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DESEMPENHO, QUALIDADE DE OVOS E SAÚDE DE POEDEIRAS COMERCIAIS ALIMENTADAS COM DIETA SUPLEMENTADA COM ÓLEO ESSENCIAL DE ORÉGANO (*Origanum vulgare*)

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CHAPECÓ, 2017.

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Orientador: Prof. Dr. Marcel Manente Boiago
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DESEMPENHO, QUALIDADE DE OVOS E SAÚDE DE POEDEIRAS COMERCIAIS ALIMENTADAS COM DIETA SUPLEMENTADA COM ÓLEO ESSENCIAL DE ORÉGANO (*Origanum vulgare*)

Elaborada por
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RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

DESEMPENHO, QUALIDADE DE OVOS E SAÚDE DE POEDEIRAS COMERCIAIS ALIMENTADAS COM DIETA SUPLEMENTADA COM ÓLEO ESSENCIAL DE ORÉGANO (O*riganum vulgare*)

AUTOR: Marcos José Migliorini
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Chapecó, 13 de Fevereiro de 2017

Esse estudo teve por objetivo avaliar os efeitos da adição do óleo essencial de orégano (OEO) na alimentação de galinhas poedeiras comerciais sobre o desempenho, qualidade de ovos frescos e armazenados em temperatura ambiente e saúde das aves. Foram utilizadas 240 poedeiras, distribuídas em um delineamento inteiramente casualizado com seis tratamentos e cinco repetições de oito aves cada. Os tratamentos consistiram de um tratamento controle com inclusão de bacitracina de zinco e cinco tratamentos sem o uso do antibiótico com níveis de inclusão de OEO de 0, 50, 100, 150 e 200mg/kg de ração, respectivamente. O experimento teve duração de 84 dias subdivididos em três ciclos de 28 dias. Nos resultados de desempenho o maior consumo de ração foi de aves dos tratamentos controle e com 200mg de OEO, sem diferenças significativas na porcentagem de postura entre os tratamentos. Nas análises de qualidade da casca dos ovos, a contagem de aeróbios mesófilos aos 70 dias apresentou aumento de UFC/ovo no tratamento com 200mg de OEO. As análises de qualidade dos ovos armazenados apresentaram diferenças significativas ($p<0,05$) no tratamento com 50mg de OEO, com menor porcentagem de casca e maior pH e maior intensidade de amarelo para coloração da gema no tratamento controle e com 200mg de OEO de ovos armazenados por 21 dias. A peroxidação lipídica mensurada pelo nível de TBARS foi reduzida na gema de ovos frescos de aves do tratamento com 200mg de OEO e de ovos armazenados no tratamento com 150mg de OEO. Os resultados dos parâmetros bioquímicos mostraram aumento significativo ($p<0,05$) nos níveis de proteínas totais e globulinas em aves do tratamento com 150mg e 200mg de OEO aos 28 dias e de albumina de aves do tratamento controle. Aos 56 dias só houve diferença significativa ($p<0,05$) para proteínas totais em aves do tratamento com 50mg de OEO. Aos 84 dias ocorreu redução significativa de proteínas totais e albumina no tratamento com 200mg de OEO, com aumento nos níveis de triglicerídeos. O OEO manteve o desempenho produtivo das aves, reduziu a peroxidação lipídica na gema de ovos frescos e armazenados e ocasionou aumento nos níveis séricos de globulina nas aves, o que reflete em possível aumento de mediadores inflamatórios e imunoglobulinas.

Palavras-Chave: ácido tiobarbitúrico, extrato herbal, peroxidação lipídica, proteínas totais, TBARS, triglicerídeos.

ABSTRACT
Master's Dissertation
Graduate Program in Animal Science
University of the State of Santa Catarina

PERFORMANCE, QUALITY OF EGGS AND HEALTH OF COMMERCIAL LAYING HENS FEEDING WITH DIET SUPPLEMENTED WITH ESSENTIAL OIL OF OREGANO (Origanum vulgare)

AUTHOR: Marcos José Migliorini
ADVISER: Marcel Manente Boiago
Chapecó, February 13, 2017

The objective of this study was to evaluate the effects of the addition of oregano essential oil (OEO) on feeding commercial hens on performance, quality of fresh eggs and stored at ambience temperature and bird health. A total of 240 laying hens were used, distributed in a completely randomized design with six treatments and five replicates of eight birds each. The treatments consisted of a control treatment with inclusion of zinc bacitracin and five treatments without the use of antibiotics with OEO inclusion levels of 0, 50, 100, 150 and 200mg / kg of feed, respectively. The experiment lasted 84 days subdivided into three cycles of 28 days. In the performance results, the highest feed intake was of birds of the control treatments and with 200 mg OEO, without significant differences in the percentage of posture between the treatments. In the egg shell quality analyzes, the counts of mesophilic aerobes at 70 days presented an increase of CFU/egg in the treatment with 200mg of OEO. The quality of the stored eggs presented significant differences ($p < 0.05$) in the treatment with 50 mg of OEO, with a lower percentage of sheel and higher pH and a higher intensity of yellow for the color of the yolk in the control treatment, and with 200 mg OEO of eggs stored for 21 days. The lipid peroxidation measured by the TBARS level was reduced in fresh egg yolk from the treatment with 200 mg of OEO and eggs stored in the treatment with 150 mg of OEO. The results of the biochemical parameters showed a significant increase ($p < 0.05$) in total protein and globulin levels in birds treated with 150mg and 200mg of OEO at 28 days and of control albumin. At 56 days there was only a significant difference ($p < 0.05$) for total proteins in birds treated with 50mg of OEO. At 84 days, there was a significant reduction of total proteins and albumin in the treatment with 200 mg OEO, with an increase in triglyceride levels. OEO maintained the productive performance of the birds, reduced the lipid peroxidation in fresh and stored egg yolk and caused an increase in serum globulin levels in birds, which reflects a possible increase of inflammatory mediators and immunoglobulins.

Key-words: Thiobarbituric acid, herbal extract, lipid peroxidation, total proteins, TBARS, triglycerides.

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1 CAPÍTULO I
REVISÃO DE LITERATURA

1.1 Introdução

A avicultura de postura apresenta amplo potencial econômico, onde a produção e o consumo de ovos no Brasil aumentam anualmente, segundo dados do relatório anual da ABPA (Associação Brasileira de Proteína animal, 2016). Nesse contexto, a avicultura de postura se destaca como atividade com ampla possibilidade e potencial de crescimento entre os maiores produtores mundiais de ovos (ALBINO et al., 2014). Sendo assim, um dos principais desafios da Zootecnia na atualidade é a produção de ovos com qualidade e que atendam as exigências dos consumidores. A obtenção de alguns destes resultados produtivos foram possíveis devidos avanços em melhoramento genético, nutrição e manejo, destacando a alimentação das aves com fundamental importância nos custos e no desempenho, quanto a parâmetros de consumo de ração, conversão alimentar, viabilidade do lote, porcentagem de postura e principalmente produção e qualidade dos ovos (ALBINO et al., 2014).

A utilização de antibióticos promotores de crescimento na alimentação por muito tempo permitiu melhores resultados em desempenho e redução na mortalidade causada por agentes patogênicos (BRENES & ROURA, 2010). Porém, a pressão do consumidor a possível resistência bacteriana resultou na proibição pela União Européia desde 2006, e desde aquele momento esperava-se que outros países tivessem essa iniciativa (SOLTAN et al., 2008). Devido a isso, as pesquisa com aditivos naturais de plantas tem demonstrado potencial para substituir os antibióticos melhoradores de desempenho (BRENES & ROURA, 2010). Os antibióticos são adicionados as rações em pequenas quantidades com a finalidade de proporcionar melhores resultados no desempenho produtivo de aves (BELLAVER, 2005). Oriundos da produção de bactérias, fungos e leveduras, atuam na inibição do crescimento de microorganismos patogênicos no tratamento ou prevenção de doenças (terapêutico e profilático) (FREITAS et al., 2001).

Óleos essenciais derivados de diferentes plantas são considerados como possíveis substitutos de antibióticos, tendo em vista que as plantas produzem metabólitos secundários que exibem propriedades bactericidas (WALLACE, 2004). São compostos complexos naturais e voláteis, os quais são caracterizados por um forte odor e formados por plantas aromáticas como metabólitos secundários (BAKKALI et al., 2008). Diferentes estudos demonstraram que

substâncias componentes de óleos essenciais exibem atividade biológica contra micro-organismos patogênicos, além de apresentarem atividade antioxidante (JANG et al., 2007).

O ovo apresenta alto potencial nutritivo e precisa ser conservado durante o período de comercialização, uma vez que podem transcorrer semanas entre o momento da postura, aquisição e consumo. A comercialização nos mercados ocorre sem refrigeração, onde compromete a conservação da qualidade interna dos ovos (PASCOAL et al. 2008).

Portanto, a pesquisa teve como objetivo avaliar a eficácia da utilização de diferentes concentrações de óleo essencial de orégano na alimentação de galinhas poedeiras semipesadas sobre o desempenho, qualidade dos ovos (física e microbiológica) e saúde das aves.

1.2 Qualidade de ovos

Na avaliação de qualidade de ovos a gravidade específica conforme metodologia de Freitas et al. (2004) é uma medida para avaliar a qualidade da casca do ovo. A avaliação interna consiste de medidas como unidade Haugh, valor de qualidade de albúmen em relação ao peso do ovo e peso de casca, albúmen e gema para cálculo de porcentagem (FREITAS et al., 2013), ainda podem ser considerados os valores de pH da gema e albúmen e coloração da gema.

