioxidants.

In physiological conditions, the endogenous antioxidant defense system includes the presence of the enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) (Halliwell and Gutterdge, 2015), which allow the elimination of excess reactive oxygen species. Among the non-enzymatic components of antioxidant defense, carotenoids such as β-carotene, lycopene and lutein, can be highlighted (Papas, 1999), which exert antioxidant functions in lipidic phases, blocking free radicals that damage lipoprotein membranes (Sies and Stahl, 1995). The enzymatic antioxidant system is the first to act, preventing the accumulation of superoxide anion radicals and hydrogen peroxide. These enzymes act in a cooperative or synergistic way to ensure global cellular protection (Day, 2009).

Exogenous antioxidants may be necessary to protect animals from oxidative stress (Pajk et al., 2006). Among the natural antioxidants in the dietthere is lycopene, a carotenoid without pro-vitamin A activity. A lipid-soluble substance composed of eleven conjugate bonds and two unconjugated double bonds, this is known as the carotenoid with the greatest sequestering ability of singlet oxygen, possibly due to the presence of two unconjugated double bonds, which gives it greater reactivity (Di Mascio et al., 1989). Studies have shown that lycopene, in addition to being a potent antioxidant and protecting protein and DNA molecules, has been extensively studied because of its actions on blood lipid components (Rao and Agarwal, 1998) and lipoprotein (LDL) (Sahin et al., 2006).

Lycopene has also been studied in diets for the most diverse species, assessing its potential on performance characteristics of animals. In juvenile Nile tilapia, lycopene reduces the negative effects of stress without altering its performance (Girao et al., 2012). In quails, Sahin et al. (2006) studied the dietary supplementation of lycopene associated with stress temperatures, both heat and thermoneutral, and found that lycopene

supplementation linearly increased feed intake, weight gain and feed efficiency under heat stress conditions. Chung et al. (2014) evaluated different inclusions of tomato processing by-products on pork quality, but found no effects on color and pH.

In this sense, lycopene shows it self as a potent antioxidant against the reactivity of oxygen species in the organism, either through the regulation of enzymes or sequestering the ROS, in addition to exerting an effect on the lipid profile of the blood. In this way, the objective of this work was to evaluate the effect of lycopene addition in the diet of barrows and gilts, regarding the performance, lipid profile of the blood and gene expression of antioxidant enzymes.

2. Materials and methods

The experiment was carried out in the swine production sector of the Experimental Farm of Iguatemi, belonging to the Agricultural Sciences Center of the State University of Maringá, located in the State of Paraná (23° 21'S, 52° 04'W and Altitude of 564m), from January 2016 to May 2016. All experimental procedures were previously submitted to the Ethical Conduct Committee on the Use of Animals in Experimentation, and were approved for execution (nº6570200815).

2.1. Animals and installation

The pigs (Piétrain x Landrace x Large White) were housed in a masonry shed, covered with asbestos cement tiles, divided into two wings, each consisting of 20 pens (3 m^2) separated by a central corridor. Each bay had a pacifier type drinker and a semi-automatic feeder, providing free access to feed and water throughout the experimental period.

The temperature was monitored by means of a digital thermometer of minimum and maximum temperature, which registered minimum was 19.62 ± 2.54ºC and maximum was 32.11 ± 3.2ºC.

2.2. Experimental design and diet

Eighty pigs were used, 40 barrows and 40 gilts, with a mean initial weight of 75.04 ± 1.6 kg. The animals were distributed in a randomized block design in a 2 x 5 factorial arrangement consisting of two sexes (male and female) and five levels of lycopene (0,

12.5, 25.0, 37.5 and 50.0 mg/kg of diet) with eight replicates and one animal per experimental unit.

The experimental diets (Table 1) were formulated based on corn, soybean meal, minerals, vitamins and additives, to meet the nutritional recommendations proposed by the National Research Council - NRC (2012). The inclusion of the product containing 10% of lycopene to the diets was replaced by 0, 125, 250, 375 and 500 mg of inert/kg of diet, corresponding to the levels of 0; 12.5; 25.0; 37.5 and 50.0 mg of lycopene/kg of diet.

2.3. Performance

The animals were weighed at the beginning and in the end of the experiment for the determination of daily gain (DG). The rations were weighed every time they were fed to the animals for the determination of daily feed intake (DFI) and feed:gain ratio (F:GR) calculation.

2.4. Gene expression of enzymes

Upon reaching the mean weight of 100 kg, the animals were fasted for 24 h and later slaughtered in the abattoir of the Maringá State University Experimental Farm. The pigs were submitted to electrical stunning (200W), and then killed by exsanguination, shaved, and gutted.

Liver tissue samples were collected immediately after slaughter. All materials used in the collection were previously treated with RNase inhibitor (RNase Zap®, Life Technologies, Brazil). The samples were conditioned in microtubes containing RNAlater® (Life Technologies, Brazil), refrigerated for 24 hours at a temperature of 2-4°C and then stored in a freezer at -18°C until the moment of RNA extraction.

Total RNA was extracted using the Trizol reagent (Invitrogen, Carlsbad CA, USA) according to the manufacturer's standards, and quantified using a spectrophotometer at 260 nm. The integrity of the RNA was evaluated in 1% agarose gel, stained with 10% ethidium bromide and visualized in ultraviolet light. Total RNA samples were treated with DNaseI (Invitrogen, Carlsbad, CA, USA) for the removal of possible genomic DNA residues, according to the manufacturer's recommendations. Complementary DNA synthesis (cDNA) was performed with the kit SuperScript^{TM} III

First-Strand Synthesis Super Mix kit (Invitrogen Corporation, Brazil), according to the manufacturer's specifications, and stored at -20°C until use.

Gene expression of the GPx, SOD and Catalase enzymes was measured by quantitative polymerase chain reaction (qPCR) in real-time using SYBR GREEN fluorescent dye (Roche, Basel, Switzerland) and LightCycler® 96 equipment (Roche, Basel, Switzerland). All analyses were performed in a volume of 25 µL and in duplicate. Cycling was performed according to the manufacturer's recommendations for the kit used.

The pairs of primers (direct and reverse) used in the amplification reactions of endogenous control (ß-actin) and target genes (SOD, GPx and catalase) were constructed based on the sequences available in the NCBI GenBank database .ncbi.nlm.nih.gov) for pig (*Sus scrofa*), using the program www.idtdna.com (Table 2). B-actin was used as an endogenous control because it presented better efficiency and greater specificity in the reaction.

The results of gene expression were generated and recorded as threshold cycle values (Ct), adjusted by the equation proposed by Coble et al. (2011), as follows:

Ct adjusted = 40 - [(mean Ct of target gene) + (median Ct of endogenous control - mean Ct of endogenous control) x (regression coefficient of target gene/regression coefficient of endogenous control)].

2.5. Plasma biochemistry

At the end of the experiment, blood was collected from the animals for the determination of urea, glucose, triglycerides, total cholesterol, HDL and LDL in the blood plasma.

The animals were first fasted for 6 hours. Blood samples were obtained by puncture of the jugular vein, according to Oliveira (2004), with the aid of needles with a length of 100 mm. The blood was collected and transferred into two tubes; one containing the anticoagulant fluoride oxalate, for glucose determination, and the other containing EDTA for the other analyses. Afterwards, the tubes were centrifuged at 3000 rpm for a period of 15 minutes, to obtain the plasma of the samples containing EDTA in the tubes and 30 minutes for samples containing fluoride oxalate. The plasma was extracted with an automatic pipette and stored in eppendorf tubes, stored at 2-8°C or cooled to -20°C, depending on the analysis protocol.

Determinations of urea, glucose, triglycerides, cholesterol and HDL were performed using the specific kits (Bioclin), and the precipitant was also used for the determination of LDL (Winer) according to standard operating procedures (SOPs) specific for each variable. Subsequently, the absorbance reading was performed on a Biochemical Analyzer (Bioplus® 2000).

2.6. Statistical analysis

The UNIVARIATE procedure was used to evaluate the presence of outliers. Subsequently, the variables were submitted to analysis of variance (ANOVA), and the effects of block, sex, lycopene and the interaction of sex and lycopene were included in the model.

The degrees of freedom regarding lycopene levels were deployed in orthogonal polynomials to obtain regression equations. Data were submitted to statistical analyzes using the Statistical Analysis System - SAS (2001). The significance level of 5% was adopted.

3. Results

3.1. Performance

No interactions ($P > 0.05$) were observed between sex and lycopene levels (Table 3). The levels of lycopene evaluated did not influence ($P > 0.05$) the performance of animals (FW, DG, DFI and F:GR) (Table 3). Sex influenced DFI ($P = 0.001$) and F:GR ($P = 0.001$), being lower for gilts.

3.2. Gene expression of enzymes

There was a significant interaction between lycopene levels and sex (Table 4) for SOD ($P = 0.018$) and catalase ($P = 0.001$). The unfolding of the interaction (Fig. 1a, b) showed a linear reduction in the gene expression of SOD and catalase in the liver only for gilts, as the dietary lycopene supplementation was increased, adjusting the equations $\hat{Y} = - 0.0125x + 0.9155$ ($R^2 = 0.50$) and $\hat{Y} = - 0.0024x + 0.2418$ ($R^2 = 0.64$), for SOD and catalase respectively. Sex influenced the expression of SOD ($P=0.001$) and catalase ($P =$

0.001), with SOD gene expression (Fig. 1c) being less expressive in gilts than in barrows with the supplementation of 50.0mg of lycopene and, catalase (Fig. 1d) being less expressive in gilts at levels of 0; 12.5 and 50.0 mg of lycopene, relative to barrows.

Supplementation of lycopene in the diet of barrows and gilts did not influence ($P > 0.05$) gene expression of the GPx antioxidant enzyme in the liver (Table 4), but was influenced by sex ($P = 0.001$), in which gilts presented lower gene expression in relation to barrows.

3.3. Plasma biochemistry

No interactions were observed ($P > 0.05$) between the factors studied (Table 5). Total cholesterol decreased ($P = 0.001$) in the blood plasma of barrows and gilts as the levels of lycopene supplementation increased in the diet (Table 5), where the ratio of this reduction is supported by the equation $\hat{Y} = - 0.134x + 78.16$ ($R^2 = 0.97$). The same behavior was observed for LDL ($P = 0.001$), represented by the equation $\hat{Y} = - 0.217x + 29.10$ ($R^2 = 0.92$).

Dietary lycopene supplementation provided improvements in the lipid profile of the blood plasma, as it increased HDL levels ($P = 0.001$), represented by the equation $\hat{Y} = 0.260x + 37.34$ ($R^2 = 0.91$). The LDL:HDL ratio decreased ($P = 0.001$) with the inclusion of lycopene in the diet ($\hat{Y} = - 0.008x + 0.793$; $R^2 = 0.94$). However, triglyceride levels were not influenced, but a trend of linear reduction ($P = 0.052$) could be observed as the concentration of lycopene increased in the diet of pigs.

Glucose and urea in blood plasma were not influenced ($P > 0.05$) by levels of supplementation of lycopene in the diet of pigs. Sex influenced the levels of urea ($P = 0.001$), triglycerides ($P = 0.001$), HDL ($P = 0.001$), LDL ($P = 0.001$) and LDL:HDL ratio ($P = 0.004$), with gilts presenting higher plasma concentrations of urea and triglycerides and lower concentrations of HDL, LDL and LDL:HDL ratio (Table 5).

4. Discussion

Were found that evaluated lycopene in diets for pigs, but tomato derivatives are the main natural sources. When using tomato silage in feed for finishing pigs, Aguilera-Sot et al. (2014) also observed that the performance variables were not influenced, as in this study, in which the inclusion of lycopene, up to 50.0 mg/kg, does not harm or benefit the performance of pigs. In this way, lycopene should be treated specifically as an antioxidant.

The difference observed (P = 0.001) between the sexes, for DFI and F:GR, is due to the gilts being more efficient than barrows. The feed conversion of pigs is influenced by sex, which is well documented, with entire males being more efficient than gilts, which in turn are more efficient than barrows (NRC, 2012).

Antioxidants are stable molecules and enable the donation of electrons to a free radical, and neutralize it, thereby reducing its ability to damage cells. These antioxidants retard or inhibit cellular damage, primarily through their free radical scavenging property (Lobo et al., 2010). One of the most frequent approaches in the literature, regarding carotenoids, is its ability to modulate gene expression. Among the most studied genes are those that code for xenobiotic metabolizing enzymes, which are responsible for the detoxification of compounds that are harmful to the body.

In this work, it was possible to observe a linear reduction in the gene expression of SOD and catalase in the liver as the inclusion of lycopene in the diet of the gilts was increased (Fig. 1 a, b). SOD is an enzyme that suppresses superoxide radicals and is considered the first line of defense against the deleterious effects of oxygen radicals in cells; also, it captures the reactive species of oxygen radicals, catalyzing the dismutation of the radical $O_2^{\circ-}$ to H_2O_2 and O_2 (Teoh-Fitzgerald and Domann, 2012). Catalase has the ability to remove hydrogen peroxide from the interior of the cells, formed by the action of SOD, in which it dismutes the hydrogen peroxide to oxygen and water, through the reaction $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$ (Agarwal et al., 2011).

However, the enzyme glutathione peroxidase was not influenced by the supplementation of lycopene in the diet of pigs (Table 4), which is part of the glutathione system, together with glutathione oxidase (GO) and glutathione reductase (GR); both act together, converting the H_2O_2 molecules into H_2O (Huber et al., 2008). The enzymatic antioxidant defense system represents the body's first line of defense and plays a key role in protecting cellular systems against oxidative damage. Therefore, gene expression can be used to assess the redox state of the organism. Depending on the intensity and duration of stress that these animals are subjected to, as well as the susceptibility of the exposed organism, both the induction and inhibition of the enzymes can be observed (Kujawska, et al., 2014).

