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MIOPATIA DORSAL CRANIAL EM FRANGOS: INFLUÊNCIA DE CONDIÇÕES METEOROLÓGICAS, CLASSIFICAÇÃO E ASPECTO DA LESÃO DURANTE PROCESSAMENTO INDUSTRIAL

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MIOPATIA DORSAL CRANIAL EM FRANGOS: INFLUÊNCIA DE CONDIÇÕES METEOROLÓGICAS, CLASSIFICAÇÃO E ASPECTO DA LESÃO DURANTE PROCESSAMENTO INDUSTRIAL[1]

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RESUMO: A miopatia dorsal cranial (MDC) causa impacto econômico na indústria de frango de corte por tornar propenso o descarte da região diagnosticada com MDC pelo serviço de inspeção. O objetivo dessa dissertação foi determinar a prevalência de MDC em matadouro frigorífico localizado no Sul do Brasil, a fim de avaliar sua correlação com as condições meteorológicas da região e as estações do ano. Além disso, foi objeto do presente estudo a pesquisa de microrganismos de interesse em saúde pública na lesão, classificação macroscópica em escores (normal, leve, moderado e severo) e avaliação do aspecto da lesão nas diferentes etapas do processamento industrial de frango griller. Para determinar a prevalência da MDC foi realizada análise do banco de dados das condenações parciais do serviço oficial de inspeção correspondente ao período de fevereiro de 2012 a agosto de 2013, totalizando 157.991.485 aves abatidas. Os dados meteorológicos da região foram obtidos na base de dados virtual do Instituto Nacional de Meteorologia – INMET, do Ministério da Agricultura, Pecuária e Abastecimento (MAPA). As estações do ano que apresentaram maiores índices de condenações parciais por MDC foram outono e inverno, 0,46 e 0,47% respectivamente. O verão diferiu ($P<0,05$) das demais estações e apresentou o menor percentual de aves condenadas por MDC (0,18%). As análises revelaram correlação positiva de magnitude média entre o percentual de aves acometidas com MDC e a umidade relativa do ar (0,41). As temperaturas apresentaram correlação negativa, também de magnitude média para temperatura mínima, temperatura máxima e temperatura compensada. Não foram observadas diferenças nas análises microbiológicas qualitativas e quantitativas entre as carcaças acometidas com MDC e grupo controle ($P>0,05$). Na avaliação visual das carcaças, inclusive as de escore severo de MDC ao final do processamento industrial, após a carcaça passar pela etapa de congelamento, foi evidente a reversão aparente na lesão hemorrágica do músculo *Anterior latissimus dorsi,* tornando-se imperceptível a lesão de MDC. Com os resultados deste estudo conclui-se que os fatores ambientais, umidade relativa do ar e temperatura média da região, influenciam na prevalência da lesão de MDC nos frangos de aviários industriais e sugere-se que outras pesquisas sejam realizadas para determinar a interferência de outros fatores ambientais e para detectar alterações sensoriais no local da lesão após processamento industrial, já que visualmente elas desaparecem ao longo do mesmo.

Palavras chave: frangos de corte, miopatia, escores, condenações.

1Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (59 p.), Março, 2019.

CRANIAL DORSAL MIOPATHY IN BROILERS: INFLUENCE OF METEOROLOGICAL CONDITIONS, CLASSIFICATION AND APPEARANCE OF THE LESIONS DURING INDUSTRIAL PROCESSING[1]

Author: Fernanda do Prado
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ABSTRACT: Cranial dorsal myopathy (CDM) causes an economic impact on the broiler industry by making it prone to discard the region diagnosed with CDM by the official inspection service. The objective of this dissertation was to determine the prevalence of CDM of broilers in a slaughterhouse located in the South of Brazil, correlating them with the meteorological conditions of the region and the seasons. Also measurements of microorganisms of interest in public health in the lesion of CDM, macroscopic classification in scores (normal, mild, moderate and severe) and evaluation of the appearance of the lesions in the different stages of industrial processing of broilers griller type were done. To determine the prevalence of CDM, the database of partial condemnations of the official inspection service for the period from February 2012 to August 2013 was performed, totalizing 157,991,484 slaughtered broilers. The meteorological data of the region were obtained from the virtual database of the National Institute of Meteorology – INMET, of the Ministry of Agriculture, Livestock and Supply (MAPA). The seasons of the year that presented higher indices of partial condemnations by CDM were autumn and winter, 0.46 and 0.47% respectively. Summer differed ($P<0.05$) from the other seasons and presented the lowest percentage of birds condemned by CDM (0.18%). The analysis revealed a positive correlation, with statistical significance of mean magnitude, between the percentage of birds affected with CDM and the relative humidity of the air (0.41). The temperatures presented a negative correlation, with statistical significance of mean magnitude, for minimum temperature, maximum temperature and temperature compensated. No differences were observed in the qualitative and quantitative microbiological analysis between carcasses affected by CDM and control group ($P>0.05$). In the visual assessment of the carcasses, including those with a severe CDM score at the end of the industrial processing, the decrese on the hemorrhagic lesion of the *Anterior latissimus dorsi* muscle became evident after the carcass went through the freezing stage, making it imperceptible to CDM lesion. The results of this study conclude that the environmental factors, air relative humidity and mean temperature in the region influence the prevalence of CDM lesion in broilers from industrial farms, and is suggest that further research be done to determine the interference of other environmental factors and to detect sensory alterations at the lesion site after industrial processing, since they disappear visually throughout the process.

Key words: broiler, myopathy, scores, condemn.

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ALD	*Anterior latissimus dorsi*
ANVISA	Agência Nacional de Vigilância Sanitária
CDPA	Centro de Diagnóstico e Pesquisa em Patologia Aviária
DIF	Departamento de Inspeção Final
INMET	Instituto Nacional de Meteorologia
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
MDC	Miopatia Dorsal Cranial
OMS	Organização Mundial da Saúde
RIISPOA	Regulamento de Inspeção Industrial e Sanitária de Produtos de Origem Animal
SIGSIF	Sistema de Informação Gerenciais do Serviço de Inspeção Federal

CAPÍTULO I

1 INTRODUÇÃO

A indústria avícola é um setor de grande importância no mercado brasileiro e está concentrada principalmente na região sul do país. Em 2017, foram abatidos 5,84 bilhões de cabeças de frango, sendo que esta região foi responsável por 60,8% do abate nacional (IBGE, 2018). O frango inteiro, comercialmente chamado de “griller”, composto da carcaça inteira sem os miúdos, é um dos produtos mais vendidos para o mercado externo. É abatido com aproximadamente 32 dias de idade e deve apresentar no produto final peso em torno de 1 kg. Sendo assim, qualquer remoção de áreas da carcaça acometidas por alguma injúria, mesmo que apresente pequena alteração, impossibilita o fluxo normal de produção do frango inteiro, sendo o restante da carcaça destinado para produção de subprodutos ou cortes de menor valor agregado.

Os progressos em genética, sanidade, nutrição e manejo, nas últimas décadas, têm contribuído de forma acentuada para o aumento da eficiência na produção de frangos de corte. Tais progressos levaram a um aumento de peso e taxa de crescimento em curto espaço de tempo, em contrapartida, tem-se observado algumas características indesejadas nas aves, como as miopatias. Estas, podem acometer o peito e/ou dorso das aves, gerando condenações e descartes de produtos, representando perdas significativas em rendimento, valor agregado e resultado operacional nos frigoríficos.

Uma das miopatias observadas é Miopatia Dorsal Cranial (MDC), descrita como uma lesão degenerativa localizada na porção cranial do dorso de frangos de corte, acometendo o músculo *Anterior latissimus dorsi*, envolvido no movimento das asas. Em 2009 no Sul do Brasil, essa lesão foi a principal causa de condenações de carcaças no inverno e a segunda maior causa durante o ano, com taxas de condenações de até 6% (Zimermann, 2011). Há relatos que em 2016 a MDC esteve entre as principais causas de condenação total de carcaças na região nordeste do Brasil (Almeida et al., 2017). Supõem-se que essa lesão possa afetar os frangos de corte em todo o mundo, pois as características da indústria avícola são muito semelhantes nas regiões de produção industrial de aves, incluindo genética, nutrição e práticas de manejo (Zimermann et al., 2012). No entanto, a etiologia e os fatores que causam esse distúrbio muscular ainda não foram elucidados

Assim, os objetivos deste trabalho foram determinar a prevalência de MDC em matadouro frigorífico localizado no sul do Brasil e sua correlação com dados meteorológicos da região. Além disso, caracterizou-se microrganismos de interesse em saúde pública e foi realizada classificação macroscópica da lesão a fim de criar-se escores. O aspecto da lesão ao longo do processamento industrial também foi avaliado.

2 REVISÃO BIBLIOGRÁFICA

2.1 Condenações de carcaças por MDC

A intensa seleção genética das linhagens de frangos, com o propósito de abater aves com menor tempo de vida e maior peso de carcaça, tem contribuído para a ocorrência de comportamentos fisiológicos anormais, como as miopatias (Pedrotti et al., 2014). Estas causam alterações na musculatura, podendo acometer o peito e o dorso das aves. Em virtude de sua aparência e alterações de qualidade, geralmente são limitantes para a indústria de carne de aves, acarretando em prejuízos econômicos ao setor (Petracci et al., 2015; Baldi et al., 2018).

O Serviço de Inspeção Federal (SIF) é responsável por inspecionar e fiscalizar a qualidade da carne de frango em escala federal, atuando diretamente nos abatedouros frigoríficos para garantir a qualidade higiênico-sanitária (Oliveira *et al*., 2016) e registra rotineiramente as lesões ou doenças identificadas no Sistema de Informação Gerenciais do Serviço de Inspeção Federal (SIGSIF).

No aspecto geral, a Instrução Normativa nº 210/98, determina que qualquer órgão ou partes de carcaças que estiverem acometidas por processo inflamatório deverão ter condenação parcial, e quando apresentarem evidência de caráter sistêmico, carcaça e vísceras deverão ser condenadas totalmente.

Recentemente o decreto nº 9013/2017 de escala Federal, trata de maneira pontual sobre condenações de carcaças apresentando miopatias, o qual determina que quando as lesões forem restritas à uma parte da carcaça ou somente a um órgão, apenas as áreas atingidas devem ser condenadas; ou quando a lesão for extensa, múltipla ou houver evidência de caráter sistêmico, as carcaças e os órgãos devem ser condenados.

