UNIVERSIDADE ESTADUAL PAULISTA – UNESP CÂMPUS DE JABOTICABAL

EFEITO SOBRE O GANHO DE PESO DIÁRIO E IMPACTO ECONÔMICO DAS LESÕES PROVOCADAS PELO *Mycoplasma hyopneumoniae* EM SUÍNOS DE TERMINAÇÃO

Maria Eugênia Silveira Ferraz

Médica Veterinária

2020

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Orientador: Prof. Dr. Luís Guilherme de Oliveira

Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Medicina Veterinária, área: Clínica Médica Veterinária.

2020

F381e

Ferraz, Maria Eugênia Silveira

Efeito no ganho de peso diário e impacto econômico das lesões provocadas pelo Mycoplasma hyopneumoniae / Maria Eugênia Silveira Ferraz. -- Jaboticabal, 2020

54 p. : tabs., fotos

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientador: Luís Guilherme de Oliveira

1. Abate. 2. Ganho de peso diário (GPD). 3. Perdas econômicas. 4. Pneumonia enzootica dos suínos. 5. qPCR. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

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TÍTULO DA DISSERTAÇÃO: EFEITO SOBRE O GANHO DE PESO DIÁRIO E IMPACTO ECONÔMICO DAS LESÕES PROVOCADAS PELO *Mycoplasma hyopneumoniae* EM SUÍNOS DE TERMINAÇÃO

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Jaboticabal, 13 de março de 2020

DADOS CURRICULARES DO AUTOR

MARIA EUGÊNIA SILVEIRA FERRAZ – Nascida em 5 de Setembro de 1992, na cidade de Patrocínio – Minas Gerais, Brasil, é Médica Veterinária formada pela Universidade Federal de Uberlândia (UFU) em 2016. Foi membro da Empresa Junior CONAVET da Faculdade Medicina Veterinária e Zootecnia da UFU, participando da organização de eventos e programas de extensão rural. Experiência em granjas de suínos por meio de estágios realizados desde 2015. Trabalho de Conclusão de Curso realizado na área de Reprodução de suínos, com o desenvolvimento de um experimento para analisar a viabilidade do toque em fêmeas suínas. E estágio supervisionado realizado na área de Nutrição de suínos, com a condução de um experimento testando ingredientes para fêmeas em lactação. Ingressou em Março de 2018 no Programa de Pós Graduação em Medicina Veterinária, área de Clínica Médica, na FCAV – UNESP Jaboticabal, como bolsista CNPq e partir de Setembro de 2018 como bolsista FAPESP, sob a orientação do Prof. Dr. Luís Guilherme de Oliveira.

“Fazer da interrupção um caminho novo, fazer da queda um passo de dança, do medo uma escada, do sono uma ponte, da procura um encontro.”

Fernando Sabino

AGRADECIMENTOS

Agradeço primeiramente à Deus e Nossa Senhora, por me confortarem e me guiarem na fé, me levando a percorrer os caminhos necessários ao meu crescimento e dando forças para concluir mais essa fase.

Mãe, mais uma vitória que eu dedico a você, sem você NADA seria possível. Obrigada por todas as batalhas que enfrentou por mim, e por ter me feito acreditar na minha capacidade de realizar com êxito tudo aquilo que me proponho.

Ao meu pai, obrigada por confiar em mim e nos meus objetivos, seu apoio foi fundamental nessa jornada.

À minha avó Catarina, que me faz sentir o amor mais puro e verdadeiro, e que nunca mediu esforços para que eu me sentisse amada e cuidada.

Ao Marcelo, que é hoje a certeza de que Deus preparou com muito carinho o meu mestrado em Jaboticabal, obrigada por tudo.

Ao Prof. Luís Guilherme, agradeço pela oportunidade de trabalhar com o que amo, pela confiança, e por ter me ensinado muito, profissionalmente e pessoalmente.

Agradeço imensamente aos colegas que se dispuseram a enfrentar esse desafio comigo pessoalmente, foi um trabalho árduo, realizado à inúmeras mãos, e todas foram essenciais. Especialmente ao Mauro e Henrique, que não mediram esforços para me ajudar e me socorrer nas muitas horas que precisei. Vocês foram demais!

Aos colegas do Laboratório de Pesquisa em Suínos: Henrique, Marina, Marcela, Karina, Gabriel, tenham a certeza de que todos vocês foram importantes para mim em algum momento nesses dois anos, obrigada pela paciência na convivência e por todos os ensinamentos.

À República Mete Marcha pela convivência diária, companheirismo e amizade. Vocês me ensinaram sobre lealdade e tolerância, e foram meu ponto de apoio nos dias cansativos e de estresse.

À Ceva Saúde Animal, pelo financiamento do projeto e por ter confiado na minha capacidade de conduzi – lo.

À PifPaf Alimentos e Granjas Vecchi, por abrirem as portas para que realizássemos as coletas, e por toda ajuda.

Aos colaboradores da Iowa State University, Daniel e César, o conhecimento de vocês foi imprescindível para o sucesso do trabalho.

Aos animais, que são a razão e a emoção disso tudo!

À Faculdade de Ciências Agrárias e Veterinárias, Câmpus de Jaboticabal pela estrutura necessária e fundamental para a realização do trabalho.

Ao Conselho Nacional de Desenvolvimento Científico – CNPq, através do programa de de Bolsas de Pós – Graduação, pelo auxílio nos primeiros meses de mestrado.

Agradeço à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), pelo apoio imprescindível para a realização do mestrado (projeto nº2018/09473-2).

SUMÁRIO

CERTIFICADO COM ISSÃO DE ÉTICA PARA EXPERIMENTAÇÃO ANIMAL

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Impacto produtivo e econômico provocado pelas consolidações pulmonares causadas pelo *Mycoplasma hyopneumoniae* em suínos na fase de terminação"**, protocolo nº 005168/18, sob a responsabilidade do Prof. Dr. Luís Guilherme de Oliveira, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 19 de abril de 2018.

Vigência do Projeto	02/05/2018 a 31/01/2020
Espécie / Linhagem	Suíno
Nº de animais	800
Peso / Idade	23 a 100 kg / 65 a 150 dias
Sexo	Ambos os sexos
Origem	Granjas comercias – SP

Jaboticabal, 19 de abril de 2018.

Fabiana Pilarski

Profª Drª Fabiana Pilarski
Coordenadora – CEUA

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EFEITO NO GANHO DE PESO DIÁRIO E IMPACTO ECONÔMICO DAS CONSOLIDAÇÕES PULMONARES CAUSADAS PELO *Mycoplasma hyopneumoniae* EM SUÍNOS DE TERMINAÇÃO

RESUMO - O objetivo deste estudo foi medir o impacto na produtividade e as consequentes perdas econômicas relacionadas a lesões pulmonares causadas por *Mycoplasma hyopneumoniae*. Quinhentos suínos de terminação, foram selecionados e pesados no início (75 dias) e no final da fase (180 dias) para avaliar o ganho médio diário (GPD). Esses animais foram avaliados no abate e as amostras coletadas para análise laboratorial, para confirmar a presença de DNA e/ou anticorpos do *Mycoplasma hyopneumoniae*. Os pulmões de cada suíno foram examinados e, com base na gravidade das lesões macroscópicas, os animais foram classificados em quatro grupos: Grupo 1 - animais sem lesões macroscópicas; Grupo 2 - 0,1 - 5% de lesões; Grupo 3 - 5,1 - 15% de lesões; Grupo 4 - >15,1% de lesões. Quatrocentos e oitenta e seis pulmões foram examinados e 68,5% (n=333) apresentaram lesões pulmonares macroscópicas. Todos os animais com lesões foram positivos para *Mycoplasma hyopneumoniae* no ELISA e no qPCR. Modelos de regressão linear (proc Glimmix) foram realizados no SAS para estimar o efeito do escore das lesões macroscópicas pulmonares no ganho de peso diário dos suínos em terminação. Múltiplas comparações entre os grupos de escores de lesões foram realizadas usando $p < 0,05$. Para cada aumento de 1% na área de lesão houve uma diminuição de 1,8 gramas no ganho de peso diário. Todos os grupos apresentaram ganho de peso diário numericamente menor quando comparados ao grupo 1 (sem lesões). O grupo 1 apresentou peso médio numericamente maior, mas não significativo ($p=0,2390$ e $p=0,2442$), quando comparado aos grupos 2 e 3. No entanto, ao comparar os grupos 4 e 1, o último apresentou peso significativamente maior ($p=0,0076$). O grupo 4 também apresentou médias de peso significativamente mais baixas quando comparado aos grupos 2 e 3 ($p=0,0370$ e $p=0,0673$). A correlação negativa encontrada entre o grupo sem lesões pulmonares e o grupo com mais de 15,1% das lesões mostrou uma diferença estatisticamente significante no GPD, o que poderia significar uma oportunidade de ganhar até US $ 6,55 por animal no momento do abate, demonstrando as perdas que a presença das lesões podem provocar no sistema de produção.