In other studies, lycopene demonstrated its potential in the up-regulation of antioxidant response elements (Van Breemen and Pajkovic, 2008), through the induction of phase II enzymes, i.e. enzymes known to play a role in detoxification, by activating

Nrf2, which activates the genes encoding phase II enzymes, including SOD, catalase, GPx (Van Breemen and Pajkovic, 2008).

In this study, a reduction in the expression of SOD and catalase enzymes was observed with the increase in the inclusion of lycopene in the diet for gilts. These findings corroborate to the results obtained by Moreira et al. (2005) and Kujawska et al. (2014), who also observed reduced hepatic activities of SOD and catalase in rats supplemented with 20 - 30 mg lycopene/kg/day and 0.5 - 2.5 mg lycopene/kg/day, respectively.

However, Kujawska et al. (2014) evaluated the effect of lycopene on N-nitrosodiethylamine-induced oxidation in rats and found that lycopene-pre-treated animals increased SOD, catalase and GPx enzyme activity and liver lipid peroxidation was reduced; however, when the animals were not induced by N-nitrosodiethylamine the activity of these enzymes was reduced. Singh et al. (2012) observed the effect of lycopene on the oxidative stress caused by diabete in humans, which resulted in the increased expression of SOD, GSH, GPx and GR enzymes with lycopene consumption. The same effect of lycopene was found by Pandir et al. (2016) on rat kidneys.

Based on previously published studies and the results obtained in this work, it can be inferred that the long-term supply of lycopene can reduce the expression of SOD and catalase in healthy animals as a result of an adjustment of the cellular redox state and exogenous antioxidants, thus influencing the ability to combat oxidative stress in the body (Da Costa et al., 2012).

In addition, it was observed that the expression of SOD and catalase enzymes behaved differently in barrows and gilts supplemented with lycopene (Figure 1.c and d). The gene expression of SOD was less expressive in gilts than barrows with the supplementation of 50.0mg of lycopene, and catalase was less expressive in gilts at levels of 0, 12.5 and 50.0 mg of lycopene, in relation to barrows. Similarly to SOD and catalase, the expression of GPx was influenced by sex (Table 4), in which gilts presented lower gene expression in relation to barrows.

Lycopene has also been assigned an antiatherogenic role, mainly due to its antioxidant capacity, which is related to the prevention of LDL oxidation (Rao, 2002). A mechanism involving the regulation of cholesterol metabolism by carotenoids was proposed by Heber and Lu (2002), supported by the fact that lycopene shares similar pathways to cholesterol synthesized in animals.

A linear reduction in total cholesterol levels was observed as a function of the increase in dietary lycopene (Table 5). The reduction of intracellular cholesterol by the

action of lycopene has been associated with a reduction in cholesterol synthesis through an inhibition of possible mechanisms involved in the reduction of intracellular cholesterol by lycopene, such as the decrease of cholesterol synthesis through the inhibition of activity and expression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, LDL receptor modulation and activation of the acyl-CoA: cholesterol acyltransferase (ACAT) enzyme activity (Palozza et al., 2012).

The maintenance of cholesterol homeostasis is achieved through a network of genes involved in cholesterol synthesis, absorption, metabolism and elimination (Palozza et al., 2012). Cholesterol is derived from LDL uptake through receptor-mediated endocytosis (Brown and Goldstein, 1986), as well as new cell synthesis (Brown and Goldstein, 1980). The cholesterol balance is maintained by distinct processes, which first involve the suppression of cholesterol synthesis within the cell by inhibiting the activity of the HMG-CoA reductase enzyme, which promotes the deacylation of HMG-CoA reductase to mevalonate (Brown and Goldstein, 1980; Istvan and Deisenhofer, 2001). There is evidence that lycopene can inhibit the synthesis of cholesterol in human macrophages by inhibiting the activity of HMG-CoA reductase and its expression (Simone et al., 2011).

The second involves the activation of ACAT, an enzyme present in the liver that catalyzes the formation of cholesterol esters from cholesterol, favoring the esterification and storage of excess cholesterol. The third process involves modulating LDL receptor activity by inhibiting the activity of HMG-CoA reductase in mammalian cells by excess cholesterol, and is associated with reduced LDL receptor activity secondary to the inhibition induced by esterol of the transcription receptor gene of LDL (Brown and Goldstein, 1986; Wang et al., 1994).

Similarly, the level of LDL reduced linearly (Table 5) as the concentration of dietary lycopene increased. This reduction can be explained by the fact that lycopene, in the blood, is transported mainly by LDL (Palozza et al., 2012). This reduction can be explained by the fact that lycopene, in the blood, is transported mainly by LDL (Palozza et al., 2012). In rabbits supplemented with 127.8 mg/kg and 10 mg/kg de lycopene, LDL and total cholesterol levels were reduce in 54.48% and 26.60% of the total cholesterol to the LDL in 54.46% and 35.10%, respectively to the levels (Verghese et al., 2008; Mulkalwar et al., 2012), as well as in rats (Hassan and Edrees, 2004). Administration of β-carotene to rats reduced the expression of HMG-CoA reductase in the liver of the animals (Moreno et al., 1995). In addition, β-carotene and lycopene suppressed the

cellular synthesis of cholesterol in macrophage culture. These data were confirmed *in vivo*, where lycopene supplementation resulted in a 14% reduction in LDL.

Lycopene had a positive effect on HDL levels (Table 5), with lycopene increasing the HDL level by 34.36%. Studies with different species showed a similar effect on HDL levels (Hassan and Edrees, 2004; Verghese et al., 2008 and Mulkalwar et al., 2012). These effects may be related to the antioxidant ability of lycopene to protect LDL from oxidation, and its role in inhibiting the activity of HMG-CoA reductase and the regulation of LDL receptor activity in macrophages (Arab and Steck 2000; Heber and Lu , 2002), as well as its powerful efficiency in the neutralization of free radicals. The LDL:HDL ratio was also reduced with dietary supplementation of lycopene, associated with a lower risk of heart disease (Millán et al., 2009).

Plasma levels of urea and glucose were not influenced by lycopene, evidencing that lycopene did not influence the absorption of carbohydrates and amino acids from the diet, since the rations were isoenergetic and isoproteic; according to Stipanuk (2004), when amino acids are supplied in excess, they need to be catabolized by the body, indicating that an increase in urea plasma concentrations is related to an elevation of amino acid catabolism (Nelson and Cox, 2011).

Plasma concentrations of urea, triglycerides, HDL and LDL were influenced by sex (Table 5). Gilts presented higher plasma concentrations of urea and triglycerides and lower concentrations of HDL, LDL and LDL:HDL ratio in relation to barrows. Differences between sex in blood lipid metabolism appear to be the result of a complex network of sex hormones action in combination with other direct or indirect modulators, possibly sex-specific, but the exact nature is not known; however, factors such as insulin, adipokines and gene expression may be involved (Wang et al., 2011).

5. Conclusion

Dietary lycopene does not influence the performance of pigs, being a potent modulator of the lipid profile, reducing the levels of total cholesterol and low density lipoproteins and increasing the high density lipoproteins, as well as reducing the gene expression of superoxide dismutase and catalase enzymes in the liver of gilts.

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Tables

Table 1

Ingredients, chemical and energetic composition of basal diet.

Ingredients (%)	Barrows	Gilts
Corn	87.14	83.89
Soybean meal 45 %	9.61	12.47
Soybean oil	0.740	0.950
Dicalcium phosphate	0.785	0.973
Limestone	0.553	0.521
Salt	0.203	0.203
L-lysine HCL 78.4 %	0.373	0.390
L-threonine 98.5 %	0.068	0.072
DL-methionine 99 %	0.036	0.053
L-tryptophan 98 %	0.022	0.017
Growth promoter [1]	0.015	0.015
Vitamin and mineral supplement [2]	0.400	0.400
Lycopene extract [3]	0.000	0.000
Inert [4]	0.050	0.050
Total	100.00	100.00
Composition		
Metabolizable energy (Mcal/kg)	3.30	3.30
Nitrogen (%)	1.86	2.03
Total Calcium (%)	0.45	0.49
Available phosphorus (%)	0.23	0.26
Potassium (%)	0.42	0.47
Sodium (%)	0.10	0.10
Chlorine (%)	0.18	0.18
SID lysine (%)	0.690	0.770
SID met + cis (%)	0.400	0.440
SID threonine (%)	0.440	0.480
SID tryptophan (%)	0.120	0.130

[1] Enramycin 0.015%.

[2] Content/kg diet; vit. A: 30000 UI, vit. D3: 5000 UI, vit. E: 120 UI, vit. K: 5 mg, vit. B12: 120 mcg, Niacin: 150 mg, Calcium pantothenate: 75 mg, folic acid: 8 mg, Choline chloride: 0.48 g, iron: 350 mg, copper 15 mg, manganese: 250 mg, Zinc: 0.75 g, iodine: 10 mg, selenium: 3 mg.

[3] Lycopene extract at a concentration of 10% included in the basal diet replacing the inert.

[4] Fine clean sand.

Table 2

Primers sequence of Glutathione peroxidase (GPx), super oxide dismutase (SOD), catalase and ß-actin sed in real-time quantitative polymerase chain reaction (PCR).

Gene	Accession Number	Primers sequence (5'-3')	Amplicon (bp)	At (ºc)
SOD	GQ913661.1	D: GTACAAAGACGGGAAATGCTG R: TGAGGGAATGTTTACTGGGTG	73	60
GPx	KJ686126.1	D:TCATTGAGAACGTAGCGTCG R: CCTCATTCTTGGCATTTTCCTG	150	60
Catalase	D89812.1	D: TTCAGGACAATCAAGGTGGG R: TGGGTGACATTATCTTCGTTGG	145	60
β-actin	XM_003124280.3	D: GGTTTATTTTGAGTGGGCAGC R: CTGGTCTCAAGTCAGTGTACAG	137	60

At - annealing temperature.

Table 3

Performance of barrows and gilts in termination phase fed diets containing different levels of lycopene.

	Barrows						Gilts							P-value			
	Lycopene (mg/kg of diet)						Lycopene (mg/kg of diet)							Sex * Lycopene	Sex	Lycopene	
Item	0	12.5	25.0	37.5	50.0	Medium	0	12.5	25.0	37.5	50.0	Medium	SEM			Lin[1]	Qua[2]
Initial weight, kg	75.12	74.93	74.19	75.13	75.44	74.96	75.07	74.96	75.01	75.22	75.3	75.11	0.130				
Final weight, kg	99.68	99.81	101.67	99.63	100.96	100.35	100.04	100.23	99.91	101.49	101.08	100.55	0.221	0.479	0.975	0.480	0.442
Daily gain, kg	0.87	0.87	0.99	0.86	0.92	0.90	0.86	0.85	0.86	0.89	0.88	0.87	0.020	0.488	0.132	0.629	0.337
Daily feed intake, kg	2.71	2.77	2.87	2.70	2.82	2.77[a]	2.46	2.39	2.45	2.53	2.51	2.47[b]	0.012	0.798	0.001	0.582	0.648
Feed:Gain ratio	3.11	3.21	2.91	3.16	3.09	3.09[a]	2.84	2.77	2.80	2.80	2.89	2.82[b]	0.026	0.331	0.001	0.744	0.169

* Different letters in line differ by F test at 5% probability.

SEM: standard error of the mean.

[1]Linear effect of lycopene levels.

[2] Quadratic effect of lycopene levels.

Table 4

Gene expression of antioxidant enzymes in the liver of barrows and gilts fed diets with different levels of lycopene.

	Barrows						Gilts							P-value			
	Lycopene (mg/kg of diet)						Lycopene (mg/kg of diet)										
Item[1]	0	12.5	25.0	37.5	50.0	Medium	0	12.5	25.0	37.5	50.0	Medium	SEM	Sex * Lycopene	Sex	Lycopene	
																Lin[2]	Qua[3]
Superoxide dismutase	1.0509	1.8675	0.8837	0.9526	1.3017	1.2113	1.2223	0.4640	0.4119	0.4925	0.4292	0.6040	0.105	0.018	0.001	0.048	0.153
Glutathione peroxidase	0.4481	0.5383	0.2383	0.4069	0.4593	0.4182[a]	0.2692	0.1555	0.1529	0.1839	0.0825	0.1688[b]	0.029	0.373	0.001	0.251	0.332
Catalase	0.7634	2.6413	0.5535	0.6790	0.7488	1.0772	0.2803	0.1682	0.1845	0.1295	0.1526	0.1830	0.195	0.001	0.002	0.006	0.183

* Different letters in line differ by F test at 5% probability.

SEM: standard error of the mean.

[1] Expressed as arbitrary unity (AU).

[2] Linear effect of lycopene levels.

[3] Quadratic effect of lycopene levels.

Table 5

Influence of lycopene on the plasma parameters of barrows and gilts from 75 to 100 kg, fed diets containing different levels of lycopene.

	Barrows						Gilts							P-value			
	Lycopene (mg/kg of diet)						Lycopene (mg/kg of diet)							Sex		Lycopene	
Item	0	12.5	25.0	37.5	50.0	Medium	0	12.5	25.0	37.5	50.0	Medium	SEM	* Lycopene	Sex	Lin[1]	Qua[2]
Glucose, mg/dL	66.00	71.06	70.64	70.64	68.33	69.33	77.38	71.06	72.63	67.50	72.25	72.16	0.489	0.068	0.083	0.403	0.934
Urea, mg/dL	15.92	14.57	14.54	15.90	15.49	15.28[b]	20.49	19.43	20.82	18.99	19.91	19.93[a]	0.196	0.442	0.001	0.812	0.468
Triglycerides, mg/dL	39.00	35.19	35.00	34.64	36.08	35.98[b]	47.25	47.13	44.31	43.25	42.83	44.95[a]	0.759	0.535	0.001	0.052	0.318
Cholesterol, mg/dL[3]	83.07	79.00	75.98	74.64	74.42	77.42[a]	74.25	73.31	72.38	71.94	68.92	72.16[b]	1.209	0.379	0.001	0.001	0.568
HDL, mg/dL[4]	37.86	38.94	46.79	49.79	48.83	44.44	37.31	39.06	42.69	48.50	48.75	43.26	2.408	0.530	0.525	0.001	0.530
LDL, mg/dL[5]	34.39	29.82	26.12	23.05	23.60	27.40[a]	25.58	22.96	19.07	16.31	15.93	19.97[b]	1.996	0.761	0.001	0.001	0.119
LDL:HDL, ratio [6]	0.91	0.77	0.56	0.46	0.48	0.64[a]	0.69	0.59	0.45	0.34	0.33	0.48 [b]	0.079	0.636	0.004	0.001	0.261

***** Different letters in line differ by F test at 5% probability.

SEM: standard error of the mean.