As carcaças e vísceras identificadas com lesões anormais ou suspeitas são retiradas da linha e encaminhadas ao Departamento de Inspeção Final (DIF), onde são julgadas e recebem o destino adequado, com o intuito de assegurar que a carne de frango e seus derivados cheguem ao consumidor sem apresentar riscos para à saúde (Freitas, 2015).

Segundo Roso e Dickel (2010) as equipes de inspeção têm detectado sob inspeção visual lesões compatíveis com miopatia dorsal cranial (MDC). Até maio de 2006 veterinários da inspeção do Ministério da Agricultura Pecuária e Abastecimento (MAPA) condenavam totalmente as carcaças acometidas. De acordo com Zimermann (2008), após este período, algumas carcaças eram condenadas parcialmente, onde eram realizados descartes do músculo afetado e adjacências, além de parte da asa e porção do peito de carcaças acometidas.

Desde outubro de 2012 tornou-se obrigatório o registro específico da miopatia dorsal cranial nos ábacos do serviço de inspeção em matadouros de aves do Rio Grande do Sul, porém nos demais estados brasileiros este registro ainda não é obrigatório. Existem dúvidas relacionadas à lesão de MDC, o que impossibilita a adoção de medidas seguras quanto ao destino correto das carcaças afetadas bem como a tomada de medidas para prevenção e controle deste problema (Zimermann, 2011).

2.2 Impactos econômicos das condenações por MDC

No ranking mundial, o Brasil é o maior exportador de carne de frango e o segundo maior produtor, atrás dos Estados Unidos da América, seguido pela União Europeia, China e Índia (ABPA, 2018). Diante da importância dessa produção, o relevante número de condenações têm provocado perdas econômicas significativas no setor (Ebling & Barsuco, 2016), similar observação foi feita por Groff et al., 2015, onde discorre que as condenações geram prejuízos aos produtores e frigoríficos, resultando em perdas de qualidade e produtividade de carne.

Um estudo realizado por Ebling & Barsuco (2016), com base nos registros do SIF/MAPA dos principais estados produtores de carne de frango do Brasil em 2011, Paraná, Santa Catarina, Rio Grande do Sul e São Paulo, demonstrou que as condenações parciais representam um valor de 2,40% e as condenações totais, 0,34% das aves abatidas. A MDC representou 0,4% das aves condenadas totalmente, impactando em prejuízo de pouco mais de 125 mil reais em 2011, montante dos quatro estados analisados. Estratificando este prejuízo entre os estados, o Rio Grande do Sul sofreu o maior impacto, com R$ 90.713,25, seguido de Santa Catarina, com R$ 23.632,20 de perda econômica devido à miopatia dorsal cranial (Ebling & Barsuco, 2016). Em pesquisa realizada em frigorífico de frangos localizado no Paraná, tendo como base de dados os relatórios mensais do SIF do ano de 2014, foi identificado um total de 1,01% de aves condenadas por miopatia dorsal cranial (Groff et al., 2015).

Segundo Zimermann et al. (2012) uma das principais causas de condenação parcial em frigoríficos de aves do sul do Brasil no inverno de 2009 foi a MDC, com percentuais médios de condenação de até 6% sobre o total de aves abatidas. Roso & Dickel (2010) avaliaram a prevalência desta miopatia e seu impacto econômico em matadouro de aves localizado no Rio Grande do Sul nos meses de abril a novembro de 2010. As condenações totais por MDC no período avaliado foram de 0,083%, representando uma perda de quase 16 mil reais no período. Para as análises das perdas por condenação parcial, os autores consideraram perda de 0,4 kg de carne para cada ave condenada por MDC no DIF e custo de produção de R$ 2,07. Somando os meses de estudo a condenação parcial gerou uma perda econômica em torno de 143 mil reais, totalizando quase 70 mil kg de asas descartadas. Os autores demonstraram que as perdas econômicas por MDC são mais proeminentes nos meses de inverno. Julho e agosto foram os meses com maiores percentuais de aves condenadas parcialmente por MDC, com 0,67% e 0,69% respectivamente. (Roso & Dickel, 2010).

Almeida et al. (2017) ao estudarem as causas de condenações de carcaças na região Nordeste do Brasil citam que a MDC esteve entre as principais causas de condenações totais de carcaças no ano de 2016, representando 1,25% do total de condenações totais. Corroborando com o descrito por Pedrotti et al. (2014), onde descreve que esta enfermidade gera condenações parciais e totais das carcaças, causando sérios prejuízos econômicos às indústrias avícolas. Os casos de condenação total ocorrem quando além de lesão no músculo *Anterior latissimus dorsi* é detectado edema difuso no tecido subcutâneo (Zimermann et al., 2011). No entanto, não foram

encontrados na literatura dados que abordem a influência das diferentes estações climáticas sobre o desenvolvimento da MDC.

2.3 Possíveis etiologias da MDC

A miopatia dorsal cranial é uma patologia que apresenta etiologia ainda desconhecida (Zimermann, 2008). Acredita-se que fatores genéticos, miopatia por exercício e deficiência de vitamina E e selênio podem estar contribuindo no desenvolvimento da enfermidade (Nesello et al., 2011). Estudos demonstram que a prevalência de MDC sofre influência das estações do ano, porém não há dados na literatura com segurança estatística sobre esta influência.

2.3.1 Fatores genéticos

Os ciclos de criação na avicultura são extremamente curtos quando comparados aos demais ciclos de outras espécies de produção de carne, o que possibilitou a rápida evolução genética nas últimas décadas, com especial enfoque no desenvolvimento muscular e ganho de peso (Zimermann 2008). Estudos relatam que aves mais pesadas associadas com as taxas de crescimento rápido em frangos de corte comerciais obtiveram aumento da incidência de miopatias peitorais e relacionam o peso das aves como um dos fatores predisponentes para o desenvolvimento de miopatia (Bauermeister et al., 2009; Kuttappan et al., 2013).

As novas linhagens comerciais apresentam alguns músculos formados por até o dobro do número de fibras musculares quando comparados às linhagens tradicionais. Estudos realizados comparando linhagens de frangos de corte evidenciaram que frangos de crescimento rápido possuem até 20% a mais, em número de fibras no músculo *Anterior latissimus dorsi*, assim como um aumento no tamanho das fibras (área transversal) quando comparados aos frangos de crescimento mais lento (Remignon et al., 1995; Scheuermann, 2008). As condições sedentárias das aves associadas ao aumento da massa muscular levam à diminuição significativa do gradiente de pressão arteriovenosa e à consequente diminuição do fluxo sanguíneo capilar, comprometendo assim o fornecimento de nutrientes e a eliminação dos metabólitos produzidos pelas fibras musculares, como o dióxido de carbono e o lactato. A deficiência na eliminação dos metabólitos induz a distúrbios iônicos, como a regulação do cálcio necessário à contração muscular, tendo como consequência o aparecimento de processos inflamatórios, necróticos e miopatias (Sosnick,1993).

2.3.2 MDC por exercício

O músculo *Anterior latissímus dorsi*, é um músculo superficial localizado na região dorsal dos frangos sendo responsável pelos movimentos do úmero durante a contração dos músculos do vôo, além de tracionar a asa caudalmente, flexionando e elevando o úmero (Zimermann, 2008).

Devido suas funções e localização, acreditava-se que o bater das asas excessivo por parte de algumas aves poderia ser uma das hipóteses para o desenvolvimento da MCD. Neste sentido, Giacomin et al. (2011) na tentativa de elucidar a causa da MDC submeteram frangos de corte a exercícios semanais (bater de asas) desde a primeira até a última semana de alojamento, totalizando seis semanas. Os resultados indicaram que as aves submetidas ao exercício não apresentaram um aumento de condenação por MDC, mas sim um aumento de condenação por miopatia peitoral profunda (Giacomin et al., 2011). Nesse caso, apesar de ser uma lesão induzida por exercício, sua origem é isquêmica, onde a miopatia desenvolve-se após exercício e ausência de espaço físico para adequada expansão, ocorrendo com maior frequência nas linhagens mais pesadas (Bianchi et al., 2006)

2.3.3 Deficiência de vitamina E e selênio

A lesão de MDC é localizada no músculo *Latissimus dorsi*, que apresenta na porção anterior fibras do tipo I, de contração lenta e na porção posterior fibras do tipo II, de contração rápida. Essas diferenças conferem às fibras musculares maior ou menor suscetibilidade a certas injúrias. As fibras brancas são mais sensíveis à deficiência de vitamina E e mais predispostas a injurias decorrentes de atividade muscular se comparadas às fibras vermelhas (Klasing, 2008).

O selênio tem um número de papéis biológicos incluindo regulação da atividade de Glutationa Peroxidase (GSH-Px), hormônios da tireóide e prostaglandinas, aprimoramento do papel da vitamina E e melhoria das funções imunológicas (Surai, 2002). O selênio (Se) e a vitamina E são componentes antioxidantes frequentemente utilizados como suplementos em ração de frango de corte (Roman et al., 2011). A associação desses dois componentes nas rações previne o aparecimento de miopatia, distrofia muscular, bem como diátese exudativa nas aves. Isso acontece devido à ação protetora desses nutrientes sobre as membranas celulares no sentido de evitar a ocorrência da lesão peroxidativa (Avanzo et al., 2001).

Zimermann (2011) experimentalmente forneceu dieta para frangos de cortes sem inclusão de vitamina E e selênio na tentativa de reproduzir a lesão de MDC. Concluiu que estatisticamente não há diferença na proporção da presença de lesão em aves com ou sem suplementação. A autora quantificou vitamina E e selênio em músculos com e sem lesão e demonstrou que os níveis de vitamina E e selênio em carcaças que experimentalmente não foram suplementadas são compatíveis com os níveis de carcaças usualmente suplementadas.

2.4 Aspectos macroscópicos das lesões de MDC

Segundo Fallavena (2009), lesões que afetam a pele, desencadeadas por diferentes causas, podem ser semelhantes entre si, caracterizando-se por aumento da espessura, alterações na coloração e na superfície da pele. Este fato dificulta o diagnóstico macroscópico destas doenças, que fica sujeito a erros pelos técnicos que realizam a inspeção nos matadouros. A MDC torna-se visível nas carcaças após o processo de depenagem sendo identificada na linha de inspeção devido alteração no aspecto da pele que recobre o músculo *Anterior*

latissimus dorsi (Figura 01), onde é observado aumento no volume subcutâneo e coloração amarelada da pele quando comparadas com carcaças sem lesão (Zimermann, 2008).

Quando a pele da carcaça afetada é removida, a lesão se caracteriza por edema subcutâneo, hemorragia muscular superficial, palidez (Figura 02), aderência, aumento da espessura e consistência, características que causam aspecto repugnante, não sendo identificada presença de odor atípico (Zimermann et al., 2012).