Palavras-chave: abate, GPD, perdas econômicas, pneumonia enzoótica, qPCR

EFFECT ON AVERAGE DAILY GAIN AND ECONOMICAL IMPACT OF LUNG CONSOLIDATION CAUSED BY *Mycoplasma hyopneumoniae* IN FINISHING PIGS

ABSTRACT - The aim of this study was to measure the impact of productivity and the consequent economical losses related to pulmonary lesions caused by *Mycoplasma hyopneumoniae*. Five-hundred finishing pigs, with approximately 75 days of age, were selected and weighed at the beggining and at the end of the phase to assess the average daily gain (ADG). These animals were evaluated at the slaughter, and samples were collected for laboratorial analysis to confirm presence of *Mycoplasma hyopneumoniae* DNA and/or antibodies. The lungs of each pig were examined and based on severity of macroscopic lung lesions the animals were classified into four groups: Group 1 – pigs without macroscopic lesions; Group 2 – 0.1-5% of lesions; Group 3 – 5.1-15% of lesions; Group 4 - >15.1% of lesions. Four-hundred eighty-six lungs were examined and 68.5% (n=333) had macroscopic lung lesions. All pigs with lesions were positive to *Mycoplasma hyopneumoniae* in ELISA and qPCR. Linear mixed regression models (proc Glimmix) were performed on SAS to estimate the effect of macroscopic lung lesion scores on the average daily gain of finishing pigs. Multiple comparisons between lesion score groups were performed using $p<0.05$. For each increase of one percent in the lesion area there was a decrease of 1.8 grams in the daily weight gain. All the groups had a numerically lower average daily gain when compared to Group 1 (no lesions). Group 1 had a numerically higher average weight, but not significant ($p=0.2390$ and $p=0.2442$), when compared to groups 2 and 3. However, when comparing groups 4 and 1, the last presented a significantly higher weight ($p=0.0076$). Group 4 also had a significantly lower weight averages when compared with groups 2 and 3 ($p=0.0370$ and $p=0.0673$). The negative correlation found between the group with no lung lesions and the group with more than 15.1% of lesions, showed a statistically difference in ADG, which could mean an opportunity to gain up to $ 6.55 per pig at slaughter.

Keywords: slaughter, ADG, economical losses, enzootic pneumonia, qPCR

LISTA DE ABREVIATURAS

°C – graus Celsius
Cq – Quantificação de ciclos
PCR: Reação em cadeia da polimerase
dNTP – Desoxirribonucleotídeos fosfatados
DNA- Ácido desoxirribonucleico
EUA – Estados Unidos da América
ELISA – Ensaio imunoenzimático
pH – Potencial hidrogeniônico
Kg- kilograma
mg- miligrama
µL- microlitro
mL – mililitro
mM – milimolar
%: Porcentagem
pb: Pares de base
qPCR – Reação em Cadeia pela polimerase quantitativa
Gapdh – gliceraldeído-3-fosfato desidrogenase
IC – Intervalo de confiança
BALT – Tecido linfoide associado aos brônquios

LISTA DE TABELAS

LISTA DE GRÁFICOS E FIGURAS

CAPÍTULO 1 – Considerações gerais

1. Introdução

Mycoplasma hyopneumoniae é uma das bactérias mais prevalentes em infecções respiratórias de suínos (Takeuti et al., 2017), sendo apontada como o principal agente causador da pneumonia enzoótica suína (PES), enfermidade de caráter crônico, caracterizada por tosse seca não produtiva, queda no ganho de peso diário dos animais, alta morbidade e baixa mortalidade (Vranckx et al., 2012; Sibila et al., 2009).

As lesões de pneumonia associadas a infecção pelo agente são as mais comumente detectadas em abatedouros de suínos, estimando-se uma prevalência mundial entre 19% a 79% (Fablet et al., 2012). Essas lesões são consideradas um dos mais importantes problemas sanitários e de bem - estar nas criações intensivas de suínos em todo o mundo (Cobanovic et al., 2016).

Apesar de não serem patognomônicas de infecções por *Mycoplasma hyopneumoniae (M.hyopneumoniae)*, lesões broncopneumônicas sugestivas de PES são segundo Merialdi et al. (2012), positivamente associadas a rebanhos soropositivos. Em estudo realizado na França, em que 125 rebanhos e 3731 pulmões foram analisados, 69,3% das lesões macroscópicas encontradas estavam associadas a PES.

A presença das lesões, também interfere no desempenho produtivo dos animais, Regula et al. (2000) observaram que ocorria uma diminuição no GPD dos animais de acordo com a severidade das lesões pulmonares, sendo que a cada 1% de aumento de lesões de consolidação, havia uma diminuição de 6,8 g/dia de ganho de peso. Outros autores também relatam uma diminuição no peso dos animais associada a severidade das lesões, além da diminuição na qualidade da carne, em razão de alterações no pH, na coloração e na retenção de água (Karabasil et al., 2017, Henninger et al., 2014). Portanto, as lesões podem provocar perdas econômicas para os produtores, pelo aumento com gasto com medicamentos,

controle e prevenção da doença, menor peso ao abate, ou maior tempo necessário para alcançar o peso de abate (Dykhuis et al., 2012).

Sabendo-se que o *M. hyopneumoniae* é mundialmente disseminado e um problema recorrente em rebanhos suínos e que lesões pulmonares são responsáveis por diminuição do desempenho dos animais com consequente queda na produtividade, o objetivo do estudo foi mensurar o impacto no GPD e consequentemente econômico, das lesões de consolidação pulmonar em suínos em terminação, associando a participação do agente nas perdas decorrentes dessas lesões.

2. Revisão de literatura

Mycoplasma hyopneumoniae (M. hyopneumoniae) é o agente primário da PES, doença respiratória crônica e altamente prevalente em grande parte das áreas produtoras, provocando grandes perdas econômicas devido ao alto gasto com medicações e à queda no desenvolvimento dos animais. Este agente também é considerado o agente primário envolvido no complexo de doenças respiratórias dos suínos e na maioria das vezes está associado a agentes secundários, como *Pasteurella multocida, Actinobacillus pleuropneumoniae, Glaserella parasuis* (Thacker, 2012).

Assim como outros micoplasmas, tem um genoma pequeno, não possui parede celular e é pleomórfico. O genoma pequeno faz com o agente tenha uma reduzida capacidade codificadora, além da presença de vias metabólicas insuficientes (Siqueira et al., 2014). Essas características refletem em um requerimento nutricional complexo, dificultando o isolamento do microorganismo, por possuírem um crescimento mais lento que outros micoplasmas suínos (Thacker et al., 2004).

Até o momento os únicos hospedeiros considerados susceptíveis ao *M. hyopneumoniae* são os suínos domésticos e os javalis, sendo que nos suínos a apresentação clínica da doença ocorre principalmente nas fases de crescimento e terminação (Pieters et al., 2019).

A patogenia acontece por meio da aderência do agente ao longo epitélio ciliar do trato respiratório dos animais por meio de adesinas, provocando ciliostase e consequente perda de cílios. Esses fatores contribuem para que ocorra uma diminuição da eficiência mucociliar e imunossupressão, predispondo os animais às infecções secundárias por outros patógenos (Pieters et al., 2019). As infecções por *M. hyopneumoniae* são clinicamente caracterizadas por tosse seca e não produtiva intermitente de intensidade variada, podendo permanecer no plantel por semanas ou meses (Sibila et al., 2009). A capacidade de modular a resposta imune do hospedeiro resulta na ineficiência da resposta inflamatória em eliminar o agente permitindo a persistência da infecção por longos períodos. Em animais

experimentalmente infectados, *M. hyopneumoniae* foi detectado até 214 dias pós-infecção (Pieters et al., 2009).

A principal forma de transmissão é o contato entre animais infectados e animais susceptíveis, os leitões ao nascimento são considerados livres da doença, não havendo evidências de transmissão vertical (Nathues et al., 2013).

Acredita-se que no período de lactação ocorra grande parte das infecções por parte de fêmeas lactantes infectadas, sendo que a duração da lactação pode ser considerado um fator de risco para colonização do *M. hyopneumoniae* em leitões (Pieters et al., 2014). A colonização pós desmame, acontece principalmente nos sistemas de produção em múltiplos – sítios, em que ocorre a mistura de lotes na creche juntamente à queda de imunidade dos animais, este tipo de infecção é responsável pela severidade da doença na fase de terminação (Sibila et al., 2007).

Em condições experimentais, na qual animais soronegativos são desafiados com altas doses infectantes de *M. hyopneumoniae*, a tosse e as lesões pulmonares podem surgir entre 7 e 14 dias pós infecção (Blanchard et al., 1992 Lorenzo et al., 2006) atingindo o máximo de severidade e extensão por volta do 28º dia pós-infecção (Villarreal et al., 2011).

Sob condições naturais, o período de incubação da doença depende de fatores como o tipo de sistema de produção, infecções concomitantes, estado imunológico do animal, virulência da cepa e pressão de infecção. Alguns fatores de risco são considerados importantes na dinâmica e gravidade da PES, como o tipo de alojamento, sistemas de ventilação, densidade de animais e clima (Giacomini et al., 2016, Vranckx et al., 2012). Em granjas clinicamente afetadas, os sinais clínicos e a soroconversão ocorrem aproximadamente de 1 a 6 semanas pós infecção (Maes et al., 2018).

As lesões provocadas pela PES estão localizadas na região cranioventral do pulmão, principalmente nas extremidades dos lobos apicais, cardíacos, intermediários e região cranioventral dos diafragmáticos. Essas lesões são bem delimitadas do tecido normal, possuem consistência de músculo e coloração de púrpura à cinza, e são levemente deprimidas em relação às áreas de pulmão normal. Em quadros respiratórios são recorrentes associações de múltiplos agentes

gerando um efeito sinérgico e produzindo lesões mais severas do que aquelas causadas por um único agente (Thacker et al., 2012).

