UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

UTILIZAÇÃO DA ULTRASSONOGRAFIA NA AVALIAÇÃO *IN VIVO* DE *WOODEN BREAST* EM FRANGOS DE CORTE SUBMETIDOS À RESTRIÇÃO ALIMENTAR

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Dissertação apresentada como um dos requisitos à obtenção do Grau de Mestre em Zootecnia

Área de Concentração Produção Animal

Porto Alegre (RS), Brasil
Março, 2018.

CIP - Catalogação na Publicação

Simões, Cristina Tonial
UTILIZAÇÃO DA ULTRASSONOGRAFIA NA AVALIAÇÃO IN VIVO DE WOODEN BREAST EM FRANGOS DE CORTE SUBMETIDOS À RESTRIÇÃO ALIMENTAR / Cristina Tonial Simões. -- 2018.
104 f.
Orientador: Sergio Luiz Vieira.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Zootecnia, Porto Alegre, BR-RS, 2018.

1. FRANGOS DE CORTE. 2. MIOPATIAS. 3. RESTRIÇÃO ALIMENTAR. 4. ULTRASSOM. I. Vieira, Sergio Luiz, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

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DISSERTAÇÃO

Submetida como parte dos requisitos
para obtenção do Grau de

MESTRE EM ZOOTECNIA

Programa de Pós-Graduação em Zootecnia
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Porto Alegre (RS), Brasil

Aprovada em: 22.03.2018
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“Na história da humanidade (e dos animais também) aqueles que aprenderam a colaborar e improvisar foram os que prevaleceram”

(Charles Darwin)

AGRADECIMENTOS

Primeiramente, agradeço a minha família; minha mãe Ester Salete, meu pai Carlos Alberto e minha irmã Aline. Vocês sempre foram e sempre serão a melhor parte de mim. O principal motivo pelo qual eu busco ser alguém melhor a cada dia.

Em memória de minhas grandes amigas Georgete Ferronato Fantin e Laís Poersch, também dedico esse trabalho. Sem a presença de vocês meu último ano de mestrado foi muito mais difícil, porém sei que hoje estão olhando por mim e comemorando essa conquista comigo. Levo sempre vocês em meu coração.

Ao professor Sergio Luiz Vieira pela confiança, por todas as oportunidades que me foram dadas, e pelos ensinamentos ao longo deste período.

Aos colegas do Aviário de Ensino e Pesquisa pelos 4 anos que estive no setor, entre estágio e mestrado. Por toda a dedicação e comprometimento com as atividades e pelos aprendizados que adquirimos juntos, além da grande amizade que construímos nesse período. Dentre estes, principalmente a minha colega de mestrado Gabriela Santiago, pela parceria, amizade e companheirismo incondicionais em tantos desafios que vivemos juntas. E à Catarina Stefanello, que por inúmeras vezes me orientou e deu força durante este período, e pela ajuda, inclusive nos estudos pré-seleção de mestrado.

Aos professores e funcionários do Programa de Pós-Graduação em Zootecnia - UFRGS. Ao CNPq pela bolsa de Mestrado e a Cobb-Vantress pelo apoio financeiro na realização desse trabalho.

Muito obrigada!

UTILIZAÇÃO DA ULTRASSONOGRAFIA NA AVALIAÇÃO *IN VIVO* DE *WOODEN BREAST* EM FRANGOS DE CORTE SUBMETIDOS À RESTRIÇÃO ALIMENTAR

Autor: Cristina Tonial Simões
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Resumo – Este estudo objetivou avaliar a utilização da ultrassonografia no diagnóstico *in vivo* de *wooden breast* (WB) em frangos de corte submetidos à restrição alimentar. Foram utilizados 1.800 frangos de corte machos Cobb x Cobb 500 distribuídos em 6 tratamentos, com 12 repetições de 25 aves cada, em um delineamento inteiramente casualizado. Os tratamentos consistiram em restrições de 50, 60, 70, 80, 90% da quantidade de ração fornecida em relação ao consumo *ad libitum* diário do tratamento controle, de 8 a 49 d. Semanalmente, as aves foram pesadas para avaliação de desempenho e foram submetidas à ultrassonografia para determinar a ecogenicidade do peito, em que os valores de cinza foram calculados a partir do histograma gerado de cada imagem. A profundidade do peito também foi mensurada. Semanalmente, de 7 a 49 d, uma ave por unidade experimental (UE) foi abatida para avaliação visual dos escores de WB, bem como para coleta de amostras de peito para análises sorológicas e de qualidade de carne. Aos 49 d, foram abatidas 5 aves por UE para avaliação do rendimento de carcaça, cortes comerciais e ocorrência de WB. Os peitos foram avaliados em escores: peito normal (0); endurecimento suave na parte cranial (1); endurecimento da parte cranial e caudal (2); endurecimento severo de todo o peito (3), endurecimento severo com presença de lesões hemorrágicas e exsudato (4). O efeito das restrições alimentares sobre desempenho e os escores de WB foi avaliado através da análise de variância. Os escores de WB foram considerados variáveis independentes e também correlacionados com as medidas de ultrassom do peito, qualidade de carne, medidas histomorfométricas e perfil sorológico das aves. O desempenho produtivo e a ocorrência de WB reduziram linearmente ($P < 0,05$) com as restrições alimentares mais severas. Os valores de ecogenicidade e profundidade do peito aumentaram ($P < 0,05$) com a severidade de WB. De 21 a 49 d, peitos com escores 3 e 4 apresentaram maior perda por cocção, diâmetro de fibra e concentração das enzimas marcadoras de lesão muscular ($P < 0,05$) comparados ao peito normal (escore 0). A ultrassonografia do peito de frangos de corte pode ser uma ferramenta auxiliar no diagnóstico da miopatia WB em aves vivas.

Palavras chave: miopatia, consumo alimentar, qualidade de carne, ultrassom.

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[1]Dissertação de Mestrado em Zootecnia – Produção Animal, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil (104 p.), Março, 2018.

UTILIZATION OF ULTRASONOGRAPHY TO DETECT THE WOODEN BREAST MYOPATHY IN LIVE BROILERS SUBMITTED TO FEED RESTRICTION[1]

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Abstract - The present study was conducted to evaluate the effectiveness of utilizing ultrasound (US) images of breast muscle to predict wooden breast (WB) myopathy in lived broilers subjected to different feed restrictions. A total of 1,800 Cobb × Cobb 500 male chicks were fed with 6 treatments and 12 replicates of 25 birds each using a completely randomized design. Birds were fed ad libitum or received 50, 60, 70, 80, and 90% of the quantity consumed by the chickens fed ad libitum. The restriction programs were applied from 8 to 49 d. All birds were banded on the first day and every week they were weighted in order to determined BWG, FCR and FI. Ultrasound images of breast muscle were weekly obtained from all birds to determine echogenicity, where mean gray values were calculated from the obtained histogram, and the breast depth was also measured. One bird per experimental unit was slaughtered every week, from 7 to 49 d, for visual evaluation of WB scores, as well as breast samples collections for serum analysis and meat quality. At 49 d, 5 birds per experimental unit were slaughtered to evaluate carcass yields and WB occurrence. Breast were evaluated as: normal breast (0), mild hardening in the upper (1), moderate hardening in the upper and/or lower part of the fillet (2), severe hardening (3), and severe hardening with hemorrhagic lesions and yellow fluid (4). The effect of feed restrictions on growth performance and WB scores was evaluated using ANOVA. The WB scores were considered independent variables and correlated with echogenicity, breast depth, meat quality, histomorphometric measurements and serologic profile of birds. Growth performance as well as carcass yield, decreased linearly ($P > 0.05$) as feed restriction was applied. The WB occurrence presented linear response to growth performance and it was lower on treatments with higher feed restrictions. Echogenicity and depth increased with WB severity ($P < 0.05$). From 21 to 49 d, the WB scores 3 and 4 also had higher cooking loss, fiber diameter, serum enzymes, whereas fiber density per area was lower ($P < 0.05$) compared to normal breasts (score 0). Ultrasound images of broilers breast can be adequately utilized to predict *in vivo* the WB of birds.

Key words: myopathy, feed intake, meat quality, ultrasound.

[1]Master of Science dissertation in Animal Science – Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (104 p.), March, 2018.

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RELAÇÃO DE ABREVIATURAS

WB	*Wooden breast*
WS	*White stripping*
CA	Conversão alimentar
CR	Consumo de ração
GP	Ganho de peso

CAPÍTULO I

INTRODUÇÃO

O aumento das taxas de crescimento muscular e consequente superior desempenho produtivo das linhagens de frangos de corte utilizadas na indústria atual são os principais fatores que têm contribuído para a maior eficiência da produção avícola. Neste contexto, se destaca a carne do peito, que é o corte comercial com maior rendimento em relação à carcaça da ave e ao peso vivo. Atrelado a esse maior rendimento, observa-se uma preocupação da indústria avícola em relação ao aparecimento de miopatias peitorais, devido às alterações que geram na aparência do produto final.

A miopatia denominada peito madeira ou *wooden breast* (WB) tem grande importância neste cenário, devido ao estranhamento que suas alterações macroscópicas geram em relação a peitos normais, apresentando também alterações estruturais das fibras musculares, que geram mudanças no seu processamento. Miopatias peitorais em frangos de corte também têm afetado a aceitação dos consumidores. Metade dos consumidores consultados na pesquisa de Kuttapan et al. (2012) não comprariam a carne que apresentasse estrias, lesão conhecida como *white stripping* (WS) de grau moderado ou severo. Devido a sua aparência visual e textura rígida, a miopatia WB também pode impactar a decisão dos consumidores. Consequentemente, muitos peitos de frango têm sido condenados no abatedouro ou perdem seu valor comercial quando são destinados à produção de industrializados, resultando em perdas econômicas para o setor (XING et al., 2017).