Por vezes, a única alteração observada na lesão de MDC é o aumento de volume no tecido subcutâneo devido à formação de exsudato. Mesmo assim a carcaça é removida na linha do Departamento de Inspeção Final, ocasionando descarte da área afetada e muitas vezes parte do peito e asas. Os critérios adequados para condenação das carcaças devem ser estabelecidos porque em algumas plantas as carcaças afetadas por MDC são descartadas erroneamente como artrite ou abscesso (Zimermann et al*.,* 2012).

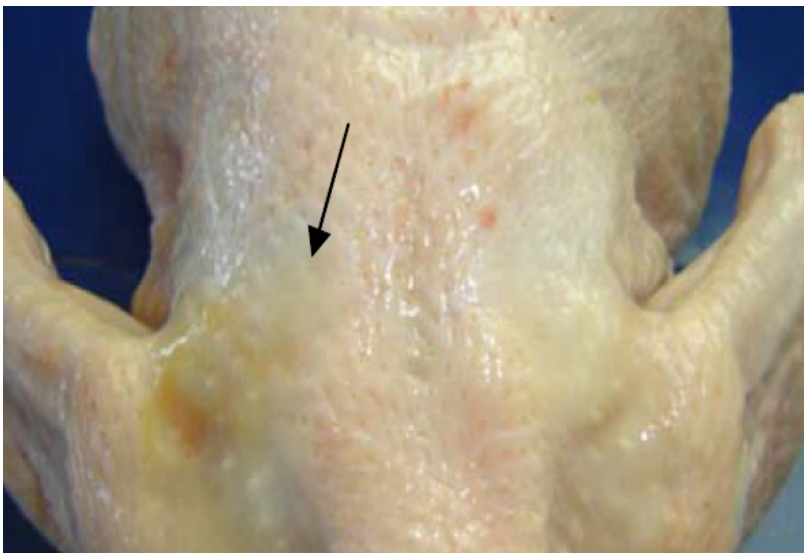

FIGURA 1: Lesão de miopatia dorsal cranial em frango de corte – aumento de volume subcutâneo e coloração amarelada da pele (Fonte: Zimermann, 2008).

FIGURA 2: Lesão de miopatia dorsal cranial após remoção da pele da região afetada – presença de edema subcutâneo, hemorragia muscular e palidez (Fonte: Zimermann, 2011).

Provin et al. (2014), ao estudar o perfil hematológico de frangos de corte acometidos pela MDC, concluiram que a mesma não compromete a saúde sistêmica do frango e afirmou que o processo inflamatório é localizado e não sistêmico.

Nesello et al. (2011) avaliaram o aspecto, a cor e a consistência do músculo *Anterior latíssimus dorsi* durante exame macroscópico em experimento realizado em aviários com elevada prevalência de MDC em 2009. E concluiram que aves com mais de 20 dias podem apresentar as mesmas características de lesões, como palidez com áreas esverdeadas, áreas hemorrágicas e por vezes aumento de volume e de consistência no músculo.

2.5 Aspectos microbiológicos da lesão de MDC

A carga microbiana das carcaças de frangos são representadas por uma microbiota oriunda, principalmente, das aves vivas ou incorporadas em qualquer uma das fases do abate (Almeida & Silva, 1992). A maioria desses agentes não são patogênicos, porém, microorganismos como *Salmonella sp., Campylobacter jejuni* e *Listeria monocytogenes* podem estar presentes, representando um risco potencial à saúde do consumidor (Silva, 1998). Segundo Boulos (1999) algumas bactérias patogênicas se destacam na maioria das infecções e toxiinfecções alimentares, apresentando maior interesse em saúde publica. Entre elas o autor cita *Listeria monocitogenes, Salmonella sp.*, *Escherichia coli*, Clostrídio Sulfito Redutor e Estafilococos coagulase positivo, como principais microorganismos que causam preocupação em relação às carnes cruas e processadas e em ambientes de plantas avícolas.

Tem-se pesquisado a presença de microorganismos em carcaças acometidas por miopatia dorsal cranial (MDC), visto que a musculatura afetada apresenta acúmulo de exsudato subcutâneo, que pode proporcionar um ótimo meio para o desenvolvimento de agentes bacterianos, justificando a importância

de pesquisas de agentes bacterianos com potencial risco à saúde pública no tecido muscular lesado (Zimermann, 2011).

Medina et al. (2010) pesquisaram agentes bacterianos de interesse em saúde pública (*Salmonella* spp*., Campylobacter jejuni subsp. jejuni, C.coli, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli,Yersinia enterocolitica e Pasteurella multocida*) em músculo afetado por MDC. De vinte amostras analisadas, apenas em uma delas foi isolado *Staphylococcus sp. não-hemolítico*. Nas demais amostras as bactérias pesquisadas não foram isoladas. Zimermann (2011) pesquisou a presença de bactérias de interesse em saúde pública no músculo *Anterior latissimus dorsi* com MDC, onde foi isolado *Staphylococcus sp.* a partir de uma das amostras analisadas. O autor pressupõe que o agente tenha sido originado de uma contaminação cruzada da pele para o músculo no momento da abertura da pele, salientando ainda que a proximidade do músculo com a pele facilita a contaminação cruzada. Este estudo sugere que não há riscos de intoxicação pelo consumo do músculo afetado com relação às bactérias *Sthaphylococcus aureus, Escherichia coli*, *Salmonella enteritidis* Enteritidis, *Listeria monocytogenes*, *Pasteurella multocida, Yersinia enterocolitica*, *Campylobacter coli* e *Campylobacter jejuni* subsp. *Jejuni* (Zimermann, 2011).

3 HIPÓTESES

H1: A prevalência de miopatia dorsal cranial sofre influência de fatores climáticos.

H2: Existem diferentes aspectos macroscópicos nas lesões de miopatia dorsal cranial o que possibilita a classificação da lesão em diferentes graus.

H3: A lesão de MCD não apresenta envolvimento de agentes relacionados com problemas de saúde pública.

H4: Durante o processamento industrial, o aumento de volume e exsudato observado na lesão de MDC regridem.

4 OBJETIVOS

4.1 Objetivo geral

Avaliar fatores relacionados à ocorrência da miopatia dorsal cranial em frangos tipo griller.

4.2 Objetivos específicos

- Correlacionar a prevalência de miopatia dorsal cranial com as variáveis meteorológicas de temperatura máxima, temperatura mínima, temperatura média compesada, umidade relativa do ar, insolação e nebulosidade.

- Avaliar microbiologicamente a presença de bactérias relacionadas à saúde pública no músculo *Anterior latissimus dors*i de aves com MDC.

- Classificar macroscopicamente a lesão de MDC e criar diferentes escores.

- Avaliar a evolução das lesões de MDC nas diferentes etapas do processamento industrial de frango tipo griller.

CAPÍTULO II

Artigo elaborado conforme as normas do periódico Poultry Science (Apêndice).

HEALTH AND DISEASE

Cranial dorsal miopathy in broilers: influence of meteorological conditions, classification and appearance of the lesions during industrial processing[1]

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ABSTRACT

The objective of was study to evaluate environmental factors related to the occurrence of cranial dorsal myopathy (CDM). Was to determine the prevalence of CDM of broilers in a slaughterhouse located in the South Brazil, correlating them with the meteorological conditions of the region and the seasons. Also measurements of microorganisms of interest in public health in the lesion of CDM, macroscopic classification in scores (normal, mild, moderate and severe) and evaluation of the appearance of the lesion in the different stages of industrial processing of broilers griller type were done. To determine the prevalence of CDM, the database of partial condemnations of the official inspection service for the period from February 2012 to August 2013 was performed. The meteorological factors of the region were obtained from the virtual database of the National Institute of Meteorology - INMET, of the Ministry of Agriculture, Livestock and Supply (MAPA). The seasons of the year that presented higher indices of partial condemnations by CDM were autumn and winter, 0.46 and 0.47% respectively. Summer differed ($P<0.005$) from the other seasons and presented the lowest percentage of birds condemned by CDM (0.18%). The analysis revealed a positive correlation, of mean magnitude, between the percentage of birds affected with CDM and the relative humidity of the air (0.417). Minimum, maximum and compensated temperatures presented a negative correlation, also of mean magnitude. No differences were observed in the qualitative and quantitative microbiological analysis between carcasses affected by CDM and the control group ($P>0.05$). In the visual assessment of the carcasses, including those with a severe MDC score at the end of the industrial processing, the regression on the hemorrhagic lesion of the *Anterior latissimus dorsi* muscle became evident after the carcass went through the freezing stage, making it imperceptible to CDM lesion. The results of this study conclude that the environmental factors, air relative humidity and mean temperature in the region influence the prevalence of MDC lesion in industrial farms, and we suggest that further research be done to determine the interference of other environmental factors and to detect sensory alterations at the lesion site after industrial processing, since they disappear visually throughout the process.

Key words: broiler, myopathy, scores, condemn.

INTRODUCTION

The poultry industry is a very important sector of the Brazilian market and is mainly concentrated in the southern region of the country. In 2017, 5.84 billion of chicken were slaughtered, where south region accounted for 60.8% of the national slaughter (IBGE, 2018). This region of Brazil is characterized by different seasons of the year, with great variations of temperature, because is the coldest region of the country, where, during the winter, frost and even snow occur in some places (Embrapa, 2018).

In winter, it seems there is an increase in the incidence of carcasses with cranial dorsal myopathy (CDM**),** causing heavy economic losses due to carcass downgrading. In 2009, this muscular lesion was found to be the main cause of downgrading in winter and the second cause in the entire year, with rates of up to 6%. Independently of the climate, a study carried out in a company in the South of Brazil reported that 0.55% of broilers processed in 2002 were downgraded monthly due to the presence CDM (Zimermann et al., 2012).

According to the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) regulations (Brasil, 2010), broiler carcasses presenting DCM must be downgraded or condemned. In general, carcasses with CDM are trimmed, with the removal of the skin (often from the neck to the cloaca), the affected and adjacent muscles, as well as the humerus and part of the thoracic muscles. Carcasses presenting extensive lesions that compromise their overall visual quality are totally condemned (Amaral, 2014).

It is possible to assume that this dorsal lesion may affect broilers worldwide, because the characteristics of the poultry industry are very similar in industrial poultry production regions, including broiler genetics, nutrition, and management practices (Zimermann et al., 2012). However, the etiology and other factors that cause this muscular disorder have not yet been elucidated.