A peroxidação lipídica que ocorre no ovo principalmente no armazenamento pode ser avaliada através da determinação de substâncias reativas ao ácido tiobarbitúrico (TBARS) (FREITAS et al., 2013). O malonaldeido é o produto resultado da peroxidação lipídica de ácidos graxos que reage com o ácido tiobarbitúrico quando aquecido e a intensidade de peroxidação é medida através de leitura em espectrofotômetro (532nm) (LUNA et al., 2010). Esses parâmetros são utilizados para avaliar a qualidade de ovos, pois auxilia na avaliação do tempo de prateleira do produto. Considerando isto a qualidade interna pode piorar devido ao tempo de armazenamento, já que, após a postura, o ovo tende a perder a qualidade de maneira contínua. Isso ocorre devido à casca possuir poros que possibilitam dentre diversos fatores, que temperatura elevada aliada a baixa umidade promovam maior ação da enzima anidrase carbônica, que acelera a dissociação do ácido carbônico em água e gás carbônico (CO_2), que resulta em perda de qualidade constante (ALBINO et al., 2014). A recomendação mais adequada de se conservar o ovo é sob refrigeração, entretanto no Brasil os ovos são comercializados em temperatura ambiente, o que compromete a conservação (PASCOAL et al., 2008).

Durante o armazenamento e processamento a peroxidação lipídica que ocorre é responsável pelo sabor e odor desagradáveis, reduzindo o potencial nutricional dos alimentos, resultando na produção de compostos tóxicos, podendo provocar sérios danos à saúde dos consumidores (KARPIŃSKA et al., 2001). A manutenção da qualidade interna depende das condições de temperatura de armazenamento e umidade relativa, estes fatores têm um efeito direto sobre a composição química, bioquímica, e física de deterioração microbiológica e de durabilidade de ovos (ARPASOVA et al., 2013). Desta forma, a inclusão dos antioxidantes, que são substâncias que visam evitar a oxidação de alimentos, atuando na conservação, retardando a deterioração, rancificação e perda de coloração, podem reduzir esta ação (BELLAVER, 2005).

A utilização de antioxidantes sintéticos, butilato dehidroxianisol (BHA) e o butilato de hidroxitolueno (BHT) parecem ser eficientes na conservação de alimentos, contudo apresentam rejeição pelo consumidor devido ao potencial carcinogênico (FREITAS et al., 2013). Isto aliado a preocupação com a possível resistência bacteriana e transferência de genes de resistência de antibióticos animais para os seres humanos, conforme já mencionado anteriormente (CASTANON, 2007), motivaram a busca de possíveis substitutos aos antioxidantes sintéticos e antibióticos. A exigência dos consumidores por alimentos livres de produtos químicos e artificiais, incluindo conservantes em alimentos, despertou maior interesse de fazer uso de produtos naturais como alternativas para aumentar a vida de prateleira dos alimentos, como plantas aromáticas e seus componentes inibidores do crescimento bacteriano têm sido estudados, onde autores destacam os óleos essenciais e outros metabólitos de plantas secundárias (CALO et al., 2015).

1.3 Óleos essenciais

Todas as plantas produzem compostos químicos orgânicos como atividade metabólica normal, estes são divididos em compostos primários, como açucares e gorduras, e metabólitos secundários ou fitoquímicos, não essenciais as funções básicas da planta, podendo atuar na proteção contra a radiação e atração de polonizadores (FRANKIC et al., 2009). Os óleos essenciais consistem em terpenos classificados em produtos químicos, como alcoóis, ácidos, esteróides, taninos, aldeídos, cetonas, saponinas entre outros, obtidos por processos de extração específicos (HASHEMI & DAVOODI, 2011; CALO et al., 2015).

A utilização de suplementos aditivos de ervas ou extratos na alimentação animal depende de muitos fatores como espécie, idade e finalidade de produção, pois a composição pode ter efeito e mecanismo de ação diferente (HASHEMI & DAVOODI, 2011). O potencial dos aditivos alimentares fitogênicos pode afetar a microflora intestinal através do controle de patógenos, melhorando a capacidade de absorção e digestão de nutrientes do intestino delgado, assim como a resposta imune e a eubiose, interação entre microorganismos, promovendo melhor desempenho aos animais (HASHEMI & DAVOODI, 2011).

Os óleos essenciais são líquidos oleosos aromáticos, voláteis, obtidos de material vegetal (flores, sementes, folhas, galhos, cascas, ervas, madeira, frutos e raízes), por processo de fermentação ou extração, mas o método de destilação é o mais utilizado (BURT, 2004). A extração da mesma parte da planta influencia na sua composição, assim como características de variedade, crescimento e período de colheita (FRANKIC et al., 2009).

Durante a destilação, a fragrância de plantas expostas à água fervente, libera seus óleos essenciais por evaporação. A quantidade de óleo essencial produzido depende de quatro critérios principais, duração do tempo de destilação, a temperatura (200 a 300ºC), a pressão de funcionamento e o mais importante, o tipo e a qualidade do material vegetal. O rendimento de óleos essenciais das plantas está entre 0,005 e 10%, essa metodologia é descrita em detalhes por Chemat & Boutekedjiret, (2015).

Os óleos são compostos complexos naturais de composição química variável, constituído principalmente de duas classes de compostos, terpenos ou terpenóides que compõem os metabólitos secundários de plantas e fenilpropanóides, que dependem do número de carbonos e variam conforme a composição em blocos de cinco carbonos (AL-KASSIE, 2009). Esses compostos orgânicos fornecem proteção as plantas, resistência a pragas e são indicadores de respostas para estímulos (VOGT, 2010). Podem conter cerca de 20-60 componentes em concentrações bastante diferentes, caracterizados por dois ou três em maiores concentrações, como os compostos fenólicos carvacrol (30%) e timol (27%) (BAKKALI et al., 2008).

Comparados aos antibióticos os óleos essenciais são produtos naturais, menos tóxicos, livres de resíduos, seguros devido ao uso em doses baixas e são possíveis substitutos aos promotores de desempenho (HASHEMI & DAVOODI, 2011). Contudo no que concerne à ação de óleos essenciais sobre aves de postura, não há resultados conclusivos a respeito do efeito sobre a fisiologia das aves, desempenho e qualidade de ovos.

Entre as alternativas se destaca o orégano (*Origanum vulgare*) membro da família *Lamiaceae*, uma planta medicinal e aromática, da qual se pode extrair o óleo essencial por processo de destilação a vapor. Tem sido estudado devido principalmente a sua composição com boa atividade antimicrobiana e antioxidante, com benefícios principalmente na redução de reações oxidativas (ARPASOVÁ et al., 2014). Essas características da composição são consideradas importantes principalmente para indústria de alimentos para promover a segurança e estabilidade lipídica de alimentos.

1.4 Atividade antimicrobiana

A atividade do orégano é atribuída principalmente aos principais componentes secundários carvacrol e timol, essas substancias modificam a permeabilidade das membranas e reagem com radicais livres tornando-os estáveis (LUNA et al., 2010). Com cerca de 78-82% da composição total do OEO, o carvacrol e timol são os principais responsáveis por atividades biológicas benéficas. Composições idênticas podem ser extraídas nas mesmas condições, a partir da mesma parte da planta, cultivada em mesmo solo, clima e época de colheita (BAKKALI et al., 2008; YESILBAG et al., 2013). A essa composição são atribuídas às principais atuações dos componentes dos óleos com ação antimicrobiana e antioxidante, com potencial conservante de alimentos para evitar a deterioração e aumentar a vida de prateleira (SOLÓRZANO-SANTOS & MIRANDA-NOVALES, 2012).

Esses compostos fenólicos carvacrol e timol presentes no OEO, assim como outras espécies de *Lamiaceae* apresentam considerável atividade microbiana e fungicida, podendo ser fornecidos de forma mais concentrada do que encontrados em sua fonte natural e em níveis maiores que antibióticos promotores de crescimento (HERNANDEZ et al., 2004). Atuam na membrana celular das bactérias inibindo sua divisão mitótica, provocando desidratação celular e impedindo a sobrevivência de bactérias patogênicas (FUKAYAMA et al., 2005).

Atividade antibacteriana dos óleos essências se deve a essa capacidade dos terpenóides e fenilpropanóides, penetrarem na membrana das bactérias e atingir a parte interna da célula por causa de sua lipofilicidade, capacidade de se dissolver (AL-KASSIE, 2009). A característica de hidrofobicidade, ou seja, capacidade de repelir dos óleos essenciais promove a separação dos lipídios da membrana celular bacteriana e mitocôndrias tornando a membrana permeável. Essa ação exibe atividade inibitória das propriedades funcionais da célula, causando vazamento do

conteúdo interno da célula, interferindo na geração de energia (ATP) e como resultado a morte celular (CALO et al., 2015).

De acordo com a literatura, o componente com maior atividade antimicrobiana do óleo essencial de orégano foi o carvacrol, com maior atividade contra bactérias Gram-positivas do que Gram-negativas (CALO et al., 2015). A atividade antimicrobiana foi verificada por Santurio et al. (2007) e Bona et al. (2012), com capacidade de alterar a permeabilidade da membrana celular microbiana (VIUDA-MARTOS et al., 2006), no controle de patógenos e melhora na digestão e absorção devido a estímulo da atividade enzimática, o que pode apresentar efeitos positivos quando utilizado em aves de postura semi-pesadas (OETTING et al., 2006).

Diferentes estudos demonstraram que substâncias componentes de óleos essenciais exibem atividade biológica contra microorganismos patogênicos, além de apresentarem atividade antioxidante (JANG et al., 2007). Neste contexto, foram observados efeitos positivos sobre a inibição de *Escherichia coli* e *Staphylococcus aureus* e diferentes sorotipos de *Salmonella* (OUSSALAH et al., 2006), populações microbianas reduzidas ao longo do tempo de armazenamento (OLIVEIRA et al., 2013).