Entre os fatores que contribuem para a severidade das lesões, estão a virulência da cepa e a capacidade do agente de modular o sistema imune do agente (Meyns et al., 2011). Estudos identificaram associações significativas entre anticorpos de mucosa e séricos com a presença e tamanho de lesões pulmonares (Fraile et al., 2010, Garcia-Morante et al., 2016), indicando que possivelmente a reação inflamatória e imune do hospedeiro estaria envolvida, ou pelo menos correlacionada à extensão e severidade das lesões pneumônicas. Entretanto, a patogenia da PES não está completamente clara, e o verdadeiro peso de cada um dos fatores na formação de lesão ainda é incerto e dificulta o controle da enfermidade em rebanhos suínos (Maes et al., 2018).

Microscopicamente, as principais lesões associadas a presença do *M. hyopneumoniae* no pulmão são broncopneumonia catarral caracterizada por grande infiltração de neutrófilos, linfócitos e macrófagos no lúmen e hiperplasia de BALTs (Hillen et al., 2014). A hiperplasia de BALT é classicamente associada a infecção por *M. hyopneumoniae,* pelo fato de que o agente possui ação mitogênica em alguns tipos de linfócitos de suíno, resultando na hiperplasia (Lopez, 2013). Casalmiglia et al. (2000) mostrou associação entre lesões histopatológicas em pulmão e a presença do *M. hyopneumoniae* no tecido, até mesmo quando as lesões ainda não eram características da infecção.

O padrão ouro para a detecção e diagnóstico do organismo é a cultura, mas seu isolamento é difícil devido ao seu crescimento demorado (Thacker, 2004), podendo ser facilmente inibido pela presença de outros micoplasmas suínos que crescem em maior velocidade (Kobisch e Friis, 1996; Thacker, 2012). Isso faz com que o isolamento seja raramente utilizado como diagnóstico, devido a grande possibilidade de resultados falso-negativos. A detecção de *M. hyopneumoniae* nos pulmões por anticorpo fluorescente ou imunohistoquímica são métodos comumente utilizados em laboratórios de diagnóstico veterinário, por serem rápidos e baratos, porém podem gerar ambiguidade de resultados, uma vez que o *M. hyopneumoniae* compartilha determinantes antigênicos com outros micoplasmas como o *M. hyorhinis e M. flocculare.* O desenvolvimento de análises por meio da reação em cadeia da

polimerase (PCR) propiciou um método mais sensível e específico para a confirmação da presença de *M. hyopneumoniae,* sendo cada vez mais utilizado no diagnóstico (Kobisch e Friis, 1996). Testes baseados em reações antígeno-anticorpo (ELISA) também estão disponíveis no mercado, com estudos que demonstram sua alta especificidade em detectar soros negativos para anticorpos, resultando em uma baixa taxa de resultados falso-positivos (Thacker, 2004).

A avaliação post mortem das lesões macroscópicas é uma das principais formas de se detectar suspeitas de animais infectados, bem como é uma das únicas formas de se avaliar a intensidade e severidade da infecção tanto experimentalmente como a campo (Garcia-Morante et al., 2016),

As lesões pulmonares são frequentemente associadas à quedas na produvitidade e na qualidade das carcaças (Teixeira et al., 2016, Permentier et al., 2015, Harley et al., 2012). Suínos sob estresse inflamatório como no caso da infecção de *M. hyopneumoniae*, tem devido a ação de citocinas, interleucinas (IL) - 1β, IL-6 e fator de necrose tumoral (TNF), redução da ingestão de alimentos, inibição da absorção de nutrientes e um aumento da taxa metabólica (Rodríguez et al., 2016, Escobar et al., 2004). Essas alterações provocam uma redução de peso corporal, e uma menor qualidade nas carcaças (Cobanovic et al., 2016).

A presença desta doença na fase de terminação prejudica a expressão do potencial genético máximo de síntese muscular e de gordura dos animais, que deixam de utilizar parte dos nutrientes disponíveis para o desenvolvimento, em áreas que naquele momento necessitam de maior atenção (Soltésová et al., 2015).

Donkó et al. (2005), afirmam que a presença de lesões respiratórias aumentou a conversão alimentar dos animais em terminação, reduzindo de forma significativa o ganho de peso diário (GPD). É relatado que a presença dessas lesões características de pneumonia pode reduzir até 7% a taxa de crescimento de animais avaliados no abate, e 20% em animais com lesões características de pleurisia (Pagot et al., 2007).

Portanto, o aumento dos valores em escores de pneumonia resultam em animais com menor peso vivo, podendo provocar efeitos negativos na qualidade da carne suína por levar a alterações nos valores de pH, na capacidade de retenção de água, coloração, perda na qualidade de cocção e alteração de sabor (Dailidavicienė

et al., 2009; Karabasil et al, 2017). Permentier et al. (2015) afirmam que a presença de lesões pulmonares pode levar à uma maior predisposição à PSE (pálido, mole, exsudativo) devido às alterações nos valores de pH de carcaças. Portanto, faz-se necessária uma investigação que possa esclarecer e quantificar o verdadeiro impacto do *M. hyopneumoniae* na produtividade dos suínos, devido ao fato de este agente estar associado à lesão pulmonar mais prevalente encontrada ao abate.

3. Referências

Blanchard, B., Vena, M., Cavalier, A., Le Lannic, J., Gouranton, J., Kobisch, M. Electron microscopic observation of the respiratory tract of SPF piglets inoculated with *Mycoplasma hyopneumoniae*. **Veterinary Microbiology**, v.30, p. 329–341, 1992.

Brewster, V. R., Maiti, H. C., Tucker, A. W., Nevel, A., 2017. Associations between EP-like lesions and pleuritis and post trimming carcass weights of finishing pigs in England. **Livestock Science** 201, 1–4. https://doi.org/10.1016/j.livsci.2017.04.012

Casalmiglia, M., Collins, J. E., Pijoan, C., 2000. Correlation between the presence of enzootic pneumonia lesions and detection of *Mycoplasma hyopneumoniae* in bronchial swabs by PCR. **Veterinary Microbiology**, 76, 299-303.

Čobanović, N. D., Karabasil, N. R., Cojkić, A. R., Vasilev, D. D., & Stajković, S. S., 2016. Carcass quality and hematological alterations associated with lung lesions in slaughter pigs. **Scientific Papers Animal Science and Biotechnologies**, 49(1), 236-240.

Dailidavičiene, J., Januškevičiene, G., Milius, J., Mieželiene, A., Alenčikiene, G., 2009. Influence of lung pathology on pig carcasses' microbiological quality and sensory parameters. **Bulletim of the Veterinary Institute** Pulawy, 433–438

Donkó, T., Kovács, M., Magyar, T., 2005. Association of growth performance with atrophic rhinitis and pneumonia detected at slaughter in a conventional pig herd in Hungary. **Acta Veterinaria Hungarica** 53, 287–298. https://doi.org/10.1556/AVet.53.2005.3.2

Dykhuis-Haden, C., Painter, T., Fangman, T., Holtkamp, D., 2012. Assessing production parameters and economic impact of swine influenza, PRRS and *Mycoplasma hyopneumoniae* on finishing pigs in a large production system. **American Association of Swine Veterinarians**, Denver, 75-76.

Escobar, J., Van Alstine, W.G., Baker, D.H., Johnson, R.W., 2004. Decreased protein accretion in pigs with viral and bacterial pneumonia is associated with increased myostatin expression in muscle. **Journal of Nutrition** 134, 3047–3053. https://doi.org/10.1093/jn/134.11.3047

Fablet, C., Marois-Crehan, C., Simon, G., Grasland, B., Jestin, A., et al. 2012. Infectious agents associated with respiratory disease in 125 farrow-to-finish pig herds: a cross-sectional study. **Veterinary Microbiology,** 157, 152-163.

Fraile, L., Alegre, A., López-Jiménez, R., Nofrarías, M., Segalés, J., 2010. Risk factors associated with pleuritis and cranio-ventral pulmonary consolidation in slaughter-aged pigs. **Veterinary Journal** 184, 326–333. https://doi.org/10.1016/j.tvjl.2009.03.029

Garcia-Morante, B., Segalés, J., Fraile, L., Pérez de Rozas, A., Maiti, H., Coll, T., Sibila, M., 2016. Assessment of *Mycoplasma hyopneumoniae*-induced pneumonia using different lung lesion scoring systems: A comparative review**. Journal of Comparative Pathology,** 154, 125–134. doi:10.1016/j.jcpa.2015.11.003

Giacomini, E., Ferrari, N., Pitozzi, A., Remistani, M., Giardiello, D., Maes, D., Alborali, G.L., 2016. Dynamics of *Mycoplasma hyopneumoniae* seroconversion and infection in pigs in the three main production systems. **Veterinary Research Communication,** 40, 81–88. https://doi.org/10.1007/s11259-016-9657

Henninger, M., Labarque, G., Fily, B., Auvigne, V, 2014. Quantification of the relation between lung lesions at slaughter and growth performance from birth to slaughter – proposal for a new synthetic indicator [in French, with English abstract]. **Journées Recherche Porcine**, 46, 183–184.

Hillen, S., von Berg, S., Köhler, K., Reinacher, M., Willems, H., & Reiner, G., 2014. Occurrence and severity of lung lesions in slaughter pigs vaccinated against *Mycoplasma hyopneumoniae* with different strategies. **Preventive Veterinary Medicine**, 113(4), 580-588.

Karabasil, N., Cobanovic, N., Vucicevic, I., Stajkovic, S., Becskei, Z., Forgách, P., Aleksic-Kovacevic, S., 2017. Association of the severity of lung lesions with carcass and meat quality in slaughter pigs. **Acta Veterinaria Hungarica** 65, 354–365. https://doi.org/10.1556/004.2017.034

Kobisch, M. and Friis, N.F. (1996) Swine mycoplasmoses. **Revue scientifique et technique** 15, 6.