Thus, the objectives of this research were to determine the prevalence of CDM in a slaughterhouse located in southern Brazil and its relationship with the regional meteorological data, in order to evaluate the correlation between the weather conditions and the percentages of CDM in the different seasons of the year. In addition, the study intended to analyze the presence of public health related microorganisms and to determine macroscopic aspect of the lesion, by grading the lesions through a score system scores, in the different stages of the industrial broilers griller type processing.

MATERIALS AND METHODS

The present study was carried out in a slaughterhouse of broiler griller type, under Federal inspection, located in the south of Brazil. The birds were approximately 30 days old, males and females, Cobb 500 strain. The data were adjusted considering the period of rearing. To determine the prevalence monthly of cranial dorsal myopathy, the database of partial convictions of the official inspection service for the period from February 2012 to August 2013 was analyzed.

The meteorological parameters of relative air humidity (%), insolation, cloudiness, average monthly compensated temperature, minimum and maximum monthly mean temperature were obtained from the Climatological Station of the city of Campos Novos, Santa Catarina State (Latitude: -27.38 S; Longitude: 51.2 W, Altitude: 946.67m), obtained from the virtual database of the National Institute of Meteorology (INMET) of the Ministry of Agriculture, Livestock and Supply (MAPA).

Microbiological Analysis

Carcasses diagnosed with CDM by the Official Inspection Service and without myopathy (control group) were collected in the slaughter line. A total of 120 carcasses were collected with CDM (40 pools) and 60 carcasses without CDM (20 pools). The carcasses were placed in a sterile polyethylene bag, placed in isothermal containers at a temperature of 4 °C and sent to

the microbiology laboratory linked to the industrial unit. Samples were collected, using three pooled carcasses, obtaining one amount of 25 g (±0.2 g) composed of the injured muscle and its adjacent ones.

Qualitative analysis were realized for *Salmonella sp*, *Listeria monocytogenes* and *Campylobacter sp*. and quantitative analysis for *Total coliforms*, *Staphylococcus aureus*, and *Escherichia coli* were carried out. The samples collected for analysis followed the methodology of Normative Instruction 62/2003 of the Ministry of Agriculture, Livestock and Supply - MAPA (Brasil, 2003).

Lesion Classification

The scores were determined in the time when the Official Inspection Service detected and deviated the CDM carcasses, according to the macroscopic changes observed in the cranial portion of the dorsum of the birds without and with removal of the skin. The appearance of the skin and subcutaneous volume was considered in the evaluation; presence of exudate in the lesion and its color; presence of bleeding in the *Anterior latissimus dorsi* (ALD) muscle and odor. Four lesion scores were determined: normal score (carcasses without apparent macroscopic changes), mild, moderate and severe score.

Macroscopic Lesion in the Industrial Processing Steps

In determine aspecto of the CDM lesions, ten deviated carcasses presenting diferentes scores CDM were identified with a seal and placed back in the processing line, and went through the pre-cooling stage, a two-stage operation - pre chiller and chiller - made in stainless steel tanks using water immersion and a continuous an endless screw system. The chiller process took about one hour, throughout the process the tanks were fed with ice. The water supply was constant and in the opposite direction to the movement of the carcasses (Brasil, 1998). Afterwards, the carcasses were suspended in the noria, classified and packaged. Afterwards, they went to a freezing tunnel until they reached the temperature of -18ºC. A

macroscopic appearance and photographic record of the lesion were performed after each stage of the processing and after thawing.

Statistical Analysis

For statistical analysis, Minitab software, version 16 was used. Initially the values were submitted to basic descriptive statistical analysis, to verify the normality of data distribution. Once the normality was confirmed, the F test was performed through analysis of variance (ANOVA). If the F statistic was significant, treatment means were separated by Tukey's test.

For the correlation analysis, the Spearman correlation coefficient was used, which measures the association degree of the variables. As a classification of the degree of correlation, the following parameter was used: values between 0.10 and 0.29 were considered low; scores between 0.30 and 0.49 considered as mean; and values between 0.50 and 1 interpreted as high. Correlations with statistical significance were those that presented $P \leq 0.05$ and degree of medium or high correlation (Cohen, 1988).

RESULTS AND DISCUSSION

Prevalence of CDM and Meteorological Data

The prevalence of cranial dorsal myopathy in the different seasons is shown in Figure 3. The seasons of the year that presented the highest rates of partial condemnations by CDM were fall and winter (0.46 and 0.47%, respectively). The summer differed significantly ($P < 0.05$) from the other seasons and presented the lowest percentage of birds condemned (0.18%). In 2012, in the autumn (march, april and may) and winter months (june, july and august), the third quartile presented a mean of 0.53 and 0.55% respectively, and in 2013 this value was higher, presenting mean values of 0.86 and 0.93% in the condemnations by CDM (Figure 3). These results corroborate with the observed by Roso and Dickel (2010), who evaluated the prevalence of CDM in the condemns of broilers slaughterhouse located in Rio Grande do Sul (Brazil), where they observed greater percentages of condemnations in the months of autumn and

winter, emphasizing months of July and August with 0.67 and 0.69% of partial condemnation. A gradual increase in carcass downgrading and condemnations due to DCM has been detected, particularly in winter, when its incidence may range from 1% (Amaral, 2014) to 6% (Zimermann et al., 2012).

Oliveira et al. (2016) did not mention any of the main causes of condemnation in broilers slaughter in Brazil from 2006 to 2011, considering the central database of the Federal Inspection Service's Management Information System (SIGSIF). This is due to the fact that it was only in October 2012 that the specific registration of cranial dorsal myopathy in the notes of the inspection service in bird slaughterhouses in Rio Grande do Sul was made compulsory. In other Brazilian states this registration is not yet mandatory (Zimermann, 2011).

According to Amaral (2014) CDM usually affects apparently healthy heavy broilers, in agreement with this study, when was observed correlation of mean magnitude between the percentage of birds affected with CDM and weight (Table 1). Authors report that heavier birds associated with rapid growth rates in commercial broilers obtained an increase in the incidence of pectoral myopathy (Bauermeister et al., 2009; Kuttappan et al., 2013). Also, Table 1 shows the correlation between the meteorological variables and the percentages of CDM presence. The analyses revealed a positive and significant correlation of mean magnitude between the percentage of birds affected with CDM and the relative humidity of the air (0.41). The temperatures presented a negative and significative correlation of mean magnitude for minimum temperature, maximum temperature and compensated temperature. It is observed that the other variables studied low correlation with the percentage of CDM.

In autumn and winter, lower values of average compensated temperature and higher values of relative air humidity were observed (Table 2). Relative humidity is a variable that is closely related to the temperature of the environment due to the effects on the birds heat losses: the higher the humidity, the lower the heat losses by the broilers. Another aspect to consider is

the higher the humidity the greater the thermal sensation, both for cold, if there is wind current, and for heat, in an environment without air ventilation (Donald, 1998). Also, relative humidity above 70% worsens litter quality and favors bacterial development, increasing sanitary challenges (Miragliotta, 2005).

According to Carvalho (2010), local meteorological conditions, such as temperature and relative air humidity, associated with management practices can influence the rates of ammonia production in the environment. High concentrations of ammonia may adversely affect feed conversion, carcass quality and bird mortality. The climatic conditions are those that most directly affect the birds, since they compromise the maintenance of optimal body temperature, which is a vital caracteristic (Oliveira et al., 2006).

In the southem Brazilian conditions, in winter some farmers reduce ventilation to save energy and keep a warm environment and this can increase ammonia and decrease oxygen levels inside the building. Meanwhile, burning wood or using natural gas to provide supplemental heat, consumes oxygen (Czarick and Fairchild, 2002).

In the commercial chicken's context, where the high growth rate increases the metabolic oxygen requirement (Geyikoglu et al., 2005) and the oxygen supply capacity is reduced, since the cardio-respiratory apparatus became relatively small (Gonzales and Macari, 2000), the lack of atmospheric oxygen can have a negative effect of great impact. Oxygen concentration in the ambient air is approximately 21%. Once oxygen concentration decreases to 17%, the resulting faster and deeper breathing reduces broiler's performance, and lower levels of oxygen can threaten the bird's life (Czarick and Fairchild, 2002). In the poultry house, excessive ammonia concentrations can inflame the bird's cornea, burn the skin, and erode its tracheal lining which can lead to respiratory diseases, decreased weight gain, and high mortality (COBB, 2008).

According to MacRae et al. (2006), increased muscle fiber and inadequate capillary supply may induce metabolic stress due to the great distance for oxygen diffusion, metabolites

and waste disposal. Besch and Kadono (1978), reported that chicken was not able to fully oxygenate their hemoglobin below 15% oxygen concentration in the air. Restoring oxygen to acceptable levels can be achieved by increasing ventilation rate. But excessive ventilation will increase the energy usage and pollutant gas emissions.

We believe that the low temperatures associated with higher relative humidity in the autumn and winter months, associated with higher concentrations of ammonia and low oxygen availability for birds, contributes to the increase in the prevalence of cranial dorsal myopathy.

Microbiological Analysis

No differences were observed in the qualitative and quantitative microbiological analyzes between carcasses affected by CDM and the control group ($P>0.05$). Table 3 shows the results of the quantitative analyzes of *Total coliforms*, *Staphylococcus aureus* and *Escherichia coli*. These values meet the requirements of Resolution of the Board - RDC N^{o}. 12/2001 for chilled or frozen meat "in natura" of broilers.

For the qualitative research of *Salmonella sp*., *Listeria monocytogenes* and *Campylobacter sp*, all the samples showed absence of these microorganisms, in compliance with the Brazilian legislation, which demands absence as a reference value for broilers cooled or frozen, in 25g of sample (RDC n 12/2001).

The results obtained in the present study corroborate those found by Medina et al. (2010), where *Salmonella spp*., *Campylobacter jejuni subsp. jejuni*, *Campylobacter coli*, *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Yersinia enterocolitica* and *Pasteurella multocida* in muscle affected by CDM of twenty samples analyzed were not isolated. In only one of the samples was isolated Staphylococcus sp. non-hemolytic. Also Zimermann (2011) concluded that there is no risk of intoxication through the intake of the lesioned *Anterior latissimus dorsi* (ALD) muscle in relation to bacterias of public health interest. According to Amaral (2017) broilers presenting dorsal cranial myopathy did not show

any abnormal hematological, biochemical or muscle pH values, indicating that the presence of this lesion does not affect their overall health status, and therefore, any health risks for consumers.