A característica que mais influencia a ação antimicrobiana dos extratos naturais é a sua elevada hidrofobicidade, habilidade de atravessar as membranas bacterianas e agir diretamente causando perda de íons e redução do potencial da membrana (PESAVENTO et al., 2015), danos a proteínas, lipídeos e organelas presentes na célula bacteriana (BAKKALI et al., 2008), causando assim a morte celular. Segundo Elgayyar et al. (2001), o óleo de orégano contendo principalmente timol e carvacrol inibiu completamente o crescimento de patógenos Gram-negativas testadas, enquanto Aligiannis et al. (2001), utilizaram duas espécies de OEO uma delas com composição de carvacrol (74,86%) o qual exibiu maior atividade contra microorganismos testados.

O ovo é considerado um dos alimentos mais completos em níveis nutritivos para o consumo humano (RÊGO et al., 2012), e portanto considerado excelente fonte de ácidos graxos, carboidratos, minerais, vitaminas e principalmente proteínas (GIAMPIETRO et al., 2008). No entanto, precisa ser conservado durante o período de comercialização para que a qualidade e composição nutricional sejam mantidas, uma vez que podem transcorrer semanas entre o momento da postura, aquisição e consumo (LOPES et al., 2012). A possível contaminação da

casca pode comprometer a segurança e qualidade de ovos armazenados e estão sujeitos à oxidação lipídica, principalmente durante o armazenamento (HAYAT et al., 2010).

1.5 Atividade antioxidante

A peroxidação lipídica é uma reação caracterizada pela formação de radicais livres, moléculas instáveis e que apresentam um elétron que tende a se associar de maneira rápida a outras moléculas de carga positiva com as quais pode reagir ou oxidar, os quais são produzidos durante o metabolismo normal e podem induzir danos no tecido em que estiverem presentes em níveis elevados, podendo ser eliminados pelos antioxidantes como enzimas ou componentes não enzimáticos antioxidantes (ZHAO et al., 2011). Como os fenóis e polifenóis são um grupo de antioxidantes naturais encontrados nas plantas, tem potencial contra danos oxidativos (KARPINSKA, et al., 2001). Antioxidantes são compostos que podem retardar ou inibir a peroxidação de lipídios ou outras moléculas, evitando o início ou propagação das reações em cadeia de oxidação. A atividade antioxidante de compostos fenólicos é principalmente devida às suas propriedades de óxido-redução, as quais podem desempenhar um importante papel na absorção e neutralização de radicais livres (DEGÁSPARI & WASZCZYNSKYJ, 2004) e a presença de grupos hidroxila (POLAT et al., 2011), que podem doar elétrons para neutralização ou seqüestro de radicais livres e quelação (transformação) de metais de transição, agindo tanto na etapa de iniciação como na propagação do processo oxidativo.

É extremamente importante notar que existe uma correlação positiva entre a atividade antioxidante conteúdo de fenólicos total das plantas. As plantas pertencentes à família *Lamiaceae*, dentre essas o orégano são muito ricas em compostos polifenólicos. As diferenças na composição do óleo volátil e no teor de fenol total do alecrim podem ser atribuído aos efeitos climáticos sobre as plantas que estão crescendo em diferentes habitats (POLAT et a., 2011).

Atividade antioxidante foi avaliada com uma mistura de óleos essenciais de orégano, alecrim, sálvia e pimenta em níveis de 50, 100, 150mg/kg em frangos de corte e verificaram efeito antioxidante na carne (TRAESEL et al., 2011). Em acordo com Young et al. (2003) e Florou-Paneri (2006) que utilizaram orégano e verificaram melhores resultados em desempenho e estabilidade oxidativa da carne do peito e coxa de frangos de corte.

A atividade antioxidante do OEO poderia atuar na qualidade e controle da estabilidade lipídica em ovos armazenados. Essa atividade da utilização do OEO na qualidade de ovos

proporcionou efeito antioxidante em ovos devido a redução de malonaldeido e aumento da estabilidade lipídica de ovos armazenados (YESILBAG et al., 2013), efeito da presença de grupos hidroxila em compostos fenólicos que atuam na redução da peroxidação lipídica (POLAT et al., 2011).

Os menores valores de malonaldeido encontrado em gema de ovos frescos de galinhas alimentadas com dieta com tomilho se devem a possível transferência dos constituintes antioxidantes do tomilho para ave através da alimentação, o que pode assim inibir a reação em cadeia envolvida na oxidação de lipídios consumidos, reduzindo assim a oxidação de produtos transferidos para a gema (BOTSOGLOU et al., 1997).

Estudos de Lee et al. (2004a, 2004b) têm demonstrado que a utilização do OEO na alimentação de aves proporcionou melhoria no desempenho e também sobre a qualidade de ovos, resultados atribuídos pelos autores às propriedades antimicrobiana e antioxidante do orégano. Em acordo, Yesilbag et al. (2013) utilizaram óleo de alecrim e orégano e observaram melhor produção e a conservação de características de qualidade interna de ovos.

Há uma escassez de resultados sobre a saúde de poedeiras submetidas à dietas com óleos essenciais. Parâmetros bioquímicos do sangue podem refletir as condições do organismo e as mudanças que ocorrem devido a fatores externos (TOGHYANI et al., 2010). Esses índices refletem a saúde das aves como indicadores do estado nutricional das aves (EL-LATIF et al., 2013), assim como pode mostrar se o tratamento ou dose usada é prejudicial (tóxica) as aves. Muitas vezes uma dieta pode ser boa para produção, mas ter efeito negativo para aves, afetando sua qualidade de vida, principalmente quando relacionado a função hepática, metabolismo protéico e lipídico.

2 CAPÍTULO II
MANUSCRITOS

Os resultados desta dissertação são apresentados na forma de dois manuscritos, com suas formatações de acordo com as orientações das revistas aos quais serão submetidos:

2.1 MANUSCRITO I

DESEMPENHO E QUALIDADE DE OVOS DE GALINHAS POEDEIRAS SUPLEMENTADAS COM DIFERENTES NÍVEIS DE ÓLEO ESSENCIAL DE ORÉGANO (*Origanum vulgare*)

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Effects of supplementation with oregano essential oil on performance of hens and quality of eggs

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ABSTRACT

The main objective of this study was to evaluate the influence of supplementation of the dietary of commercial semi-heavy laying hens with oregano essential oil (OEO) on the performance and physical, chemical and microbiological quality of fresh eggs and eggs stored for 21 days. The 240 laying hens (59 weeks' age) were distributed according to a complete randomized block design consisting on six treatments (eight hens in each group) and five trials. The dietary of each group was constituted by the control (without OEO supplemented with zinc bacitracin) and the basal ration supplemented with the addition of 0, 50, 100, 150 and 200 mg/kg of OEO without zinc bacitracin. In addition, a sample of 4 eggs from each group was randomly collected to determine the egg quality immediately (2 eggs) and after 21 days of storage (2 eggs) at room temperature (25±2°C). Results showed that the feed consumption increased ($p<0.05$) during the treatment with zinc bacitracin and the supplementation with 200 mg/kg of OEO, but there was not observed significant difference in the posture percentage for all the treatments. Quality analyses of the stored eggs showed significant difference ($p<0.05$) resulting in a lower eggshell percentage and a higher yolk pH for the treatment with the addition of 50 mg/kg of OEO. It was also obtained a higher yellow intensity in the egg yolk for the control treatment and the supplementation with 200 mg/kg of OEO. The total mesophilic count per egg was higher after 70 days of treatment with the addition of 200 mg/kg of OEO. Regarding the thiobarbituric acid reactive substances (TBARS) in the egg yolks, they were lower ($p<0.05$) for the fresh eggs with addition of 200 mg/kg of OEO and for the stored eggs supplemented with 150 mg/kg of OEO. It was possible to conclude that the supplementation of the ration of the hens with 150 mg/kg of OEO can be useful to the maintenance of the quality and to extend the shelf life of eggs.

Key-words: herbal extract, lipid peroxidation, *Origanum vulgare*.

INTRODUCTION

Eggs are rich in fatty acids and proteins. Generally, in Brazil, eggs are stored outside the refrigerator during the period of commercialization. This practice affects the inner quality of eggs due to the fact that the egg yolks are highly susceptible to lipid oxidation during storage (Hayat et al., 2012). Oxidative stability of eggs is directly related to the composition of laying hens' diet. Dietary supplemented with antioxidants may be a useful alternative to improve egg oxidative stability (Yesilbag et al., 2013). In this respect, the use of natural products such as plant extracts has been considered as valuable alternatives to antibiotic feed additives (Akyurek & Yel, 2011).

Essential oils are natural volatile compounds formed by aromatic plants as secondary metabolites (Bakkali et al., 2008). These compounds provide beneficial effects in food products from animal origin due to the antioxidant potential which result from the hydroxyl groups of their phenolic compounds (Yesilbag et al., 2013). For instance, different combinations of essential oils of oregano, thyme, rosemary and curcuma longa have been applied on experimental diets of laying hens. As a result, they improved the production performance and had positive effects on the oxidative stability of the eggs (Radwan et al., 2008). Among these compounds, the oregano (*Origanum vulgare*) is considered an important source of antimicrobial and antioxidant molecules (Arsapova et al., 2014). A major drawback relies on the fact that there are not many available studiesregarding the addition of pure oregano essential oil (OEO) in the diets of laying hens. Therefore, this study seeks to evaluate the effects of the addition of OEO in experimental diets of laying hens on performance and physical, chemical and microbiological quality of fresh eggs and eggs after 21 days of storage at room temperature.

MATERIAL AND METHODS

Hens and duration of the experiment

There were used 240 commercial semi-heavy laying hens (59 weeks old). Theywere allocated in an experimental shed type Californian equipped with galvanized wire cages. The animals were fed (through feeders) and received water (*nipple* drinking) *ad libitum* during all experimental period. The experiment lasted for 84 days, which were subdivided into three periods of 28 days.