Lopez, A. Sistema Respiratório, Mediastino e Pleuras, 2013. In: MCGAVIN, M., ZACHARY, J.F. (Eds.). **Bases da Patologia em Veterinária** 5. ed. Rio de Janeiro, Elsevier, 9, 461-541.

Lorenzo, H., Quesada, O., Assuncao, P., Castro, A., Rodriguez, F. Cytokine expression in porcine lungs experimentally infected *with Mycoplasma hyopneumoniae*. **Veterinary Immunology and Immunopathology**, v. 109, p. 199–207, 2006.

Maes, D., Sibila, M., Kuhnert, P., Segalés, J., Haesebrouck, F., & Pieters, M. (2018). Update on Mycoplasma hyopneumoniae infections in pigs: knowledge gaps for improved disease control**. Transboundary and emerging diseases,** *65*, 110-124.

Merialdi, G., Dottori, M., Bonilauri, P., Luppi, A., Gozio, S., Pozzi, P., Spaggiari, B., Martelli, P., 2012. Survey of pleuritis and pulmonary lesions in pigs at abattoir with a focus on the extent of the condition and herd risk factors. **Veterinary Journal**, 193, 234–239. https://doi.org/10.1016/j.tvjl.2011.11.009

Meyns, T., Van Steelant, J., Rolly, E., Dewulf, J., Heasebrouk, F., Maes, D. A cross-sectional study of risk factors associated with pulmonary lesions in pigs at slaughter. **The Veterinary Journal,** 187, 388–392, 2011.

Mores, M. A. Z., 2006. Anatomopatologia e microbiologia de lesões pulmonares responsáveis por condenações de carcaças em suínos.

Nathues, H., Doehring, S., Woeste, H., Fahrion, A., Doherr, M., Grosse Beilage, E. Individual risk factors for *Mycoplasma hyopneumoniae* infections in suckling pigs at the age of weaning. **Acta Veterinaria Scandinavica**, v. 55, 44, 2013. Disponível em: https://doi.org/10.1186/1751-0147-55-44.

Pacce, V. D., de Oliveira, N. R., Jorge, S., & Dellagostin, O. A. (2019). Occurrence of *Mycoplasma hyopneumoniae* in slaughter pigs from Southern Brazil. **Brazilian Journal of Veterinary Research and Animal Science**, 56(1), e150072-e150072.

Pagot, E., Pommier, P., Keïta, A., 2007. Relationship between growth during the fattening period and lung lesions at slaughter in swine. **Revue de Medecine Veterinaire,** Toulouse, 158, 253–259.

Permentier, L., Maenhout, D., Deley, W., Broekman, K., Vermeulen, L., Agten, S., Verbeke, G., Aviron, J., Geers, R., 2015. Lung lesions increase the risk of reduced meat quality of slaughter pigs. **Meat Science**, 108, 106–108. https://doi.org/10.1016/j.meatsci.2015.06.005

Pieters, M., Maes, D., 2019. Mycoplasmosis. In: Zimmerman, J., Karriker, L., Ramirez, A., Schwartz , K., Stevenson , G., Zhang, J. (Eds.), **Diseases of Swine,** Wiley, 863-883.

Regula, G., Lichtensteiger, C. A., Mateus-Pinilla, N. E., Scherba, G., Miller, G. Y., Weigel, R. M., 2000. Comparison of serologic testing and slaughter evaluation for assessing the effects of subclinical infection on growth in pigs. **Journal of American Veterinary Medical Association**, 217, 888–895. https://doi.org/10.2460/javma.2000.217.888

Rodríguez, F., Batista, M., Hernández, J. N., Afonso, A. M., Poveda, J. B., 2016. Relationship Between Expression of Interleukin-5 and Interleukin-13 by Epithelial Cells and Bronchiolar Changes in Pigs Infected with *Mycoplasma hyopneumoniae.* **Journal of Comparative Pathology**, 154, 165–168. https://doi.org/10.1016/j.jcpa.2016.01.007

Sibila, M., Nofrarias, M., Lopez-Soria, S., Segalés, J., Valero, O., Espinal, A., Calsamiglia, M. Chronological study of *Mycoplasma hyopneumoniae* infection, seroconversion and associated lung lesions in vaccinated and non-vaccinated pigs. **Veterinary Microbiology**, v. 122, p. 97-107, 2007.

Sibila, M., Pieters, M., Molitor, T., Maes, D., Haesebrouck, F., Segalés, J. Current perspectives on the diagnosis and epidemiology of *Mycoplasma hyopneumoniae* infection. **The Veterinary Journal**, v. 181, p. 221–231, 2009.

Silva, A., Paganini, F., Acosta, J., Rocha, P., Mistura, H., Marcon, E., Simon, V., Casagrande, H. Estudo do perfil das doenças respiratórias nas regiões Sul, Sudeste e Centro-oeste do Brasil. In: Congresso Brasileiro De Veterinários Especialistas Em Suínos, 2001, Porto Alegre. **Resumos...**Porto Alegre: Associação Brasileira de Veterinários Especialistas em Suínos, p.58-67, 2001.

Siqueira, F. M., Gerber, A. L., Guedes, R. L. M., Almeida, L. G., Schrank, I. S., Vasconcelos, A. T. R., Zaha, A., 2014. Unravelling the transcriptome profile of the swine respiratory tract Mycoplasmas. **Plos One** 9, 12.

Šoltésová, H., Nagyová, V., Tóthová, C., Nagy, O., 2015. Haematological and blood biochemical alterations associated with respiratory disease in calves. **Acta Veterinaria Brno,** 84, 249–256. https://doi.org/10.2754/avb201584030249

Szeredi, L., Cságola, A., Dán, Á., Dencső, L., 2015. Vascular lesions and pneumonia in a pig fetus infected by porcine circovirus type 2. **Acta Veterinaria Hungarica**, 63, 215–222.

Takeuti, K. L., de Barcellos, D. E., de Andrade, C. P., de Almeida, L. L., & Pieters, M., 2017. Infection dynamics and genetic variability of *Mycoplasma hyopneumoniae* in self-replacement gilts. **Veterinary microbiology**, 208, 18-24.

Thacker, E. L., Minion, C., 2012. Mycoplasmosis. In: Zimmerman, J. J., Karriker, L. A., Ramirez, A., Schwartz, K. J., Stevenson, G. W. J. (Eds), **Diseases of Swine**, 10a Ed., Ames, EUA, 779-797.

Thacker, E.L. (2004) Diagnosis of *Mycoplasma hyopneumoniae*. **Journal of Swine Health and Production**, 12, 252-254.

Vicente, A. F., Catto, D., Allendorf, S. D., Garcia, K. C. O. D., Antunes, J. M. A. P., Appolinario, C. M., Peres, M.G., Megid, J., 2013. Soropositividade para *Mycoplasma hyopneumoniae* em suínos abatidos em frigoríficos da região central do estado de São Paulo. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia,** 65, 1899–1903. https://doi.org/10.1590/S0102-09352013000600045

Villarreal, I., Maes, D., Vranckx, K., Calus, D., Pasmans, F., Haesebrouck, F. Effect of vaccination of pigs against experimental infection with high and low virulence *Mycoplasma hyopneumoniae* strains. **Vaccine,** v. 29, p. 1371–1375, 2011.

Vranckx, K., Maes, D., Marchioro, S. B., Villarreal, I., Chiers, K., Pasmans, F., & Haesebrouck, F. (2012). Vaccination reduces macrophage infiltration in bronchus-

associated lymphoid tissue in pigs infected with a highly virulent *Mycoplasma hyopneumoniae* strain. **BMC veterinary research**, 8(1), 24

CAPÍTULO 2 - Lung consolidation caused by *Mycoplasma hyopneumoniae* has a negative effect on productive performance and economic revenue in finishing pigs

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Abstract

This study aimed to measure the impact of productivity and the consequent economic losses related to lung lesions caused by *M. hyopneumoniae*. Five-hundred 75 days-old pigs were selected and weighed at the beginning and at the end of the finishing phase to assess the average daily gain (ADG). These animals were evaluated at the slaughter, and samples were collected for laboratory analysis to confirm the presence of *M. hyopneumoniae* DNA. The lungs of each pig were examined and classified into groups based on the extension of macroscopic lung lesions. Four-hundred eighty-six lungs were examined and 68.5% (n=333) had macroscopic lung lesions. All pigs with lesions were positive for *M. hyopneumoniae* in qPCR. Linear mixed regression models (proc Glimmix) were performed on SAS to estimate the effect of macroscopic lung lesion scores on the ADG of finishing pigs. All pairwise comparisons among lesion score groups were performed using $p<0.05$. For each increase of one percent in the lesion area, there was a decrease of 1.8 grams in the daily weight gain. All the groups had a numerically lower ADG when compared to Group 1 (no lesions). The economic analysis was performed by simulation on Excel to estimate and compare the financial performance of the different lung lesion score groups. The negative correlation found between the group with no lung lesions and the group with more than 15.1% of lesions, showed a statistical difference in ADG, which could mean an opportunity to gain up to $ 6.55 per pig at slaughter. The presence of lesions causes the animals to decrease their productive potential, causing financial loss and generating impacts on the production system.

Keywords: ADG, enzootic pneumonia, financial losses, qPCR, slaughter.

Introduction

Swine respiratory diseases cause a considerable production and economic impact in the swine industry due to decreased feed efficiency and growth rate, high morbidity, and mortality, increased cost of medications, and lower carcass quality (Szeredi et al., 2015).