[1]Linear effect of lycopene levels.

[2] Quadratic effect of lycopene levels.

[3] $\hat{Y} = -0.134x + 78.16$ ($R^2 = 0.97$).

[4] $\hat{Y} = 0.260x + 37.34$ ($R^2 = 0.91$).

[5] $\hat{Y} = -0.217x + 29.10$ ($R^2 = 0.92$).

[6] $\hat{Y} = -0.008x + 0.793$ ($R^2 = 0.94$).

Figure

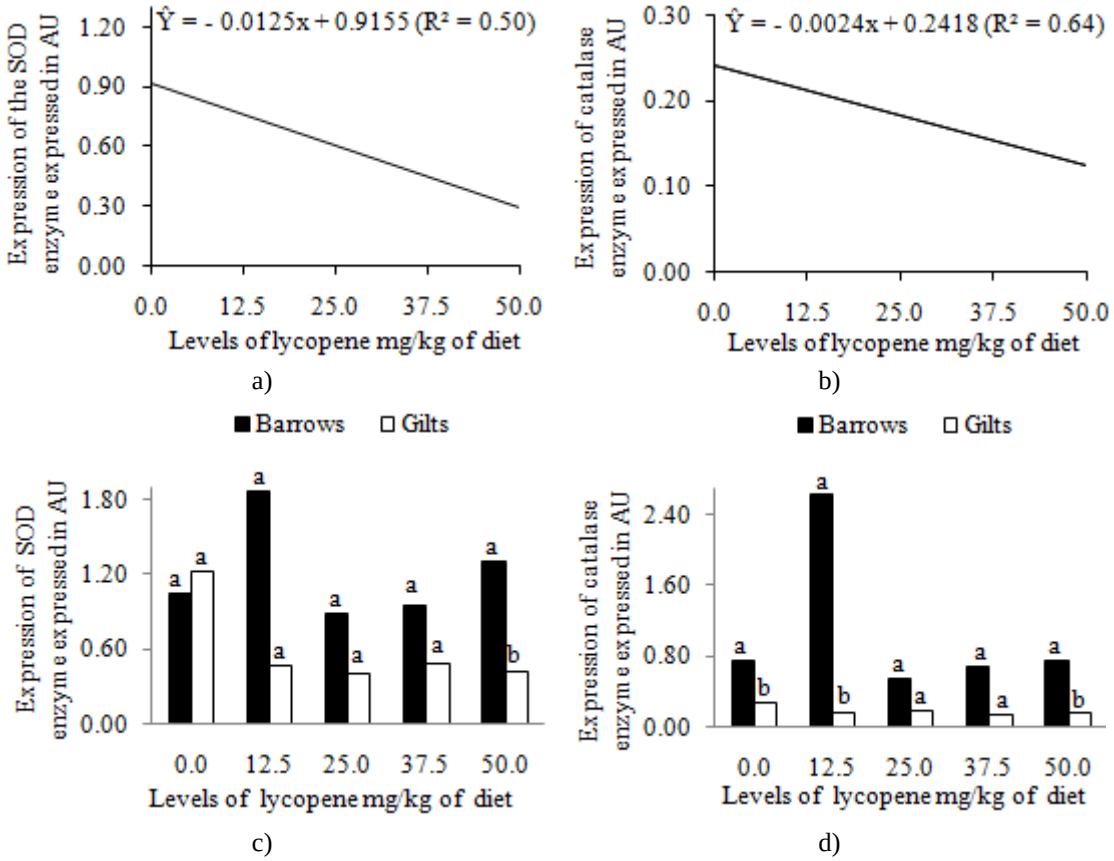

Fig. 1. Effect of levels lycopene on the gene expression of antioxidant enzymes in expressed as arbitrary unity (AU). A) superoxide dismutase (SOD) and b) catalase in the liver of gilts pig; and differences in the gene expression of thec) SOD and d) catalase enzymes in the liver of barrows and gilts (5% F test).

IV – LICOPENE ACTS IN PROTECTION AGAINST THE OXIDATION OF MUSCLE AND HEPATIC TISSUE OF PIGS

ABSTRACT

The objective of this study was to evaluate the effect of different levels of lycopene supplementation on the quantitative carcass characteristics, meat quality, lipid concentration oxidation product and antioxidant potential in the meat and liver of, barrows and gilts, in the termination phase. A total of 40 barrows and 40 gilts were distributed in a 2 x 5 factorial scheme consisting of two sexes (barrows and gilts) and five levels of lycopene (0, 12.5, 25.0, 37.5 and 50.0 mg kg^{-1} of diet). In addition, four storage periods (0, 24, 48 and 72 h), at 4ºC, were added to the model for the *longissimus lumborum* muscle. The inclusion of lycopene in the diet of barrows and gilts reduced the thawing loss and was effective in the protection against oxidation of *longissimus lumborum* muscle and liver. In which the best results for these variables were obtained with the supplementation of 50.0 mg of lycopene kg^{-1} of diet.

Keywords: antioxidants, DPPH, oxidation, TBARS

1. Introduction

Meat is a food that is rich in proteins and lipids and is highly susceptible to oxidation (Devatkal, Thorat, & Manjunatha, 2012). Oxidation processes occur even before slaughter, and intensify after slaughter, processing and storage of meat. After slaughter, oxidative processes in the meat are initiated mainly by lipid peroxidation and the oxidation of proteins, due to the production of reactive oxygen species, mainly caused by cellular rupture, contact with oxygen and light, presence of heme iron, temperature and irradiation (Papuc, Goran, Predescu, & Nicorescu, 2017).

The lipid oxidation process is one of the main factors related to meat deterioration, affecting nutritional and sensorial characteristics (Karakaya, Bayrak, & Ulusoy, 2011), due to the formation of free radicals and unpleasant odors (Simitzis, Symeon, Charismiadou, Bizelis, & Deligeorgis, 2010). Pork is highly susceptible to these oxidative changes, due to the high content of polyunsaturated fatty acids (Yi, Haug, Nyquist, & Egelandsdal, 2013).

The recent advances in research point to the possibility of attenuating the chemical substances in meat products through interventions with antioxidants (Engel, Ratel, Bouhlel, Planche, & Meurillon, 2015), making it an effective way to minimize the development of oxidative rancidity and extend the shelf life and nutritional quality of meat (Rajauria, Jaiswal, Abu-ghannam, & Gupta, 2013).

Antioxidants can be supplemented in animal diets or directly in processed meat (Moroney, O'Grady, O'Doherty, & Kerry, 2012). Dietary sources of antioxidants are essential when the body is exposed to a high degree of stress by radical superoxide anions and hydrogen peroxide (Jiang & Xiong, 2016), since excess free radicals react with membrane lipids and proteins to induce cellular and tissue damage (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006).

Synthetic antioxidants are under increasing oversight due to potential genotoxic effects (Blaszczyk, Augustyniak, & Skolimowski, 2013; Jiang & Xiong, 2016). Thus, there is a worldwide trend in using natural antioxidants (Kumar, Yadav, Ahmad, & Narsaiah, 2015) both as a supplement in animal diet and in processed products. The lycopene is a natural antioxidant, which is a member of the carotenoid family and is known to be a potent antioxidant that protects cells against damage caused by reactive oxygen species (Agarwal, Shen, Agarwal, & Rao, 2001; Rao & Rao 2007), because it has a greater ability to capture singlet oxygen molecules, compared to other pigments such as α-carotene, β-carotene and α-tocopherol (Di Masci, Kaiser, & Sies 1989). Also, they prevent lipid peroxidation by eliminating the initiating radicals (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003).

In pigs, Pajk et al. (2006) evaluated the effect of lycopene, present in tomato pulp, and observed an effective reduction in oxidative stress, reducing lipid oxidation and protecting mononuclear cells from increased blood damage to DNA.

The scientific literature is scarce in relation to the effects of the dietary supplementation of lycopene for pigs, in the termination phase, in relation to meat quality. Thus, the objective of this study was to evaluate the effect of different levels of lycopene on the quantitative carcass characteristics, meat quality, concentration of lipid oxidation products and antioxidant potential in the meat and liver of, barrows and gilts, in the termination phase.

2. Materials and methods

The experiment was carried out in the swine production sector of the Experimental Farm of Iguatemi, belonging to the Agricultural Sciences Center of the State University of Maringá, located in the State of Paraná (23° 21'S, 52° 04'W and Altitude of 564m), from January 2016 to May 2016. All experimental procedures were previously submitted to the Ethical Conduct Committee on the Use of Animals in Experimentation, and were approved for execution (nº6570200815).

2.1 Animals and installation

The pigs (Piétrain x Landrace x Large White) were housed in a masonry shed, covered with asbestos cement tiles, divided into two wings, each consisting of 20 pens ($3m^2$) separated by a central corridor. Each bay had a pacifier type drinker and a semi-automatic feeder, providing free access to feed and water throughout the experimental period.

The temperature was monitored by means of a digital thermometer of minimum and maximum temperature, which registered minimum was 19.62 ± 2.54ºC and maximum was 32.11 ± 3.2ºC.

2.2. Experimental design and diet

For the quantitative parameters of the carcass, meat quality and relative body weight, and determination of the concentrations of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and thiobarbituric acid reactive substances (TBARS) in the liver, a 2 x 5 factorial arrangement was used consisting of two sexes (male and female) and five levels of lycopene (0, 12.5, 25.0, 37.5 and 50.0 mg kg^{-1} of diet), with eight replicates and one animal per experimental unit. Additionally, for the variables DPPH and TBARS of the *logissimus lumborum* muscle, a factorial arrangement 2 x 5 x 4 was used, composed of two sexes (males and females), five levels of lycopene (0, 12.5, 25.0, 37.5 and 50.0 mg kg^{-1} of diet) and four meat storage periods (0, 24, 48 and 72 h).

Eighty pigs were used, 40 barrows and 40 gilts, with a mean initial weight of 75.04 ± 1.6 kg. The experimental diets (Table 1) were formulated based on corn, soybean meal,

minerals, vitamins and additives, to meet the nutritional recommendations proposed by the National Research Council - NRC (2012). The inclusion of the product containing 10% of lycopene to the diets was replaced by 0, 125, 250, 375 and 500 mg of inert kg^{-1} of diet, corresponding to the levels of 0; 12.5; 25.0; 37.5 and 50.0 mg of lycopene kg^{-1} of diet.

2.3. Quantitative carcass characteristics, meat quality and relative weight of organs

At the end of the trial period, the pigs were fasted for 24 h and weighed to obtain live weight at slaughter; these were later slaughtered in the abattoir of the Maringá State University Experimental Farm. The pigs were submitted to electrical stunning (200W), and then killed by exsanguination, before being shaved and gutted.

The gutted carcasses were divided in the middle in the longitudinal direction and later weighed and cooled (2 ± 1ºC per 24 hours). The carcasses were individually evaluated according to Bridi & Silva (2009) guidelines, for the hot carcass weight (HCW), cold carcass weight (CCW), hot carcass yield (HCY), cold carcass yield (CCY), carcass weight loss in cooling (CWLC), ham yield (HY), backfat thickness (BT) and depth of *logissimus lumborum* muscle (LL depth).

BT and LL depth were measured in the left half carcass, 24 hours *post-mortem*, with the aid of a pachymeter, in the region of insertion of the last thoracic vertebra with the first lumbar, six centimeters from the midline of the carcass (point P2). From the values of BT and LL depth, lean meat yield (LMY) using the equation proposed by Irgang et al. (1998), as follows: LMY (%) = 60 - ((BT mm x 0.58) + (LL depth mm x 0.10)). The liver, kidneys and abdominal fat were weighed to obtain the relative weight, calculated based on the HCW.

The pH of the meat was measured in the LL muscle with the aid of a HI 99163 digital portable pH meter (Hanna Instruments), at the height of the last rib, 45 minutes after slaughter (initial pH) and 24 hours after the beginning of cooling (final pH), following the recommendations of Bridi & Silva (2009).

For qualitative evaluations, three samples (2.5 cm thickness) of the LL muscle were used, in the region of insertion of the last thoracic vertebra with the first lumbar, in the caudal-cranial direction, as described by Bridi & Silva (2009). The first sample was used for color evaluation, the second for drip loss (DL) and the third for the determination of thawing loss (TL) cooking (CL) and shear force (SF).

DL was evaluated according to the technique described by Boccard et al. (1981). The TL was obtained using the weight difference of the frozen sample and after storage for 24 hours at 4ºC. The CL was obtained by the weight difference of the thawed sample and after baking in a preheated oven at 170ºC until the internal temperature of 71ºC was reached (Bridi & Silva, 2009).

Baked muscle samples were used to determine SF. Six subsamples with a cylindrical shape (1.27cm in diameter) were drawn longitudinally towards the muscle fibers (Ramos & Gomide, 2012). The SF was evaluated perpendicularly to the orientation of the muscle fibers with the Warner-Bratzler blade adapted in the Stable Mycro Systems TA-XT2i texturometer, using the pre-test speed of 5mm/sec in the 2mm/sec test and the 5mm/sec post-test, as recommended for pork meat.