Treatments

The poultries were randomly distributed in a completely randomized design with six treatments (five repetitions with eight birds per cage), totalizing thirty installments. The basal ration (Table 1) was formulated based on nutritional values and in accordance with the requirements established by the Brazilian Poultry and Pork Tables (Rostagno et al, 2011), with the inclusion (2%) of commercial nucleus. The control treatment (CT) consisted of the basal ration with performance improver (30 mg of zinc bacitracin/kg of ration). Meanwhile, five treatments (T0, T50, T100, T150 and T200) were carried out with poultries that received basal ration free of performance improver supplemented with the addition of five levels of OEO (0, 50, 100, 150 and 200 mg/kg), respectively. The OEO was diluted in soy oil and subsequently the mixture was mixed with grounded corn in a vertical mixer (500 kg). The animals were exposed daily to 16 h oflight throughout the experiment.

OEO extraction and characterization

The oregano leaves were purchased from a wholesaler located in São Paulo, Brazil. The OEO was extracted from dehydrated *O. vulgare* through steam distillation methodology, as described by Chemat and Boutekedjiret (2015). The material was placed in an extraction flask and the distillation was kept for a period of 2 hours. It was calculated an average yield of the extraction of 0.8%. The OEO characterization was performed by using a gas chromatograph Varian Star 3400CX (CA, EUA) equipped with a flame ionization detection (GC-FID), as well as the qualitative analyses of the compounds were performed by using a gas chromatograph Shimadzu QP2010 Plus coupled with a mass spectrometer (GC/MS, Shimadzu Corporation, Kyoto, Japan). The analyses revealed the existence of 35 compounds present in the OEO although five of them represent the majority (54.56%) as presented in Table 2.

Performance parameters of the hens

During each period of the experiment, the daily average consumption of feed by hen was monitored (g/bird/day). The daily number of eggs was also registered and the average performance of the hens after each period was estimated (%/day). Feed conversion was evaluated as kg of feed per dozen eggs and kg of feed per kg of eggs. Eggs were weighted in the last three days of each period and the average daily egg production was estimated (g/bird/day). The number of dead hens was used to evaluate the feasibility (%).

Quality parameters of the eggs

To evaluate the quality of the eggs, a sample composed by four eggs of each group studied was collected. Two of these eggs were used immediately after the collect (fresh eggs) and two eggs were stored in a cellulose tray at room temperature (25±2°C) for a period of 21 days. Specific gravity of the eggs was determined according to Freitas et al. (2004). Eggshell strength (kgf) was measured with a texture analyzer (TA.XT plus). Albumen height was measured with a tripod Micrometer. The Haugh units (HU) were calculated from albumen height and egg weight according to the following equation (Haugh, 1937): $HU = 100 \log (H + 7.57 - 1.7 W^{0,37})$, in which H corresponds to the albumen height (mm) and W stands for the egg weight (g).

The yolk index (YI) was estimated with a digital paquimeter as the ratio between the height (mm) and the diameter of the yolk (mm). Yolk color was determined with a colorimetric array (DSM) and colorimeter(Minolta CR-400).There were evaluated the parameters of luminosity (L*), red intensity (a*) and yellow intensity (b*).Yolks were separated from albumen and the eggshells were washed and dried at room temperature for 48 hours. After drying, they were weighted and yolk, albumen and eggshell percentages were obtained. The yolk and albumen pHs were measured with a digital pHmeter (Testo 205).

Lipid peroxidation was determined according to Giampietro et al. (2008) by measuring thiobarbituric acid reactive substances (TBARS) at 532 nm, formed during the decomposition of lipid peroxides using a spectrophotometer. The compound 1,1,3,3 tetramethoxypropane (TMP) was used as a TBARS standard. Results were expressed as mg TMP/kg of yolk.

Eggs were randomly collected in the beginning of the experiment (without storage), and on days 35 and 70 to carry out the microbiological analyses. Each sample was composed by a group of four eggs per repetition. Eggs surfaces were washed with peptone water according to a methodology adapted from Gentry and Quarles (1971). After the serial dilution of the samples, 1 ml of them was inoculated in Petrifilm plates (3M) to evaluate the Mesophilic Aerobic Bacteria, *Escherichia coli* and total coliforms following 3M's guidelines. Incubation was performed at 37º C for 48 hours and bacterial colonies were enumerated with a colony counter following Petrifilm system (3M). Results were expressed as the decimal logarithm of colony forming units by egg.

Statistical analysis

Data were submitted to Analysis of Variance (ANOVA) and Tukey's Test ($p<0.05$) with the software SAS (Statistical Analysis System).

RESULTS

Productive performance

Hens in the control treatment (TC) with zinc bacitracin and feed with 200 mg/kg of OEO (T200) have consumed most of their daily ration in relation to the treatments T0 and T150 (Table 3). It was not observed significant difference between treatments for the other performance parameters (conversion rate/dozen eggs, conversion rate/daily ration consumed, percentage of laying and weight of eggs).

Quality of eggs

The total coliforms count on the eggshells did not show significant difference on days 0, 35 and 70 for all the treatments studied (Table 4). *Escherichia coli*was not found in any treatment. The Mesophilic Aerobic Bacteria from eggshells was higher after 70 days for the T200 treatment in relation to TC, T0 and T50 treatments ($p<0.05$). However, it was not observed significant difference between T200, T100 and T150 (Table 4).

There were not observed significant statistical difference ($p>0.05$) in the following parameters for the fresh eggs after the experiment: specific gravity, Haugh units, yolk index, yolk pH, albumen pH, eggshell strength, yolk color (L*, a* e b*) and yolk, eggshell and albumen percentages (Table 5). However, for the samples stored for 21 days, there occurred statistical differences ($p<0.05$) in the yolk pHs for the T50 treatment in relation to the others (Table 6). In addition, the treatments T0 and T200, the eggshell percentage resulted in higher values relative to T50. Nevertheless, there were not statistical differences between these two treatments and TC, T100 and T50.In what concerns the yolk color, only the yellow intensity (b*) differed for the treatment T100 ($p<0.05$) which resulted in lower values compared to the other treatments (see Table 6).

It was not obtained statistical difference between T200, T50 and T150 for the TBARS of the fresh eggs ($p<0.05$). However, the results obtained after the treatments TC, T0 and T100 were statistically higher for these eggs. For the stored eggs, the T50 and T100 treatments furnished higher results in relation to the others (Table 6).

DISCUSSION

Eggs production was not affected by the different treatments. Actually, it was observed that the addition of OEO in the hens' diets did not affect the production in relation to the control treatment (TC). The only treatment that resulted in an increase of the ration consumption was the T200, similarly in result obtained for quails (Yesilbag et al., 2013). According to Kamel et al. (2001), herbs and extract from different plants have a positive effect on the appetite and digestion of the hens. In addition, they possess antimicrobial properties (Al-Kassie, 2009). Nevertheless, the addition of leaves of oregano in the feed of turkeys resulted in a lower feed intake (Bampidis et al., 2005). A number of factors such as species of the animal, age and levels of OEO can affect the feed intake. For instance, when 150 mg/kg were added to the diet of broiler chickens, the effect was positive. However, 300 mg/kg had a negative effect in the same study (Kirkpinar et al., 2011). It was observed in a study involving a mixture composed by 1.0 and 2.0 g/kg of volatile oils supplemented to the basal diet of cockerels that there was an increase of the feed consumption due to the odor and palatability (Tollba et al., 2010).

Regarding the other performance parameters, it was not obtained statistical difference in this study. Similar results were obtained for laying hens with the addition of 5 g/kg of oregano for 56 days (Botsoglou et al., 2005). According to these authors, these effects can be ascribed to the diet composition, the use of healthy hens, a clean environment and a moderate density of hens. In addition, variations in the effects of the supplementation of OEO can be a result of differences in the composition and of the concentration of components with specific biological activity (Amad et al., 2011).

To evaluate the microbiological quality of the eggshells, the higher results for the Mesophilic Aerobic Count per egg was obtained for the T200 treatment after 70 days. This microbial load may be related to the temperature, environment and contact of the eggs with faces (Englmaierová et al., 2014). In other study that evaluated the influence of OEO on the control of bacteria, the positive effect was ascribed to the presence of carvacrol, the main component in the composition of the oil (Aligiannis et al., 2001). The antibacterial compounds present in the oil act on the cell membrane causing deleterious effects on the cytoplasmic activity and preventing the survival of pathogenic bacteria (Lambert et al., 2001). This effect was not observed in the eggshells in the present study. This behavior suggests that further studies with OEO are

necessary, since that there was not the presence of *E. coli*and the total coliforms count did not diminish after the treatments.

Inner quality of fresh eggs did not change after all the treatments. In what concerns the stored eggs, it was observed an increase in the pH of the yolk for the T50 treatment. This result may be affected by the increase of the lipid peroxidation due to the storage (Botsoglou et al., 1997). The alkaline ions from the albumen migrate and are replaced by hydrogen ions in the yolk, increasing the pH of the yolk and diminishing the pH of the albumen (Shang et al., 2004). The eggshell percentage was reduced for the T50 treatment. This may enable gaseous changes with the environment with a higher loss of carbonic gas to the environment during the storage (Leandro et al., 2006). This result may be ascribed to the influence exerted by the OEO on the metabolic activity of the beneficial bacteria in the intestine. That influence affects the efficiency of the absorption of Calcium (Ca) and Magnesium (Mg) (Bozkurt et al., 2012a). Phenolic compounds, such as carvacrol and thymol, exhibit antibacterial activity and thus, may affect both beneficial and pathogenic bacteria (Fukayma et al., 2005). In another study, it was observed an increase of the weight, thickness and strength of the eggshell due to a higher retention and availability of nutrients in the hens' intestine during the eggshell formation (Bozkurt et al., 2012b). However, this behavior may be age dependent as suggested in the literature (Bozkurt et al., 2016).

Some authors have related changes in the color of egg yolks when the diet was supplemented with rosemary, oregano and saffron. They have suggested that some compounds of the herbs have migrated to the yolk (Botsoglou et al., 2005).The yellow color of the yolks is related to the amount of xanthophyll in the diet and to the antioxidant activity of the pigments, like carotene and xanthophyll that protect the lipids from oxidation (An et al., 2010; Gül et al., 2012).The increase in the feed consumption for eggs stored after the TC and T200 treatments may be related to the values of the yellow intensity in the yolk as a result of the higher availability of carotenes from the diet.