Lesion Classification

The carcasses selected to define the different CDM scores presented macroscopic changes in the appearance of the skin covering the ALD muscle. This portion of the skin presented a yellowish color, sometimes greenish, with an increase in the subcutaneous volume when compared to the normal carcass. There was no presence of atypical odor in carcasses affected by CDM, corroborating with Zimermann study from 2012. After removal of the skin from the dorsum it was possible to observe the presence of citrus yellow exudate and gelatinous appearance near the ALD muscle. The ALD muscle in general presented an increase in volume and hemorrhagic areas involving its inferior surface and the surface of the *Rhomboideus superficialis* muscle, which is located below the ALD.

The musculature adjacent to the anterior latissimus dorsi muscle was dissected and it was observed that the CDM lesion is well located, generally not affecting the wings of the birds. The results of the present study differ from those reported by Roso and Dickel (2010), where the authors affirm that the wing is the main part reached by CDM.

The different CDM scores were established based on these macroscopic characteristics; considered normal score the carcass without apparent lesion of CDM. The lesion was classified as mild, showing a slightly yellowish skin color in the cranial region of the dorsum (Fig. 4 - A) and in the internal aspect, exudate with a citrus yellow coloration, ALD without hemorrhage (Figure 4 - B). The lesion classified as moderate had external appearance, increased volume in the region between the wing and the neck, skin with yellowish color (Figure 4 - C) and in the internal aspect, a large amount of exudate with citrus yellow color attached to the skin and between the wing and neck region (Figure 4 - D). The lesion classified as severe presented on

the external aspect, increased volume in the region between the wing and the neck, skin with yellowish green color, areas with dark coloration in the region humerals, due to the presence of hemorrhage in the ALD muscle (Figure 4 - E). In the internal aspect, a large amount of exudate with citrus yellow or greenish coloration, superficial hemorrhages in the ALD muscle and thickness increment of the ALD muscle (Figure 4 - F).

CDM in the Industrial Processing Steps

The images of Figure 03 represent the lesion aspect of CDM classified as "severe" in the different stages of the industrial processing of frozen broiler griller. At the beginning of the process, there is an increase in volume and a greenish appearance on the skin that covers the back (Figure 5 - A). After rebounding the skin, the muscle with hemorrhage and presence of exudate of greenish color (Figure 5 - B) is noted. After the carcass passes through the immersion tank in ice water (pre-chiller stage) (Figure 5 - C) a reduction in the musculature hemorrhage and decrease of the exudate is observed. At the end of the cooling phase (chiller) the exudate is no longer observed and there is a regression in the hemorrhagic aspect of the ALD muscle (Figure 5 - D). At the end of industrial processing (Figure 5 - E), after the carcass went through the packing and freezing stage, regression on the hemorrhagic lesion of the *Anterior latissimus dorsi* muscle becomes evident, turning into imperceptible the CDM lesion, even after the product is thawed (Figure 5 - F).

Considering these observations, we believe that it is possible to maintain part in the carcass, since the lesion regression was evidenced and there are no implications for the public health.

CONCLUSIONS AND APPLICATIONS

It is concluded that relative air humidity and average temperature of the region, influence the prevalence of cranial dorsal myopathy lesion in industrial broiler production system. Was also concluded that there are no risks of the presence of public health microorganisms.

The macroscopic appearance of the cranial dorsal myopathy lesions regresses after the carcass goes through all steps of the industrial broiler slaughter processing, suggesting that the lesions may become imperceptible at the end of industrial processing.

It's suggest that further research should be conducted to determine the interference of other environmental factors, especially regarding air quality in the prevalence of the lesion and to study sensorial changes at the lesion site after industrial processing.

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Table 1. Correlation between meteorological data and percentage of cranial dorsal myopathy from a industrial plant from South Brazil

	Weight	T.Min.	T.Max.	T. Comp	RH.	Insolation	Cloudiness
Broilers CDM[1], %	0.47	-0.30	-0.34	-0.32	0.41	-0.23	-0.050
P – value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0035

T. Min.= Average monthy minimal temperature, T. Max.= Average monthy maximal temperature, T. Comp.= Average compensated temperature, RH.= Relative air humidity, [1] Cranial dorsal myophathy.

Table 2. Incidence of cranial dorsal myophathy (CDM) in broilers slaughtered in an industrial plant from South Brazil, in two years and by season and meteorological data.

Item	CDM, %	Weight, g.	T.comp. °C	RH, %	Insolation, h.	Cloudiness, d.
Year						
2012	0.30[b]	1413,0[b]	16.95	75.40[b]	209.29[a]	5.11
2013	0.43[a]	1435,0[a]	16.99	80.76[a]	189.67[b]	5.49
Season						
Summer	0.18[c]	1406,0[b]	20.40[a]	72.60[c]	238.35[a]	5.34
Autumn	0.46[a]	1446,0[a]	18.34[b]	79.36[b]	203.69[b]	5.10
Winter	0.47[a]	1413,6[b]	12.50[c]	83.35[a]	170.40[b]	5.39
[1]Spring	0.36[b]	1426,0[b]	16.31[b]	73.25[c]	149.75[bc]	5.26
Mean	0.39	1423,8	16.97	77.70	200.89	5.26
SEM	0.0017	0,077	1.27	3.34	34.20	0.88
Prob						
Year	<0.0001	<0,0001	0.9017	<0,0001	0,0386	0.0812
Season	<0.0001	<0,0001	<0.0001	<0.0001	<0.0001	0.6931
Year *vs* Season	0.5456	<0,0001	0.0618	0.6523	0.4401	0.8753

T.comp.= Average compensated temperature, RH=Humidity, h = hours d= tenths [a>b>c>d] Means with different subscripts in the same column are significant different, by Tukey test, 5%.

[1] Data from spring are just from year 2012.

Table 3. Quantitative microbiological analyzes in muscle ALD.

Microorganism	Without MDC	With MDC	P value
Total coliforms	7.8×10^3 CFU/g	8.8×10^2 CFU/g	0.19
Staphylococcus aureus	1.5×10^2 CFU/g	5×10^1 CFU/g	0.45
Escherichia coli	6.4×10^3 CFU/g	3.3×10^2 CFU/g	0.35

CFU= Colony Forming Units

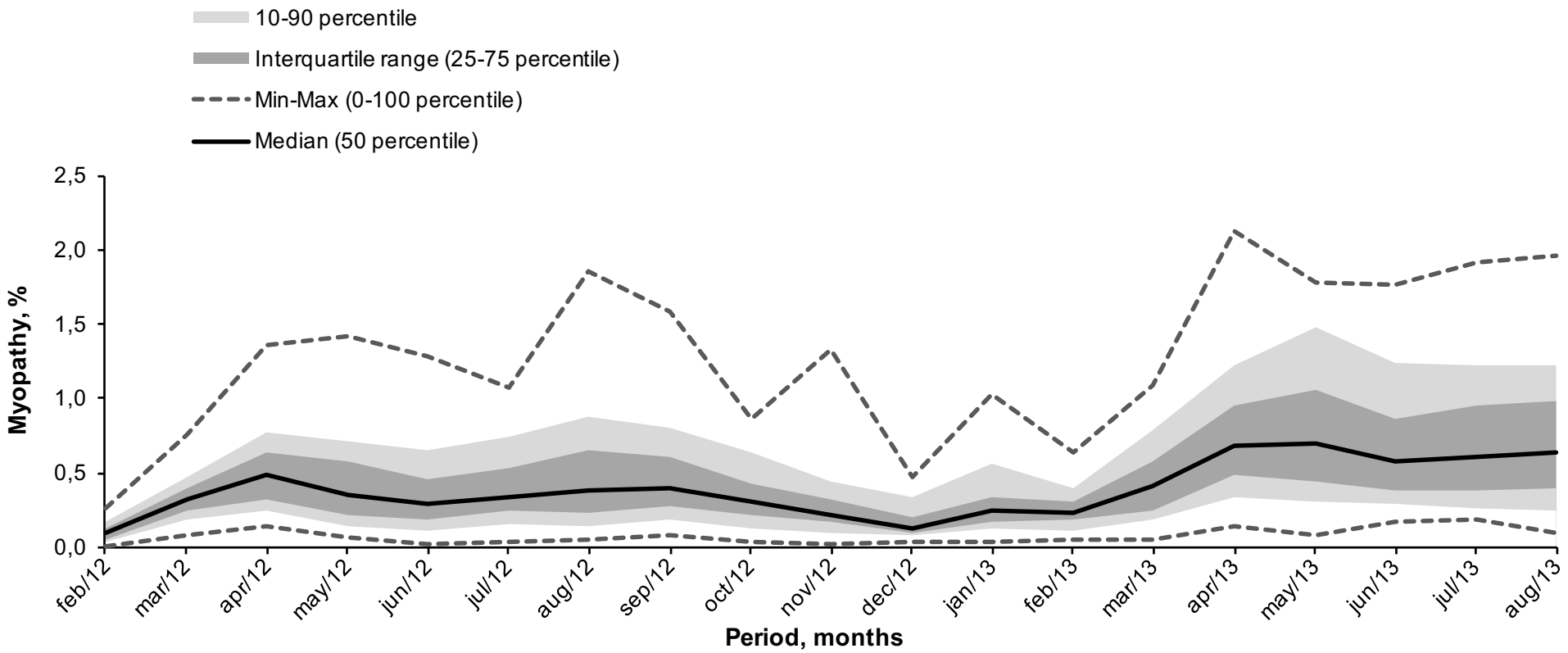

Figure 3. Prevalence of montly cranial dorsal myophaty, in a industrial plant from South Brazil in the period of February 2012 until August 2013.