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the etiological pathogen of enzootic pneumonia (EP), which is a chronic respiratory disease of pigs and highly distributed across most swine producing countries (Maes et al., 2018). The condition causes significant financial losses by increasing medication costs and reducing growth performance. Also is one of the main players in the porcine respiratory complex disease (Takeuti et al., 2017) and frequently associated with secondary pathogens, such as *Pasteurella multocida, Actinobacillus pleuropneumoniae, Glasserella parasuis* (Thacker et al., 2012).

Enzootic pneumonic lesions mostly occur at the cranioventral lung areas, mainly bilaterally in the apical, cardiac, and intermediate lobes and sometimes in the anterior parts of the diaphragmatic lobes (Garcia-Morante et al., 2016). The lesions are demarcated from healthy tissue, and consist of purple and grey areas of pulmonary consolidations with a moderately firm consistency (Tamiozzo et al., 2011).

Swine affected by enzootic pneumonia eat less than necessary for its maintenance and development. The lack of nutrients leads to the incapacity to express its maximum genetic potential for muscle synthesis and fat accumulation as they have areas of highest demand. (Karabasil et al., 2017). Such metabolic alterations reduce live weight and decrease carcass quality (Čobanović et al., 2016).

Studies conducted in Brazil in the decade of the 80's (Piffer et al., 1985) indicated that pigs that presented more than 10% of lung consolidations at slaughter had a reduction of 9.3% in their growth. Donkó et al. (2005) also claimed that respiratory lesions increased feed conversion ratio of finishing pigs, reducing the average daily gain (ADG) significantly. These lesions can reduce up to 7% the growth rate of slaughter animals (Pagot et al., 2007).

Therefore, increased values in pneumonia scores result in animals with lower live weight at slaughter time and may cause negative effects on the pork quality by leading to changes in pH values, water holding capacities, color, flavor, and cooking quality loss (Dailidavicienė et al., 2009). Permentier et al. (2015) showed that the presence of lung lesions is a potential risk factor for obtaining PSE meat (pale, soft, exudative) due to the alterations in its pH values. This study aimed to measure the economical and productive impact of lung consolidation lesions on finishing pig, associating the participation of the agent in the losses arising from these lesions.

Materials and Methods

Animals, housing and experimental design

This prospective field study was conducted on a farm with 9000 finishing pigs, located in Minas Gerais - Brazil, between December 2018 and March 2019. The pigs came from different sow farms with all-in/all-out system, positive for *M. hyopneumoniae*, who had no relevant health problems. The animals were vaccinated at the farrowing farms at 21 days of age with a bivalent vaccine against *M. hyopneumoniae* and Circovirus (PCV-2), Porcilis®

PCV M HYO from MSD Animal Health. The animals were maintained in collective pens with about 100 pigs per pen and had free access to water and feed *ad libitum*. Two hundred and fifty males and females were conveniently selected from the same batch at the beginning of the finishing phase at 75 days of age. The pigs were identified with ear tags and weighed to assess the finishing ADG. This study was approved by the Ethics Committee on the Use of Animals (Protocol 005168/18) from the School of Agricultural and Veterinarian Sciences, São Paulo State University (Unesp).

Weighing and assessment of finishing average daily gain (ADG)

The animals were weighed at the beginning of the finishing phase, at 75 days of age (d0), and a week before the slaughter (d105). From the weight data, it was possible to assess the ADG of each individual throughout the period. The ADG was calculated based on this equation:

$$ADG = \frac{d105 - d0}{Days\ on\ finishing\ phase}$$

Sampling procedures

The samples were collected at the slaughterhouse located in the state of Minas Gerais (southeast region of Brazil). Macroscopic lung lesions were assessed as described below, and lung fragments with lesions were taken from each animal for quantitative PCR (qPCR) and histopathology. Thus, the objective of testing by qPCR those lungs showing consolidation was to confirm the contribution of *M. hyopneumoniae* in the formation of such lesions. The

lung samples for qPCR testing were stored in RNAse and DNAse-free microtube (Corning, EUA) at -80°C until processing. The lung tissues for histopathology were stored in buffered formalin 10%. Trained veterinaries collected all the samples.

Macroscopic lung lesions evaluation

The lungs of all the pigs were evaluated for macroscopically visible lesions with the App Ceva LungProgram® (Ceva Animal Health, France), by one trained veterinarian to avoid individual variations. The App is based on two methods, the first one, each lobe was scored from 0 to 4 for pulmonary consolidations according to the method described by Madec & Kobisch (1982), according to the affection of the lobe (%) (Table 1). Moreover, the score found was corrected by the App according to the method established by Christensen et al. (1999) which the percentage of each lobe affected area is multiplied by the relative lobe weight. Since each lobe does not represent an equal part of the total volume of the lung, the following relative percentages are assigned (Christensen, 1999). Contribution of each lobe at total lung volume: Right apical lobe = 11%, right cardiac lobe = 10%, right diaphragmatic lobe = 34%, left apical lobe = 5%, left cardiac lobe = 6%, left diaphragmatic lobe = 29%, intermediate lobe = 5%. Based on the extension of macroscopic lung lesions, the animals were classified into four groups: Group 1 – pigs without macroscopic lesions; Group 2 – 0.1-5% of lesions; Group 3 – 5.1-15% of lesions; Group 4 - >15,1% of lesions.

DNA extraction and conventional PCR for the mammals-gapdh gene

For DNA extraction of lung samples that had macroscopic lesions, an in house extraction protocol was used as described by (Kuramae-Izioka, 1997) using 0.2g of lung fragment. Also, ultrapure water (Promega, EUA) was used as negative control. After the extraction, the purity and quantity of the extracted material were measured in a spectrophotometer Nanodrop® (Thermo Fisher, EUA), and the samples that were in standard, were stored at -20°C until forwarded to the qPCR. To accurately assess the quality of the sample, 260/280 or 260/230 should be analyzed; pure nucleic acids usually produce a ratio of 260/280 = 1.8 and a ratio of 260/280 of = 2.0.

To avoid the presence of inhibitors and false-negative results on the qPCR, the extracted DNA samples were submitted to a cPCR using the *gapdh*-F primer (5'-CCTTCATTGACCTCAACTACAT-3') and *gapdh*-R (5'-CCAAAGTTGTCATGGATGACC3'), which flank a fragment of the mammals-*gapdh* gene (Birkenheuer et al., 2003). After the reaction, the product was subjected to horizontal electrophoresis on 1% agarose gels stained with Ethidium Bromide (Life Technologies™, USA) in TEB pH 8.0 running buffer at a current of 90V/50mA for 40 min. Then, the gel was visualized using an ultraviolet light transilluminator (Chemi-Doc, Bio-Rad®).

Detection and quantification of M. hyopneumoniae DNA by qPCR

The qPCR assay was adapted from the protocol published by Fourour et al. (2018). Primers and the hydrolysis probe used in the reaction target the multicopy gene of the p102 adhesion protein of *M. hyopneumoniae* (GenBank: AE017332.1). The nucleotide sequences of

the initiators were tested using Blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and did not show homology with other swine pathogens genetic material. The nucleotide sequences of the sense and antisense initiators and the hydrolysis probe (Fourour et al., 2018), are listed below:

Sense primer: 5'-TAAGGGTCAAAGTCAAAGTC-3'

Anti-sense primer: 5'- AAATTAAAAGCTGTTCAAATGC-3'

Hydrolysis probe 5'- FAM-AACCAGTTTCCACTTCATCGCC-§BHQ2-3'

The reaction was composed of 1X of Master mix Go taq® (Promega, Madison, EUA), 0.5 µM of each primer (Invitrogen, EUA), 0.3µM hydrolysis probe (IDT, Iowa City-EUA), ultrapure water q.s. and 1 µL of DNA template per sample totaling 10 µL of reaction. The qPCR was performed on CFX-96 Thermal Cycler (Biorad - EUA) under the following conditions: an initial denaturation cycle at 95°C for 3 minutes, followed by 39 cycles of 95°C for 15 seconds and extension at 55.7 °C for 1 minute. The samples were tested in duplicate, and to the validation of the results, the difference between the copies Cqs should be lower than 0.5 cycles.

Absolute quantification

Quantification was performed using serial 10-fold dilutions (starting at 10^7 until 10^1 copies⁄ µL) of synthetic DNA (GBlock®, IDT, Iowa City, IA, USA) containing the 150 bp fragment amplified by the primer pair used in the qPCR. The fragment was diluted according to the manufacturer's instructions until the starting concentration of 10^7

copies⁄µL. Quantification data based on the standard curve generated was only validated if the reaction efficiency was between 90 % and 105 % (Bustin et al., 2009).

Histopathology

The fragments were collected in the areas between lesions and healthy lobes, and the location of the lesions varied among the animals. The tissue slides were stained with hematoxylin and eosin (HE) and the histopathological lesions were graded from 0 to 4: 0 = no lesions; 1 = interstitial pneumonia or purulent bronchopneumonia; 2 = light to moderate infiltrates neutrophils, lymphocytes, and macrophages into alveoli and airways; 3 = perivascular or peribronchiolar lymphoplasmacytic hyperplasia, type II pneumocyte hyperplasia and presence of edema in the alveoli and 4 = the same lesions of 3 plus the presence of perivascular and peribronchiolar lymphoid nodes. Lesions 1 and 2 are not specific, while lesions 3 and 4 are considered specific for enzootic pneumonia (Casalmiglia et al., 2000).