Uma vez que a miopatia leva ao maior enrijecimento da musculatura peitoral, muitos estudos buscam investigar a presença de WB e a sua relação com alterações na qualidade da carne de peito. Textura, cor e pH do músculo são algumas das características avaliadas e que podem ser influenciadas em peitos afetados por graus severos de WB (KUTAPPAN et al., 2017). Acredita-se ainda, que devido às lesões causadas pela miopatia, a alteração na estrutura das fibras musculares torne esses músculos mais sensíveis ao corte, apresentando menor resistência à força de cisalhamento, quando comparados a peitos normais, embora isto ainda não tenha sido comprovado em estudos recentes, como os de Soglia et al (2017) e Mudalal et al. (2014).

Ainda sobre a textura do peito, o estudo de Lee et al. (2014) buscou aprimorar técnicas de avaliação de textura na musculatura peitoral, com a metodologia de MORS (Meullenet-Owens *razor shear force*) e MORSE (Meullenet-Owens *razor shear energy*), que vem sendo amplamente utilizada. Outras pesquisas (KUTTAPPAN et al., 2013; PETRACCI et al., 2014) sugerem que miopatias causam efeitos negativos no metabolismo das fibras e consequentemente levam a alterações patológicas na musculatura peitoral que afetam negativamente a qualidade da carne.

Embora ainda não seja claramente atribuída a uma causa única e específica, a miopatia WB é alvo de pesquisas que visam relacionar sua ocorrência com fatores ligados à nutrição, genética e velocidade de crescimento (TROCINO et al., 2015; ZAMBONELLI et al., 2017; RADAELLI et al., 2017), por exemplo. A idade em que as lesões começam a aparecer nas aves também é uma informação relevante quanto à etiologia da miopatia. Estudos que possibilitem o acompanhamento da evolução das lesões ao longo da vida das aves podem contribuir na elaboração de novas estratégias de controle. Portanto, informações que venham a esclarecer as causas e propor soluções para esta miopatia são imprescindíveis, visto que sua etiologia e estabelecimento ainda são desconhecidos. Estratégias nutricionais têm sido adotadas como forma de reduzir a incidência e a severidade de miopatias em lotes de frangos de corte. Entretanto, a maioria dos estudos que visaram reduzir a miopatia, como o de Cruz et al. (2017), têm demonstrado que fatores nutricionais que afetam o ganho de peso das aves, como a suplementação de níveis crescentes de lisina digestível em dietas a base de milho e farelo de soja, acabam que indiretamente aumentando a incidência de WB, uma vez que estão altamente relacionados com o aumento do ganho de peso e rendimento de peito das aves.

A suplementação de elementos com função antioxidante, como o selênio, também já foi citada como alternativa de redução das lesões causadas pelas miopatias, já que devido a sua ação antioxidante, a suplementação de selênio poderia proteger os tecidos do estresse oxidativo causado pelas miopatias (SIHVO et al., 2014). Porém, seus resultados ainda são controversos, visto que a suplementação de níveis crescentes de selênio na dieta também pode aumentar o desempenho produtivo das aves e consequentemente agravar o aparecimento das lesões (CEMIN et al., 2018).

A restrição alimentar, utilizada como alternativa de manejo para reduzir a ocorrência de miopatias pode apresentar resultados satisfatórios sobre a integridade do tecido muscular. Desta forma, manejos que realizam algum tipo de restrição alimentar, com a consequente redução do ganho de peso, podem representar mudanças na ocorrência de WS e WB (TROCINO et al., 2015). Porém, o ponto chave neste caso, será encontrar uma forma de utilização da restrição alimentar como controle do ganho de peso e ocorrência de miopatias, sem prejudicar o desempenho produtivo já considerado satisfatório pelas linhagens comerciais mais utilizadas na indústria.

A velocidade do crescimento do músculo *Pectoralis major* durante o desenvolvimento das aves pode estar relacionada ao surgimento destas lesões teciduais, entretanto o diagnóstico das miopatias é evidenciado apenas nas carcaças. Percebe-se, então, a necessidade do desenvolvimento de novas metodologias e ferramentas capazes de identificar a presença de WB em aves vivas. Dentre os principais métodos confiáveis de diagnóstico por imagem utilizados na medicina veterinária e humana destaca-se a ultrassonografia, que possibilita a validação de exames *in vivo* como metodologia para diagnóstico de lesões em diferentes órgãos e tecidos animais. Essa metodologia é descrita por Pillen (2010) como uma técnica não invasiva de avaliar músculos normais ou que apresentam alguma alteração patológica em tempo real e que vem sendo amplamente utilizada desde a década de 50.

Desta forma, acredita-se que a utilização de imagens ultrassonográficas do músculo *Pectoralis major* de aves vivas auxiliará na compreensão do desenvolvimento muscular em animais utilizados em programas genéticos, facilitando a seleção de características como o ganho de peso dentro de um lote de aves comerciais. Medidas de avaliação destas imagens poderão ser utilizadas como ferramentas para identificar tecidos comprometidos pela miopatia, sendo possível também, através de um acompanhamento periódico, determinar as fases de desenvolvimento destas lesões. Case et al. (2012) já obtiveram sucesso utilizando ultrassom para medir profundidade de peito em perus. Entretanto, o número de publicações que abordam esta metodologia em frangos de corte ainda é escasso.

Para tanto, a presente pesquisa teve como objetivo avaliar o efeito de restrições alimentares quantitativas sobre o desempenho produtivo, a ocorrência de WB em frangos de corte de 8 a 49 dias de idade, bem como correlacionar os graus de WB com a qualidade da carne, perfil sorológico das aves e parâmetros histológicos da carne do peito. Objetivou-se também testar uma nova metodologia para diagnóstico *in vivo* da miopatia através da análise de imagens do peito obtidas por ultrassonografia em aves vivas.

REVISÃO BIBLIOGRÁFICA

Miopatia *wooden breast*

O alto rendimento da carne de peito é uma característica comum e desejada entre as principais linhagens de frangos de corte que estão inseridas no mercado avícola. Segundo Zuidhof et al. (2014), entre os anos de 1957 e 2005 houve um incremento de 79% e de 85% do músculo *Pectoralis major* de frangos de corte machos e fêmeas, respectivamente. Em decorrência disso, diferentes linhagens têm apresentado crescente ocorrência de miopatias peitorais, como *wooden breast* (WB) (PETRACCI & CAVANI, 2012), que ocorre na musculatura peitoral maior e, eventualmente, no músculo peitoral menor de frangos de corte.

Além de apresentarem maior peso e espessura (KUTAPPAN et al., 2017), observa-se que peitos acometidos com graus mais avançados de WB apresentam um enrijecimento da musculatura, com protuberâncias ao longo da porção ventral da musculatura peitoral ou, em casos mais severos, ao logo de toda a superfície da carne de peito. Assim, esta miopatia se caracteriza macroscopicamente por apresentar diferentes graus de dureza na superfície do músculo *Pectoralis major*, com possível aparecimento de áreas pálidas (SIHVO et al., 2014). A presença de exsudato translúcido, bem como de lesões hemorrágicas e petéquias também chama a atenção quando da visualização de peitos mais severamente acometidos, assim como descrito por Kutappan et al. (2016).

Visto que a aparência visual é um fator de qualidade decisivo quando da escolha do produto final pelo consumidor, qualquer alteração em relação ao aspecto normal da carne pode levar a sua rejeição no momento da compra (KUTAPPAN et al., 2013). Neste contexto, há uma expressiva preocupação em relação às perdas econômicas decorrentes da condenação e redução no preço da carne de peito que as lesões características de WB podem trazer ao mercado avícola.

A busca por estratégias que visam diminuir a ocorrência desta miopatia se dá através do estudo aprofundado de todos os fatores que estão associados ao seu surgimento. Portanto, há uma maior importância na condução de pesquisas que contribuam para esclarecer sua etiologia e as alterações que ocorrem no peito da ave com o decorrer da idade.

Alterações celulares

Pesquisas sugerem que a miopatia WB está associada à outra alteração muscular denominada estriação branca ou *white stripping* (WS) que, através de avaliações histológicas, está usualmente associada à degeneração muscular e alterações miopáticas abaixo da área com estriação. Peitos com WS possuem perda das estriações transversais, variabilidade no tamanho da fibra, degeneração e lise das fibras, mineralização moderada, regeneração, infiltração de células mononucleares, lipidose, inflamação intersticial e fibrose (KUTTAPPAN et al., 2012; 2013a; PETRACCI et al., 2013). Para Radaelli et al. (2016), há pouca diferença entre imagens histológicas de peitos afetados com

WS e WB de frangos abatidos aos 46 d, sugerindo uma forte relação entre o aparecimento das duas miopatias, podendo estarem presentes simultaneamente no peito, com perda da estriação, necrose e variabilidade no tamanho das fibras e infiltração de linfócitos e macrófagos.

As miopatias peitorais também estão associadas a uma menor densidade capilar no tecido muscular, podendo ser observada uma redução no fornecimento de nutrientes, oxigênio e também na remoção mais lenta de ácido lático nos músculos, levando a danos musculares e ao aparecimento das lesões características de miopatia (BILGILI et al., 2013). Para Hoving-Bolink et al. (2000) há correlação negativa entre densidade capilar e rendimento de peito em frangos machos adultos saudáveis, sugerindo como consequência o menor aporte de oxigênio na musculatura. Isso possivelmente pode ocorrer em peitos que apresentam WB, devido à correlação positiva que existe entre o aparecimento da miopatia e o peso e maior rendimento do peito. Ainda, Velleman & Clark (2015) associaram WB à hipertrofia das fibras musculares em frangos de crescimento rápido, com consequente diminuição do número de capilares adjacentes, gerando estresse oxidativo e afetando assim a regeneração da fibra muscular.