Lipid peroxidation is one of the main causes of spoilage in food products (Olmedo et al., 2014). In the current study, it was observed that lipid peroxidation was reduced in the yolk of fresh eggs for the T200 treatment and in the eggs after 21 day of storage for the T150 treatment. This possible transfer of the antioxidants compounds of the OEO to the yolk resulted in eggs with

higher antioxidant properties involved in the reduction of the amount of malondialdehyde (Botsoglou et al., 2005).

The antioxidant effect has been ascribed to carvacrol and thymol in other study involving oregano and sage leaf oils due to the reduction in the concentration of malondialdehyde in the yolk of stored eggs (Bozkurt et al., 2012b). According to the literature, lipid peroxidation was reduced in eggs stored under refrigeration temperatures, but the reduction was not affected by the duration of the storage. This behavior suggests the transfer of antioxidant compounds of OEO through feeding (Florou-Paneri et al., 2005). In addition, the concentration of malondialdehyde was reduced in yolks of refrigerated eggs stored for 30 days of hens fed with rosemary and oregano (Yesilbag et al., 2013). The influence exerted by the oils on the properties of the eggs may be ascribed to the presence of phenolic compounds that consist in a hydroxyl group acting as hydrogen donators to the peroxide radicals. This action retards the formation of hydrogen peroxides (Farag et al., 1989).

In summary, from the results obtained in the present study, it can be concluded that the OEO exerted an antioxidant effect that reduced the lipid peroxidation in both fresh eggs and eggs stored for 21 days. It was not observed statistical difference between the production performance of the hens. However, for the T200 treatment, there was an increase in the feed consumption of the hens. A global analysis leaded to the conclusion that the supplementation of the ration with 150 mg/kg of OEO may be a useful alternative to maintenance of the quality and to increase the shelf life of the eggs.

Ethics Committee: This study has been approved by the Ethics Committee in Animal Research of the Santa Catarina State University - UDESC. Protocol Number: CEUA 1.39.15.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Table 1 Percentage and calculated composition of the experimental rations used for the treatments of laying hensadopted in the present study.

Ingredients	**Composition (%)**
Corn	63.34
Soybean meal	22.16
Soy oil	1.30
Calcic limestone	11.20
Vitamin and Mineral Core *	2.00
Total	100.00
Calculated composition	
Crude protein (%)	15.00
Metabolic energy(kcal/kg)	2.85
Phosphorus available (%)	0.28
Calcium (%)	4.00
Digestible methionine + cysteine (%)	0.69
Digestible methionine (%)	0.35
Digestible lysine (%)	0.68

* Product composition (kg): folicacid 54,00 mg, nicotinicacid 1.000,00 mg, pantothenicacid 680,00 mg, biotin 2,70 mg, calcium 80,00/160,00 g, cobalt 27,0 mg, cooper 6.000,00 mg, choline 10,00 g, iron 5.000,00 mg, phytase 20,00 ftu, fluorine 650,00 mg, phosphorus 42,00 g, iodine 40,00 mg, manganese 2.500,00 mg, mineral matter 900,00 g, methionine 38,00 g, selenium 10,00 mg, sodium 95,00 g, humidity 120,00 g, vitamin A 374.000,00 UI, vitamin B1 40,00 mg, vitamin B12 1.000,00 mcg, vitamin B2 200,00 mg, vitamin B6 54,00 mg, vitamin D3 75.000,00 UI, vitamin E 1.500,00 UI, vitamin K 100,00 mg andzinc 4.000,00 mg.

Table 2 Composition of the Oregano essential oil (*Origanum vulgare)*.

Components	**Composition (%)**
Sabinene	3.09
β-Myrcene	1.13
α-terpinene	4.99
p-Cymene	3.73
β-Phellandrene	1.11
γ-Terpinene	9.41
trans-Sabinene Hydrate	2.97
Terpinolene	1.67
Linalool	1.32
cis-Sabinene Hydrate	12.38
cis menth-2-en-1-ol	1.40
4-Terpineol	14.05
α-Terpineol	3.31
Carvacrol methylether	1.54
Linalyl acetate	4.18
Thymol	9.54
Carvacrol	9.18
trans-Caryophyllene	3.22
Bicyclogermacrene	1.85
Others*	8.18

*Others: Percentage composition luzir tem 1% (5-Metal-3-exalou, α-thujene, α-pinene, β-pinene, cyclobutanol, α-phellandrene, limonene, α-methyladamantanemethylamine, β-ocymene, trans menth-2-en-1-ol, endo-borneol, cispiperitolacetate, thymolmethylether, gamma-terpinene, α-humulene, spathulenol, caryophyllene oxide).

Table 3 Results obtained for feed consumption (FC, g/hen/day), feed conversion (FCV, kg/dozenand kg/kg) of produced eggs, posture percentage (PP) and egg weight (EW, g/hen/day).

Treatments	FC	FCV (kg/dz)	FCV (kg/kg)	PP	MO
TC	116.0a	1.60	2.03	87.46	57.15
T0	109.2b	1.64	2.08	80.71	53.04
T50	111.6ab	1.55	1.98	86.83	56.57
T100	111.0ab	1.58	1.99	84.80	55.88
T150	108.8b	1.61	2.02	81.04	53.70
T200	114.8a	1.66	2.07	83.77	55.71
p-value	0.0021*	0.7826^{ns}	0.8301^{ns}	0.4946^{ns}	0.4270^{ns}
CV (%)	2.55	8.08	6.58	7.93	6.49

[A, B] Different letters in the same column differ statistically by Tukey Test (5%); Coefficient of Variation (CV). No significant (ns); TC: control treatment with 30 mg of zinc bacitracin; T0: 0 mg/kg of OEO; T50: 50 mg/kg of OEO; T100: 100 mg/kg of OEO; T150: 150 mg/kg of OEO; T200: 200 mg/kg of OEO; OEO: Oregano essential oil; *($p<0.05$).

Table 4 Results obtained for the total coliforms count and the mesophilic aerobic count (CFU/egg) in the eggshell of fresh eggs in days 0, 35 and 70.

	Total coliforms (CFU/egg)			Mesophilic aerobic count (CFU/egg)		
	Days			Days		
	0	35	70	0	35	70
TC	$0.3X10^{1}$	$0.4X10^{1}$	$0.1X10^{1}$	$9.3X10^{3}$	$15.6X10^{3}$	$9.8X10^{3}$b
T0	$0.4X10^{1}$	$0.5X10^{1}$	$0.2X10^{1}$	$6.6X10^{3}$	$29.9X10^{3}$	$8.0X10^{3}$b
T50	$0.4X10^{1}$	$0.9X10^{1}$	$0.2X10^{1}$	$8.3X10^{3}$	$21.6X10^{3}$	$10.4X10^{3}$b
T100	$0.3X10^{1}$	$0.1X10^{1}$	$0.3X10^{1}$	$6.9X10^{3}$	$16.0X10^{3}$	$14.7X10^{3}$ab
T150	$0.2X10^{1}$	$0.3X10^{1}$	$0.4X10^{1}$	$8.1X10^{3}$	$31.7X10^{3}$	$14.8X10^{3}$ab
T200	$0.4X10^{1}$	$0.7X10^{1}$	$0.4X10^{1}$	$10.7X10^{3}$	$25.7X10^{3}$	$22.1X10^{3}$a
p-value	0.9703^{ns}	0.5803^{ns}	0.4774^{ns}	0.3324^{ns}	0.4589^{ns}	0.0099*
CV (%)	115.68	101.62	106.90	32.99	59.25	37.25

[A, B] Different letters in the same column differ statistically by Tukey Test (5%); Coefficient ofVariation (CV). Non significant (NS); Colony forming unities (CFU); TC: control treatment with 30 mg of zinc bacitracin; T0: 0 mg/kg of OEO; T50: 50 mg/kg of OEO; T100: 100 mg/kg of OEO; T150: 150 mg/kg of OEO; T200: 200 mg/kg of OEO; OEO: Oregano essential oil; *($p<0.05$).

Table 5 Results obtained for the following analyses: specific gravity (SG), Haugh unity (HU), yolk index (YI), yolk pH (YpH), albumen pH (ApH), eggshell strength (ES. kgf), yolk percentage (YP), eggshell percentage (EP), albumen percentage (AP), color array (CA), luminosity (L*), red intensity (a*) and yellow intensity (b*) of fresh eggs.

Parameters	TC	T0	T50	T100	T150	T200	p value	CV (%)
SG	1.091	1.093	1.090	1.089	1.091	1.090	0.3547^{ns}	0.22
HU	82.13	84.13	83.78	85.17	81.13	84.97	0.0941^{ns}	2.93
YI	0.469	0.476	0.497	0.480	0.471	0.473	0.7383^{ns}	20.04
YpH	6.03	6.05	6.08	6.02	6.06	6.11	0.3559^{ns}	0.67
ApH	8.51	8.48	8.56	8.50	8.51	8.57	0.9356^{ns}	1.78
ES	4701	4956	4971	4940	5566	5183	0.1096^{ns}	9.13
YP	26.35	26.31	27.19	26.48	26.67	26.61	0.5118^{ns}	2.87
EP	10.22	10.30	10.07	10.06	10.41	10.02	0.2958^{ns}	3.02
AP	63.43	63.42	62.69	63.46	62.91	63.48	0.5718^{ns}	1.37
CA	7.2	7.1	7.1	7.0	6.9	7.1	0.8594^{ns}	5.71
L*	57.40	58.39	59.83	59.10	59.72	59.54	0.1390^{ns}	2.61
a*	-1.50	-1.89	-2.31	-1.95	-1.89	-2.14	0.1646^{ns}	23.96
b*	41.88	42.96	44.69	43.77	43.88	42.91	0.2428^{ns}	4.18
TBA	2.29a	1.98ab	1.69bcd	1.75bc	1.25cd	1.18d	0.0010*	15.62

Coefficient of variation (CV); Non significant (NS).TC: control treatment with 30 mg of zinc bacitracin; T0: 0 mg/kg of OEO; T50: 50 mg/kg of OEO; T100: 100 mg/kg of OEO; T150: 150 mg/kg of OEO; T200: 200 mg/kg of OEO; OEO: Oregano essential oil; TBA: concentration of malondialdehyde (mg TMP/kg yolk) of the yolks; *($p<0.05$).