For color evaluation, six Minolta lightness measurements were performed (L*, a*, and b*) using a portable colorimeter CR-400 Konica Minolta's (settings: Illuminant D65; 0° viewing angle; and 4 auto-average). The components L* (lightness), a* (red-green) and b* (yellow-blue) were expressed using the CIELAB color system.

2.4. Determination of thiobarbituric acid reactive substances (TBARS)

The evaluation of lipid oxidation by thiobarbituric acid was carried out by means of determination of the reactive substances to thiobarbituric acid, value in samples of hepatic tissue at 0 hours and for LL muscle, at periods 0, 24, 48 and 72 hours after slaughter. Samples were collected and stored in liquid nitrogen until analysis, where they were thawed at room temperature and monitored until reaching the temperature of 4ºC, the then were packed in plastic film and distributed randomly for storage under refrigeration at 4ºC under different storage periods. The procedures were performed according to the improved methodology of Juncher et al. (2001).

The aldehydes were extracted from the LL muscle through homogenization with 10 mL of a solution containing trichloroacetic acid (7.5%)/propyl gallate (0.1%)/EDTA (0.1%) with a 5 g Sample. Then, the solution with the sample was homogenized with an Ultra Turrax for 1min and the solution was filtered on qualitative filter paper. An aliquot of 2 mL was withdrawn from the extracted solution, which was conditioned with 1 mL of the thiobarbituric acid solution.

After mixing the solution with the extract, it was allowed to react by boiling at 100°C for 15 minutes, followed by cooling in ice water for 5 minutes. Subsequently, the

samples were centrifuged at 3000 rpm during 10 minutes for precipitation of the particles, followed by reading the absorbance at a wavelength of 532 nm.

For the extraction of the aldehydes from the liver, the same procedure described for the LL muscle was used, with some modifications. For extraction, 15 mL of extractive solution and 5 g of sample were used. From the extracted solution, a 1.5 mL aliquot was taken and packed with 1.5 mL of the thiobarbituric acid solution.

For the analysis of the results, a standard curve was used with 1,1,3,3 tetraethoxypropane (TEP) and distilled water. All results were expressed as mg of malonaldehyde Eq. kg^{-1} of tissue.

2.5. Determination of Total Antioxidant Activity by DPPH radicals

The DPPH analysis is based on the capture of the 2,2-diphenyl-1-picrylhydrazyl radical by antioxidants, producing a decrease in absorbance at 515 nm, according to the methodology described by Brand-Williams et al. (1995) and Li et al. (2005), with modifications, and using a 0.06 mM DPPH solution.

The analyzes were carried out on LL samples at 0, 24, 48 and 72 hours after slaughter and in hepatic tissue samples at zero hours. Samples were collected and stored in liquid nitrogen until analysis, where they were thawed at room temperature and monitored until reaching the temperature of 4ºC, the then were packed in plastic film and distributed randomly for storage under refrigeration at 4ºC under different storage periods.

For the meat samples, 5 g of the comminuted sample was used and then 15.0 mL of methanol was added, being homogenized in an Ultra Turrax for 1 minute and then filtering the extract with the aid of a qualitative paper filter. From extraction in the dark, 200, 400 and 600 μL aliquots of the test tube extract were transferred and 2 mL of the 0.06 nM DPPH solution was added. The tubes were left in the dark for 30 minutes to stabilize the absorbance. The effective concentration which inhibited 50% of the initial concentration of the DPPH radical, with subsequent readings at 515 nm. The results were obtained as follows:

% Inhibition DPPH = [(Abs control - Abs sample) / Abs control] × 100

For the liver tissue samples, the same procedure was used, with some changes. Here, 2.5 g of liver and 17.5 mL of methanol were used for the extraction and aliquots of 50, 100 and 150 μL of the extract were used for the readings.

2.6. Statistical analysis

The UNIVARIATE procedure was used to evaluate the presence of outliers. Subsequently, the variables were submitted to analysis of variance (ANOVA), and the effects of block, sex, lycopene and the interaction of sex and lycopene were included in the model for the quantitative and meat quality characteristics of DPPH and TBARS of the liver. Additionally, the effects of the storage period and the sex x period, lycopene x period and period x sex x lycopene interactions were included in the model, for the evaluation of DPPH and TBARS of the LL muscle.

The degrees of freedom regarding lycopene levels and/or storage period were deployed in orthogonal polynomials to obtain regression equations. Data were submitted to statistical analyzes using the Statistical Analysis System - SAS (2001). The significance level of 5% was adopted.

3. Results

3.1. Quantitative and qualitative characteristics carcass and relative weight of organs

The quantitative characteristics of the carcass and relative weight of the organs did not show any interactions (P>0.05) between the factors evaluated (Table 2). Likewise, the levels of lycopene in the diet did not influence (P>0.05) these variables. There was a difference in sex, whereby the barrows presented higher HCY (P=0.049), CCY (P=0.023), BT (P=0.001) and abdominal fat (P=0.001).

There were no interactions (P>0.05) between the factors evaluated (Table 3) in relation to the qualitative characteristics of the LL muscle. pH45 and pH24 were not influenced (P>0.05) by lycopene levels. DL and CL also did not differ between treatments (P>0.05), implying in a effect positive whit lycopene supplementation on what did not result in increased water losses of the LL muscle. TL linearly reduced (P=0.024) as a function of lycopene levels, reducing by up to 17.46% in relation to the treatment without lycopene, adjusting the equation $\hat{Y}$= - 0.0244x + 6.9282 (R^2= 0,89). For the SF, a tendency of reduction (P=0.064) was observed with an increase of the lycopene supplementation in the diet.

The color of the meat was not influenced (P>0.05) by the levels of lycopene in the diet, but a color difference between the sexes was observed for the variables a*

(*P*=0.001) and b* (*P*=0.045), in which the barrows showed higher staining intensity for the red-green and blue-yellow component, respectively.

3.2. Determination of the total antioxidant activity by the DPPH radical and lipid oxidation by thiobarbituric acid (TBARS) of the LL muscle

An interaction was observed (*P*=0.006) between storage periods and lycopene levels in the diet for the LL muscle (Table 4). The unfolding of the interaction revealed a reduction in lipid oxidation as the dietary lycopene supplementation was increased for all evaluated periods (Fig. 1. a) in which the ajusted equations $\hat{Y}$= - 0.0001x + 0.0799 (R^2= 0.92), $\hat{Y}$= - 0.0002x + 0.0994 (R^2= 0.85), $\hat{Y}$= - 0.0003x + 0.1224 (R^2 = 0.97), $\hat{Y}$= - 0 , 0003x + 0.1453 (R^2 = 1.00), for periods 0, 24, 48 and 72 hours, respectively, were obtained. For an analysis of the interaction within each lycopene level, linear and quadratic equations were adjusted over the evaluated periods, but the linear model better explained the observed data, in which a linear increase in lipid oxidation was obtained with increasing days (Fig. 1. b), and adjusting the equations $\hat{Y}$= 0.0009x + 0.0793 (R^2= 0.91), $\hat{Y}$= 0.0008x + 0.0781 (R^2= 0.88), $\hat{Y}$= 0 , 0008x + 0.0757 (R^2= 0.88), $\hat{Y}$= 0.0007x + 0.0769 (R^2= 0.89), $\hat{Y}$= 0.0007x + 0.0750 (R^2= 0.89), for the levels 0; 12.5; 25.0; 37.5 and 50.0 mg of lycopene, respectively.

No interactions were observed (*P*>0.05) for the inhibition of the DPPH radical in meat. However, the DPPH radical was influenced by the storage period and levels of lycopene supplementation in the diet (Table 5). The inhibition of the DPPH radical in the meat was reduced up to 72 h, represented by the adjustment of the equations $\hat{Y}$= 0.0594x + 39.70 (R^2 = 0.97) and $\hat{Y}$= 0.0005x^2 - 0.0956x + 39.99 (R^2= 1.00), where the derivation of the quadratic equation overestimated the storage time (95.6 h).

In relation to the level of dietary lycopene supplementation, there was an increase (*P*=0.001) in the capture of DPPH radicals by antioxidants in the meat, represented by the equation $\hat{Y}$= 0.04279x + 36.50 (R^2= 0.96).

3.3. Determination of the total antioxidant activity by the DPPH radical and lipid oxidation by the thiobarbituric acid (TBARS) of the liver

No interactions were observed (*P*>0.05) between the factors evaluated (Table 6). Lipid oxidation of the liver was reduced by the supplementation of lycopene in the diet

of pigs, being sustained by the equations $\hat{Y}$= - 0.0006x + 0.3211 (R^2= 0.84), $\hat{Y}$= 0.0000x^2 - 0.0014x + 0.3261 (R^2= 0.98), and which derivation of this equation resulted in a level of 34.47 mg of lycopene kg^{-1} of diet.

The capture of the DPPH radical by antioxidants in the liver was increased (*P*=0.001) (Table 6), resulting in an increase in the antioxidant power exerted by lycopene in the liver, due to the increase in dietary supplementation of lycopene ($\hat{Y}$= 0.0115x + 5.98; R^2= 0.84). The concentrations of TBARS and DPPH in the liver were influenced by sex (*P*=0.001), in which the gilts presented the lower production of malonaldehyde and greater capture of the DPPH radical by antioxidants in relation to barrows.

4. Discussion

The quantitative characteristics of carcass and relative body weight were not influenced by the dietary supplementation of lycopene (Table 2). However, there were sex differences for the variables HCY, CCY, LMY, BT and abdominal fat. This may be related to the fact that sex directly influences the body composition of pigs (Patience, Rossoni-Serão & Gutiérrez, 2015).

In this study, the inclusion in the evaluated concentrations of lycopene in the diet did not alter the color of the LL muscle, suggesting that lycopene can be inserted into the diet without influencing the instrumental color parameters of the LL muscle. The same was observed by Chung et al. (2014) who evaluated the effect of lycopene on pigs supplemented with by-products of tomato processing, and did not observe any effects of inclusion on the color and pH of pork. DL and CL did not differ between treatments, which suggests that lycopene supplementation did not result in elevated water losses of the LL muscle in these processes. However, the TL showed a reduction of up to 17.46%. This reduction of muscle TL, as the inclusion of lycopene in the diet has been increased, can be explained by the antioxidants preserving the integrity of the muscle cell membrane, which reduces the loss of water.

Although the shear force was not influenced by the increase in lycopene levels in the diet (Table 3), a reduction and trend was observed (*P*=0.064), which may be related to antioxidants, improving meat softness, since they avoid the oxidation of sarcoplasmic proteins (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004), these are the main enzymes responsible for *rigor mortis* (μ-calpain and *m*-calpain) and have a cysteine residue that can be oxidized. By avoiding the oxidation of calpain, this contributes to a

greater proteolysis during the maturation of the meat, making it soft. The shear force obtained in this study is in an extreme softness range, including the maximum value (30.11 N). According to Lyon & Lyon (1991), the LL of the pig can be classified in the same way as the *pectoralis major* of the chicken. Thus, according to the ranking of the authors mentioned above, all samples from this study are classified in extremely soft samples (<35.50 N).

Shelf life of *in natura* or processed pork meat presents fundamental importance to the food industry. In this way, research is needed to optimize the time and quality of meat products. One of the alternatives has been antioxidant supplementation in the animal diet (Moroney, O'Grady, O'Doherty, & Kerry, 2012), since besides protecting derived from animal origin against oxidative stress, they can optimize the shelf life of animal origin products. In this study, a reduction of lipid oxidation was observed as dietary lycopene supplementation was increased in all of the evaluated periods: 0, 24, 48 and 72 hours (Fig. 1. a). In addition, lipid oxidation increased over the long storage days for all lycopene levels studied: 0, 12.5, 25.0, 37.5 and 50.0 mg kg^{-1} (Fig. 1. b).

The increase in lipid oxidation resulted in high TBARS values, which are usually associated with meat odor and unpleasant taste (American Meat Science Association - AMSA, 2012), as they are based on the development of a thiobarbituric acid reaction with saturated aldehydes (2-enals and 2-dienais), produced in the termination phase of lipid oxidation. As in this present study, malonaldehyde concentrations were reduced in the LL muscle, Girao et al. (2012) also observed that the inclusion of lycopene extract (10% lycopene) in the juvenile diet of Nile tilapias inhibited the negative effects of containment stress, avoiding the elevation of malonaldehyde concentrations in the fillet.

Lipidic oxidation is a process that involves the deterioration of fatty acids with oxygen, through a chain reaction mechanism, forming lipid hydroperoxides and other byproducts responsible for the development of rancidity and deterioration of food (Shahidi & Zhong, 2010). Also, meat pork is highly susceptible to lipidic oxidation (Juntachote, Berghofer, Siebenhandl, & Bauer, 2006). According to Sindelar (2003), the minimum lipid oxidation was 0.008 and 0.117 mg MDA Eq. Kg^{-1} for pork meat. Values above the minimum lipid oxidation were observed in the present study, from 48 h of storage, only for the samples with the inclusion of 0 and 12.5 mg of lycopene, but after 72 h, the meat samples of all the treatments presented values above the minimum suggested oxidation by Sindelar (2003).

This oxidation reduction can be explained by the mode of action of the antioxidants, preventing lipid peroxidation in function of elimination of the initiating radicals, or by acting as a bond catalyst, such as metal ions, to prevent the initiation of radical generation or by the decomposition of peroxides. Thus, they cannot be reconverted into radical initiators by performing chain breaks to avoid the continuous uptake of hydrogen by active radicals (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003).

There is a growing amount of evidence indicating the protective role of antioxidants in the industry, in order to retard lipid oxidation and prolong the lifespan of *in natura* meat pork (Liu, Shi, Ibarra, Ibarra, & Xue, 2008; & Abu Ghannam, 2011; Karakaya, Bayrak, & Ulusoy, 2011; Jiang & Xiong, 2016).