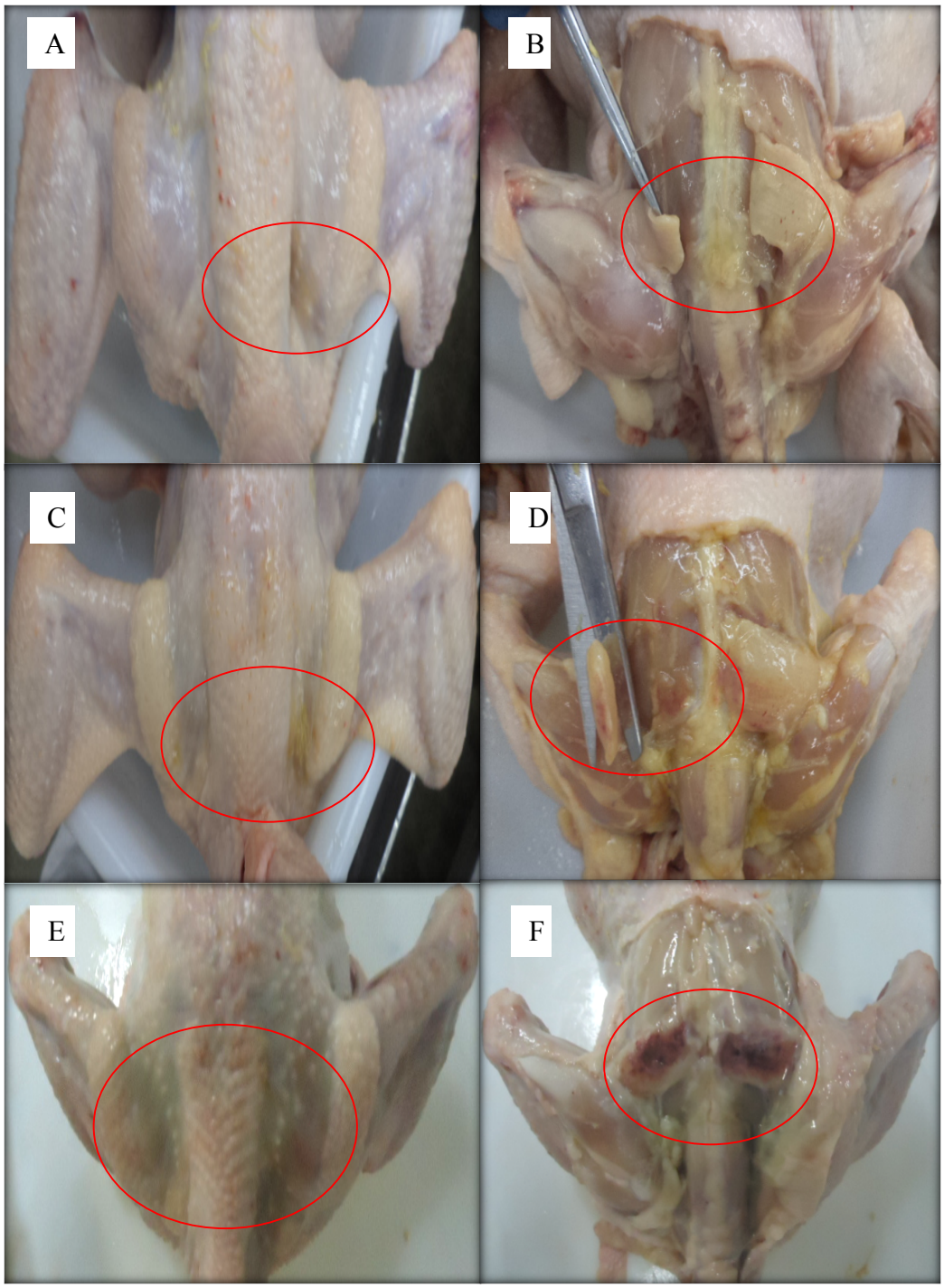

Figure 4. Broiler carcasses and anterior latissimus dorsi (ALD) muscles. Mild score: external appearance (A), internal aspect (B). Moderate score: external aspect (C), internal aspect (D). Severe score: external aspect (E), internal aspect (F).

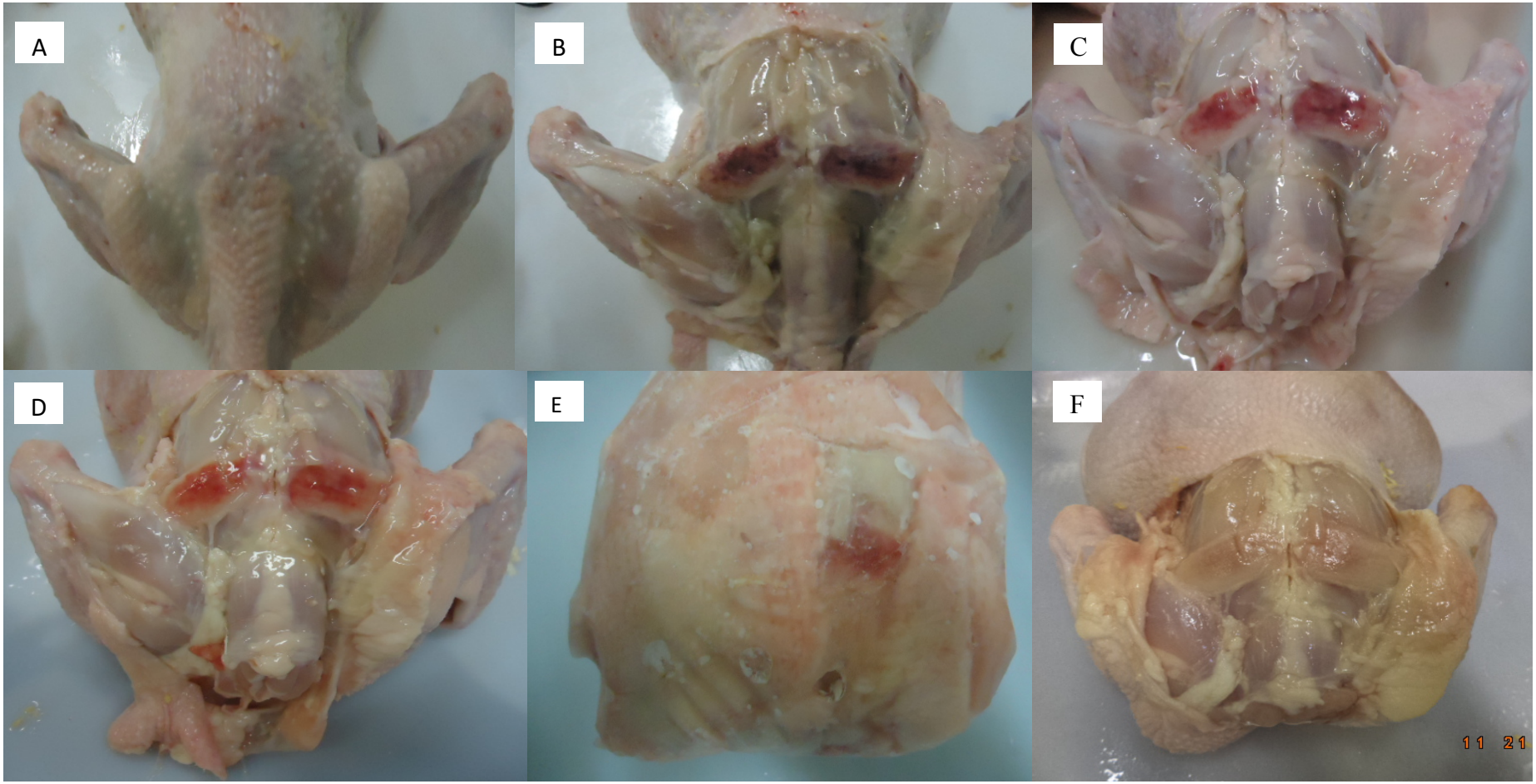

Figure 5. Aspect of MDC lesion classified with severe score in different stages of industrial processing. A - external appearance: carcass removed from the carcass in the line of the final inspection department. B - internal aspect: carcass removed in the line of the final inspection department. C - casing removed at the pre-chiller outlet. D - casing removed in chiller output. E - industrial processing end - carcass withdrawn at freezing tunnel exit. F - Thawed product.

CAPÍTULO III

5 CONSIDERAÇÕES FINAIS

Os dados deste trabalho demonstram que as variáveis meteorológicas de temperatura e umidade relativa do ar estão correlacionadas com o desenvolvimento da MDC, abrindo a possibilidade de estudos mais específicos sobre fatores ambientais nos aviários, como as condições de criação e interferência dos níveis de amônia nos parâmetros fisiológicos das aves.

A miopatia dorsal cranial ocasiona perdas econômicas na avicultura industrial, especialmente em plantas produtoras de frango tipo griller, onde na linha do Departamento de Inspeção Final ocorre a remoção da área acometida, mesmo que apresente pequena alteração. Esta remoção impossibilita o fluxo normal de produção do frango inteiro, sendo o restante da carcaça destinado para produção de subprodutos ou cortes de menor valor comercial.

Considerando que não há risco de contaminação cruzada para outras carcaças devido à MDC e que a legislação brasileira sobre miopatias aborda que a parte acometida deve ser descartada devido ao seu aspecto visual de qualidade do produto, é pertinente que novos estudos em plantas frigoríficas sejam realizados considerando a classificação da lesão em escores visando melhor aproveitamento da carcaça e estudos que avaliem a miopatia dorsal cranial nas etapas finais do processamento industrial, visto que a lesão regride visualmente no decorrer do processamento.

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7 APÊNDICE

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Age, sex, breed, and strain or genetic stock of animals used in the experiments shall be specified. Animal care guidelines should be referenced if appropriate.
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When describing the composition of diets and vitamin premixes, the concentration of vitamins A and E should be expressed as IU/kg on the basis of the following equivalents:
Vitamin A
1 IU = 0.3 μg of all-trans retinol
1 IU = 0.344 μg of retinyl acetate
1 IU = 0.552 μg of retinyl palmitate
1 IU = 0.60 μg of β-carotene
Vitamin E
1 IU = 1 mg of dl-α-tocopheryl acetate
1 IU = 0.91 mg of dl-α-tocopherol
1 IU = 0.67 mg of dl-α-tocopherol
In the instance of vitamin D3, cholecalciferol is the acceptable
term on the basis that 1 IU of vitamin D3 = 0.025
μg of cholecalciferol.
In the instance of vitamin D3, cholecalciferol is the acceptable term on the basis that 1 IU of vitamin D3 = 0.025 μg of cholecalciferol.
The sources of vitamins A and E must be specified in parentheses immediately following the stated concentrations.
Statistical Analysis: Biology should be emphasized, but the use of incorrect or inadequate statistical methods to analyze and interpret biological data is not acceptable. Consultation with a statistician is recommended. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or orthogonal contrasts, is not sufficient. A statement of the results of statistical analysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar experiments separately.
The experimental unit is the smallest unit to which an individual treatment is imposed.