Portanto, ao passo que aumenta a severidade da miopatia WB, ao analisar a estrutura tecidual da musculatura são encontradas áreas caracterizadas por infiltrado inflamatório, necrose das fibras musculares com substituição por tecido conjuntivo adjacente e desorganização da estrutura fibrilar, com alta variabilidade no tamanho das fibras musculares e menor densidade capilar (WOLD et al., 2017).

Padrões de qualidade de carne

Peitos de frangos de corte que apresentam algum grau de WB são frequentemente questionados quanto aos efeitos negativos que trazem a qualidade da carne, sugerindo que as funções fisiológicas não estão normais nas áreas afetadas da musculatura. Estudos recentes chegaram a resultados semelhantes quanto ao maior valor de pH em peitos com WS e WB quando comparados a peitos normais (TASONIERO et al., 2016; KUTAPPAN et al., 2017; CAI et al., 2018;). O maior pH da carne é explicado por fatores anormais e estressantes que ocorrem pré e pós abate, impedindo a diminuição do pH no processo de transformação do músculo em carne, podendo estar relacionados com nutrição, genética ou manejo. No caso de WB, esse aumento pode ser um resultado da redução do potencial glicolítico da musculatura, associado com a deficiência do metabolismo de carboidratos encontrado em músculos *post mortem* que apresentam a miopatia (AGUIRRE et al., 2018).

Comparando peitos de frango não acometidos pela miopatia e peitos com diferentes graus de WB, verificou-se que o aumento do tecido conjuntivo intersticial com consequente fibrose, observado em peitos com WB por Soglia et al. (2017) e Sihvo et al. (2014), pode exercer efeito significativo sobre a textura da carne. Porém, o efeito disto sobre a resistência ao corte não tem sido encontrado quando a força de cisalhamento é comparada entre peitos normais e peitos com WB (MUDALAL et al., 2014; CAI et al., 2018), possivelmente por essa análise ser realizada com amostras de peito cozido, ou seja, é possível que a desnaturação das proteínas musculares devido ao cozimento da carne diminua a diferença de textura entre peitos normais e peitos com WB. Por outro lado, quando a técnica de MORS foi utilizada para comparar a textura entre peitos com WB e peitos normais, Chatterjee et al. (2016) observaram maior força e energia de cisalhamento (MORS e MORSE, respectivamente) em peitos com WB, sugerindo que peitos com WB exigem maior força para serem cortados, além de que, esta análise é feita em peitos crus. Essa diferença de resultados encontrados com relação à resistência da carne pode ser devida à metodologia utilizada em cada estudo, o que sugere a necessidade de novos estudos para aperfeiçoar metodologias já conhecidas.

Contudo, a capacidade de retenção de água de peitos de frango com WB parece ser uma maneira consolidada de avaliação de qualidade da carne, visto que grande parte das pesquisas realizadas encontraram resultados semelhantes, onde amostras de peitos com WB apresentaram maior perda por cocção do que amostras de peitos normais (MUDALAL et al. 2014; SOGLIA et al., 2016; AGUIRRE et al., 2018). Esta redução na capacidade de reter água também pode ser explicada pelo aumento de tecido conjuntivo adjacente às fibras musculares e diminuição das proteínas fibrilares, levando à perda da integridade tecidual.

Perfil sorológico das aves

As análises hematológicas são ferramentas clínicas muito eficientes, utilizadas como diagnóstico de distúrbios metabólicos nos animais, inclusive em aves de produção. Neste contexto, o dano muscular causado pelas miopatias WS e WB, bem como de outras doenças musculares, pode refletir em marcadores bioquímicos, como enzimas, por exemplo. Abasht et al. (2016) identificaram quais marcadores bioquímicos tem maior importância na distinção dos perfis de aves afetadas e não afetadas por WB. Segundo os autores, os níveis elevados de dissulfureto de cisteína e glutationa peroxidase nos tecidos afetados são marcadores biológicos de degeneração muscular e estresse oxidativo, visto que refletem a exposição do organismo aos radicais livres.

A ruptura das células causada pela miopatia pode resultar no aumento dos níveis de enzimas séricas como creatina fosfoquinase (CK), alanina aminotransferase (ALT), aspartato transaminase (AST) e lactato desidrogenase (LDH) (LUMEIJ, 2008; KUTTAPPAN et al., 2013b). Já que essas são enzimas intracelulares consideradas bons marcadores de dano muscular, podem auxiliar na identificação de aves que apresentem a miopatia WB. Sendo assim, acredita-se que esta análise pode ser utilizada em associação com outros métodos que prevejam miopatias peitorais em aves vivas.

Etiologia

Existe um forte componente não genético que pode estar relacionado ao aparecimento de WB e dos fatores que desencadeiam o processo de ruptura das fibras musculares do peito. Inicialmente, citada por Mutryn et al. (2015) como miopatia nutricional, a influência de fatores nutricionais na ocorrência de miopatias é geralmente associada com baixos níveis de selênio e vitamina E na dieta, sendo sua apresentação clínica mais comum a estriação branca na musculatura esquelética, além da necrose, degradação, mineralização e regeneração das fibras.

Atrelado a isso, existe um número expressivo de estudos recentes que relacionam maiores taxas de crescimento com diferentes distúrbios musculares como a baixa densidade capilar, estresse metabólico e redução do potencial glicolítico. Apesar de ter sido associada a diferentes fatores como gênero (TROCINO et al., 2015), idade (KUTAPPAN et al., 2015), genética (ZAMBONELLI et al., 2017) e nutrição (TROCINO et al., 2015; CRUZ et al., 2017), a maioria destes fatores demonstra que o peso corporal ao abate é diretamente relacionado com o aparecimento das lesões (KUTAPPAN et al., 2016).

Sendo assim, é possível que haja uma forte relação de causa-efeito entre o peso das aves e a ocorrência das lesões. Kutappan et al. (2013) observaram diferenças na ocorrência de miopatias durante o abate atribuídas ao peso vivo de frangos de corte. Além disso, devido a sua correlação com o ganho de peso, também consequente da idade das aves, estas lesões têm sido mais observadas em frangos de corte a partir dos 21 dias de idade, quando a taxa de crescimento é maior, e foram encontradas em mais de 50% das aves avaliadas no estudo de Mutryn et al. (2015). Portanto, quanto maior o peso da ave e sua carcaça, maior a possibilidade de o peito apresentar lesões de WB. Há, ainda, a necessidade de desenvolver estudos para identificar a complexa origem e causa das miopatias peitorais de frangos de corte de rápido crescimento.

Idade das aves e restrição alimentar

No que diz respeito à idade do aparecimento das lesões, ainda há poucos estudos que comparem a incidência de miopatias em aves de diferentes idades. Kutappan et al. (2017) concluíram que graus severos de WS e WB estão associados com aves mais pesadas e também mais velhas, quando em seu estudo, a severidade das duas miopatias aumentou significativamente entre a sexta e nona semana de idade dos frangos. De acordo com Radaelli et al. (2016), a severidade das lesões musculares aumenta com a idade do animal e também a redução do número de capilares periféricos às fibras musculares.

A restrição alimentar passou a ser estudada a partir do momento em que a presença de WB foi atribuída a maior taxa de crescimento e menor idade de abate das linhagens atuais de frangos de corte. Vista como uma ferramenta de controle do crescimento corporal, a restrição alimentar pode ser uma das alternativas para redução de diferentes danos metabólicos e musculares em aves de produção. Boostani et al. (2010) observaram que frangos de corte submetidos à restrição alimentar de 7 a 21 dias de idade apresentaram mesmo ganho de peso e menor conversão alimentar aos 42 dias do que aves que foram sempre alimentadas *ad libitum*. No mesmo estudo de Radaelli et al. (2017) aves submetidas à restrição alimentar de 13 a 21 dias apresentaram diminuição da incidência de degeneração das fibras musculares em relação às aves que receberam alimentação à vontade. Por outro lado, após o período de restrição alimentar, as aves apresentaram um crescimento compensatório que também acarretou em danos importantes às fibras musculares.

O ganho de peso compensatório de aves anteriormente submetidas à restrição alimentar também foi observado no estudo de Butzen et al. (2013). Estes autores afirmam que a utilização de restrições alimentares quantitativas pode ser pensada como estratégia de manejo para reduzir o ganho de peso inicial, sem afetar o desempenho final de machos e fêmeas da linhagem Cobb, assim como o peso do peito pode ser recuperado em frangos de corte abatidos aos 42 dias quando comparadas a aves que receberam alimentação à vontade durante toda sua vida produtiva.

Em contrapartida, restrições alimentares realizadas em frangos de corte machos nos primeiros 14 dias de idade causaram efeitos negativos à carcaça, como aumento da deposição de gordura, necrose e má organização das fibras musculares (VELLEMAN et al., 2010). Desta forma, é importante que as novas pesquisas considerem a idade, o manejo de restrição alimentar escolhido e a combinação destes dois fatores como variáveis que afetam o rendimento do peito e a ocorrência de WB, e que podem contribuir para o entendimento da evolução desta e de outras miopatias, tendo em vista em diminuir os efeitos negativos e os graus de severidade que estas trazem ao peito, que é o corte comercial de maior importância na produção avícola.

Utilização de imagens ultrassonográficas da musculatura peitoral de frangos de corte

A ultrassonografia em tempo real é uma técnica não invasiva e eficaz de estimar características de conformação corporal nos animais. Dados obtidos em animais vivos, a partir da ultrassonografia para avaliação de características de carcaça, possibilitam a avaliação de um número maior de animais dentro de uma população (CREWS & KEMP, 2002). Diferentes autores já propuseram a utilização do método para correlacionar imagens obtidas pelo ultrassom com o peso de peito e suas proporções em relação à carcaça de frangos de corte (GAYA et al., 2006; OVIEDO et al., 2007). Além disso, as imagens ultrassonográficas podem identificar a presença de diversas alterações teciduais, como edema, inflamação, acúmulo de gordura, fibrose e calcificação (CASE et al., 2012; PILLEN et al., 2010).