Data analysis

Statistical analysis was done using individual pigs as the experimental units. A regression model (proc GLM) was performed on SAS (Statistical Analysis System) to estimate the linear relationship between macroscopic lung lesion scores, measured at the slaughterhouse, and the ADG of pigs during the finishing phase. A regression coefficient

representing the mean change in ADG associated with one unit increase in macroscopic lung lesion scores was reported. Additionally, animals were classified into four groups, based on the extension of macroscopic lung lesions at the slaughterhouse: Group 1 – pigs without macroscopic lesions; Group 2 – 0.1-5% of lesions; Group 3 – 5.1-15% of lesions; Group 4 - >15.1% of lesions. A linear mixed regression model (proc Glimmix) was performed on SAS to estimate and compare the ADG associated with each group. All multiple pairwise comparisons, as well as 95% confidence intervals between lesion score groups, were performed. Dunn's Test was used to estimate the association between SQs (copies/μL) and lung lesions score.

Economical analysis

The economic analysis was performed on Excel to estimate and compare the financial performance of the different lung lesion score groups. The financial profit of each lung lesion score group was based on productivity parameters of study units and calculated by the difference of the revenue and the variable cost of each group.

The following productivity parameters were collected for each lung lesion score group: average days on feed (days/group), finishing mortality (% of dead pigs), finishing ADG (kg/day), average weight at the beginning of the finishing (kg/pig). The revenue was calculated by the market pig price ($/kg), the annual income from pig ($/pig sold), annual revenue from pigs ($/group), for a group of 1000 animals. Variable cost was calculated by the price of weaned pigs ($/pig placed), price of diet ($/kg of feed), animal health vaccine costs ($/pig placed), animal health antimicrobial costs and labor and farm management ($/pig

placed), for a group of 1000 animals. The economic profit difference among all lung lesion score groups was reported.

Results

From the 500 selected animals, fourteen were excluded from the study due to their deaths or loss their identification earrings and problems during sample collection at the slaughterhouse, which were mainly related to the processing speed of slaughter. Therefore, only 486 pigs were included in the ADG and macroscopic lesions analysis. For the laboratory analysis by PCR real-time, and histopathology, only samples from animals with any macroscopic lung lesions (n=333) were used. Laboratory analysis was done to confirm *M. hyopneumoniae* presence in the pulmonary lesions observed.

Macroscopic lung lesions scoring

Only 31.5% of the 486 lungs examined had no macroscopic lung lesions, while 68.5% (IC95%: 63.97%-72.23%) or 333 animals, presented areas affected by pulmonary consolidation at slaughter. The mean value found was 3,84% of affected lung, and the highest value was 27,9% of affected lung. Classification results are shown in Table 2.

Linear association between macroscopic lesions and average daily gain (ADG)

The regression showed a negative linear association between the ADG and macroscopic lesions scores. The regression coefficient reported was -0.00189, which means that for each increase of one percent in the lesion area, there was a decrease of 1.8 grams in the daily weight gain.

Average Daily Gain (ADG)

All the groups had a numerically lower ADG when compared to group 1 (no lesions). Group 1 had a numerically higher average weight, but not significant (p=0.2390 and p=0.2442) when compared to groups 2 and 3. However, when comparing groups 4 and 1, group 1 presented a significantly higher weight (p=0.0076). Group 4 also had a considerably lower weight average when compared with groups 2 and 3 (p=0.0370 and p=0.0673). The statically significant differences in ADG between animals showing different lung lesions scores are shown in Figure 1.

Detection/Quantification of M. hyopneumoniae by qPCR

From the 333 tested lung samples (with macroscopic lesions), 100% were positive by qPCR for the fragment of the p102 gene. The averages of starting quantification values (SQ) are detailed in Table 2. No statistically significant difference was found (P>0.05) between SQs (copies/µL) and lung lesions score, using Dunn's Test.

Histopathology

Microscopic findings revealed suggestive lesions of *M. hyopneumoniae* on most animals. From the 333 animals included in this analysis, 129 (38.73%) were graded 3 e 116 (34.83%) were grade 4, both grades classified as consistent with enzootic pneumonia lesions. While 57 animals (17.11%) were grade 2, 29 (8.7%) were grade 1, and only 2 animals showed no lesions.

Economical analysis

From the association found between ADG and lung lesions score, it was possible to calculate the economic losses that these animals had at slaughter as a result of the disease. This descriptive analysis was made by the simulation method, using productivity parameters, variable costs, and revenue. Some values were the same to all groups of animals, since they were from the same barn, as average days on feed (105 days), finishing mortality (3.5%), and variable costs. Other productivity parameters (finishing ADG/group (kg/day), average weight at the beginning of the finishing/group (kg/pig) were also included in the analysis (Table 2). The variable costs considered in this analysis were the price of weaned pigs ($11.33/pig), the price of diet ($0.20/kg), animal health vaccine costs ($0.81/pig), animal health antimicrobial costs and labor and farm management ($2.39/pig). The market pig price considered (U$1.24), was according to what was established in October 2019 in Minas Gerais state (Cepea/Usp – 2019). The total variable costs, the revenue, and the economic profit difference among groups are presented in Table 3.

Regarding the economic profit, the comparison between animals with more than 15% of lesions (group 4) and animals without lung lesions (group 1), was the one that showed the highest difference.

Discussion

This study focused on assessing the financial losses caused by lung lesions in animals with *M. hyopneumoniae* infection in a finishing herd. The mean prevalence of consolidation lesions was 68% (IC95%: 63.97%-72.23%). Accordingly, previous studies in Brazil showed 72.4% and 80.3% of macroscopic lung lesions prevalence at the slaughterhouse (Baraldi et al., 2019; Galdeano et al., 2019).

The presence of *M. hyopneumoniae* was detected in lung fragments of all animals evaluated by qPCR, as well as compatible gross and microscopic lung lesions of the sampled animals (except for two animals that did not show microscopic lesions). The pathological process of the lung lesions formation is not clear and different factors can affect it, such as the strain characteristics and immune responses of the animal (Hillen et al., 2014; Meyns et al., 2007). Co-infection with other swine respiratory pathogens such as Swine influenza virus (SIV), *Actinobacillus pleuropneumoniae* and Porcine Circovirus- 2 (PCV2), or even subsequent infections with different strains, were also shown to increase macroscopic lung lesions (Villarreal et al., 2009, Fablet et al., 2012, Fourour et al., 2018).

Thus, lung lesions are often associated with declines on productivity (Teixeira et al., 2016; Permentier et al., 2015; Harley et al., 2012). Pigs under inflammatory stress as in the case of *M. hyopneumoniae* infection, decrease the feed intake due to the action of cytokines, interleukins (IL) -1β, IL-6, and Tumor Necrosis Factor- α (TNF- α), macro and micronutrient

deficiencies may also occur, exacerbating the initial disease and predisposing the animals to secondary infections (Rodríguez et al., 2016; Escobar et al., 2004; Almeida et al., 2020). These metabolic factors contribute to reduced growth rate, feed conversion efficiency, and quality in carcasses (Čobanović et al., 2015; Stukelj et al., 2015; Szeredi et al., 2015; Donko et al., 2005).

This study reported that for each increase of one percent in the lesion area, there was a decrease of 1.8 g in the ADG, meaning that an animal with 10% of lung lesions, decreased 18 grams in the ADG throughout the finishing phase, similar with Pagot et al. (2007) that showed for each increase of 1% in the lesion area, there was a decrease of 0.7% in the weight. Moreover, regarding degrees of lung lesion extension, it was observed that group 4, composed by animals that presented more than 15% of lung affection, had a lower ADG when compared to all other groups. Other authors reported a decrease in the animal weight associated with the severity of lung consolidation lesions (Karabasil et al., 2017; Henninger et al., 2014).

Karabasil et al. (2017) evaluated the animals in three groups according to the level of the lesions: no lesions, mild lesions and moderate to severe pneumonia, showed that the average weight at slaughter was 118 kg, 115.10 kg and 112.50 kg, demonstrating a significant difference between groups. Divergent results were found in which no consistent impacts of development in animals associated with increased pulmonary lesions were shown (Escobar et al., 2002). However, these authors evaluated animals an experimental infection with *M. hyopneumoniae,* which may have resulted from the absence of other pathogens in the lesions formation, that generally co-exist with *M. hyopneumoniae* under commercial conditions, and is known the synergism with others pathogens in the formation of the lesions.

Vaccination is one of the most used practices under filed conditions aiming to reduce deleterious effects of lung lesions in the productive results, and different commercial *M. hyopneumoniae* vaccines are available (Maes et al., 2018). In this study, the animals were vaccinated, which proves that vaccines do not prevent infection and the pathogen transmission, as previously suggested by other studies (Arsenakis et al., 2018). Thus, commercial vaccines are effective in reducing clinical signs but demonstrate only partial protection against the lesion development (Haesebrouck et al., 2004), which is the main factor involved in ADG losses.

Regula et al. (2000), after analyzing animals from a vaccinated property, found a decline of 6.8g/day for each 1% in the lesion area. Different results can occur mainly due to the age of infection and measurement of ADG since older animals on the finishing phase have less weight variation than younger animals and may have less significant associations with lung lesions.

Another point is the age of infection, because apparently, those who develop pneumonia in a later stage, spend part of their lives without the disease, and therefore have a better performance than the animals infected at an earlier age, even though they still have active lesions at the slaughter (Morris et al., 1995). Meanwhile, the youngest infected animals, at the developmental stage, may have the most compromised growth (Sitjar et al., 1996).

Even though enzootic pneumonia is a recurring problem in pig farms, few studies show the real economic and productive impacts of the disease. It is also known that the presence of lesions causes the animals to decrease their productive potential, resulting in financial loss, which has implications on the production system (Brewster et al., 2017; Dykhuis et al., 2012). The economical descriptive analysis showed that the participation of *M.*

hyopneumoniae in the formation of lung consolidation lesions could cause economic losses of $ 6.55 in animals with more than 15% of lung lesions.