The efficiency of lycopene as an antioxidant can be observed from the results revealed by the DPPH radical scavenging test (Table 5), where all meat samples showed a greater capacity to eliminate the DPPH radical, due to the increase of dietary lycopene. The antioxidant power exerted by the lycopene in the meat increased as a result of the elevation of this in the diet of pigs. Similar results were obtained by Liu et al. (2008), who reported an increase in elimination capacity as a result of dietary vitamin E supplementation. The DPPH radical elimination assay also revealed that the antioxidant power exerted by lycopene on meat was influenced by the storage period (Table 5). Inhibition of the DPPH radical was reduced over the 72 hours evaluated.

In the case of lycopene, the antioxidant action is based on the ability to extinguish singlet oxygen ($^1O_2$) and other oxygenated species, resulting in cellular protection against oxidative damage; that is, lycopene intercepts oxidative species before damage. By physically extinguishing $^1O_2$ by lycopene, the a deactivation occurs by transferring the excitation energy of $^1O_2$ to the lycopene molecule, and this causes the lycopene to reach the triplet state and the energy of the excited lycopene is dispensed through vibrational interactions, recovering the state of the carotenoid (Krinsky, 1998). This is due to its conjugated polyene structure, which is responsible for this reaction, making the lycopene a potent antioxidant.

Among the methods used to control lipid oxidation, the use of antioxidants is the most effective, convenient and economical means. In addition to protecting products from deterioration, this additive can also be used for health promotion because of its ability to protect the body against oxidative damage (Shahidi & Zhong, 2010). This can be confirmed by the results obtained in this study, such as dietary supplementation of

lycopene, in addition to reducing lipid oxidation and increasing the antioxidant power in the meat, had a similar effect on the liver.

Lipid oxidation of the liver was reduced by the supplementation of lycopene to the pig diet, in which supplementation of 34.47 mg of lycopene kg^{-1} of diet resulted in a lower oxidation of hepatic tissue (Table 6). This shows that lycopene can be a potent antioxidant in eliminating free radicals *in vivo*. Due to its polyene structure, lycopene provides an electron-rich system, being an eligible target for electrophilic reagents. Thus, it shows high reactivity against free radicals and oxygen (Krinsky, 1998). Similar results were observed by Sun et al. (2015), where the dietary supplementation of lycopene reduced the malonaldehyde content in the liver.

The capture of the DPPH radical increased linearly in the liver of the pigs (Table 6), due to the inclusion of lycopene in the diet, providing an increase of the total antioxidant power in 7.08%. Similar values were found by Damgaard et al. (2014), in which the pig liver DPPH was 9.90%. Studies have suggested a dose-dependent effect on radical removal DPPH by antioxidants(Balakrishnan, Prasad, Rai, Velappan, Subbanna & Narayan, 2011).

In the liver, the variables TBARS and DPPH were influenced by sex, and the gilts presented lower lipid oxidation and higher capture of the 2,2-diphenyl-1-picryl-hydrazyl radical by antioxidants in relation to barrows. However, there are no reports in the literature that evaluated the effect of lycopene supplementation on swine and its effect on the MDA content of swine muscle and liver to help explain this action on sex.

This effectiveness of lycopene in protecting LL meat and liver of pigs against oxidation (Table 4, 5 and 6) is of great importance because lipid oxidation is a process in which unsaturated fatty acids react with oxygen and free radicals, through a chain reaction mechanism giving rise to the formation of lipid hydroperoxides and other by-products, such as aldehydes, responsible for rancidity and spoilage of food (Shahidi & Zhong, 2010). Pork meat and its products are highly sensitive to lipid oxidation (Juntachote, Berghofer, Siebenhandl, & Bauer, 2006), which can mainly influence meat quality attributes such as color, taste, texture and nutritional values (Halliwell & Gutteridge, 2015; Karakaya, Bayrak, & Ulusoy, 2011), which are determining factors for the consumer.

5. Conclusion

The inclusion of lycopene in the diet of barrows and gilts reduced the thawing loss and was effective in the protection against oxidation of *longissimus lumborum* muscle and liver. In which the best results for these variables were obtained with the supplementation of 50.0 mg of lycopene kg^{-1} of diet

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Tables

Table 1

Ingredients, chemical and energetic composition of basal diet.

Ingredients (%)	Barrows	Gilts
Corn	87.14	83.89
Soybean meal 45 %	9.61	12.47
Soybean oil	0.740	0.950
Dicalcium phosphate	0.785	0.973
Limestone	0.553	0.521
Salt	0.203	0.203
L-lysine HCL 78.4 %	0.373	0.390
L-threonine 98.5 %	0.068	0.072
DL-methionine 99 %	0.036	0.053
L-tryptophan 98 %	0.022	0.017
Growth promoter [1]	0.015	0.015
Vitamin and mineral supplement [2]	0.400	0.400
Lycopene extract [3]	0.000	0.000
Inert [4]	0.050	0.050
Total	100.00	100.00
Composition		
Metabolizable energy (Mcal kg $^{-1}$)	3.30	3.30
Nitrogen (%)	1.86	2.03
Total Calcium (%)	0.45	0.49
Available phosphorus (%)	0.23	0.26
Potassium (%)	0.42	0.47
Sodium (%)	0.10	0.10
Chlorine (%)	0.18	0.18
SID lysine (%)	0.690	0.770
SID met + cis (%)	0.400	0.440
SID threonine (%)	0.440	0.480
SID tryptophan (%)	0.120	0.130

[1] Enramycin 0.015%.

[2] Content kg $^{-1}$diet; vit. A: 30000 UI, vit. D3: 5000 UI, vit. E: 120 UI, vit. K: 5 mg, vit. B12: 120 mcg, Niacin: 150 mg, Calcium pantothenate: 75 mg, folic acid: 8 mg, Choline chloride: 0.48 g, iron: 350 mg, copper 15 mg, manganese: 250 mg, Zinc: 0.75 g, iodine: 10 mg, selenium: 3 mg.

[3] Lycopene extract at a concentration of 10% included in the basal diet replacing the inert.

[4] Fine clean sand.

Table 2

Quantitative carcass characteristics and organs relative weight of, barrows and gilts, from 75 to 100 kg, fed diets containing different levels of lycopene.

Item	Barrows						Gilts							*P*-value			
	Lycopene (mg kg^{-1} of diet)					Medium	Lycopene (mg kg $^{-1}$ of diet)					Medium	SEM	Sex x Lycopene	Sex	Lycopene	
	0	12.5	25.0	37.5	50.0		0	12.5	25.0	37.5	50.0					Lin[1]	Qua[2]
Hot carcass weight, Kg	78.88	78.80	81.55	79.85	81.32	80.08	79.98	79.08	77.64	81.41	79.98	79.62	0.329	0.279	0.557	0.160	0.712
Hot carcass yield, %	82.17	82.75	83.33	83.16	83.28	82.94[a]	76.99	76.53	75.04	78.75	77.33	76.93[b]	0.098	0.524	0.049	0.375	0.641
Cold carcass weight, Kg	76.40	76.42	78.53	77.47	78.80	77.52	82.64	82.15	81.97	82.75	82.07	82.32	0.344	0.400	0.454	0.124	0.705
Cold carcass yield, %	79.59	80.24	80.25	80.68	80.72	80.30 [a]	79.54	79.50	79.22	79.91	79.36	79.5 [b]	0.126	0.855	0.023	0.265	0.742
Carcass weight loss in cooling, %	3.13	3.03	3.69	2.99	3.07	3.18	3.75	3.22	3.35	3.28	3.30	3.38	0.083	0.558	0.293	0.371	0.903
Ham yield, %	29.27	29.30	29.09	29.27	29.63	29.31	30.23	29.84	29.60	29.21	30.13	29.80	0.126	0.906	0.117	0.944	0.155
Lean meat yield, %	57.54	56.25	58.24	56.66	58.04	57.35[b]	59.21	59.39	59.10	58.93	58.81	59.09 [a]	0.212	0.646	0.004	0.784	0.065
Backfat thickness, cm	1.56	1.80	1.46	1.65	1.53	1.60[a]	1.24	1.19	1.22	1.28	1.24	1.23 [b]	0.035	0.767	0.004	0.627	0.650
LL depth, cm	6.07	5.93	6.35	6.25	6.11	6.14	6.40	6.31	6.20	6.34	5.93	6.24	0.052	0.569	0.451	0.546	0.361
Relative weight of liver, %	1.739	1.716	1.636	1.735	1.603	1.686	1.694	1.711	1.685	1.657	1.759	1.701	0.010	0.361	0.717	0.484	0.669
Relative weight of kidney, %	0.393	0.400	0.381	0.383	0.367	0.385	0.393	0.439	0.366	0.422	0.401	0.404	0.008	0.713	0.203	0.519	0.817
Relative weight of abdominal fat, %	1.833	1.739	1.630	1.872	1.992	1.813 [a]	1.312	1.346	1.401	1.414	1.525	1.400 [b]	0.044	0.524	0.001	0.055	0.122

* Different letters in line differ by F test at 5% probability.

SEM: standard error of the mean.

LL dpth: Depth of *longissimus lumborum.*

[1] Linear effect of lycopene levels.

[2] Quadratic effect of lycopene levels.

Table 3

Qualitative characteristics of the *longissimus lumborum* muscle of, barrows and gilts (75-100 Kg), fed diets containing different levels of lycopene.

Item	Barrows						Gilts							*P*-value			
	Lycopene (mg kg $^{-1}$ of diet)					Medium	Lycopene (mg kg $^{-1}$ of diet)					Medium	SEM	Sex x Lycopene	Sex	Lycopene	
	0	12.5	25.0	37.5	50.0		0	12.5	25.0	37.5	50.0					Lin[1]	Qua[2]
pH45	6.34	6.31	6.36	6.30	6.30	6.32	6.28	6.20	6.53	6.41	6.43	6.37	0.031	0.745	0.627	0.506	0.689
pH24	5.75	5.82	5.71	5.79	5.76	5.77	5.75	5.72	5.90	5.73	5.85	5.79	0.012	0.838	0.595	0.474	0.838
Drip loss, %	5.35	5.64	4.93	5.08	4.75	5.15	5.14	4.89	4.92	3.93	4.47	4.67	0.152	0.915	0.269	0.199	0.950
Minolta L*	56.57	55.70	57.77	58.33	59.04	57.48	57.21	57.06	56.43	56.83	54.84	56.47	0.193	0.880	0.147	0.613	0.880
Minolta a*	6.50	6.26	7.14	6.54	7.02	6.69[a]	5.27	5.41	5.31	5.56	5.61	5.43[b]	0.093	0.524	0.001	0.258	0.975
Minolta b*	3.34	3.13	3.85	3.71	3.37	3.48[a]	3.22	3.36	2.99	3.17	2.55	3.06[b]	0.085	0.388	0.045	0.531	0.232
Thawing loss, %[3]	7.28	6.65	5.83	5.81	5.41	6.20	6.77	6.70	6.20	6.34	6.19	6.44	0.229	0.846	0.524	0.024	0.571
Cooking loss, %	27.48	26.53	26.57	24.89	25.94	26.28	24.51	24.72	24.34	25.31	24.33	24.64	0.165	0.806	0.111	0.548	0.909
Shear force, N	30.11	29.91	29.22	26.87	25.89	28.44	28.15	28.05	27.65	27.26	27.36	27.65	0.193	0.655	0.592	0.064	0.635

* Different letters in line differ by F test at 5% probability.

SEM: standard error of the mean.

[1] Linear effect of lycopene levels.

[2] Quadratic effect of lycopene levels.

[3] $\hat{Y} = - 0.0244x + 6.9282$ ($R^2 = 0.89$).

Table 4

Dietary levels of lycopene for barrows and gilts (75-100 kg) and their effects on lipid oxidation by the TBARS (mg MDA Eq. kg^{-1}) methodology of the *longissimus lumborum* muscle at different storage periods.

Periods (hours)	Barrows						Gilts						
	Lycopene (mg kg^{-1} of diet)					Medium	Lycopene (mg kg^{-1} of diet)					Medium	SEM
	0	12.5	25.0	37.5	50.0		0	12.5	25.0	37.5	50.0		
0	0.0803	0.0787	0.0775	0.0769	0.0762	0.0779	0.0797	0.0804	0.0784	0.0791	0.0783	0.0792	0.001
24	0.1009	0.0985	0.0923	0.0936	0.0869	0.0944	0.0993	0.0957	0.0917	0.0942	0.0920	0.0946	0.002
48	0.1237	0.1184	0.1175	0.1101	0.1073	0.1154	0.1210	0.1169	0.1165	0.1108	0.1068	0.1144	0.003
72	0.1470	0.1431	0.1401	0.1339	0.1289	0.1386	0.1431	0.1393	0.1335	0.1292	0.1270	0.1344	0.003
Medium	0.1108	0.1081	0.1050	0.1033	0.1010		0.1130	0.1097	0.1069	0.1036	0.0998		
	P-value[1]		Linear	Quadratic									
Periods x Sex x Lycopene	0.704												
Periods x Lycopene	0.006												
Sex x Periods	0.099												
Sex x Lycopene	0.635												
Lycopene	0.001		0.001	0.848									
Periods	0.001		0.001	0.001									
Sex	0.213												

TBARS: Substances reactive with thiobarbituric acid.

SEM: standard error of the mean.

[1]Effect of *P* at 5% significance.

Table 5

Dietary levels of lycopene for, barrows and gilts (75-100 kg), and their effects on the % of inhibits of DPPH radical, in the *longissimus lumborum* muscle.