O estudo de Case et al. (2012) já obteve sucesso ao sugerir a utilização do ultrassom como ferramenta viável para correlacionar a profundidade do peito com o rendimento desta musculatura em perus. A partir de imagens ultrassonográficas do músculo *Pectoralis major* de aves vivas, seria possível identificar as fases de desenvolvimento e surgimento da miopatia, o que auxilia na compreensão do desenvolvimento muscular dos frangos de corte e os principais mecanismos que regulam a formação e alteração das fibras musculares. Além disso, a utilização dessa ferramenta como forma de predizer a existência da miopatia em aves vivas se daria a partir de medidas feitas pelo aparelho.

Espera-se que peitos acometidos possam apresentar diferença de ecogenicidade, por exemplo, em relação a peitos normais, uma vez que esta medida, normalmente utilizada em medicina humana e veterinária, está relacionada com lesão tecidual, apresentando-se numericamente mais elevada quando há uma maior concentração de tecido conjuntivo fibroso nos tecidos submetidos a esta avaliação por imagem. Dados da medicina humana podem ser úteis para a obtenção de metodologias a serem utilizadas na avicultura. Um estudo feito por Sholten et al. (2003) encontrou maior ecogenicidade em imagens de ultrassom obtidas da musculatura de crianças com distrofia muscular e miopatias congênitas.

A ultrassonografia pode contribuir com pesquisas da área avícola através do reconhecimento da miopatia no animal vivo, podendo vir a ser uma nova ferramenta de diagnóstico de WB, possibilitando o desenvolvimento de estratégias na seleção dos animais. Assim, novas metodologias e procedimentos operacionais podem surgir para melhoria da utilização de imagens de ultrassom para avaliar características da carne de peito de frangos, sobretudo da miopatia WB.

HIPÓTESES E OBJETIVOS

Hipóteses

Restrições alimentares podem influenciar o desempenho produtivo, o peso do peito e as características das células musculares de frangos de corte em função da diferente taxa de crescimento.

Reduções no ganho de peso e crescimento muscular do peito de frangos de corte podem diminuir os danos às células musculares, reduzindo a ocorrência de *wooden breast.*

A ecogenicidade, obtida através de imagens de ultrassom podem ser uma ferramenta de avaliação *in vivo* das alterações que ocorrem na musculatura peitoral de frangos de corte.

Maiores graus da miopatia *wooden breast* estão relacionados a alterações de enzimas sorológicas, histomorfométricas e na qualidade da carne.

Objetivos

Avaliar o efeito de restrições alimentares quantitativas sobre o desempenho produtivo, a ocorrência de WB em frangos de corte de 8 a 49 dias de idade, bem como correlacionar os graus de WB com a qualidade da carne, perfil sorológico das aves e parâmetros histológicos da carne do peito. Adicionalmente, o projeto propõe criar uma ferramenta de avaliação *in vivo* da miopatia e identificar em que idade ela predominantemente surge e pode ser avaliada.

CAPÍTULO II[1]

Artigo elaborado conforme as normas do periódico Poultry Science

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PROCESSING AND PRODUCTS

FEED RESTRICTION AND WOODEN BREAST

An in vivo evaluation of the effects of feed restriction regimens on wooden breast

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ABSTRACT A study was conducted to evaluate the effectiveness of utilizing ultrasound (US) images of breast muscle to predict wooden breast (WB) myopathy in live broilers subjected to different feed restriction programs. A total of 1,800 Cobb × Cobb 500 slow feathering male chicks were fed 6 treatments and 12 replicates of 25 birds each using a completely randomized design. Birds were fed ad libitum or received 50, 60, 70, 80, and 90% of the quantity consumed by the chickens fed ad libitum on the previous day. The programs were applied from 8 to 49 d to induce differences on growth rate. From 8 to 49 d, US images of breast muscle were weekly obtained from all birds to determine echogenicity. One bird per pen was slaughtered every week, breast was weighed and visual degrees of WB were provided as: 0 (normal), 1 (mild hardening in the upper), 2 (moderate hardening in the upper and/or lower), 3 (severe hardening), and 4 (severe hardening with hemorrhagic lesions and yellow fluid). From 21 to 49 d, serum was collected for serologic profile and samples of breast were collected for meat quality and histomorphometric analysis. In the overall period and weekly, BW gain and breast yield decreased linearly ($P < 0.05$) when broilers were subjected to feed restrictions. Echogenicity of breast fillets increased according to WB severity ($P < 0.05$). The WB scores 3 and 4 presented higher ($P < 0.05$) echogenicity at 35, 42, and 49 d. From 21 to 49 d, WB scores 3 and 4 also had higher cooking loss, fiber diameter, serum creatine kinase and lactate dehydrogenase, whereas fiber density per area was lower ($P < 0.05$) compared to normal breasts. The increasing feed consumption improved broiler growth and breast yield as well as induced the severity of WB and breast muscles presented increased echogenicity according to the WB severity. Ultrasound images can be adequately utilized to predict the WB of broilers in vivo.

Key words: broiler, fiber diameter, serum enzymes, ultrasound, wooden breast

INTRODUCTION

The wooden breast (**WB**) myopathy is a meat quality problem leading to downgrades, which can be visually detected. It has been negatively affecting the poultry industry due to rejection by the customer and, therefore, has been demanding the redirection of the affected breast fillets to further processing into lower value products. Increased cooking loss, lower water retention and reduced protein functionality of WB meat are further issues that demand changes in breast meat processing methods when compared to normal breast meat that also diminish profitability (Trocino et al., 2015).

In the presence of WB, the *Pectoralis major* muscle exhibits varying degrees of fiber necrosis, polyphasic myodegeneration with regeneration and accumulation of interstitial connective tissue or fibrosis (Shivo et al., 2014), variability in fiber size, immune cell infiltration and extensive collagen deposition (Kuttappan et al., 2013b; Velleman and Clark, 2015). Despite the increased research on the WB problem, there has not been any important finding on this issue that could lead to its resolution so far. A significant correlation, however, has been shown between WB and bird growth rate (Trocino et al., 2015; Cruz et al., 2017). Therefore, feed restriction has been thought as a method capable of reducing the incidence of WB.

One limitation of the studies devoted to understand factors that trigger the WB is the prediction of this myopathy in birds at an early age, before they can visually present it. Ultrasound (US) is a consolidated diagnosis method of muscle issues in human and veterinary medicine, which also has been speculated as a tool to evaluate breast muscle characteristics in lived birds (Gaya et al., 2006; Oviedo et al., 2007; Case et al., 2012). In addition, echogenicity, which is a light intensity measurement calculated in a grey scale, presents a high correlation with the amount of interstitial fibrous tissue and fat, and is a reliable measure to identify muscle damages and determine the severity of lesions in breast meat or in animals presenting muscular disorders (Pillen et al., 2009). Thus, it is possible that the tissue changes caused by WB lesions could present a similar effect on echogenicity, allowing its identification at early ages, and therefore be used to anticipate the amount of birds affected at processing. This could also be used as a tool in genetic selection against WB.

If the increased metabolic rate presently noticed by ad libitum fed birds is a main triggering cause of WB, therefore, there would be a decrease in growth rate that corresponds to the inhibition of processes that initiate it. The objective of the present study was to gradually feed restrict broilers in order to obtain different degrees of WB affection such that the use of US images of breast muscle could early detect changes that can predict WB in vivo.

MATERIALS AND METHODS

All procedures used in this study were approved by the Ethics and Research Committee of the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Birds and Experimental Diets

A total of 1,800 one-day-old, slow-feathering Cobb × Cobb 500 male broiler chicks, vaccinated for Marek's disease at the hatchery (BRF, Lajeado, Brazil) and averaging 44 ± 1.1 g were randomly placed into 72 floor pens (1.65 × 1.65 m; 9.2 birds/m^2). Bedding material was rice hulls and individual pens were equipped with a 15 kg capacity tube feeder and 3 nipple drinkers. Average temperature was 32ºC at placement being reduced by 1ºC every 2 d until 23ºC to provide comfort throughout the study with the use of thermostatically controlled heaters, fans and foggers. Lighting was continuous until 7 d of age, with a 14L:10D cycle used afterwards. Birds had ad libitum access to water.

Birds were individually weighed and identified in the neck with plastic numbered tags and allocated into 6 treatments with 12 replications of 25 birds each in a completely randomized design. Broilers were fed pelleted diets formulated using Brazilian commercial nutrient and energy practical levels in a 5-phases feeding program composed by pre-starter (1 to 7 d), starter (8 to 21 d), grower I (22 to 35 d), grower II (36 to 42 d), and finisher (43 to 49 d) diets. These diets had, respectively, 3,030; 3,120; 3,200; 3,220 and 3,200 kcal/kg AME_n; 23.8; 21.9; 20.3; 19.7 and 18.7 % CP; 1.05; 0.96; 0.86; 0.79 and 0.74 % Ca, 0.59; 0.47; 0.46; 0.42 and 0.38 % Av. P, and 1.31; 1.20; 1.12; 1.07 and 1.02 % digestible lysine (**dig. Lys**).

All birds were ad libitum fed from 1 to 7 d as well as programs that restricted feed intake by 50, 60, 70, 80, and 90% when compared to the ad libitum fed birds were applied from 8 to 42 d. The provided feed and its leftovers were daily weighted in all pens from ad libitum fed birds, which was used to calculate the amount of feed provided for the feed restriction treatments.