For group-fed animals, the group of animals in the pen is the experimental unit; therefore, groups must be replicated. Repeated chemical analyses of the same sample usually do not constitute independent experimental units. Measurements on the same experimental unit over time also are not independent and must not be considered as independent experimental units. For analysis of time effects, use time-sequence analysis.Usual assumptions are that errors in the statistical models are normally and independently distributed with constant variance. Most standard methods are robust to deviations from these assumptions, but occasionally data transformations or other techniques are helpful. For example, it is recommended that percentage data between 0 and 20 and between 80 and 100 be subjected to arc sin transformation prior to analysis. Most statistical procedures are based on the assumption that experimental units have been assigned to treatments at random. If animals are stratified by ancestry or weight or if some other initial measurement should be accounted for, they should include a blocking factor, or the initial measurement should be included as a covariate. A parameter [mean (μ), variance (σ2)], which defines or describes a population, is estimated by a statistic (x, s2). The term parameter is not appropriate to describe a variable, observation, trait, characteristic, or measurement taken in an experiment. Standard designs are adequately described by name and size (e.g., “a randomized complete block design with 6 treatments in 5 blocks”). For a factorial set of treatments, an adequate description might be as follows: “Total sulfur amino acids at 0.70 or 0.80% of the diet and Lys at 1.10, 1.20, or 1.30% of the diet were used in a 2 × 3 factorial arrangement in 5 randomized complete blocks consisting of initial BW.” Note that a factorial arrangement is not a design; the term “design” refers to the method of grouping experimental units into homogeneous groups or blocks (i.e., the way in which the randomization is restricted). Standard deviation refers to the variability in a sample or a population. The standard error (calculated from error variance) is the estimated sampling error of a statistic such as the sample mean. When a standard deviation or standard error is given, the number of degrees of freedom on which it rests should be specified. When any statistical value (as mean or difference of 2 means) is mentioned, its standard error or confidence limit should be given. The fact that differences are not “statistically significant” is no reason for omitting standard errors. They are of value when results from several experiments are combined in the future. They also are useful to the reader as measures of efficiency of experimental techniques. A value attached by “±” to a number implies that the second value is its standard error (not its standard deviation). Adequate re-porting may require only 1) the number of observations, 2) arithmetic treatment means, and 3) an estimate of experimental error. The pooled standard error of the mean is the preferred estimate of experimental error. Standard errors need not be presented separately for each mean unless the means are based on different numbers of observations or the heterogeneity of the error variance is to be emphasized. Presenting individual standard errors clutters the presentation and can mislead readers.

For more complex experiments, tables of subclass means and tables of analyses of variance or covariance may be included. When the analysis of variance contains several error terms, such as in split-plot and repeated measures designs, the text should indicate clearly which mean square was used for the denominator of each F statistic. Unbalanced factorial data can present special problems. Accordingly, it is well to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions concerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was designed; they should form the basis for comparing treatment means. Nonorthogonal contrasts may be evaluated by Bonferroni t statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally arranged. Fixed-range, pairwise, multiple-comparison tests should be used only to compare means of treatments that are unstructured or not related. Least squares means are the correct means to use for all data, but arithmetic means are identical to least squares means unless the design is unbalanced or contains missing values or an adjustment is being made for a covariate. In factorial treatment arrangements, means for main effects should be presented when important interactions are not present. However, means for individual treatment combinations also should be provided in table or text so that future researchers may combine data from several experiments to detect important interactions. An interaction may not be detected in a given experiment because of a limitation in the number of observations.

The terms significant and highly significant traditionally have been reserved for $P < 0.05$ and $P < 0.01$, respectively; however, reporting the P-value is preferred to the use of these terms. For example, use ". . . there was a difference ($P < 0.05$) between control and treated samples" rather than ". . . there was a significant ($P < 0.05$) difference between control and treated samples." When available, the observed significance level (e.g., $P = 0.027$) should be presented rather than merely $P < 0.05$ or $P < 0.01$, thereby allowing the reader to decide what to reject. Other probability (α) levels may be discussed if properly qualified so that the reader is not misled. Do not report P-values to more than 3 places after the decimal. Regardless of the probability level used, failure to reject a hypothesis should be based on the relative consequences of type I and II errors. A "nonsignificant" relationship should not be interpreted to suggest the absence of a relationship. An inadequate number of experimental units or insufficient control of variation limits the power to detect relationships. Avoid the ambiguous use of $P > 0.05$ to declare nonsignificance, such as indicating that a difference is not significant at $P > 0.05$ and subsequently declaring another difference significant (or a tendency) at $P < 0.09$. In addition, readers may incorrectly interpret the use of $P > 0.05$ as the probability of a β error, not an α error.

Present only meaningful digits. A practical rule is to round values so that the change caused by rounding is less than one-tenth of the standard error. Such rounding increases the variance of the reported value by less than 1%, so that less than 1% of the relevant information contained in the data is sacrificed. Significant digits in data reported should be restricted to 3 beyond the decimal point, unless warranted by the use of specific methods.

Results and Discussion: Results and Discussion sections may be combined, or they may appear in separate sections. If separate, the Results section shall contain only the results and summary of the author's experiments; there should be no literature comparisons. Those comparisons should appear in the Discussion section. Manuscripts reporting sequence data must have GenBank accession numbers prior to submitting. One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experiments are adequately replicated. The results of experiments must be replicated, either by replicating treatments within experiments or by repeating experiments.

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

Appendix: A technical Appendix, if desired, shall follow the Discussion section or Acknowledgments, if present. The Appendix may contain supplementary material, explanations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel computer programs or mathematical computations would be appropriate. The Appendix will not be a repository for raw data.

References: Citations in Text: In the body of the manuscript, refer to authors as follows: Smith and Jones (1992) or Smith and Jones (1990, 1992). If the sentence structure requires that the authors' names be included in parentheses, the proper format is (Smith and Jones, 1982; Jones, 1988a,b; Jones et al., 1993). Where there are more than two authors of one article, the first author's name is followed by the abbreviation et al. More than one article listed in the same sentence of text must be in chronological order first, and alphabetical order for two publications in the same year. Work that has not been accepted for publication shall be listed in the text as: "J. E. Jones (institution, city, and state, personal communication)." The author's own un- published work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and un- published data must not be included in the References section.

References Section: To be listed in the References section, papers must be published or accepted for publication. Manuscripts submitted for publication can be cited as "personal communication" or "unpublished data" in the text.

Citation of abstracts, conference proceedings, and other works that have not been peer reviewed is strongly discouraged unless essential to the paper. Abstract and proceedings references are not appropriate citations in the Materials and Methods section of a paper.

In the References section, references shall first be listed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the authors' names. As with text citations, two or more publications by the same author or set of authors in the same year shall be differentiated by adding lowercase letters after the date. The dates for papers with the same first author that would be abbreviated in the text as et al., even though the second and subsequent authors differ, shall also be differentiated by letters. All authors' names must appear in the Reference section. Journals shall be abbreviated according to the conventional ISO abbreviations given in journals database of the National Library of Medicine. One-word titles must be spelled out. Inclusive page numbers must be provided. Sample references are given below. Consult recent issues of Poultry Science for examples not included below.

Article: Bagley, L. G., and V. L. Christensen. 1991. Hatchability and physiology of turkey embryos incubated at sea level with in- creased eggshell permeability. Poult. Sci. 70:1412–1418.

Bagley, L. G., V. L. Christensen, and R. P. Gildersleeve. 1990. Hematological indices of turkey embryos incubated at high altitude as affected by oxygen and shell permeability. Poult. Sci. 69:2035–2039.

Witter, R. L., and I. M. Gimeno. 2006. Susceptibility of adult chickens, with and without prior vaccination, to challenge with Marek's disease virus. Avian Dis. 50:354–365. doi:10.1637/7498-010306R.1

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

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

Federal Register: Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Regis. 69:10137–10151.
Other: Choct, M., and R. J. Hughes. 1996. Long-chain hydrocarbons as a marker for digestibility studies in poultry. Proc. Aust. Poult. Sci. Symp. 8:186. (Abstr.)
Dyro, F. M. 2005. Arsenic. WebMD. Accessed Feb. 2006. http:// www.emedicine.com/neuro/topic20.htm.
El Halawani, M. E., and I. Rosenboim. 2004. Method to enhance reproductive performance in poultry. Univ. Minnesota, as- signee. US Pat. No. 6,766,767.
Hruby, M., J. C. Remus, and E. E. M. Pierson. 2004. Nutritional strategies to meet the challenge of feeding poultry without antibiotic growth promotants. Proc. 2nd Mid-Atlantic Nutr. Conf., Timonium, MD. Univ. Maryland, College Park.
Luzuriaga, D. A. 1999. Application of computer vision and electronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gainesville.
Peak, S. D., and J. Brake. 2000. The influence of feeding program on broiler breeder male mortality. Poult. Sci. 79(Suppl. 1):2. (Abstr.).

Tables

Tables must be created using the MS Word table feature and inserted in the manuscript after the references section. When possible, tables should be organized to fit across the page without running broadside. Be aware of the dimensions of the printed page when planning tables (use of more than 15 columns will create layout problems). Place the table number and title on the same line above the table. The table title does not require a period. Do not use vertical lines and use few horizontal lines. Use of bold and italic typefaces in the table body should be done sparingly; such use must be defined in a footnote. Each table must be on a separate page. To facilitate placement of all tables into the manuscript file (just after the references) authors should use “section breaks” rather than “page breaks” at the end of the manuscript (before the tables) and between tables. Units of measure for each variable must be indicated. Papers with several tables must use consistent format. All columns must have appropriate headings. Abbreviations not found on the inside front cover of the journal must be defined in each table and must match those used in the text. Footnotes to tables should be marked by superscript numbers. Each footnote should begin a new line. Superscript letters shall be used for the separation of means in the body of the table and explanatory footnotes must be provided [i.e., “Means within a row lacking a common superscript differ ($P < 0.05$).”]; other significant P-values may be specified. Comparison of means within rows and columns should be indicated by different series of superscripts (e.g., a,b, . . . in rows; x–z . . . in columns) The first alphabetical letter in the series (e.g., a or A) shall be used to indicate the largest mean. Lowercase superscripts indicate $P \leq 0.05$. Uppercase letters indicate $P \leq 0.01$ or less.

Probability values may be indicated as follows: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, and $\dagger P \leq 0.10$. Consult a recent issue of Poultry Science for examples of tables.

Figures

To facilitate review, figures should be placed at the end of the manuscript (separated by section breaks). Each figure should be placed on a separate page, and identified by the manuscript number and the figure number. A figure with multiple panels or parts should appear on one page (e.g., if Figure 1 has parts a, b, and c, place all of these on the same page). Figure captions should be typed (double spaced) on a separate page.