Dykhuis et al. (2012), analyzing the association between *M. hyopneumoniae*, PRRS, and swine influenza virus in American swine herds, confirmed that the most tremendous economic and productive losses occurred in the presence of the causative agent of swine enzootic pneumonia. These authors found an increase in the cost of growing and finishing animals from $ 9.69 to $ 10.12 per head while *M. hyopneumoniae* was associated with swine influenza virus (SIV). Similarly, Gillespie (2013), attributed a loss of $ 7.92 in a herd free from the agent, after having an outbreak of *M. hyopneumoniae*.

Eradication programs have evolved and adapted to current production systems, *herd closure and medication* consisting of stopped replacement entry and antibiotic treatment, and *whole herd medication*, using antibiotics without herd closure, are the most commonly used methods for *M. hyopneumoniae* eradication (Pieters et al., 2019, Holst et al., 2015). This alternative can bring economic benefits by lowering the costs of medication and vaccination and improving the performance of pigs in daily weight gain, feed conversion, and mortality. Certainly, the longer the herd remains negative, the greater the financial advantage on downstream performance (Silva et al., 2019).

Conclusions

Under the study's condition, lung consolidation lesions were negatively correlated with ADG, considering that for each increase of 1% in the lesion area, there was a decrease of 1.8 grams. Animals in the most affected group, with more than 15% of lesions, when compared to

group 1, animals with no lesions, can be more profitable to the farmer, being worth an additional $ 6.55 per pig. This study also highlighted that depending on the presence and severity of lung lesions, the economic profit is significantly downgraded, resulting in economic losses to the production system.

Animal Welfare

Permission to conduct the field study was obtained from the Ethics Committee on the Use of Animals (Protocol #005168/18), and consent was obtained from the owner of the herd.

Declaration of Interest

Although three of the authors are employed by CEVA Animal Health, in Brazil, a company that partially sponsored this work, the employments did not cause any bias concerning the study.

Acknowledgments

Ceva Animal Health Brazil and a grant from São Paulo Research Foundation – FAPESP (#2018/09473-2) funded this study. The authors are grateful for the kindness and collaboration of the producer and thank the technical managers for their cooperation in providing the valuable data used in this study.

References

Almeida, H. M., Mechler-Dreibi, M. L., Sonálio, K., Ferraz, M. E. S., Storino, G. Y., Rezende, F. B., de Oliveira, L. G., 2020. Cytokine expression and *Mycoplasma hyopneumoniae* burden in the development of lung lesions in experimentally inoculated pigs. Vet. Microbiol., 108647.

Arsenakis, I., 2018. Different vaccination protocols with bacterins as a means to control *M. hyopneumoniae* infections in peri-weaned and fattening pigs. Doctoral dissertation, Ghent University.

Baraldi, T. G., Cruz, N. R. N., Pereira, D. A., Galdeano, J. V. B., Gatto, I. R., Silva, A. F. D., de Oliveira, L. G., 2019. Antibodies against *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae* and influenza virus and their relationships with risk factors, clinical signs and lung lesions in pig farms with one-site production systems in Brazil. Prev. Vet. Med., 171, 104748.

Brewster, V.R., Maiti, H.C., Tucker, A.W., Nevel, A., 2017. Associations between EP-like lesions and pleuritis and post trimming carcass weights of finishing pigs in England. Livest. Sci. 201, 1–4. https://doi.org/10.1016/j.livsci.2017.04.012

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., Wittwer, C. T., 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clini. Chem., 55, 611–22.

Casalmiglia, M., Collins, J. E., Pijoan, C., 2000. Correlation between the presence of enzootic pneumonia lesions and detection of *Mycoplasma hyopneumoniae* in bronchial swabs by PCR. Vet. Microbiol., 76, 299-303.

Cepea-USP, P. A., 2010. Centro de estudos avançados em economia aplicada-Esalq/Usp. Avalilable from: http://www. cepea. esalq. usp. br/pib/ (accessed Out 23, 2019).

Christensen, G., Sorensen, V., Mousing, J., 1999. Diseases of the respiratory system. Diseases of Swine. In: B Straw, SD' Allaire, W Mengeling, DJ Taylor, 8º Eds., Iowa State University Press, Ames, 913-940.

Čobanović, N. D., Karabasil, N. R., Cojkić, A. R., Vasilev, D. D., & Stajković, S. S., 2016. Carcass quality and hematological alterations associated with lung lesions in slaughter pigs. Sci. P. Ani. Sci. and Biotech., 49, 236-240.

Dailidavičiene, J., Januškevičiene, G., Milius, J., Mieželiene, A., Alenčikiene, G., 2009. Influence of lung pathology on pig carcasses' microbiological quality and sensory parameters. Bull. Vet. Inst. Pulawy 53, 433–438.

Donkó, T., Kovács, M., Magyar, T., 2005. Association of growth performance with atrophic rhinitis and pneumonia detected at slaughter in a conventional pig herd in Hungary. Acta Vet. Hung. 53, 287–298. https://doi.org/10.1556/AVet.53.2005.3.2

Dykhuis-Haden, C., Painter, T., Fangman, T., Holtkamp, D., 2012. Assessing production parameters and economic impact of swine influenza, PRRS and *Mycoplasma hyopneumoniae* on finishing pigs in a large production system. Denver: American Association of Swine Veterinarians, 75-76.

Escobar, J., Van Alstine, W.G., Baker, D.H., Johnson, R.W., 2004. Decreased protein accretion in pigs with viral and bacterial pneumonia is associated with increased myostatin expression in muscle. J. Nutr. 134, 3047–3053. https://doi.org/10.1093/jn/134.11.3047

Escobar, J., Van Alstine, W.G., Baker, D.H., Johnson, R.W., 2002. Growth performance and whole-body composition of pigs experimentally infected with *Mycoplasma hyopneumoniae*, Journal of Animal Science, 80, 384–391. https://doi.org/10.2527/2002.802384x

Fablet, C., Marois, C., Dorenlor, V., Eono, F., Eveno, E., Jolly, J.P., Le Devendec, L., Kobisch, M., Madec, F., Rose, N., 2012. Bacterial pathogens associated with lung lesions in slaughter pigs from 125 herds. Res. Vet. Sci. 93, 627–630. https://doi.org/10.1016/j.rvsc.2011.11.002

Fourour, S., Fablet, C., Tocqueville, V., Dorenlor, V., Eono, F., Eveno, E., Kempf, I., Marois-Créhan, C., 2018. A new multiplex real-time TaqMan® PCR for quantification of *Mycoplasma hyopneumoniae, M. hyorhinis* and *M. flocculare*: exploratory epidemiological investigations to research mycoplasmal association in enzootic

pneumonia-like lesions in slaughtered pigs. J. Appl. Microbiol. 125, 345–355. doi:10.1111/jam.13770

Galdeano, J. V. B., Baraldi, T. G., Ferraz, M. E. S., de Souza Almeida, H. M., Mechler-Dreibi, M. L., Costa, W. M. T., de Oliveira, L. G. Cross-sectional study of seropositivity, lung lesions and associated risk factors of the main pathogens of Porcine Respiratory Diseases Complex (PRDC) in Goiás, Brazil. Porci. Health Manag., 5, p. 23, 2019.

Garcia-Morante, B., Segalés, J., Fraile, L., Pérez de Rozas, A., Maiti, H., Coll, T., Sibila, M., 2016. Assessment of *Mycoplasma hyopneumoniae*-induced pneumonia using different lung lesion scoring systems: A comparative review. J. Comp. Pathol. 154, 125–134. doi:10.1016/j.jcpa.2015.11.003

Gillespie, T., 2013. *Mycoplasma* infection costs in a naïve population. In: Allen D. Leman Swine Conference. p. 51.

Haesebrouck, F., Pasmans, F., Chiers, K., Maes, D., Ducatelle, R., Decostere, A., 2004. Efficacy of vaccines against bacterial diseases in swine: What can we expect? Vet. Microbiol. 100, 255–268. https://doi.org/10.1016/j.vetmic.2004.03.002

Harley, S., More, S., Boyle, L., O'Connell, N., Hanlon, A., 2012. Good animal welfare makes economic sense: Potential of pig abattoir meat inspection as a welfare surveillance tool. Ir. Vet. J. 65, 1–12. https://doi.org/10.1186/2046-0481-65-11

Henninger, M.; Labarque, G.; Fily, B.; Auvigne, V, 2014. Quantification of the relation between lung lesions at slaughter and growth performance from birth to slaughter – proposal for a new synthetic indicator [in French, with English abstract]. Journées Rech. Porcine, 46, 183–184.

Hillen, S., Von Berg, S., Köhler, K., Reinacher, M., Willems, H., Reiner, G., 2014. Occurrence and severity of lung lesions in slaughter pigs vaccinated against *Mycoplasma hyopneumoniae* with different strategies. Prev. Vet. Med., 113, 580-588.

Holst, S., Yeske, P., & Pieters, M., 2015. Elimination of *Mycoplasma hyopneumoniae* from breed-to-wean farms: a review of current protocols with emphasis on herd closure and medication. J Swine Health Prod., 23(6), 321-330.

Karabasil, N., Cobanovic, N., Vucicevic, I., Stajkovic, S., Becskei, Z., Forgách, P., Aleksic-Kovacevic, S., 2017. Association of the severity of lung lesions with carcass and meat quality in slaughter pigs. Acta Vet. Hung. 65, 354–365. https://doi.org/10.1556/004.2017.034

Kuramae-Izioka, 1997. A rapid, easy and high yield protocol for total genomic DNA isolation from *Colletotrichum gloeosporioides* and *Fusarium oxysporum* for RAPD. Rev. Unimar 19, 683–689.