Growth Performance, Carcass Yield, Myopathy Scoring

Chicks were individually weighed into groups of 25 birds per pen before placement. Body weight gain, feed intake (**FI**) and feed conversion ratio corrected for the weight of dead birds (**FCR**) were weekly determined from 8 to 49 d. At 49 d, 5 birds were randomly selected from each pen (n = 360) and processed for carcass and commercial cuts evaluation. Broilers were fasted for 8 h and individually weighed prior to slaughter. Birds were humanely rendered insensible using electrical stunning (45 V for 3 s), then bled through a jugular vein cut for 3 min, scalded at 60ºC for 45 s, and lastly defeathered. Evisceration was manually performed and carcasses were statically chilled in ice for approximately 3 h. Eviscerated carcasses (without feet and neck) were hung for 3 min to remove excess water prior to weighing. Commercial cuts were performed by a crew of industry-trained personnel into bone-in drumsticks, thighs, wings as well as deboned breast fillets and tenders. Abdominal fat was weighed separately. Carcass yield was expressed relative to live weight while commercial cuts and abdominal fat were expressed as percentage of the eviscerated carcass. Breast fillets and tenders were manually deboned from the carcasses. Wooden breast evaluations were performed on boneless and skinless breasts.

Deboned fillets were separated into groups by the presence or absence of WB. Breast fillets were submitted to a 4-subject panel evaluation to provide scores of WB as: normal breast (score 0); mild hardening in the upper (score 1); moderate hardening in the upper and/or lower part of the fillet (score 2); severe hardening (score 3), and severe hardening with hemorrhagic lesions, increased volume, and presence of yellow fluid (score 4).

Weekly Measurements: Ultrasonography, Serum, Myopathies and Meat Quality

All birds were submitted to ultrasonography every week from 8 to 49 d using a Logiq E equipment (GE Healthcare, Little Chalfont, United Kingdom) with a probe (L8-18i-RS) placed on the skin surface parallel to the keel as well as cranial and caudal to the ribcage. The image frequency was 18 MHz with 64% brightness. Mean gray values were calculated from the obtained histogram (black to white scale from 0 to 255, respectively) using Adobe Photoshop (Adobe Systems Inc, San Jose, CA, USA) to determine echogenicity (Pillen et al., 2009). The breast depth was also taken through the images using an Image ProPlus software (Media Cybernetics Inc, Bathesda, MD, USA) and according described to Case et al. (2012).

One bird from each pen was weekly randomly taken in order to collect blood for serological analyses as well as to assess myopathies and other meat quality parameters from 21 to 49 d. Birds were euthanized using the same methodology used to evaluate carcass yield as shown above. Blood was collected immediately after a jugular vein cut; blood was then centrifuged (2,500 RPM for 10 min). Obtained serum was used to estimate plasma activities of creatine kinase (**CK**) (Randox, Crumlin, County Antrim, UK), alanine aminotransferase (**ALT**) and lactate dehydrogenase (**LDH**) (Sigma Aldrich, Poole, Dorset, UK), as well aspartate aminotransaminase (**AST**) (Thermo Electron Corporation, Runcorn, Warrington, UK).

Breast meat color was assessed using the CIELAB trichromatic system as lightness (**L***), redness (**a***), and yellowness (**b***) values from the surface of *Pectoralis major* using a chromometer (CR-400, Konica Minolta, New Jersey, USA). Breast meat pH was measured at 24 h postmortem from the homogenates of meat with a pH meter (HI 221, Hanna Instruments, Ottawa, Canada) coupled with a glass electrode.

Water holding capacity was measured using meat cubes (2 g each) laid between two filter paper circles placed on glass plates, with a 10 kg weight for 5 min. Difference between initial and final weight corresponded to the drip loss and was expressed in percentage (Zhang et al., 2006). The right side of breast meat was placed into plastic bags and cooked in boiling water (82 to 85ºC) until 72ºC of internal temperature (Boccard et al., 1981) in order to measure cooking loss. After room temperature, samples were weighted, and the difference between the weights before and after cooking corresponded to cooking loss.

Shear force and shear energy were assessed with 4 measurements (2 cranial and 2 medial) conducted in raw breast fillets using a TA.XT2 Texture Analyzer with a Meullenet-Owens Razor Blade (Texture Technologies Corp. and Stable Micro Systems Ltd., Hamilton, MA). This blade was placed with muscle fibers longitudinally to determine Meullenet-Owens razor shear force (**MORS**) and Meullenet-Owens razor shear energy (**MORSE)** (Cavitt et al., 2004; Meullenet et al., 2004;Tijare et al., 2016). Other three samples per breast were cut in small slices (2 × 2 × 1 cm) and placed with the muscle fibers longitudinally oriented to the probe to evaluate work of shear and firmness using a TA.XT2 Texture Analyzer with a

Warner-Bratzler probe (Texture Technologies Corp. and Stable Micro Systems Ltd., Hamilton, MA).

Analysis of muscle structure consisted of dimensional morphometric measurements as minimum fiber diameter and fiber density (fiber/cm^2). Thus, samples (~2 cm^3) were collected from the same area of each breast muscle from one bird per replication. Samples were oriented for transverse fiber sectioning and serial cryostat sections (10 µm; −20ºC) were cut and stained with hematoxylin and eosin (Cumming et al., 1994). Muscle fiber size was estimated by measuring the minimum fiber diameter (µm) of 100 fibers, and the density of muscle fibers (fibers/cm^2) was estimated by point-counting stereology using 500 points. Both analyses were estimated using the Image ProPlus software (Media Cybernetics Inc, Bathesda, MD, USA). The partial volume of muscle tissue (vol, %) was estimated by point-counting stereology using the M42 Testing System (Weibel, 1979).

Statistical Analysis

Data were analyzed using the GLM procedure of SAS Institute (2009; SAS Inst. Inc., Cary, NC). Significance was accepted at $P < 0.05$. Data were submitted to a one-way ANOVA and mean differences were separated using Tukey's HSD test (Tukey, 1991). Feed restriction was used as an independent variable. The obtained scores of WB afterwards were considered as independent variables to evaluate meat quality parameters, echogenicity, serologic enzymes, and histomorphometric measurements according to broilers' age. Pearson's correlation analysis was conducted using the CORR procedure.

RESULTS

Effects of feed restriction on performance, carcass and breast yields, breast myopathies and echogenicity are presented in Table 1. Feed restriction applied at 50, 60, 70, 80, and 90%

of the ad libitum daily FI was linearly adjusted with the overall cumulative FI (Y = - 57.369x + 5,956; R^2 = 0.98; P < 0.0001). Effects were linear in BW (Y = - 37.317x + 3,597; R^2 = 0.98; P < 0.0001), FCR (Y = - 0.0026x + 1.902; R^2 = 0.53; P < 0.0001), carcass yield (Y = - 0.088x + 82.91; R^2 = 0.63; P < 0.0001) and breast fillets (Y = - 0.0048x + 26.30; R^2 = 0.63; P < 0.0001). Effects of feed restriction programs were not observed in the echogenicity at 7 (64.4), 14 (73.8), 21 (74.4), 28 (79.8), and 35 d (80.6); however, it was lower at 42 and 49 d (P < 0.001) in broilers feed restricted at 50% and 60% of the ad libitum.

Scores of WB were not different (P > 0.05) between broilers fed ad libitum and 90% of FI, but birds fed ad libitum had higher (P < 0.0001) WB score when compared to birds that received 50, 60%, 70 and 80% of FI (Table 1). Feed restriction was also negatively correlated with cumulative BW gain, breast weight, breast yield, and WB scores at 49 d as (0.993, 0.920, 0.791, and 0.826, respectively; P < 0.0001). The WB scores had positive correlation (P < 0.0001) with BW, BW gain and breast yield, (0.821, 0.816, and 0.627, respectively; P < 0.0001).

Occurrences of WB among feed restriction programs and broiler age are shown in Figure 1. There were no evidences of WB at 7 d (all were scored 0); however, scores 0 and 1 were observed at 14 d. The WB scores ranged from 58.3 to 100% and 91.7 to 100% at 42 and 49 d, respectively. Occurrences of WB score 4, showing severe hardening with hemorrhagic lesions, increased at 42 and 49 d, when broilers had 3,079 and 3,477 g of BW, respectively (P < 0.05). At 49 d, breast fillets with WB score 0 were not found and birds fed 50% of the ad libitum FI had only 8.33% of this score. At 21 and 28 d, the WB score 4 also was not observed in the evaluated breast fillets.

Echogenicity of breast muscle increased with WB severity (P < 0.005) and bird age, as presented in Table 2. Echogenicity of breast muscle evaluated in lived broilers at 35, 42, and 49 d was higher (P < 0.05) in breast fillets classified with WB scores 3 and 4 compared to the score 1 and normal breasts were not verified. The WB scores had similar echogenicity at 21 and 28 d (*P* > 0.05).

Depth of breast fillets was and WB scores are presented in Table 3. Means of breast fillets depth increased as birds aged in parallel with WB severity (*P* < 0.0001). Breast fillets classified with WB scores 3 and 4 presented higher (*P* < 0.001) depth at 42 and 49 d compared to breasts with WB score 1.

Color measurements and pH of breast fillets at 24 h postmortem are presented in Table 4. All scores of WB had similar pH from 21 to 42 d (*P* > 0.05); however, at 49 d, breasts with WB score 4 had higher (*P* < 0.004) pH (5.7) when compared to scores 1 (5.2) and 2 (5.3). Breast fillets scored 4 presented higher (*P* < 0.001) L*, a*, and b* values at 35 (55.6, 13.9, and 15.9, respectively) and at 42 d (55.5, 13.8, and 16.2, respectively) compared to breasts with WB score 1 at 35 d (51.3, 11.4, and 13.3, respectively) and at 42 d (51.4, 11.9, and 12.9, respectively). Firmness and water-holding capacity did not differ from the WB scores (*P* > 0.05) in all evaluated ages. However, breast fillets with WB scores 3 and 4 had higher cooking loss (*P* < 0.001) when compared to the normal breast at 35 and 42 d. At 49 d, fillets classified with WB scores 3 and 4 presented higher (*P* < 0.001) cooking loss compared to breasts with WB score 1 and 2 (Table 5).