Table 6 Results obtained for the following analyses: specific gravity (SG), Haugh unity (HU), yolk index (YI), yolk pH (YpH), albumen pH (ApH), eggshell strength (ES. kgf), yolk percentage (YP), eggshell percentage (EP), albumen percentage (AP), color array (CA), luminosity (L*), red intensity (a*) and yellow intensity (b*) in eggs stored for 21 days at room temperature.

Parameters	**TC**	**T0**	**T50**	**T100**	**T150**	**T200**	**p value**	**CV (%)**
SG	1.047	1.047	1.021	1.038	1.046	1.055	0.0743^{ns}	0.94
HU	23.26	34.08	46.94	28.45	26.17	36.53	0.0509^{ns}	36.52
YI	0.399	0.354	0.35	0.33	0.338	0.346	0.5938^{ns}	6.62
YpH	6.12b	6.23b	6.57^{a}	6.11b	6.19b	6.18b	0.0005*	2.34
ApH	9.43	9.41	9.23	9.39	9.37	9.37	0.0605^{ns}	0.37
ES	5115	5063	3499	4406	4519	4999	0.0757^{ns}	19.68
YP	28.92	27.8	29.07	29.13	28.04	28.37	0.6760^{ns}	5.54
EP	9.99ab	10.37a	9.13b	9.77ab	9.85ab	10.18a	0.0204*	5.33
AP	61.09	61.95	61.82	60.92	62.14	61.45	0.8080^{ns}	2.65
CA	6.6	6.9	7.2	6.7	7.3	7.2	0.1765^{ns}	7.22
L*	60.27	58.59	58.43	59.83	62.53	63.19	0.0729^{ns}	4.81
a*	-1.12	-0.48	-0.72	-0.86	-0.69	-0.6	0.7549^{ns}	92.01
b*	57.09a	52.61ab	54.55ab	47.14b	5.47ab	56.55a	0.0203*	8.35
TBA	2.05ab	2.16a	2.13^{a}	1.89ab	1.51b	1.77ab	0.0170*	15.57

[a. b] Different letters in the same column differ statistically by Tukey Test (5%); Coefficient of variation (CV); Non significant (NS). TC: control treatment with 30 mg of zinc bacitracin; T0: 0 mg/kg of OEO; T50: 50 mg/kg of OEO; T100: 100 mg/kg of OEO; T150: 150 mg/kg of OEO; T200: 200 mg/kg of OEO; OEO: Oregano essential oil; TBA: concentration of malondialdehyde (mg TMP/kg yolk) of the yolks; *($p<0.05$).

2.2 MANUSCRITO II

ÓLEO ESSENCIAL DE ORÉGANO (*Origanum vulgare*) NA ALIMENTAÇÃO DE POEDEIRAS E SEUS EFEITOS SOBRE A SAÚDE DAS AVES

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Oregano essential oil (*Origanum vulgare*) in the feeding of laying hens and your effects on animal health

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ABSTRACT

This study evaluated the effect of oregano essential oil added to the feed of commercial laying hens. Based on animal health, the research was focused on the analysis of changes in biochemical parameters linked with hepatic function, and protein and lipid metabolism. There were investigated 240 birds (59 weeks old) distributed in a completely randomized design of six treatments (five repetitions with eight birds). The experiments were constituted by a control treatment (CT) with the inclusion of zinc bacitracin and five treatments (T0, T50, T100, T150 and T200) with the inclusion of different concentrations of oregano essential oil (OEO: 0, 50, 100, 150, and 200 mg/kg), respectively. During the experiment, the seric levels of total proteins, albumin, globulin, triglycerides, cholesterol, uric acid, alanine aminotransferase (ALT) and alkaline phosphatase were analyzed. After 28 days of feeding, an increase on seric levels of total proteins and globulins was observed on groups T150 and T200, as well as an increase on albumin levels on group TC. Afterward 84 days of feeding, a significantly reduction on total proteins and albumin was observed on group T200, as well as an increase on seric triglycerides levels. The addition of 200 mg/kg of oregano essential oil on the feed of commercial laying hens leads to an increase of globulins concentration in 28 days of treatment. The result may be considered an effect on inflammatory response, which increases the seric immunoglobulins and proteins of acute phase.

Key words: albumin, cholesterol, globulin, total proteins, triglycerides.

INTRODUCTION

The search for new alternatives of food supplementation on poultry production in order to improve health, and quantitative and qualitative productivity is considered one of main priorities of poultry industry (Gerzilov *et al.*, 2015). During many years, the use of antibiotics as growth promoters in poultry feeding allowed better results in performance and mortality reduction caused by pathogenic agents due to the inhibition of microorganism growth (Yegani & Korver, 2010). However, the consumption of animal products with antibiotics residues, as well as the indiscriminate use of antibiotics leads to the development of resistant microorganisms, reinforcing the need of an alternative source of supplements (Windisch *et al.*, 2008).

Essential oils are widely accepted by consumers due to its healthy promoting properties. In the field of animal feeding, the natural extract presents potent antimicrobial properties and acts as a growth promoter to be added in poultry feed (Hashemipour *et al.*, 2016). These additives have demonstrated interesting results on bird production and development (Brenes & Roura, 2010) acting directly on control and maintenance of physiology and metabolic functions.

The antioxidant and bactericidal actions of essential oils contribute with animal healthy development. These outstanding properties are linked with the presence of active compounds in their composition that inhibit the growth of some microorganisms (Alipour *et al.*, 2015). The antimicrobial activity of oregano essential oil (OEO) is due to the action of phenolic compounds, such as carvacrol and thymol (Fukayama *et al.*, 2005). These antioxidant substances act on bacterial cell membrane impeding the mitotic division and causing cell dehydration, therefore preventing the survival of avian pathogenic bacteria. A study conducted by Oetting *et al.* (2006) indicated that essential oil possesses antioxidant and antimicrobial activity, acting in the control of pathogenic microorganisms.

Essential oils can act as stimulant agents of the immune system against acute or chronic inflammatory processes that can be characterized by an increase on seric globulin levels (Rosa Neto & Carvalho, 2009), which can express the metabolic and nutritional animal status (Zhu *et al.*, 2014). Moreover, essential oils may improve the digestion and the absorption of nutrients by enzymatic stimulation and exert positive effects when used in laying hens. It is important to emphasize that the response of essential oils as supplements added in the ration is dependent of the level, the composition and the combinations of compounds (Zhang *et al.*, 2005). Based on the assumption that the influence of essential oils on metabolism and health of laying hens remains

little studied, this study intended to evaluate the change in the seric biochemical profile of animals fed with ration supplemented with different concentrations of OEO.

MATERIAL AND METHODS

Animals and local of experiment

The procedure applied in this experimental research used 240 commercial semi-heavy laying hens (59 weeks old), that were allocated in an experimental shed type Californian equipped with galvanized wire cages. The animals were fed (trough feeders) and received water (*nipple* drinking) *ad libitum* during all experimental period, and were exposed to 16 h of light during the experiment.

Experimental design

The poultries were randomly distributed in a completely randomized design with six treatments (five repetitions with eight birds), totalizing thirty installments. The basal ration (Table 1) was formulated based on nutritional values and in accordance with requirements established by the Brazilian Poultry and Pork Tables (Rostagno et al., 2011), with inclusion (2%) of commercial nucleus. The control treatment (CT), consisted in the basal ration with performance improver (30 mg of zinc bacitracin/kg of ration). Meanwhile, five treatments (T0, T50, T100, T150 and T200) with poultries that received basal ration free of performance improver supplemented with the addition of five levels of OEO (0, 50, 100, 150 e 200 mg/kg), respectively. The OEO was diluted in soy oil and subsequently the mixture was mixed with ground corn in a vertical mixer.

OEO extraction and characterization

The OEO was extracted from dehydrated *Origanun vulgare* through steam distillation methodology, descript in details in the literature (Chemat & Boutekedjiret, 2015). The material was placed in an extraction flask and the distillation was kept for a period of 2 hours. It was calculated an average of the extraction yield of 0.8%. The OEO characterization was performed using a gas chromatograph Varian Star 3400CX (CA, EUA) equipped with a flame ionization detection (GC-FID), as well as the qualitative analyses of compounds were performed using a gas chromatograph Shimadzu QP2010 Plus coupled a mass spectrometer (GC/MS, Shimadzu

Corporation, Kyoto, Japan). The analyses revealed the existence of 35 compounds present in the OEO although five of them represent the majority (54.56%) as presented in Figure 1.

Sample collection

The experimental period lasted 84 days, and it was divided in three cycles of 28 days. At the end of each cycle (days 28, 56 and 84) the blood samples (approximately 2 mL) were collected through the branchial vein of two poultries by repetition (totalizing 10 laying hens by treatment), and the samples were allocated in tubes without anticoagulant. After, the blood samples were centrifuged (3500 rpm during 10 min) in order to obtain serum, subsequently stored at -20 ºC until utilization.

Biochemical analyses

The seric levels of total proteins (g/dL), albumin (g/dL), glolubin (g/dL), triglycerides (mg/dL), cholesterol (mg/dL), uric acid (mg/dL), alanine aminotransferase (ALT) (U/L) and alkaline phosphatase (U/L) were evaluated in a semi-automated BioPlus (Bio-200) using commercial kits. Globulins values were calculated based on the total proteins levels subtracted from albumin levels.