• Figure Size. Prepare figures at final size for publication. Figures should be prepared to fit one column
(8.9 cm wide), 2 columns (14 cm wide), or full-page width (19 cm wide).
• Font Size. Ensure that all type within the figure and axis labels are readable at final publication size.
A minimum type size of 8 points (after reduction) should be used.
• Fonts. Use Helvetica or Times New Roman. Symbols may be inserted using the Symbol palette in Times New Roman.
• Line Weight. For line graphs, use a minimum stroke weight of 1 point for all lines. If multiple lines are to be distinguished, use solid, long-dash, short-dash, and dotted lines. Avoid the use of color, gray, or
shaded lines, as these will not reproduce well. Lines with different symbols for the data points may also be used to distinguish curves.
• Axis Labels. Each axis should have a description and a unit. Units may be separated from the descriptor by a comma or parentheses, and should be consistent within a manuscript.
• Shading and Fill Patterns. For bar charts, use different fill patterns if needed (e.g., black, white, gray, diagonal stripes). Avoid the use of multiple shades of gray, as they will not be easily distinguishable in print.
• Symbols. Identify curves and data points using the following symbols only: □, ■, ○, ●, ▲, ▼, n, ,, e, r, +, or ×. Symbols should be defined in a key on the figure if possible.
• File Formats. Figures can be submitted in Word, PDF, EPS, TIFF, and JPEG. Avoid PowerPoint files and other formats. For the best printed quality, line art should be prepared at 600 ppi. Grayscale and color images and photomicrographs should be at least 300 ppi.
• Grayscale Figures. If figures are to be reproduced in grayscale (black and white), submit in grayscale. Often color will mask contrast problems that are apparent only when the figure is reproduced in grayscale.
• Color Figures. If figures are to appear in color in the print journal, files must be submitted in CMYK color (not RGB).
• Photomicrographs. Photomicrographs must have their unmagnified size designated, either in the caption or with a scale bar on the figure. Reduction for publication can make a magnification power designation (e.g., 100×) inappropriate.
• Caption. The caption should provide sufficient information that the figure can be understood with excessive reference to the text. All author-derived abbreviations used in the figure should be defined in the caption.
• General Tips. Avoid the use of three-dimensional bar charts, unless essential to the presentation of the data. Use the simplest shading scheme possible to present the data clearly. Ensure that data, symbols, axis labels, lines, and key are clear and easily readable at final publication size.
Color Figures. Submitted color images should be at least 300 ppi. The cost to publish each color figure is $995; a surcharge for color reprints ordered will be assessed. Authors must agree in writing to bear the costs of color production after acceptance and prior to publication of the paper. The form “Color Charge Agreement” is available on the journal web site (http://ps.fass.org) and should be completed and returned to PSA Headquarters upon submission.
Miscellaneous Usage Notes
Abbreviations. Abbreviations shall not be used in the title, key words, or to begin sentences, except when they are widely known throughout science (e.g., DNA, RNA) or are terms better known by abbreviation (e.g., IgG, CD).

A helpful criterion for use of abbreviation is whether it has been accepted into thesauri and indexes widely used for searching major bibliographic databases in the scientific field. Abbreviations may be used in heads within the paper, if they have been first defined within the text. The inside back cover of every issue of the journal lists abbreviations that can be used without definition. The list is subject to revision at any time, so authors should always consult the most recent issue of the journal (or the updated list at http://ps.fass.org/) for relevant information. Abbreviations are allowed when they help the flow of the manuscript; however, excessive use of abbreviations can confuse the reader. The suitability of abbreviations will be evaluated by the reviewers and editors during the review process and by the technical editor during editing. As a rule, author-derived abbreviations should be in all capital letters. Terms used less than three times must be spelled out in full rather than abbreviated. All terms are to be spelled out in full with the abbreviation following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbreviations shall be used consistently thereafter, rather than the full term. The abstract, text, each table, and each figure must be understood independently of each other. Therefore, abbreviations shall be defined within each of these units of the manuscript.

Plural abbreviations do not require “s.” Chemical symbols and three-letter abbreviations for amino acids do not need definition. Units of measure, except those in the standard Poultry Science abbreviation list, should be abbreviated as listed in the CRC Handbook for Chemistry and Physics (CRC Press, 2000 Corporate Blvd., Boca Raton, FL 33431) and do not need to be defined. The following abbreviations may be used without definition in Poultry Science.

A	adenine
ADG	average daily gain
ADFI	average daily feed intake
AME	apparent metabolizable energy
AMEn	nitrogen-corrected apparent metabolizable energy
ANOVA	analysis of variance
B	cell bursal-derived, bursal-equivalent derived cell
bp	base pairs
BSA	bovine serum albumin
BW	body weight
C	cytosine
cDNA	complementary DNA
cfu	colony-forming units
CI	confidence interval
CP	crude protein
cpm	counts per minute
CV	coefficient of variation
d	day
df	degrees of freedom
DM	dry matter
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetate
ELISA	enzyme-linked immunosorbent antibody assay
EST	expressed sequence tag
g	gram
g	gravity
G	guanine

GAT	glutamic acid-alanine-tyrosine
G:F	gain-to-feed ratio
GLM	general linear model
h	hour
HEPES	N-2-hydroxyethyl piperazine-N′-ethane-sulfonic acid
HPLC	high-performance (high-pressure) liquid chromatography
ICU	international chick units
Ig	immunoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IU	international units
i.v.	intravenous
kb	kilobase pairs
kDa	kilodalton
L	liter*
L:D	hours light:hours darkness in a photoperiod
m	meter
µ	micro
M	molar
MAS	marker-assisted selection
ME	metabolizable energy
MEn	nitrogen-corrected metabolizable energy
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
min	minute
mo	month
MS	mean square
n	number of observations
N	normal
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NRC	National Research Council
NS	not significant
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
pfu	plaque-forming units
QTL	quantitative trait loci
r	correlation coefficient
r2	coefficient of determination, simple
R2	coefficient of determination, multiple
RFLP	restriction fragment length polymorphism
RH	relative humidity
RIA	radioimmunoassay
RNA	ribonucleic acid
rpm	revolutions per minute
s	second
s.c.	subcutaneous
SD	standard deviation
SDS	sodium dodecyl sulfate

SE	standard error
SEM	standard error of the mean
SRBC	sheep red blood cells
SNP	single nucleotide polymorphism
T	thymine
TBA	thiobarbituric acid
T	cell thymic-derived cell
TME	true metabolizable energy
TMEn	nitrogen-corrected true metabolizable energy
Tris	tris(hydroxymethyl)aminomethane
TSAA	total sulfur amino acids
U	uridine
USDA	United States Department of Agriculture
UV	ultraviolet
vol/vol	volume to volume
vs.	versus
wt/vol	weight to volume
wt/wt	weight to weight
wk	week
yr	year

*Also capitalized with any combination, e.g., mL.

International Words and Phrases. Non-English words in common usage (defined in recent editions of standard dictionaries) will not appear in italics (e.g., in vitro, in vivo, in situ, a priori). However, genus and species of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. German nouns shall begin with capital letters.

Capitalization. Breed and variety names are to be capitalized (e.g., Single Comb White Leghorn).

Number Style. Numbers less than 1 shall be written with preceding zeros (e.g., 0.75). All numbers shall be written as digits. Measures must be in the metric system; however, US equivalents may be given in parentheses.

Poultry Science requires that measures of energy be given in calories rather than joules, but the equivalent in joules may be shown in parentheses or in a footnote to tables. Units of measure not preceded by numbers must be written out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically. Measures of variation must be defined in the Abstract and in the body of the paper at first use. Units of measure for feed conversion or feed efficiency shall be provided (i.e., g:g).

Nucleotide Sequences. Nucleotide sequence data must relate to poultry or poultry pathogens and must complement biological data published in the same or a companion paper. If sequences are excessively long, it is suggested that the most relevant sections of the data be published in Poultry Science and the remaining sequences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submission of sequence data to one of the databases. The following statement should appear as a footnote to the title on the title page of the manuscript. “The nucleotide sequence data reported in this paper have been submitted to GenBank Submission (Mail Stop K710, Los Alamos National Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN.” Publication of the description of molecular clones is assumed by the editors to place them in the public sector.

Therefore, they shall be made available to other scientists for research purposes. Nucleotide sequences must be submitted as cameraready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow Poultry Science guidelines.

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

Use the slant line only when it means “per” with numbered units of measure or “divided by” in equations. Use only one slant line in a given expression (e.g., g/d per chick). The slant line may not be used to indicate ratios or mixtures.

Use “to” instead of a hyphen to indicate a range. Insert spaces around all signs (except slant lines) of operation (=, –, +, ×, >, or <, etc.) when these signs occur between two items. Items in a series should be separated by commas (e.g., a, b, and c). Restrict the use of “while” and “since” to meanings related to time. Appropriate substitutes include “and,” “but,” or “whereas” for “while” and “because” or “although” for “since.” Leading (initial) zeros should be used with numbers less than 1 (e.g., 0.01). Commas should be used in numbers greater than 999.

Registered (®) and trademark (™) symbols should not be used, unless as part of an article title in the References section. Trademarked product names should be capitalized.

Supplemental Information (Online)

The following information is available online and updated regularly. Please refer to these pages when preparing a manuscript for submission.

Journal Title Abbreviations. A list of standard abbreviations for common journal titles is available online (http://ps.fass.org/misc/ifora.dtl).

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

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Manuscript Central Instructions. Manuscripts are submitted online (http://mc.manuscriptcentral.com/psa). Full user instructions for using the Manuscript Central system are available on the Mansuscript Central home page.

VITA

Fernanda do Prado, filha de Elizandro Miranda do Prado e Maria Inês Ferreira do Prado, nascida em 30 de março de 1990, em Canoinhas, Santa Catarina. Realizou o ensino fundamental na Escola de Educação Básica Irmã Maria Felícitas, ensino médio no Colégio Estadual Santa Cruz e em 2008 ingressou no curso de Medicina Veterinária na Universidade do Contestado – UNC, ambas instituições localizadas na sua cidade natal.

Em 2012, na cidade de Videira – SC, realizou estágio curricular em medicina veterinária na empresa BRF – Brasil Foods, onde conquistou o primeiro emprego em frigorífico de aves (frangos e codornas).

Em 2014 atuou em frigorífico de bovinos e suínos, nos estados do Mato Grosso e Minas Gerais. Em 2015 na cidade de Porto Alegre, atuou como médica veterinária em hipermercados da rede Walmart do Brasil e ingressou no curso de Especialização em Produção, Higiene e Tecnologia de Alimentos de Origem Animal da Universidade Federal do Rio Grande do Sul.

Em 2017, ingressou no Mestrado em Zootecnia, na área Produção Animal na Universidade Federal do Rio Grande do Sul, no qual foi submetido à banca de defesa de Dissertação em março de 2019, sob orientação da professora Dr^{a}. Andréa Machado Leal Ribeiro.