The severity of WB was also related with shear force (MORS) and shear energy (MORSE) of breast fillets from broilers at different ages. Shear force and shear energy, measured in cranial and medial sections of raw breast fillets, were lower (*P* < 0.001) in WB scores 2, 3, and 4 when compared to normal breasts at 42 d. At 42 d, fillets classified as WB score 4 presented lower (*P* < 0.05) cranial MORS when compared to normal breasts, 13.8 and 17.2 N, respectively. Raw breast fillets scored 4 (99 N.mm) also presented lower (*P* < 0.05) cranial MORSE when compared to normal breasts (171 N.mm). However, means of MORS and MORSE of raw breast fillets were similar ($P > 0.05$) among WB scores at 21, 28, 35, and 49 d.

There were significant effects of WB scores on histomorphometric measurements (Table 6). The density of muscle fibers (fibers/cm^3) decreased with WB severity ($P < 0.001$). From 21 to 35 d, breast fillets classified with WB scores 0 and 1 showed higher ($P < 0.001$) density of muscle fibers and lower fiber diameter ($P < 0.001$) per area compared to WB score 3. At 49 d, also breast fillets with WB score 1 had higher fiber/cm^3 and lower fiber diameter ($P < 0.001$) compared to the WB scores 3 and 4.

The concentration of all serum enzymes increased as WB scores were higher at all ages, except at 21 d (Table 7). The concentration of ALT was not significantly different according to the WB scores in all evaluated ages. However, serum concentration of AST, CK and LDH increased with the severity of WB myopathy from 28 to 49 d ($P < 0.001$). Higher CK serum concentration ($P < 0.001$) was observed in broilers that had WB score 4 compared to other WB scores from 28 to 49 d. From 28 to 49 d, LDH concentration also was higher in breasts classified as score 3 and 4 when compared to scores 0, 1 and 2 ($P < 0.001$). At 28 d, the concentration of AST was the highest in score 3, while from 35 to 49 d it was the highest in score 4 when compared to lower severe scores ($P < 0.001$).

The WB myopathy was positively correlated ($P < 0.001$) with echogenicity, depth, cooking loss, fiber diameter and lightness values of breast muscle of male broilers from 21 to 49 d. The WB was positively correlated ($P < 0.001$) with echogenicity at 21 (0.510), 28 (0.531), 35 (0.470), 42 (0.430) and 49 d (0.548) as well as with breast depth ($P < 0.001$) when values were 0.501, 0.851, 0.848, 0.773, and 0.614 at 21, 28, 35, 42, and 49 d, respectively. A negative correlation, however, was observed with fiber density per area and MORSE of raw breast fillets of broilers from 21 to 49 d ($P < 0.001$) (Table 8).

DISCUSSION

In the current study, linear impacts on BW gain and FCR were observed when feed restriction was applied at 50, 60, 70, 80, and 90% of the ad libitum daily FI. These results were in line with Urdaneta-Rincon and Leeson (2002) and Boostani et al. (2010) that fed birds in a limited period per day or at 5, 10, and 15% feed restriction levels. In parallel, carcass and yields of commercial cuts also decreased when broilers were submitted to feed restrictions.

The correlation between WB scores and growth rate and breast yield is high in the presently grown broiler (Trocino et al., 2015; Cruz et al., 2017). In the present study, WB occurrence was maximized when feed intake and BW gain were highest. Moreover, the severity of WB was reduced with birds having reduced BW gain as has been earlier shown when 80% of feed restriction from 13 to 28 d was used (Radaelli et al., 2017) or when growth was restricted by feeding Lys deficient diets (Cruz et al., 2017).

The use of US images may be a convenient method to detect normal and pathological muscle tissues in animals (Crews and Kemp, 2002; Pillen, 2010). The utilization of the echogenicity scale to predict WB in live broilers, however, has not been used as of yet. Since this method is not invasive, it allows the in vivo evaluation of the growth-related changes in breast muscle as bird's age. Pillen et al. (2009) previously used the mean gray scale expressed as a value between 0 and 255 to calculate the echo intensity. These authors observed that the muscle echo intensity was significantly correlated to the amount of fibrous tissue found in dog muscle.

The increased echogenicity due to WB severity in broiler chickens corroborates the fact that lesions as well as other changes in muscle tissue can be correlated to higher echo measurements, which is probably due to the muscle cell replacement with fat and connective tissue. Sholten et al. (2003) observed that children with muscular dystrophy, spinal muscle atrophy, and congenital myopathies had increased echogenicity using US images. In the present study, the correlation between echogenicity at 21 d and 49 d and between 28 d and 49 d was 0.26 ($P < 0.05$) and 0.30 ($P < 0.05$), respectively.

Depth estimations of *Pectoralis major* using US were conducted according to Case et al. (2012) and showed that this method was well correlated with breast meat yields. This has been previously shown as a feasible selection criterion to obtain improvements in breast weight (Gaya et al., 2006). In the present study, correlations between WB scores and breast depth were also positive including at different ages, and have demonstrated that an increased WB severity was associated to a higher breast depth.

In the current study, higher WB scores negatively affected meat quality parameters, such as cooking loss and work of shear. Several authors (Kuttappan et al., 2013b; Petracci et al., 2014; Sihvo et al., 2014) have suggested that the WB myopathy are related to a dysfunction in fiber metabolism. High cooking loss was previously associated to protein denaturation and the shrinkage of muscle fibers (Huff-Lonergan and Lonergan, 2005). In the present study, cooking loss was higher in breast fillets with WB scores 3 and 4 when compared to normal breasts at 35, 42, and 49 d. Tijare et al. (2016) also found similar results when non-marinated fillets with both severe lesions of WB were compared to normal fillets. Cooking losses were associated to the reduction in water-holding capacity (Soglia et al., 2016; Kuttappan et al., 2017).

Normal breast fillets presented higher work of shear than those with WB scores 2, 3 and 4 as well as higher MORS and MORSE. These were previously used to predict the texture of breast meat (Lee et al., 2014). Normal fillets had higher MORSE and MORS values when compared to other WB scores at 42 d. These results paralleled those by Tijare et al. (2016), who found that non-marinated normal fillets had higher MORSE values than other WB scores in male broilers at 6 weeks.

The histopathology of WB lesions was previously shown in a positive correlation with macroscopically visual lesions (Kutappan et al., 2013b; Soglia et al., 2016). Histomorphometric measurements of *Pectoralis major* in the present study showed to be positively correlated with high WB scores. The number of muscle fibers per cm² obtained was higher in breast samples with reduced WB severity from 21 to 49 d. In this context, Soglia et al. (2016) also found that fibers decreased in number but had higher size in breast samples affected by severe WB lesions. In both cases, the decreased muscle fiber number could be associated to a result of severe fibroses with abundant connective tissue and increasing of intramuscular fat, leading to a decrease in the number of fibers by analyzed area. Additionally, the minimum fiber diameter of myofibers evaluated in the present study increased according to WB severity in all ages until 49 d. This corroborates the hypothesis that the increased *Pectoralis major* thickness contains larger myofibers, as well as Sihvo et al. (2014) observed higher variability in muscle fibers size in histological samples with lesions of WB.

Hematological analyses are very useful and common clinical tools used for the diagnosis of various disease conditions in animals. Muscle damage observed in WB as well as other muscular diseases can be diagnosed by the observation of the serum enzyme profile. The WB affected muscle presents disrupted cells resulting in increasing levels of serum enzymes such as CK, ALT, AST, and LDH (Lumeij, 2008; Kuttappan et al., 2013). Macrae et al. (2006) reported that these intracellular enzymes are good markers of muscle damage and this analysis can be used in association to other methods to predict breast myopathies in vivo. The presence of WB lesions could be also explored as a factor that results higher concentration of these serum enzymes. In the present study, higher serum CK, AST, and LDH concentrations were observed in breast fillet with WB scores 3 and 4 when compared to WB score 0 and 1.

It can be concluded from the findings presented in this study that feed restriction impairs live performance as well as breast meat yields at any level used, but can gradually reduces the incidence and degree of severity of WB. Therefore, feed restriction can be used as a tool in the study of this myopathy. Serum enzymes, such as AST, CK and LDH are highly correlated with WB; however, their increase is a consequence of muscle damage and, therefore, has a limited use in the prediction of birds that will present WB at processing ages. The use of US, on the other hand, can early detect alterations, and therefore, is a better tool when prediction of WB becomes necessary, such as in genetic selection programs.

ACKNOWLEDGEMENTS

The authors acknowledge the funding from Conselho Nacional de Pesquisa (CNPq – Brasilia, DF, Brazil) and Cobb-Vantress for their support in this project.

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Table 1. Effects of feed restriction on performance, echogenicity, breast yield and means of wooden breast of broiler from 8 to 49 d

Item	BW gain	FCR	Carcass[1]	Breast fillets[2]	Tenders	WB score[3]	Echogenicity[4]					
							14 d	21 d	28 d	35 d	42 d	49 d
Restriction program[5]												
Ad libitum	3,632^{a}	1.663^{b}	83.7^{a}	26.4^{a}	5.40^{a}	3.82^{a}	76.2	76.9	82.9	84.8	97.9^{a}	98.1^{a}
90%	3,198^{b}	1.669^{b}	81.8^{b}	24.7^{ab}	5.38^{ab}	2.94^{ab}	75.2	75.4	81.6	81.6	92.7^{ab}	92.8^{ab}
80%	2,836^{c}	1.676^{b}	80.5^{c}	24.6^{b}	5.38^{ab}	2.27^{b}	74.5	75.2	80.9	81.6	89.8^{ab}	91.7^{abc}
70%	2,471^{d}	1.699^{b}	79.6^{cd}	23.7^{c}	5.32^{ab}	2.00^{bc}	73.5	73.9	79.3	79.7	87.6^{ab}	91.3^{abc}
60%	2,105^{e}	1.749^{a}	79.4^{cd}	22.2^{cd}	5.27^{ab}	1.04^{cd}	71.6	72.8	78.1	79.3	86.7^{ab}	87.0^{bc}
50%	1,748^{f}	1.793^{a}	79.2^{d}	20.4^{d}	5.12^{b}	0.47^{d}	71.8	72.2	75.8	76.7	82.5^{b}	83.4^{bc}
Mean	2,665	1.708	80.7	23.5	5.31	1.91	73.8	74.4	79.8	80.6	89.5	90.7
SEM	76.12	0.007	0.22	0.29	0.028	0.004	0.89	1.41	1.27	1.29	1.23	1.10
P-value	0.001	0.001	0.001	0.001	0.033	0.001	0.6177	0.9399	0.5970	0.4252	0.0033	0.005

^{a,d}Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Eviscerated carcass as a percentage of body weight at 49 d (n = 360).