Periods (hours)	Barrows					Medium	Gilts					Medium	SEM
	Lycopene (mg kg $^{-1}$ of diet)						Lycopene (mg kg $^{-1}$ of diet)						
	0	12.5	25.0	37.5	50.0		0	12.5	25.0	37.5	50.0		
0	38.771	39.107	39.012	40.768	40.733	39.678	39.343	39.809	40.284	41.284	40.923	40.329	0.407
24	36.675	37.764	38.274	37.451	38.989	37.831	37.227	37.606	38.219	38.304	39.311	38.133	0.358
48	34.938	36.375	36.541	37.011	37.083	36.390	35.045	36.578	36.958	37.503	37.788	36.774	0.421
72	34.349	35.816	36.181	36.401	36.549	35.859	34.063	35.177	35.213	36.532	36.919	35.581	0.438
Medium	36.183	37.266	37.502	37.908	38.339		36.420	37.293	37.669	38.406	38.735		

	P-value[1]	Linear	Quadratic	Ajusted Equations
Periods x Sex x Lycopene	0.507			
Periods x Lycopene	0.962			
Sex x Periods	0.587			
Sex x Lycopene	0.950			
Lycopene	0.001	0.001	0.362	$\hat{Y} = 0.0428x + 36.50$ ($R^2 = 0.96$)
Periods	0.001	0.001	0.018	$\hat{Y} = - 0.0594x + 39.70$ ($R^2 = 0.97$); $\hat{Y} = 0.0005x^2 – 0.0956x + 39.99$ ($R^2 = 1.00$)
Sex	0.278			

DPPH: radical 2,2-diphenyl-1-picryl-hydrazide.

SEM: standard error of the mean.

[1] Effect of *P* at 5% significance.

Table 6

Levels of lycopene for barrows and gilts (75-100 kg), and their effects on the lipid oxidation by TBARS methodology (mg MDA Eq. Kg^{-1}) and % of inhibits of DPPH radical, of liver.

Item	Barrows						Gilts							*P*-value			
	Lycopene (mg kg^{-1} of diet)					Mediu	Lycopene (mg kg^{-1} of diet)					Mediu	SEM	Sex x Lycopen	Sex	Lycopene	
	0	12.5	25.0	37.5	50.0		0	12.5	25.0	37.5	50.0					Lin[1]	Qua[2]
TBARS[3]	0.3302	0.3205	0.3094	0.2996	0.3027	0.3125[a]	0.3197	0.3069	0.2939	0.2894	0.2938	0.3007[b]	0.006	0.984	0.004	0.001	0.038
DPPH[4]	5.8202	5.9244	6.2461	6.3839	6.3642	6.1478[b]	6.0372	6.2588	6.5662	6.5934	6.5325	6.3976[a]	0.111	0.958	0.003	0.001	0.068

* Different letters in line differ by F test at 5% probability.

SEM: standard error of the mean.

DPPH: radical 2,2-diphenyl-1-picryl-hydrazide.

TBARS: thiobarbituric acid reactive substances.

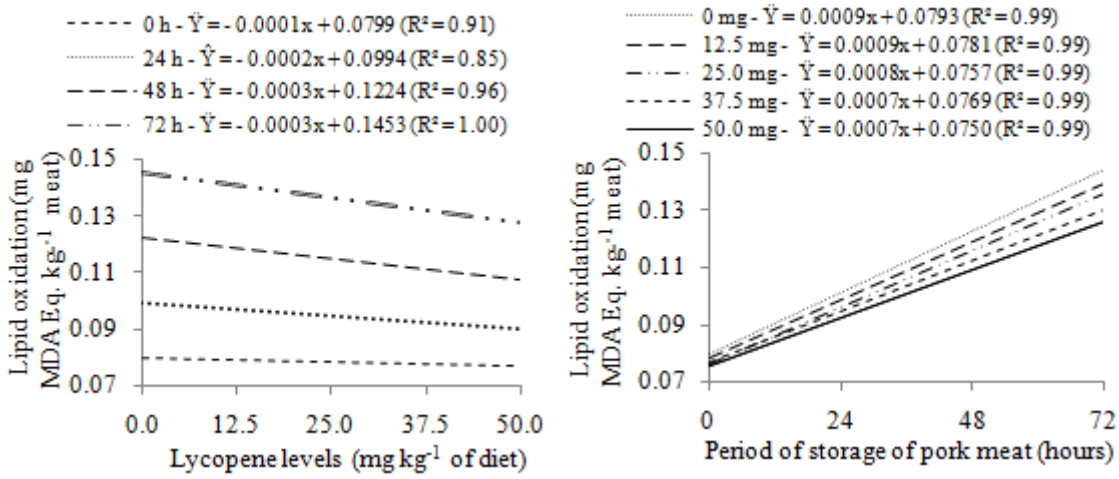
[1]Linear effect of lycopene levels.

[2] Quadratic effect of lycopene levels.

[3] $\hat{Y} = - 0.0006x + 0.3211$ ($R^2 = 0.84$). $\hat{Y} = 0.00002x^2 - 0.0014x + 0.3261$ ($R^2 = 0.98$).

[4] $\hat{Y} = 0.0115x + 5.98$ ($R^2 = 0.84$).

Figure



a) b)

Fig. 1. Measurement of lipid oxidation in terms of thiobarbituric acid reactive substances (TBARS). a) at different periods of storage in relation to levels of lycopene supplementation; b) at each level of lycopene supplementation over the storage periods of the *longissimus lumborum* muscle.

V- LYCOPENE STIMULATES THE IMMUNE RESPONSES OF PIGS IN THE FINISH PHASE

ABSTRACT

Objective: Evaluate the supplementation of different levels of lycopene on the immune responses of, barrows and gilts, in the finish phase.

Methods: 40 barrows pigs and 40 gilts were used, with a mean initial weight of 75.04 ± 1.6 kg. A randomized block design was used in a 2 x 5 factorial scheme, consisting of two sexes (males and females) and five levels of lycopene (0, 12.5, 25.0, 37.5, 50.0 mg/kg of diet). Were evaluated the effects of supplementation of different levels of lycopene on the immunite responses. The variables were submitted to the statistical analysis adopting the level of significance of 5%.

Results: There was no interaction (P>0.05) between the factors evaluated on plasma proteins. Increasing lycopene in the diet of pigs increased to plasma albumin (P=0.012). There was no interaction (P>0.05) between the factors evaluated on the leukocyte profile or the hematocrit. As lycopene levels increased in the diet the lymphocyte concentration increased linearly (P=0.045). The neutrophil concentration and the neutrophil:lymphocyte ratio were influenced (P<0.05) by the levels of lycopene in the diet, resulting in a lower concentration of neutrophils at lycopene level of 17.49 mg/kg and the lowest neutrophil:lymphocyte ratio at the lycopene level of 16.46 mg/kg. Eosinophils were also influenced (P=0.050) by the supplementation of lycopene in the diet, in which level of 22.69 mg of lycopene/kg resulted in a greater response of eusinophils to dietary lycopene. There was an interaction (P=0.011) between the collection period and the lycopene levels for the production of anti-BSA IgG, resulting in higher production of anti-BSA IgG with the supplementation of up to 20.06 mg of lycopene/kg of diet. The production of IgG anti-BSA increased throughout the study period at all levels of lycopene supplementation.

Conclusion: The supplementation of lycopene in the diet of, barrows and gilts, from 75 to 100 kg, altered their immune responses, with the best response at the supplementation level of 20.06 mg/kg of diet.

Key words: immunoglobulins, leukocytes, IgG, carotenoids, barrows, gilts

INTRODUCTION

The high density of animals and environmental conditions in modern pig production systems cause pigs to be exposed to a significant number of pathogenic microorganisms, bacteria, viruses and parasites. As a result, dietary nutrients that could be targeted for growth are redirected to the immune system to provide protection against pathogenic microorganisms, directly interfering with the animal's productive potential.

The addition of antioxidants and natural immunostimulants in feed is considered as a potential way to improve animal health and performance [1]. In the absence of an infectious picture, the need to elaborate an immune response, by itself, is capable of affecting productive capacity. However, the cost of activating the immune system involves a nutrient energy cost and the functioning of the immune system depends on a very complex mechanism, which is subject to the influence of internal and external factors [2].

Among the antioxidants, carotenoids are one of these external factors that can affect the immune system [2], because they present a broad spectrum of biological effects and act as immunomodulators, influence the function of T and B cells, induce differentiation and inhibit the proliferation of certain cells [3], and are able to influence both humoral immunity and cellular immunity [1]. Among the carotenoids, lycopene is considered to have the greatest sequestering ability of singlet oxygen, possibly due to the presence of two unconjugated double bonds, which gives it greater reactivity than other carotenoids [4].

Lycopene stimulates the immune system, where it acts against oxidative damage to the DNA of lymphocytes [5]. It stimulates lymphocytes, increasing the production of IL-2 and interferon-gamma (INF-δ), a potent activator of T lymphocytes [6]. It also influences the production of immunoglobulins, increasing blood levels of IgA, IgG and IgM, and enhancing immunity [7], stimulating the communication between cells and raising the immune response [8].

In addition, lycopene is a potent antioxidant, as well as an inhibitor of proinflammatory and prothrombotic factors [9]. Lycopene suppresses inflammation in several tissues, inhibiting the formation of proinflammatory cytokines and chemokines in macrophages [10, 11], in addition to controlling chronic immune and inflammatory processes, and delaying the maturation of dendritic cells [12].

The immunomodulatory role of lycopene in the immune system has been well studied, but little is known in relation to the effect of supplementation of lycopene on the animal response in pigs. Thus, the present study aimed to evaluate the effect of supplementation of different lycopene levels on the immunite responses of pigs, barrows and gilts, in the termination phase.

MATERIALS AND METHODS

The experiment was carried out in the swine production sector of the Experimental Farm of Iguatemi, belonging to the Agricultural Sciences Center of the State University of Maringá, located in the State of Paraná (23° 21'S, 52° 04'W and Altitude of 564m), from January 2016 to May 2016. All experimental procedures were previously submitted to the Ethical Conduct Committee on the Use of Animals in Experimentation, and were approved for execution (nº6570200815).

Animals and installation

The pigs (Piétrain x Landrace x Large White) were housed in a masonry shed, covered with asbestos cement tiles, divided into two wings, each consisting of 20 pens ($3m^2$) separated by a central corridor. Each bay had a pacifier type drinker and a semi-automatic feeder, providing free access to feed and water throughout the experimental period.

The temperature was monitored by means of a digital thermometer of minimum and maximum temperature, which registered minimum was 19.62 ± 2.54ºC and maximum was 32.11 ± 3.2ºC.

Experimental design and diet

Eighty pigs were used, 40 barrows and 40 gilts, with a mean initial weight of 75.04 ± 1.6 kg. The animals were distributed in a randomized block design in a 2 x 5 factorial arrangement consisting of two sexes (male and female) and five levels of lycopene (0, 12.5, 25.0, 37.5 and 50.0 mg/kg of diet), with eight replicates and one animal per experimental unit.

The experimental diets (Table 1) were formulated based on corn, soybean meal, minerals, vitamins and additives, to meet the nutritional recommendations proposed by the National Research Council - NRC [13]. The inclusion of the product containing 10% of lycopene to the diets was replaced by 0, 125, 250, 375 and 500 mg of inert/kg of diet, corresponding to the levels of 0; 12.5; 25.0; 37.5 and 50.0 mg of lycopene/kg of diet.

Determination of hematocrit and leukocyte profile

The animals were first fasted for 6 hours, and blood samples were obtained by puncture of the jugular vein, as described by Oliveira et al [14]. After harvest, the samples were placed in an automatic homogenizer for 5 minutes. Subsequently, the samples were transferred to capillary microtubes and centrifuged at 10,000rpm for 5 minutes. The percentage of hematocrit was measured using a specific scale.

For determination of the leukocyte profile, a blood smear was prepared on glass slides, and then stained using the May Grunwald-Giemsa method. The differential count was performed under an optical microscope with an immersion objective, and the cells were classified into lymphocytes, eosinophils, monocytes, neutrophils and basophils, calculating the proportion of each 100 cells counted per pig.

Total proteins and fractions

At the end of the experiment, blood was collected for the determination of total proteins (TP), albumin (AB) and globulins (GL) in blood plasma.

Blood samples were collected according to the procedures described above. After collection, the blood was immediately sent to the FEI Swine Laboratory, and centrifuged at 3000 rpm for a period of 15 minutes to obtain the plasma, which was extracted by automatic pipette and stored in microtubes, stored at cooled to -20°C, acording on the analysis protocol.

TP and AB determinations were performed using specific kits (Bioclin®) according to the standard operating procedure (SOP) specific to each variable. Subsequently, the absorbance reading was performed on a Biochemical Analyzer (Bioplus® 2000). The GL quantification was performed by means of the difference between AB and TP, an the AB:GL ratio was also calculated.

Antibovine Serum Albumin (anti-BSA) IgG production

On days 0 and 12 of the experimental period the pigs were immunized with 1 mg of a bovine serum albumin solution (BSA) (A3912, Sigma-Aldrich) diluted in 0.5 mL phosphate-buffered saline (PBS) and 0.5 mL of adjuvant by subcutaneous immunization [15]. Complete Freund's adjuvant (F5881, Sigma-Aldrich) was used at the first inoculation and the incomplete Freund's adjuvant (F5506 Sigma-Aldrich) was used in second inoculation (Figure 1).

Blood samples were collected on days 0, 12 and 24 of the experimental period by means of puncture of the jugular vein, according to Oliveira et al [14], and transferred to test tubes. After collection, the blood was immediately sent to the FEI Swine Laboratory, where the samples were centrifuged at 3000 rpm for a period of 15 minutes to obtain the serum. The serum was extracted with the aid of an automatic pipette and stored in microtubes at -20ºC.