Statistical analyzes

At first, the experimental data were ~~firstly~~ analyzed descriptively, and it was calculated the measure of central tendency (mean) and data dispersion (standard deviation). Moreover, all variables were submitted to the Shapiro Wilk test to verify if the data were normally distributed. The data was log transformed to meet the assumptions of normality before the application of analyses of variance (ANOVA). The comparison of the means between groups in each day of observation (days 28, 56 and 84) for all evaluated biochemical variables, followed by Tukey post hoc test ($p<0.05$) was carried out with the statistical software R, v.2.15.2. (R Development Core Team, 2012).

RESULTS AND DISCUSSION

The results showed significant differences on total proteins, albumin, globulin and cholesterols levels for the treatments as presented in Tables 2 and 3), in which the total proteins

levels showed a significant difference on 3 days of collection. A significant increase (p=0.002) on total protein levels was observed on groups T150 and T200 compared with other groups (Table 2) on day 28, while on day 56 only the group T50 showed an increase on total proteins compared with groups CT and T0. On day 84, the animals from group T100 showed an increase on seric total protein levels, while the group T200 showed a reduction in relation to CT group.

Regarding the seric albumin levels, the results of the treatments showed significant differences on days 28 and 84. On day 28 (p=0.03), the poultries of group CT showed higher levels than those of the group T150, while the group T0 showed higher levels than group T100 (p=0.04).Regarding albumin levels (Table 2), the group T200 showed an increase on globulin concentrations compared with groups CT, T0 and T50, as well as the groups T100 and 150 showed an increase compared with groups CT and T50.

The seric triglycerides levels (Table 3) differs for the studied groups only on day 84 (p<0.001), wherein the group T200 showed an increase compared with groups CT, T50 and T100. No differences were observed regarding the cholesterol, uric acid, ALT and alkaline phosphatase levels (p>0.05) (Table 3 and 4).

The seric biochemical analyses of poultries revealed that the higher doses of OEO changes the proteic metabolism increasing the seric levels of globulins, in accordance to results observed by Alp *et al.* (2012). This author detected an increase on globulin levels in broilers with use of 300 mg/kg of OEO on the feed.

Albumin and globulin comprises the most important seric proteins, exerting important physiological functions, such as: transport of substances (hormones, vitamins, minerals and lipids), maintenance of plasmatic osmotic pressure and immunity (Polat *et al.*, 2011). The increase on total proteins levels, as well as on globulin levels in treated poultries with OEO may be attributed to a direct influence on proteic metabolism linked to immune system (Zhu *et al.*, 2014).

According to Moomivand *et al.* (2015), the compounds present in the essential oils can stimulate the proteic synthesis and the immune system, protecting the cells against oxidation. These effects were observed by Tollba *et al.* (2010) using a mixture of 1.0 and 2.0 g/kg of oils (thyme, oregano, cinnamon and piper), with a simultaneous increase on albumin and globulin levels and a reduction on lipids content in chickens. According to these authors, the increase on globulin levels is linked to the immunostimulants effects, important to poultries immunity

(Ghazalah & Ali, 2008), i.e., the augmentation is linked with a possible response to inflammatory process.

The basal seric levels of total proteins in poultries varies from 3.0 to 6.0 mg/dL, and values above 6.0 mg/dL, such as observed in this present study, may occur due to dehydration or increase on total globulins levels, hyperglobulinemia associated with diseases or chronic bacterial infections. However, in female poultries, the concentration of total proteins increases before the oviposition, which may be attributed to estrogen induction in the ovary, raising globulin levels (Hasegawa *et al.*, 2002). In this study, the increase on seric levels of total proteins may be partially explained by possible hormonal effects associated to immunostimulatory effect of OEO, increasing the globulins levels.

Albumin is a protein responsible by the transport of fatty acids, minerals, uric acid, vitamins and hormones (Maciel *et al.*, 2007). According to Traesel *et al.* (2011b), the reduction of seric total proteins (albumin and globulin) associated to increase on seric triglycerides levels suggests hepatic insufficiency (but not hepatic disease), for the reason that albumin synthesis occurs only in the liver.

The increase on seric triglycerides levels on day 84 was observed in the poultries that received 200 mg of OEO/kg of ration, similarly to results observed by Bolukbasi *et al.* (2006) in broilers supplemented with thyme oil. According to these authors, it occurs due to an increase on metabolism of proteins, fatty and carbohydrate, associated with an increase on triglycerides concentration (Sirvydis *et al.*, 2003). According to Traesel *et al.* (2011a), the essential oils can produce some toxic effects when administered in higher doses. Thus, it is necessary to define safety levels in order to avoid poultries health damage.

In a study conducted by Toghyani *et al.* (2016), it was observed an increase on seric triglycerides levels in poultries fed *ad libitum*. This occurs due to an increase on hepatic very low-density lipoprotein (VLDL) in serum, the main triglyceride transporter. It may be considered an indicator of changes in hepatic secretion of VLDL and hepatic lipogeneses (synthesis of triglycerides and fatty acids). Other explication may be linked with the catabolism of fatty acids and regulation of energetic metabolism that affects directly the physiological responses.

According to Melo *et al.* (2016), the addition of new elements to a ration may cause an increase on mobilization of triglycerides from tissues to circulation. Concentrations of triglycerides and cholesterol in poultries that received ration with natural additives may be linked

with antioxidant and antibacterial properties (Gálik *et al.,* 2015). Some factors contribute for these results, such as age, sex, type of poultries, nutritional and physiological status, as well as the differences on composition of compounds (Moomivand *et al.,* 2015; Akbari & Mehran, 2014). However, this increase on triglycerides levels on poultries is not considered a negative effect on bird health.

Bampidis *et al.* (2005) observed the absence of differences regarding plasmatic cholesterol levels in turkeys fed with dried oregano leaves, similarly to results observed in this study. Moreover, in a diet for broilers containing carvacrol and thymol (Lee *et al.*, 2003) and in a diet for laying hens containing a mixture of essential oils (Bozkurt *et al.,* 2012) no difference was observed on cholesterols levels. This non-significant effect may be associated to OEO components that were ineffective in the inhibition of the enzyme 3-hidroxi-3-methyl-glutaril-CoA reductase (HMG-CoA reductase), which is a limitant on cholesterol synthesis (Khattak *et al.,* 2014).

Study conducted by Basmacioglu-Malayoglu *et al.* (2010) related that thymol and carvacrol may exhibit hypocholesterolemic effects through inhibition of HMG-CoA reductase. Moreover, a reduction on lipid content and total cholesterol levels in a diet containing rosemary oil for broiler can be attributed to thymol and carvacrol compounds (Polat *et al.,* 2011). However, despite the presence of thymol and carvacrol on OEO used in this work, none inhibition was verified on seric triglycerides levels. Probably, the absence of inhibition is associated with the quantities of OEO applied in this study.

No differences regarding seric uric acid levels, as well as on ALT and alkaline phosphatase levels were observed for the studied groups. According to Zhu *et al.* (2014), hepatic damages or increase on permeability of hepatic cells increase the seric ALT levels. This increase was not observed in the present study. The alkaline phosphatase activity produced by many organs and tissues, such as the liver, is an important marker of bony metabolism, and the age of poultry may increase or decrease its activity (Zhu *et al.,* 2014). The activity of alkaline phosphatase activity did not differ for the investigated treatments, therefore, the applied treatment did not cause hepatic injury.

Based on the results, it is noted that the use of 200 mg of OEO/kg of ration leads to an increase on seric globulin levels on day 28. The observation may be considered an immunostimulatory effect of OEO. For further days of treatment, the use of OEO increases the

seric triglycerides levels. In summary, it is concluded that treatment with OEO exerts beneficial effects on bird health, and not possesses toxic effects on tested doses.

Ethics Committee: This project was approved by Ethics Committee on animal research of Santa Catarina State University (UDESC), under protocol number 1.39.15.

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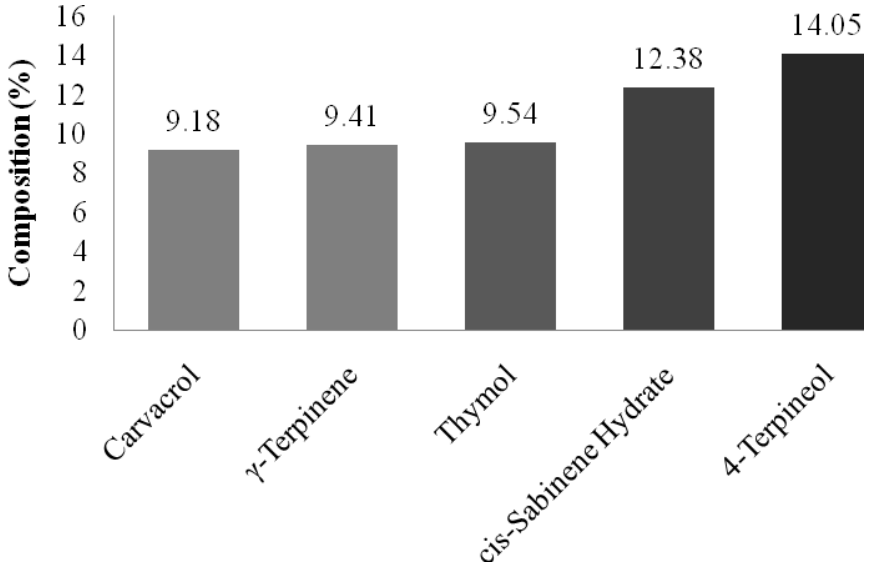

Figure 1. Composition of *Origanum vulgare* essential oil (oregano) obtained by gas chromatograph.

Table 1: Percentual and calculated composition of experimental ration used to the treatment of laying hens.