Madec, F.; Kobisch, M., 1982. Bilan lesionnel des poumons de porcs charcutiers a l'abattoir. Journees Rech Porcine, 14, 405-412.

Maes, D.; Sibila, M.; Kuhnert, P.; Segalés, J.; Haesebrouck, F.,; & Pieters, M., 2018. Update on Mycoplasma hyopneumoniae infections in pigs: knowledge gaps for improved disease control. Transbound. Emerg. Dis. 65, 110-124.

Meyns, T., Van Steelant, J., Rolly, E., Dewulf, J., Haesebrouck, F., Maes, D., 2011. A cross-sectional study of risk factors associated with pulmonary lesions in pigs at slaughter. Vet. J. 187, 388–392. https://doi.org/10.1016/j.tvjl.2009.12.027

Morris, C.R., Gardner, I.A., Hietala, S.K., Carpenter, T.E., Anderson, R.J., Parker, K.M., 1995. Seroepidemiologic study of natural transmission of *Mycoplasma hyopneumoniae* in a swine herd. Prev. Vet. Med. 21, 323–337. https://doi.org/10.1016/0167-5877(94)00384-U

Pagot, E., Pommier, P., Keïta, A., 2007. Relationship between growth during the fattening period and lung lesions at slaughter in swine. Rev. Med. Vet. (Toulouse). 158, 253–259.

Permentier, L., Maenhout, D., Deley, W., Broekman, K., Vermeulen, L., Agten, S., Verbeke, G., Aviron, J., Geers, R., 2015. Lung lesions increase the risk of reduced meat quality of slaughter pigs. Meat Sci. 108, 106–108. https://doi.org/10.1016/j.meatsci.2015.06.005

Pieters, M., Maes, D., 2019. Mycoplasmosis. In: Zimmerman, J., Karriker, L., Ramirez, A., Schwartz , K., Stevenson , G., Zhang, J. (Eds.), Diseases of Swine. Wiley, 863-883.

Piffer, I., Freitas, A. D., Munaro, N., Soncini, R., 1985. Efeito das afecções pulmonares, observadas no abate, sobre o desenvolvimento dos suínos. In: Anais... 2º Congresso brasileiro de veterinários especialistas em suínos. Abraves, Rio de Janeiro, 2, 105-106.

Regula, G., Lichtensteiger, C.A., Mateus-Pinilla, N.E., Scherba, G., Miller, G.Y., Weigel, R.M., 2000. Comparison of serologic testing and slaughter evaluation for assessing the effects of subclinical infection on growth in pigs. J. Am. Vet. Med. Assoc. 217, 888–895. https://doi.org/10.2460/javma.2000.217.888

Rodríguez, F., Batista, M., Hernández, J.N., Afonso, A.M., Poveda, J.B., 2016. Relationship Between Expression of Interleukin-5 and Interleukin-13 by Epithelial Cells and Bronchiolar Changes in Pigs Infected with *Mycoplasma hyopneumoniae*. J. Comp. Pathol. 154, 165–168. https://doi.org/10.1016/j.jcpa.2016.01.007

Silva, G. S., Yeske, P., Morrison, R. B., Linhares, D. C. L., 2019. Benefit-cost analysis to estimate the payback time and the economic value of two *Mycoplasma hyopneumoniae* elimination methods in breeding herds. Prev. Vet. Med. 168, 95–102. https://doi.org/10.1016/j.prevetmed.2019.04.008

Sitjar, M., Noyes, E. P., Simon, X., Pijoan, C., 1996. Relationships among seroconversion to *Mycoplasma hyopneumoniae*, lung lesions, and production parameters in pigs. J. Swine Heal. Prod. 4, 273–277.

Štukelj, M., Plut, J., Toplak, I., 2015. Serum inoculation as a possibility for elimination of porcine reproductive and respiratory syndrome (PRRS) from a farrow-to-finish pig farm. Acta Vet. Hung.,63, 389–399.

Szeredi, L., Cságola, A., Dán, Á., Dencső, L., 2015. Vascular lesions and pneumonia in a pig fetus infected by porcine circovirus type 2. Acta Vet. Hung., 63, 215–222.

Takeuti, K. L., de Barcellos, D. E. S. N., de Lara, A. C., Kunrath, C. F., Pieters, M., 2017. Detection of *Mycoplasma hyopneumoniae* in naturally infected gilts over time. Vet. Microbiol. 203, 215–220. doi:10.1016/j.vetmic.2017.03.025

Tamiozzo, P. J., Pelizza, B. R., Carranza, A. I., Ambrogi. A., 2011. Monitoramento da presença de *Mycoplasma hyopneumoniae* em granjas de suínos durante a implementação de programas de erradicação. Ciência Rural, 1-7.

Teixeira, D. L., Harley, S., Hanlon, A., O'Connell, N.E., More, S.J., Manzanilla, E.G., Boyle, L.A., 2016. Study on the association between tail lesion score, cold carcass weight, and viscera condemnations in slaughter pigs. Front. Vet. Sci. 3, 1–7. https://doi.org/10.3389/fvets.2016.00024

Thacker, E. L., Minion, C., 2012. Mycoplasmosis. In: Diseases of Swine, 10a Ed., Zimmerman, J. J., Karriker, L. A., Ramirez, A., Schwartz, K. J., Stevenson, G. W. John Wiley & Sons, Ames, EUA, 779-797.

Villarreal, I., Maes, D., Meyns, T., Gebruers, F., Calus, D., Pasmans, F., Haesebrouck, F., 2009. Infection with a low virulent *Mycoplasma hyopneumoniae* isolate does not protect piglets against subsequent infection with a highly virulent *M. hyopneumoniae* isolate. Vaccine, 27, 12, 1875-1879.

Table 1. Score and extension of pulmonary consolidation lesions in each pulmonary lobe.

Scores	Extension of lobe lung lesions (% of affected lung area) (Madec & Kobisch, 1982)
0	Lobe without lesions
1	<25% of affected lung area
2	26 a 50% of affected lung area
3	51 a 75% of affected lung area
4	>76% of affected lung area

Table 2. Results of macroscopic lung lesions, qPCR and productive parameters.

Groups of lesions	**References values in % of lung consolidations**	**Pigs per group**	**Average of ADG (kg/day)**	**Average weight at the beginning of the finishing (kg/pig)**	**Average SQ values (copies/uL)**
1	0%	153	0.912	51.702	
2	0,1 - 5%	207	0.899	51.725	2.1×10^4
3	5,1 - 15%	96	0.898	51.516	2.4×10^4
4	> 15,1%	30	0.860	51.686	1.8×10^4

SQ: starting quantification value (copies/uL)

Table 3. Results of the economical analysis based on revenue, total variable costs and productivity parameters.

Groups of lesions	Revenue ($/group)	Total variable costs ($/group)	Economic profit ($/group)	Difference from baseline ($/group)
1	176.451	14.530	161.921	Baseline*
2	174.817	14.530	160.287	1.633
3	174.572	14.530	160.042	1.879
4	169.893	14.530	155.363	6.557

*****The baseline is the Group 1, animals without lesions.

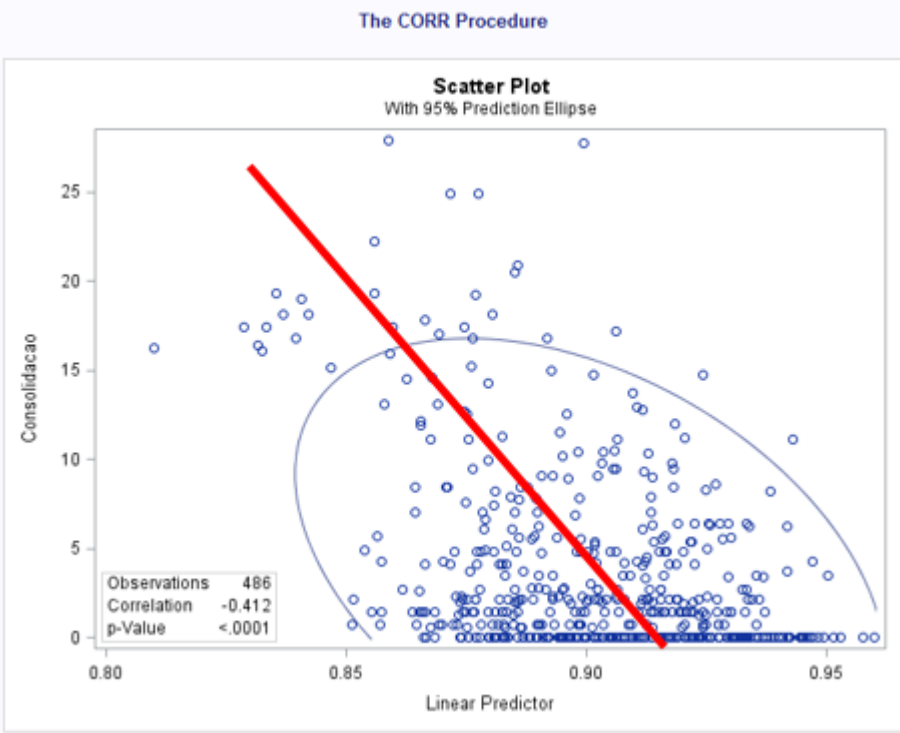

Figure 1. ADG obtained by group according to lung lesion area and respective 95% confidence interval.