Determination of anti-BSA IgG production was determined using the indirect ELISA technique. The 96-well ELISA plates were coated with a solution (100 μL/well) containing 0.01 mg BSA/mL of carbonate buffer (pH 9.6). The plates were then incubated at 4°C for 16 h. A blocking solution was used at 100 μL/well, and the plate was incubated for 1 h at 37°C. Serum samples were tested in triplicate (100 μL/well, 1:10000) and incubated in a greenhouse for 2 h at 37ºC. IgG antipig conjugated to rabbit-produced peroxidase (100 μL/well, 1:30000, SAB3700420, Sigma-Aldrich) was added. And incubated again with 100 μL/well of the 3,3', 5,5' substrate, - Tetramethylbenzidine (TMB) (T0440, Sigma-Aldrich) was added for 30 min at 27°C. The reaction was stopped with the use of HCl (2M, 100 μL/well).

The anti-BSA IgG production was quantified by means of absorbance reading, with measurements being performed on an ELISA plate reader (SMP500 - 13334-RCPP coupled to SoftMaxR Pro 5 serial number), at 450 nm and the results were expressed as an optical density. Between each step listed, the plates were washed three times with a NaCL and Tween-20 wash solution. To determine the optimal dilution of the serum and the antipig IgG for taking readings, a series of dilutions was tested. Serum was tested in the dilutions (1:1000, 1:5000, 1:10000, 1:15000, 1:20000) and the IgG antipig tested at the dilutions (1:5000, 1:10000, 1:15000, ; 1:30000; 1:50000), the best readings being obtained at the concentrations 1:10000 of serum and 1:30000 of IgG antipig.

Statistical analysis

The UNIVARIATE procedure was used to evaluate the presence of outliers. Subsequently, the variables were submitted to analysis of variance (ANOVA), and the effects of block, sex, lycopene and the interaction of sex and lycopene were included in the model. Additionally, for the anti-BSA IgG production, the evaluation effect of days and the sex x days, lycopene x days and days x sex x lycopene interactions were included in the model.

The degrees of freedom regarding lycopene levels and/or storage period were deployed in orthogonal polynomials to obtain regression equations. Using the quadratic and or linear models and the Linear Response Plateou (LRP) model was also adjusted, choosing the best fit model for the data. Data were submitted to statistical analyzes using the SAS (Statistical Analysis System, version 9.0). The significance level of 5% was adopted.

RESULTS

Leukocyte profile and hematocrit

There was no interaction (P>0.05) between sex and lycopene levels on leukocyte profile and hematocrit (Table 2). The lymphocyte profile was influenced by the level of lycopene in the diets of barrows and gilts, with a linear increase in lymphocyte concentration as lycopene level increased (P=0.045) ($\hat{Y}$= 0.1382x + 68.81; R^2= 0.58).

Neutrophils were also influenced by lycopene level in the diet (P=0.003), according to the equations $\hat{Y}$= - 0,1225x + 19,87 (R^2= 0,35) and $\hat{Y}$= 0.0110x^2 - 0.6710x + 23.26 (R^2= 0.97), and the model LRP ($\hat{Y}$ = - 0.66x + 23.75; R^2= 1.00), where the association of the models quadratic and LRP, resulted in a lower percentage of neutrophil at the level of 17.49 mg of lycopene/kg of diet (Figure 2.A). The neutrophil:lymphocyte ratio was also influenced by the lycopene levels (P=0.005), according to the equations $\hat{Y}$= - 0.0026x + 0.328 (R^2= 0.33) and $\hat{Y}$= 0.00024x^2 - 0.146x + 0.4024 (R^2= 0.97), and the LRP model, $\hat{Y}$= - 0.01x + 0.4114 (R^2= 1.00), where the association of the models quadratic and LRP resulted in a lower neutrophil:lymphocyte ratio at the level of 16.46 mg of lycopene/kg of diet (Figure 2.B).

The eosinophils presented a quadratic response (P=0.050) as a function of dietary lycopene supplementation, according to the equation $\hat{Y}$= - 0.0016x² + 0.0726 + 1.79 (R^2= 0.69), resulting in a greater response of eosinophils to dietary lycopene at the level of 22.69 mg of lycopene/kg of diet. The other parameters, such as basophils, monocytes and hematocrit were not influenced (P>0.05) by dietary lycopene levels. Sex only influenced the monocytes (P=0.047), with gilts having a lower concentration than barrows.

Total proteins and fractions

There was no interaction (P>0.05) between sex and lycopene levels (Table 3) on total proteins (P=0.971), AB (P=0.131), GL (P=0.821), or the AB:GL ratio (P=0.459). The inclusion of lycopene in the diet of, barrows and gilts altered the plasma protein fractions, with a linear increase (P=0.012) in plasma albumin concentration as lycopene level increased, according to the equation $\hat{Y}$= 0.0095x + 3.165 (R^2= 0.84).

The TP, GL and AB:GL ratio were not influenced (P>0.05) by lycopene levels. However, sex influenced the plasma protein fractions, with gilts having higher concentrations of TP (P=0.001) and GL (P=0.001) and lower concentrations of AB (P=0.049) and AB:GL (P=0.001) in relation to barrows.

Anti-BSA IgG production

There was an interaction (P=0.011) between collection periods and lycopene levels for anti-BSA IgG production (Table 4). The result of this interaction (Table 5) showed better adjust for the quadratic model (R^2= 0.95) than the linear model (R^2= 0.57), at 24 days of collection, represented by the equation $\hat{Y}$= - 0.0005x² + 0.0310x + 0.5268 (R^2= 0.95), with the highest concentration of IgG being observed at the level of 30.98 mg of lycopene/kg of diet. The LRP model was also adjusted to the data ($\hat{Y}$= 0.02x + 0.5333; R^2= 0.99), with the highest concentration of IgG being observed at the level of 20.0 mg of lycopene/kg of diet. Among the models adjusted for IgG concentration in relation to lycopene level, the broken line model presented the highest coefficient of determination (R^2= 0.99) (Figure 3).

Anti-BSA IgG production increased over the study period at all levels of lycopene supplementation (Figure 4), and can be observed by the equations $\hat{Y}$= 0.0215x - 0.0071 (R^2= 0.98) $\hat{Y}$ = 0.0330x - 0.0333 (R^2 = 0.94), $\hat{Y}$= 0.0442x - 0.0553 (R^2 = 0.94), $\hat{Y}$ =

0.0406x - 0.04628 (R^2 = 0.95), $\hat{Y}$ = 0.0391x - 0.0391 (R^2= 0.96), for lycopene levels (0, 12.5, 25.0, 37.5 and 50.0 mg), respectively.

DISCUSSION

The total proteins correspond to about 6-7% of plasma, and among the main plasma proteins this the albumin corresponding to about 50-60%, followed by globulins (40-50%) and fibrinogen (1-4%) [16]. These plasma proteins are modified by acute phase response, factores stressors, as well as by age, nutrition, race, sex, and reproductive status [17]. Therefore the correct interpretation of the protein fractions is very useful to evaluate the state of health of the animals. In this study, no changes were observed in PT concentrations (P> 0.050) by dietary lycopene supplementation. Protein levels may be altered by a protein or a single amino acid deficiency in the diet, which may lead to a decrease in serum protein content, mainly due to the reduction of albumin [18] which did not occur in this study.

However, the plasma protein fraction was influenced by the inclusion of lycopene in the diet of pigs (Table 3), as the plasma albumin concentration increased as a function of the dietary supplementation of lycopene. The concentration of albumin is influenced by the transport of various lipophilic compounds (bilirubin, long chain fatty acids, iodinated thyroid hormones, corticosteroids, drugs) and of divalent cations [6]. The synthesis of this protein is influenced mainly by protein and amino acid nutrition, colloidal osmotic pressure, hormones action of growth, thyroid and insulin and the disease states (19). However, globulins were not influenced by lycopene levels. Globulins are associated with the immune response. An increase in globulin fractions may result in an increase in immunoglobulins [19], as in cases of acute and chronic liver disease, and autoimmune infections and diseases [6]. A reduction in the globulin fraction may be the result of a congenital nutritional or immunological deficiency [19].

The AB:GL ratio was not influenced by dietary lycopene. The AB:GL ratio indicates the inflammatory state [7]. To be considered normal, the AB:GL ratio should be slightly above 1, as a low AB:GL ratio may reflect an overproduction of globulins or a deficit of albumin production. In contrast, selective loss of albumin from the circulation and a high AB:GL ratio suggest a deficiency in immunoglobulin production [18].

Plasma proteins are easily influenced by physiological, nutritional, sexual, environmental and genetic factors [6, 17]. In this study gilts presented higher

concentrations of TP and GL, and lower concentrations for AB and AB:GL in relation to barrows. The higher levels of TP in gilts may be due to a higher nutritional requirement for proteins in relation to barrows [13], which can directly influence blood levels.

The hematocrit concentration (Table 2) was not influenced by the dietary levels of lycopene and no differences were found in relation to sex. The hematocrit indicates the proportion of blood that is occupied by the red blood cells in a whole blood sample, and the observed values were within the range recommended for pigs, which can vary from 26 to 41% according to Friendship and Henry [20].

Leukocytes are the first line of defense of the immune system and can be classified according to their nuclear morphology in mononuclear cells (T lymphocytes, B lymphocytes, exterminating or natural killer cells, monocytes, macrophages and dendritic cells) or polymorphonuclear cells (neutrophils, eosinophils, basophils and mast cells).

In the present study, the percentage of lymphocytes increased with the supplementation of lycopene in the diet of pigs. Similar results were observed by Watzal et al [21] in which daily intake of tomato juice (lycopene source) by healthy men increased lymphocyte proliferation and cytotoxicity of natural killer cells. Furthermore, Yamaguchi et al [22] indicate that lycopene may modulate the potential of cytokine production in T cells or indirectly activate T cells. Regarding lymphocytes, little is known about how lycopene influences the processes of activation, proliferation and differentiation in immunoglobulin secreting plasmocytes (antibodies). It is known that diets enriched with lycopene and other carotenoids result in an increase in B lymphocytes and elevated levels of serum IgG after 7 days of treatment, suggesting that carotenoid-based dietary supplementation may increase B lymphocyte production and the concentration of circulating immunoglobulins [23].

Neutrophils represent the first line of defense and are often associated with subclinical and clinical infections [24]. In this study, the lowest concentration of neutrophils was obtained at the level of 17.49 mg of lycopene/kg of diet (Figure 2.A). Neutrophils together with macrophages are sources of active oxygen species (AOS), and play an important role in cellular immunity. Neutrophils use myeloperoxidase to transform hydrogen peroxide resulting from the conversion of superoxide anions by the enzyme superoxide dismutase, into the hypochlorite ion, a highly active bactericide whereas macrophages generate a hydroxyl radical [2]. The AOS level balance is maintained using endogenous and exogenous antioxidants including carotenoids [2], with lycopene being the predominant carotenoid in plasma.

The neutrophil/lymphocyte ratioare inflammatory biomarkers used as prognostic factors in various inflammatory diseases and infections [25]. In this study, lycopene supplementation in the diet of pigs influenced the neutrophil/lymphocyte ratio and resulted in a lower ratio at the level of 16.46 mg of lycopene/kg of diet for the lowest neutrophil/lymphocyte ratio (Figure 2.B). Likewise, eosinophils were influenced by the levels of lycopene supplementation in the diet where at a level of 22.69 mg of lycopene/kg of diet, there was a greater response of eusinophils to dietary lycopene.

The specific antibody response to ovalbumin is a characteristic of humoral immunity, whereas stimulation of lymphocyte proliferation causes cellular immunity [1]. An interaction (P=0.011) was observed between collection periods and lycopene levels for the production of anti-BSA IgG (Table 4). IgM is the first antibody formed when white blood cells are initially exposed to an antigen, ans when exposed to an antigen for a second time, the pig builds very high levels of antibodies, mostly in the class of IgG and the white blood cells switch from synthesizing IgM to synthesizing IgG after continued antigen exposure. The Serum IgG is considered the major constituent of blood immune globulin and this antibody plays a major role in defending against antigens [26]

Anti-BSA IgG production increased over the study period at all levels (0, 12.5, 25.0, 37.5 and 50.0 mg) of lycopene supplementation (Figure 4). Luo et al [27] found that lycopene can increase blood levels of IgA, IgG and IgM, improving the immunity of rats with cancer. Similarly Neyestani et al [28] observed that dietary supplementation of lycopene stimulates the formation of specific antibodies, mainly IgG isotype, and inferred that lycopene is not only a common immune enhancer because it stimulates immune response (Anti-atherogenic) with no effect on the specific (pro-atherogenic) T cell dependent response.

Lycopene supplementation had a beneficial effect on the long-term production of anti-BSA IgG, with increased production observed only on the 24 th day of collection, and stimulation of humoral immunity following supplementation of up to 20.06 mg of lycopene/kg of diet (Figure 3). When it comes to an immune mediator, it is not always the highest concentration of nutraceutical supplementation that promotes the best immune response [29]. Silva et al [30] tested three levels of vitamin E (30, 65 and 100 mg/kg) in broiler chicken and observed that the intermediate concentration promoted the highest production of antibodies against Newcastle disease. Likewise, it was observed with of the lycopene supplementation in which the intermediate level of 20.06 mg of lycopene/kg of diet provided the best response to the anti-BSA IgG production.

CONCLUSION

The supplementation of lycopene in the diet of, barrows and gilts, from 75 to 100 kg, had an improvement in the immune response, in which the supplementation of 20.00 mg/kg of diet produced the best response.