Ingredients	**Composition (%)**
Corn	63.34
Soybean meal	22.16
Soy oil	1.30
Calcitic limestone	11.20
Vitamin and Mineral Core *	2.00
Total	100
Calculated composition	
Crude protein (%)	15.00
Metabolic energy(kcal/kg)	2.85
Phosphorus available (%)	0.28
Calcium (%)	4.00
Digestible methionine + cysteine (%)	0.69
Digestible methionine (%)	0.35
Digestible lysine (%)	0.68

* Product composition (kg): folic acid 54,00 mg, nicotinic acid 1.000,00 mg, pantothenic acid 680,00 mg, biotin 2,70 mg, calcium 80,00/160,00 g, cobalt 27,0 mg, cooper 6.000,00 mg, choline 10,00 g, iron 5.000,00 mg, phytase 20,00 ftu, fluorine 650,00 mg, phosphorus 42,00 g, iodine 40,00 mg, manganese 2.500,00 mg, mineral matter 900,00 g, methionine 38,00 g, selenium 10,00 mg, sodium 95,00 g, humidity 120,00 g, vitamin A 374.000,00 UI, vitamin B1 40,00 mg, vitamin B12 1.000,00 mcg, vitamin B2 200,00 mg, vitamin B6 54,00 mg, vitamin D3 75.000,00 UI, vitamin E 1.500,00 UI, vitamin K 100,00 mg and zinc 4.000,00 mg.

Table 2: Seric levels of total proteins, albumin and globulin on days 28, 56 and 84 post-treatment.

Variables		**Mean ± DP**						**P value**
		TC	T0	T50	T100	T150	T200	
Total proteins (g/dl)	Day 28	7.18b (±0.77)	7.52b (±1.43)	6.98b (±0.61)	7.78b (±1.63)	10.78a (±3.64)	11.32a (±2.89)	0.002*
	Day 56	6.22b (±0.53)	6.38b (±0.78)	7.68a (±0.92)	7.17ab (±0.89)	7.02ab (±0.25)	7.07ab (±0.86)	0.020*
	Day 84	6.33b (±0.63)	6.60b (±0.64)	5.35b (±0.34)	6.67a (±1.33)	5.65b (±0.32)	5.30c (±0.60)	0.003*
Albumin (g/dl)	Day 28	2.70a (±0.43)	2.35ab (±0.87)	1.97ab (±0.14)	2.02ab (±0.18)	1.82b (±0.38)	2.05ab (0.45)	0.020*
	Day 56	1.92 (±0.54)	1.90 (±0.51)	2.13 (±0.24)	1.62 (±0.59)	2.18 (±0.50)	2.15 (±0.14)	0.250^{ns}
	Day 84	1.87ab (±0.10)	2.07a (±0.12)	1.87ab (±0.36)	1.65b (±0.28)	1.73ab (±0.16)	1.87ab (±0.12)	0.040*
Globulin (g/dl)	Day 28	4.32c (±0.51)	5.17b (±1.54)	5.02c (±0.69)	5.77ab (±1.70)	8.97ab (±3.72)	9.27a (±3.26)	0.001*
	Day 56	4.30 (±0.99)	4.48 (±1.14)	5.55 (±1.04)	5.55 (±1.16)	4.83 (±0.54)	4.92 (±0.79)	0.150^{ns}
	Day 84	4.47 (±0.59)	4.53 (±0.67)	3.48 (±0.65)	5.02 (±1.37)	3.92 (±0.34)	3.43 (±0.55)	0.050^{ns}

[a, b, c] Different letters in the same line differs statistically between groups by Tukey post hoc test (5%). Standard deviation (DP), non-significative (NS); Group CT: control treated with 30 mg of zinc bacitracin; Group T0: 0% of oregano essential oil (OEO); group T50: 50 mg/kg of OEO; group T100: 100 mg/kg of OEO; Group T150: 150 mg/kg of OEO; Group T200: 200 mg/kg of OEO. * $p<0.05$.

Table 3: Seric levels of triglycerides, cholesterols and uric acid (mg/dL) on days 28, 56 and 84 post-treatment.

Variable		Mean ± DP						P value
		TC	T0	T50	T100	T150	T200	
Triglycerides (mg/dl)	Day 28	998.33 (±158.16)	1089.83 (±188.94)	992 (±118.98)	1069.33 (±220.44)	1049 (±56.85)	995.83 (±237.84)	0.870^{ns}
	Day 56	879.17 (±341.68)	878.00 (±210.65)	916.83 (±171.15)	723.17 (±320.22)	874.17 (±153.93)	834.17 (±183.20)	0.790^{ns}
	Day 84	986.17b (±151.42)	1097.67ab (±308.19)	708.17c (±134.56)	684.67c (±119.55)	987ab (±101.89)	1340.83a (±236.47)	0.001*
Cholesterol (mg/dl)	Day 28	97.33 (±14.15)	103.50 (±34.55)	96.83 (±31.75)	102.67 (±29.99)	96.83 (±24.70)	103.33 (±29.62)	0.990^{ns}
	Day 56	89.50 (±21.58)	82.17 (±19.36)	115.67 (±43.29)	102.50 (±40.27)	119.17 (±36.41)	113.83 (±42.07)	0.370^{ns}
	Day 84	85.33 (±35.56)	100.50 (±35.57)	115.17 (±36.50)	116.67 (±36.52)	92.50 (±19.46)	80.00 (±20.67)	0.440^{ns}
Uric acid (mg/dl)	Day 28	5.53 (±1.30)	5.88 (±1.48)	4.93 (±1.01)	4.18 (±1.78)	6.00 (±1.84)	5.38 (±1.11)	0.290^{ns}
	Day 56	5.67 (±1.60)	4.22 (±1.25)	4.97 (±0.96)	5.73 (±1.70)	5.70 (±2.15)	3.85 (±1.35)	0.160^{ns}
	Day 84	8.93 (±2.99)	6.82 (±1.95)	6.87 (±2.21)	7.20 (±1.40)	8.03 (±2.01)	7.18 (±1.61)	0.470^{ns}

[a, b, c] Different letters in the same line differs statistically between groups by Tukey post hoc test (5%). Standard deviation (DP), non-significative (NS); Group CT: control treated with 30 mg of zinc bacitracin; Group T0: 0% of oregano essential oil (OEO); group T50: 50 mg/kg of OEO; group T100: 100 mg/kg of OEO; Group T150: 150 mg/kg of OEO; Group T200: 200 mg/kg of OEO. * $p<0.05$.

Table 4: Seric levels of alanine aminotransferase (ALT) and alkaline phosphatase (U/L) on days 28, 56 and 84 post-treatment.

Variable		Mean ± DP						P value
		TC	T0	T50	T100	T150	T200	
ALT(U/L)	Day 28	13.73 (±4.96)	12.33 (±5.82)	14.67 (±6.31)	12.33 (±3.61)	16.17 (±10.85)	13.83 (±7.68)	0.190^{ns}
	Day 56	11.83 (±2.56)	10.50 (±0.84)	9.33 (±3.20)	10.17 (±4.12)	8.50 (±3.56)	8.00 (±3.63)	0.340^{ns}
	Day 84	8.50 (±3.62)	8.50 (±2.66)	8.33 (±5.32)	8.50 (±3.33)	8.17 (±2.56)	7.83 (±2.71)	0.990^{ns}
Alkaline phosphatase (U/L)	Day 28	178.50 (±70.94)	255.00 (±79.63)	248.50 (±98.32)	215.00 (±115.49)	247.50 (±115.89)	213.83 (±34.45)	0.670^{ns}
	Day 56	193.17 (±72.73)	142.00 (±25.50)	124.00 (±53.58)	179.50 (±78.31)	187.33 (±68.51)	170.00 (±56.75)	0.330^{ns}
	Day 84	180.50 (±47.09)	142.00 (±44.69)	165.33 (±71.60)	175.17 (±56.87)	199.83 (±78.01)	203.67 (±78.90)	0.590^{ns}

Standard deviation (DP), non-significative (NS); Group CT: control treated with 30 mg of zinc bacitracin; Group T0: 0% of oregano essential oil (OEO); group T50: 50 mg/kg of OEO; group T100: 100 mg/kg of OEO; Group T150: 150 mg/kg of OEO; Group T200: 200 mg/kg of OEO. * $p<0.05$.

3 CONSIDERAÇOES FINAIS

O óleo essencial de orégano pode ser utilizado na alimentação de galinhas poedeiras sem efeitos negativos sobre o desempenho produtivo das aves substituindo o antibiótico. Na qualidade de ovos, sua utilização provou ser eficiente em retardar a peroxidação lipídica da gema de ovos frescos e armazenados por 21 dias em temperatura ambiente, aumentando assim sua viabilidade e tempo de prateleira. O efeito do óleo essencial de orégano na alimentação das aves apresentou efeito sobre o perfil bioquímico com aumento nos níveis séricos de globulina nas aves, com possível resposta imune estimulante, além disso, não foi tóxico para as aves e, portanto tem efeito benéfico na saúde das aves.

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Cetea UDESC
Comitê de Ética em Experimentação
Centro de Ciências Agroveterinárias

CARTA DE APROVAÇÃO

O Comitê de Ética em Experimentação Animal da UDESC analisou o(s) projeto(s):

Protocolo: 1.39.15
Título: Uso do óleo essêncial de orégano na alimentação de podeiras comerciais e seus efeitos sobre o desempenho e qualidade dos ovos.
Coordenador/Pesquisador: Marcel Manente Boiago

O Comitê de Ética em Experimentação Animal (CETEA) APROVOU o(s) projeto(s) acima relacionado(s) em seus aspectos éticos e metodológicos, para utilização de animais em pesquisa, de acordo com as diretrizes e normas nacionais e internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008 que disciplina a criação e utilização de animais em atividades de ensino e pesquisa no Brasil.

Lages, 02 de outubro de 2015.

Prof. Ubirajara Maciel da Costa
Coordenador do CETEA/UDESC