[2]Skinless boneless *Pectoralis major* as proportion of carcass.

[3]Wooden breast (WB) scores from 8 to 49 d were square root transformed to present normal distribution of the residual; however, values are real means (n = 432).

[4]Echogenicity, mean gray values were calculated from the obtained histogram (black to white scale from 0 to 255, respectively) of *Pectoralis major* muscle in live broilers (n = 72).

[5]Feed restricted birds had 50, 60, 70, 80, or 90% of the ad libitum consumption on the previous day; programs were applied from 8 to 49 d (n = 1,800).

Table 2. Echogenicity of *Pectoralis major* muscle of live broilers as they aged[1]

Age, d	WB score[2]					SEM	*P*-value
	0	1	2	3	4		
21	72.6	71.2	78.3	74.4	.	1.41	0.446
28	78.9	82.2	76.6	82.1	.	1.27	0.441
35	.	77.6[b]	79.1[ab]	88.0[a]	81.0[ab]	1.28	0.058
42	.	86.8[b]	88.6[b]	93.3[ab]	98.1[a]	1.23	0.005
49	.	85.3[b]	90.0[ab]	97.4[a]	94.8[a]	1.09	0.006

[a,b]Means with different superscript letters differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Echogenicity, mean gray values were calculated from the obtained histogram (black to white scale from 0 to 255, respectively) using Adobe Photoshop (n = 360).

[2]Valueless cells are due to the absence of the corresponding WB score.

Table 3 Depth of *Pectoralis major* muscle of live broilers as they aged

Age, d	WB score[1]					SEM	*P*-value
	0	1	2	3	4		
21	15.3[b]	18.1[a]	19.7[a]	19.7[a]	.	0.38	0.001
28	15.3[c]	17.5[b]	18.5[b]	20.8[a]	.	0.31	0.001
35	.	17.2[c]	19.8[b]	21.5[b]	24.0[a]	0.38	0.001
42	.	19.6[c]	23.2[b]	25.2[ab]	25.8[a]	0.42	0.001
49	.	20.4[c]	24.7[b]	27.1[a]	26.0[ab]	0.42	0.001

[a,c]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Valueless cells are due to the absence of the corresponding WB score.

Table 4. Color and pH of breast fillets as broilers aged[1]

Age, d	Item	WB score[2]					SEM	*P*-value
		0	1	2	3	4		
21	pH	7.2	7.1	7.2	7.3	.	2.70	0.430
	L*	50.0	47.6	50.8	51.8	.	1.02	0.388
	a*	11.4[b]	11.6[b]	13.2[ab]	13.8[ab]	.	1.6.1	0.002
	b*	12.3	17.1	12.6	14.8	.	1.95	0.506
28	pH	6.9	7.0	7.2	7.1	.	5.02	0.146
	L*	51.5[bc]	51.0[c]	53.6[ab]	54.2[a]	.	4.80	0.001
	a*	11.8[b]	13.0[ab]	13.0[ab]	14.0[a]	.	1.26	0.002
	b*	12.6[b]	13.0[b]	15.4[a]	16.3[a]	.	1.56	0.001
35	pH	6.6	6.6	6.6	6.7	6.8	3.60	0.283
	L*	51.3[b]	51.5[b]	52.7[b]	53.7[ab]	55.6[a]	5.44	0.001
	a*	11.4[b]	11.8[b]	11.6[b]	12.9[ab]	13.9[a]	1.36	0.001
	b*	13.3[b]	13.5[b]	15.3[ab]	15.4[ab]	15.9[a]	1.59	0.001
42	pH	6.2	6.3	6.2	6.3	6.4	2.85	0.173
	L*	51.4[c]	51.1[c]	52.4[bc]	54.3[ab]	55.5[a]	5.40	0.001
	a*	11.9[b]	12.0[b]	12.1[b]	12.7[ab]	13.8[a]	1.23	0.001
	b*	12.9[b]	13.3[b]	14.7[ab]	15.9[a]	16.2[a]	1.60	0.004
49	pH	.	5.3[c]	5.5[bc]	5.6[ab]	5.7[a]	0.02	0.001
	L*	.	53.2[b]	53.6[b]	56.7[a]	58.5[a]	0.42	0.001
	a*	.	10.0	10.3	10.6	10.6	0.28	0.748
	b*	.	14.5[b]	15.5[ab]	16.9[a]	16.1[ab]	0.26	0.025

[a,c]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Color CIELAB trichromatic system as lightness (L*), redness (a*), and yellowness (b*) from the surface of *Pectoralis major* muscle using a chromometer.

[2]Valueless cells are due to the absence of the corresponding WB score.

Table 5. Meat quality parameters of breast fillets as broilers aged

Age, d	Item	WB score[1]					SEM	*P*-value
		0	1	2	3	4		
21	CL[2], %	0.9	1.4	1.3	2.0	.	0.75	0.051
	WHC[3], %	91.8	86.9	91.8	89.1	.	15.50	0.579
	Work of shear, N/mm.sec	13.2	14.9	11.1	12.0	.	0.62	0.841
	Firmness, N/mm	1.6	2.0	1.5	1.6	.	0.64	0.769
28	CL, %	1.3	1.7	1.9	1.9	.	0.51	0.088
	WHC, %	93.2	94.0	94.0	92.8	.	2.97	0.608
	Work of shear, N/mm.sec	66.7	68.0	63.3	61.2	.	21.60	0.748
	Firmness, N/mm	64.8	60.6	62.7	57.3	.	20.20	0.570
35	CL, %	1.5[d]	2.1[cd]	2.6[bc]	3.6[b]	4.8[a]	0.57	0.001
	WHC, %	94.2	94.5	94.6	94.4	93.2	3.50	0.861
	Work of shear, N/mm.sec	17.5	18.9	15.6	13.1	14.7	42.21	0.265
	Firmness, N/mm	2.2	2.5	2.0	1.7	2.0	0.44	0.359
42	CL, %	1.6[c]	2.5[bc]	3.1[ab]	4.0[a]	4.3[a]	0.45	0.001
	WHC, %	95.5	94.8	95.1	95.0	92.8	3.54	0.167
	Work of shear, N/mm.sec	28.3[a]	22.4[ab]	21.1[b]	20.9[b]	16.0[b]	0.32	0.001
	Firmness, N/mm	3.5	2.8	2.6	2.6	2.6	0.49	0.712
49	CL, %	.	2.3[b]	3.6[b]	6.4[a]	7.5[a]	0.32	0.001
	WHC, %	.	91.5	91.1	90.8	90.4	0.36	0.779
	Work of shear, N/mm.sec	.	19.2	16.0	14.4	13.8	0.87	0.161
	Firmness, N/mm	.	2.4	2.1	1.9	1.8	0.10	0.251

[a,c]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Valueless cells are due to the absence of the corresponding WB score.

[2]CL = Cooking loss.

[3]WHC = Water-holding capacity.

Table 6. Histomorphometric measurements of breast meat as broilers aged

Age, d	Item	WB score[1]					SEM	*P*-value
		0	1	2	3	4		
21	Fiber diameter[2], µm	13.62[b]	13.35[b]	16.00[a]	16.81[a]	.	14.70	0.001
	Density, fibers/cm^2	1,214[a]	1,246[a]	904.2[b]	782.1[b]	.	35.55	0.001
	Vol[3], %	53.95[ab]	48.36[ab]	44.16[b]	45.19[b]	.	19.42	0.001
28	Fiber diameter, µm	13.74[b]	14.91[b]	17.61[a]	16.63[a]	.	16.39	0.001
	Density, fibers/cm^2	1,185[a]	1,072[a]	1,195[a]	742.7[b]	.	73.20	0.001
	Vol, %	41.42	43.00	39.28	43.69	.	20.35	0.001
35	Fiber diameter, µm	15.16[c]	15.43[c]	16.45[bc]	17.86[a]	17.29[ab]	13.60	0.001
	Density, fibers/cm^2	1,066[a]	968.7[ab]	869.8[bc]	740.3[cd]	633.9[d]	33.01	0.001
	Vol, %	45.19[b]	45.23[b]	46.13[b]	53.49[a]	40.47[b]	22.59	0.001
42	Fiber diameter, µm	15.32[b]	16.10[b]	18.95[a]	19.71[a]	21.08[a]	20.71	0.001
	Density, fibers/cm^2	965.9[a]	724.1[b]	632.6[bc]	572.7[bc]	493.8[c]	49.79	0.001
	Vol, %	.	61.30[ab]	60.77[ab]	59.16[b]	64.66[a]	18.41	0.001
49	Fiber diameter, µm	.	16.55[c]	20.18[b]	22.67[a]	21.81[ab]	3.34	0.001
	Density, fibers/cm^2	.	826.1[a]	555.1[b]	433.2[c]	447.3[c]	102.53	0.001
	Vol, %	.	58.95[ab]	57.44[ab]	53.04[b]	62.85[a]	10.41	0.012

[a,d]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Valueless cells are due to the absence of the corresponding WB score.

[2]Minimum fiber diameter in µm.

[3]Vol = partial volume of muscle tissue in %.