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TABLES

Table 1. Ingredients, chemical and energetic composition of basal diet

Ingredients (%)	**Barrows**	**Gilts**
Corn	87.14	83.89
Soybean meal 45 %	9.61	12.47
Soybean oil	0.740	0.950
Dicalcium phosphate	0.785	0.973
Limestone	0.553	0.521
Salt	0.203	0.203
L-lysine HCL 78.4 %	0.373	0.390
L-threonine 98.5 %	0.068	0.072
DL-methionine 99 %	0.036	0.053
L-tryptophan 98 %	0.022	0.017
Growth promoter [1)]	0.015	0.015
Vitamin and mineral supplement [2)]	0.400	0.400
Lycopene extract [3)]	0.000	0.000
Inert [4)]	0.050	0.050
Total	100.00	100.00
Composition		
Metabolizable energy (Mcal/kg)	3.30	3.30
Nitrogen (%)	1.86	2.03
Total Calcium (%)	0.45	0.49
Available phosphorus (%)	0.23	0.26
Potassium (%)	0.42	0.47
Sodium (%)	0.10	0.10
Chlorine (%)	0.18	0.18
SID lysine (%)	0.690	0.770
SID met + cis (%)	0.400	0.440
SID threonine (%)	0.440	0.480
SID tryptophan (%)	0.120	0.130

[1)] Enramycin 0.015%.

[2)] Content/kg diet; vit. A: 30000 UI, vit. D3: 5000 UI, vit. E: 120 UI, vit. K: 5 mg, vit. B12: 120 mcg, Niacin: 150 mg, Calcium pantothenate: 75 mg, folic acid: 8 mg, Choline chloride: 0.48 g, iron: 350 mg, copper 15 mg, manganese: 250 mg, Zinc: 0.75 g, iodine: 10 mg, selenium: 3 mg.

[3)] Lycopene extract at a concentration of 10% included in the basal diet replacing the inert.

[4)] Fine clean sand.

Table 2. Blood leukocyte profile and hematocrit of, barrows and gilts, from 75 to 100 kg, fed diets containing different levels of lycopene

Item	Barrows					Medium	Gilts					Medium	SEM	P-value			
	Lycopene (mg/kg of diet)						Lycopene (mg/kg of diet)							Sex x Lycopene	Sex	Lycopene	
	0	12.5	25.0		50.0		0	12.5	25.0	37.5	50.0					Lin[1)]	Qua[2)]
Lymphocyte, (%) [3)]	63.96	72.63	78.40	73.92	75.31	72.84	68.87	71.17	72.19	73.42	72.60	71.65	1.719	0.747	0.568	0.045	0.193
Monocyte, (%)	7.27	8.04	7.39	7.12	5.20	7.00 [a]	3.13	5.93	5.79	6.79	6.06	5.54[b]	0.629	0.352	0.048	0.369	0.088
Neutrophil, (%)[4)]	23.62	13.89	10.25	14.18	17.26	15.84	23.89	16.71	16.04	15.74	17.24	17.92	1.868	0.946	0.233	0.024	0.003
Basophil, (%)	3.84	2.82	1.91	2.47	1.12	2.43	2.11	3.17	3.03	2.88	1.95	2.63	0.348	0.321	0.624	0.636	0.295
Eosinophil, (%)[5)]	1.31	2.62	2.06	2.31	1.11	1.88	2.00	3.02	2.96	1.16	2.15	2.26	0.313	0.520	0.778	0.479	0.050
Neu:Lin, ratio [6)]	0.47	0.20	0.13	0.21	0.25	0.25	0.35	0.27	0.24	0.22	0.30	0.28	0.042	0.596	0.519	0.041	0.005
Hematocrit, (%)	32.21	33.57	30.33	34.15	32.64	32.58	34.45	32.36	34.94	33.81	32.66	33.64	0.605	0.099	0.154	0.496	0.934

* Different letters in line differ by F test at 5% probability.

SEM: standard error of the mean.

[1)] Linear effect of lycopene levels.

[2)] Quadratic effect of lycopene levels.

[3)] $\hat{Y} = 0.1382x + 68.81$ ($R^2 = 0.58$).

[4)] $\hat{Y} = -0.1225x + 19.87$ ($R^2 = 0.35$). $\hat{Y} = 0.0110x^2 - 0.6710x + 23.26$ ($R^2 = 0.97$).

[5)] $\hat{Y} = -0.0016x^2 + 0.0726 + 1.79$ ($R^2 = 0.69$).

[6)] $\hat{Y} = -0.002592x + 0.328$ ($R^2 = 0.33$). $\hat{Y} = 0.00024x^2 - 0.146x + 0.4024$ ($R^2 = 0.97$).

Table 3. Total proteins and fractions of barrows and gilts, from 75 to 100 kg, fed diets containing different levels of lycopene

Item	Barrows						Gilts						SEM	P-value			
	Lycopene (mg/kg of diet)					Medium	Lycopene (mg/kg of diet)					Medium		Sex x Lycopene	Sex	Lycopene	
	0	12.5	25.0	37.5	50.0		0	12.5	25.0	37.5	50.0					Lin[1)]	Qua[2)]
Total protein, mg/dL	7.07	6.50	6.68	6.34	6.49	6.62 [b]	7.82	7.59	7.56	7.19	7.73	7.58 [a]	0.251	0.971	0.001	0.279	0.307
Albumin, mg/dL [3)]	3.36	3.43	3.68	3.39	3.62	3.49 [a]	3.16	3.07	3.26	3.55	3.58	3.27 [b]	0.112	0.430	0.117	0.023	0.899
Globulin, mg/dL	3.83	2.73	3.18	2.96	2.88	3.11 [b]	4.38	4.38	4.30	3.89	4.15	4.33 [a]	0.334	0.648	0.001	0.115	0.429
AB:GL ratio, mg/dL	0.95	1.26	1.50	1.18	1.41	1.26 [a]	0.72	0.93	0.80	1.17	0.99	0.90 [b]	0.121	0.527	0.009	0.080	0.348

* Different letters in line differ by F test at 5% probability.

SEM: standard error of the mean.

[1)] Linear effect of lycopene levels.

[2)] Quadratic effect of lycopene levels.

[3)] $\hat{Y} = 0.0071x + 3.23$ ($R^2 = 0.89$).

Table 4. Levels of lycopene for, barrows and gilts, from 75 to 100 kg, on the production of IgG in different collection periods

Periods (Days)	Barrows					Medium	Gilts					Medium	SEM
	Lycopene (mg/kg of diet)						Lycopene (mg/kg of diet)						
	0	12.5	25.0	37.5	50.0		0	12.5	25.0	37.5	50.0		
0	0.0122	0.0145	0.0181	0.0156	0.0140	0.0149	0.0172	0.0289	0.0204	0.0160	0.0180	0.0201	0.002
12	0.3214	0.2311	0.3808	0.2781	0.3348	0.3092	0.0925	0.2750	0.2722	0.3566	0.3065	0.2606	0.036
24	0.5889	0.7980	1.2893	0.9661	0.9301	0.9145	0.4714	0.8316	0.8722	1.0169	0.9825	0.8349	0.102
Medium	0.3075	0.3479	0.5627	0.4199	0.4263		0.1937	0.3785	0.3883	0.4632	0.4356		

	P-value[1]	Linear	Quadratic
Day x Sex x Lycopene	0.557		
Day x Lycopene	0.011		
Day x Sex	0.218		
Sex x Lycopene	0.625		
Lycopene	0.000	0.001	0.010
Day	0.001	0.001	0.001
Sex	0.257		

[1] Effect of P at 5% significance.

* NS = not significant.

** Equations adjusted for the collection periods: 0 day – NS; 12 day – NS; 24 day - $\hat{Y} = 0.00823x + 0.668914$ ($R^2 = 0.57$). $\hat{Y} = - 0.0005x^2 + 0.03098x + 0.526780$ ($R^2 = 0.95$).

*** Equations adjusted for the lycopene levels (mg/kg of diet): 0 mg - $\hat{Y} = 0.0215x – 0.0071$ ($R^2 = 0.98$); 12.5 mg - $\hat{Y} = 0.0330x – 0.0333$ ($R^2 = 0.94$); 25 mg - $\hat{Y} = 0.0442x – 0.0553$ ($R^2 = 0.94$); 37.5 mg - $\hat{Y} = 0.0406x – 0.0462$ ($R^2 = 0.95$); 50 mg - $\hat{Y} = 0.0391x – 0.0391$ ($R^2 = 0.96$).

FIGURES

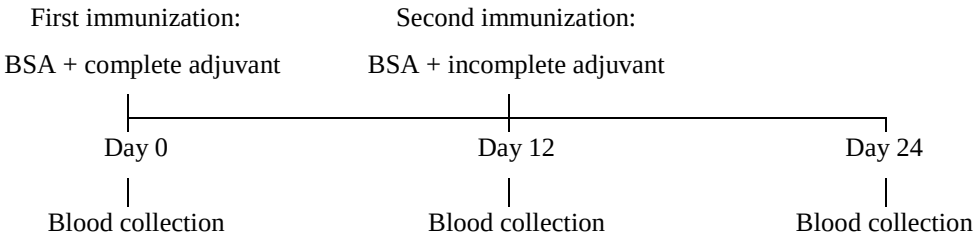
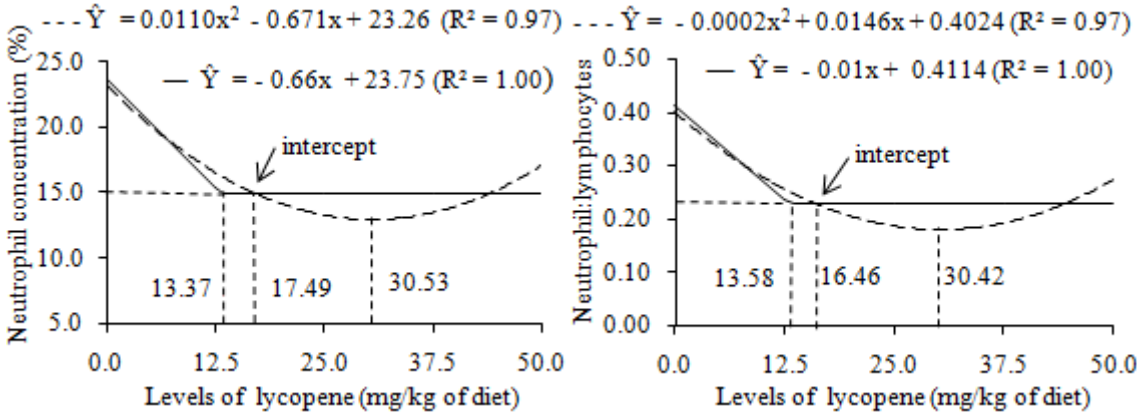


Figure 1. Immunization and blood collection protocol for determination of Antibovine Serum Albumin (anti-BSA) IgG by Elisa indirect.



A) B)

Figure 2. A) Neutrophil concentration and B) neutrophil:lymphocytes ratio in the blood of pigs, barrows and gilts, with diets containing different levels of lycopene.

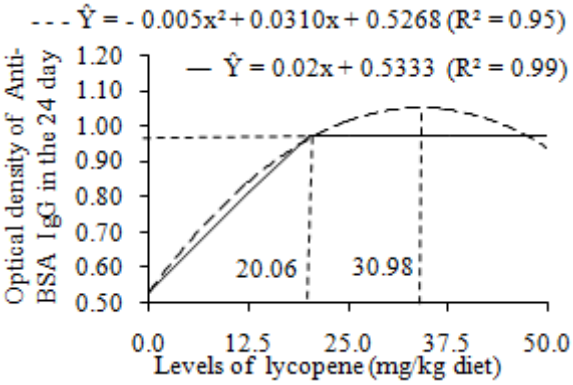


Figure 3. Absorbance reading of Anti-BSA IgG in the ELISA, at 24 days after starting of the supplementation of different lycopene levels for barrows and gilts, from 75 to 100 kg.

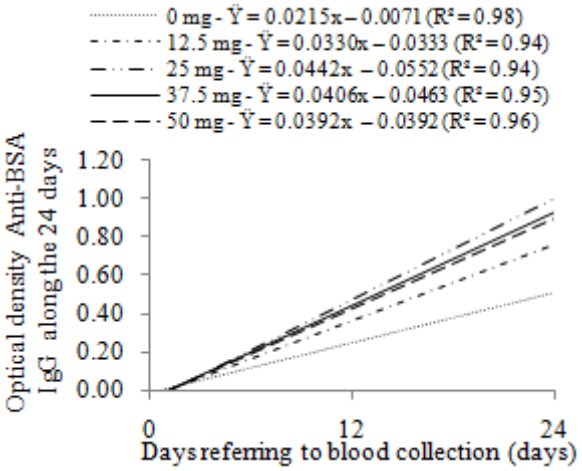


Figure 4. Absorbance reading of Anti-BSA IgG in ELISA, along the 24 days after starting the supplementation different lycopene levels for barrows and gilts, from 75 to 100 kg.

VI- CONSIDERAÇÕES FINAIS

A suplementação licopeno na dieta de suínos, machos castrados e fêmeas não influenciou o desempenho e as características quantitativas de carcaça, dessa forma, o licopeno deve ser tratado como um antioxidante, o que pode ser importante, principalmente, pelos seus efeitos biológicos.

O licopeno demonstrou ser efetivo na proteção contra a oxidação do músculo *longissimus lumborum* e fígado, e reduziu a perda de liquido no descongelamento da carne. Além de ser um potente antioxidante, resultando em uma redução da expressão gênica das enzimas superóxido dismutase e catalase no fígado de fêmeas suínas.

O licopeno é potente modulador do perfíl lipídico sanguíneo de suínos machos e fêmeas, reduzindo linearmente os níveis de colesterol total e lipoproteínas de baixa densidade e aumentou as lipoproteínas de alta densidade, assim como um potente modulador do sistema imune, promovendo alterações no perfíl leucocitário e na produção de IgG.

Com base nos resultados obtidos, pode-se concluir que a maior eficiência do licopeno como antioxidante obtida com a suplementação de 50,0 mg de licopeno/kg de ração para a qualidade da carne, fígado e perfil lipídico e 20,0 mg de licopeno/kg de ração apresentou a melhor resposta imune.