Table 7. Serum enzymes concentration according to the wooden breast score and age of broilers, U/L

Age, d	Item	WB score[1]					SEM	*P*-value
		0	1	2	3	4		
21	ALT[2]	3.80	2.80	3.00	1.80	.	0.59	0.405
	AST[3]	334.3	537.3	290.5	461.5	.	96.9	0.574
	CK[4]	7,852	8,949	10,550	12,231	.	36.5	0.327
	LDH[5]	3,913	4,582	4,744	6,662	.	32.7	0.121
28	ALT	3.80	3.10	2.20	1.60	.	0.78	0.262
	AST	273.2[b]	274.8[b]	289.5[b]	482.6[a]	.	35.2	0.001
	CK	7,022[b]	7,679[b.]	9,720[b]	1,5042[a]	.	53.4	0.001
	LDH	4,064[b]	4,293[b]	5,075[ab]	6,134[a]	.	32.7	0.003
35	ALT	5.35	2.94	3.08	2.63	3.80	1.23	0.438
	AST	266.6[b]	284.4[b]	472.1[ab]	543.4[ab]	657.7[a]	85.2	0.006
	CK	6,148[c]	7,857[c]	9,401[bc]	15,465[b]	29,274[a]	87.1	0.001
	LDH	3,745[b]	3,969[b]	4,035[b]	5,344[b]	9,172[a]	69.3	0.001
42	ALT	1.33	1.93	2.08	4.93	7.27	1.78	0.080
	AST	338.5[c]	344.4[c]	679.8[b]	890.0[ab]	960.5[a]	95.5	0.035
	CK	7,148[b]	8,610[b]	11,383[b]	22,489[a]	30,212[a]	72.4	0.001
	LDH	4,193[c]	4,411[c]	5,100[bc]	7,563[ab]	10,122[a]	54.2	0.001
49	ALT	.	2.73	3.24	7.00	5.73	1.79	0.253
	AST	.	305.3[b]	496.5[b]	856.7[ab]	1,335[a]	84.5	0.001
	CK	.	5,553[c]	10,651[c]	19,961[b]	31,905[a]	1,636	0.001
	LDH	.	3,265[c]	4,018[bc]	5,702[ab]	7,652[a]	365.8	0.001

[a,c]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

[1]Valueless cells are due to the absence of the corresponding WB score.

[2]ALT = Alanine aminotransferase

[3]AST = Aspartate aminotransferase.

[4]LDH = Lactate dehydrogenase.

[5]CK = Creatine kinase.

Table 8. Pearson correlation among wooden breast scores and evaluated parameters as broilers aged

Age, d	Echogenicity[1]	Depth[2]	Cooking loss	Density, fiber/cm[2]	Fiber diameter	Creatine kinase[3]	MORSE cranial[4]	b*[5]
21	0.510***	0.501***	0.546***	-0.301***	0.450***	0.220	-0.300**	0.380
28	0.531***	0.851***	0.454***	-0.224***	0.536***	0.529***	-0.305**	0.667***
35	0.470***	0.848***	0.763***	-0.483***	0.429***	0.716***	-0.333**	0.528***
42	0.430***	0.773***	0.552***	-0.268***	0.428***	0.710***	-0.286**	0.549***
49	0.548***	0.614***	0.754***	-0.715***	0.658***	0.747***	-0.217**	0.469***

[1]Echogenicity, mean gray values were calculated from the obtained histogram (black to white scale from 0 to 255, respectively) of *Pectoralis major* muscle in live broilers (n = 360).

[2]Depth of breast fillets according to birds age.

[3]Serum concentration of creatine kinase.

[4]MORSE = Meullenet-Owens razor shear energy of raw breast fillet (cranial region).

[5]Color measurement as yellowness (b*) from the surface of *Pectoralis major* muscle using a chromometer.

$^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

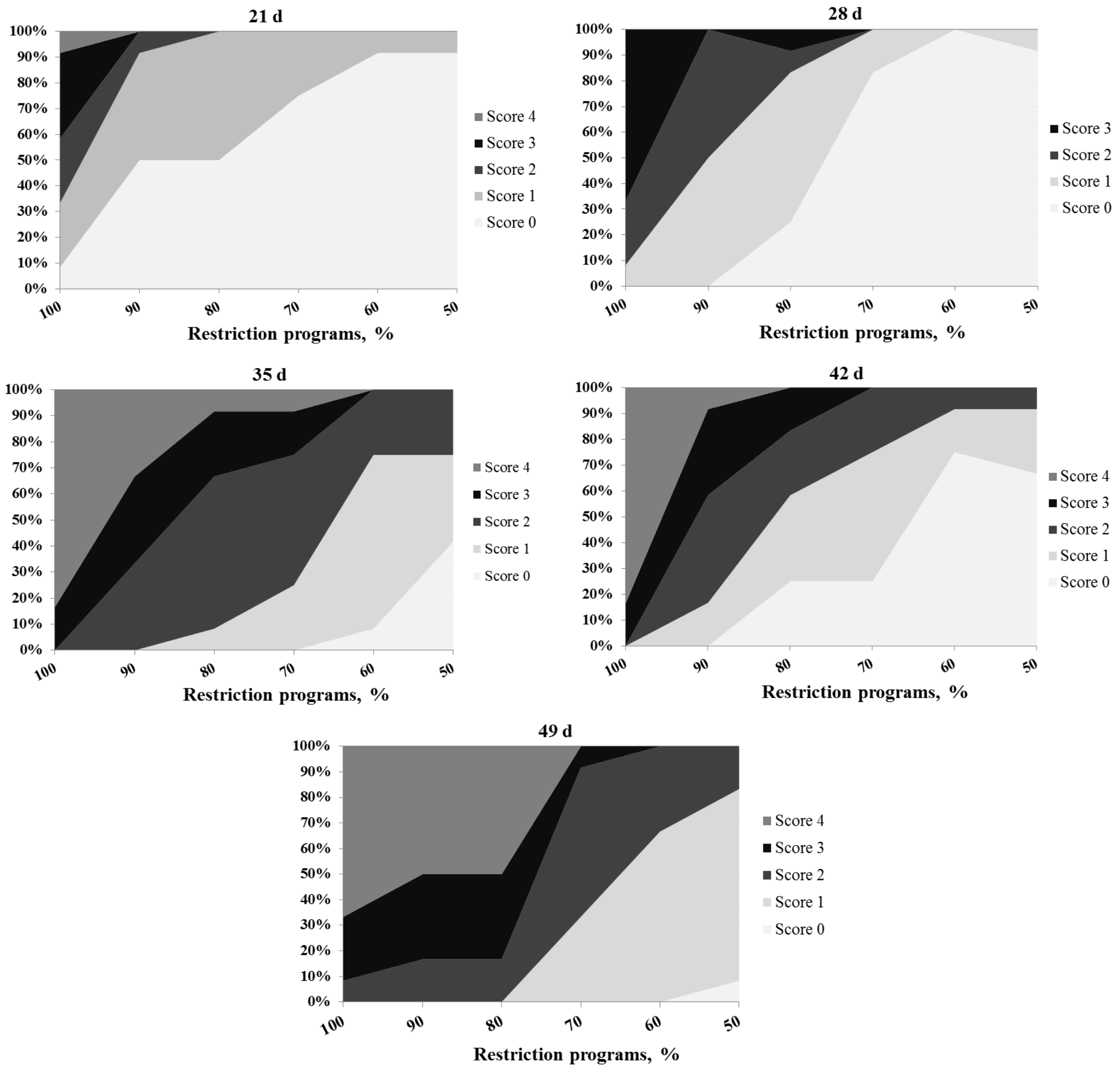

Figure 1. Wooden breast occurrence in breast fillets of broilers from 21 to 49 d. Wooden breast scores were: normal (score 0), mild hardening in the upper part of the fillet (score 1), moderate hardening in the upper and/or lower part of the fillet (score 2), severe hardening (score 3), and severe hardening with hemorrhagic lesions, increased volume, and presence of yellow fluid (score 4)

CAPÍTULO III

CONSIDERAÇÕES FINAIS

O presente trabalho envolve a restrição alimentar como alternativa para reduzir a ocorrência de WB em frangos de corte até os 49 dias. É possível observar uma redução linear no ganho de peso das aves conforme o consumo de ração foi reduzido, com consequente diminuição da média de WB obtida através dos escores das lesões. Apesar de apresentarem maior ganho de peso, rendimento de carcaça e de peito, aves que foram alimentadas *ad libitum* durante o período também apresentaram maior média de WB no período acumulado, reiterando a hipótese de que há uma forte relação entre ganho de peso e surgimento de miopatias. Entretanto, restrições alimentares menos severas, como as de 90 e 80% de consumo, podem ser consideradas, devido ao seu desempenho similar ao de aves alimentadas à vontade, e também por proporcionarem uma média de WB inferior, podendo trazer menor impacto para a indústria avícola. O presente trabalho contribui para aumentar o leque de alternativas de manejo nutricional a serem adotadas com o propósito de reduzir a ocorrência de WB em lotes comerciais de frangos de corte. Novos estudos que considerem a restrição alimentar como alternativa de manejo devem considerar também a melhor idade em que as aves podem ser submetidas ao menor consumo de ração, e também como essa pode ser adotada no dia a dia de granjas industriais.

A metodologia de ultrassom utilizada como ferramenta de diagnóstico da miopatia *in vivo*, demonstrou resultados satisfatórios que devem ser considerados em estudos futuros. Peitos com maior escore de WB apresentaram maior ecogenicidade em relação a peitos normais de 35 a 49 dias. Por se tratar de uma técnica pioneira na produção avícola, a correlação entre WB, ecogenicidade e profundidade, que apresentou valores entre médios e fortes, reforça a confiabilidade da metodologia escolhida.

Os resultados observados neste estudo também contribuíram para um maior conhecimento sobre as alterações causadas pela miopatia WB sobre a qualidade de carne e paramêtros histomorfométricos do peito de frangos. Resultados consistentes com relação a sua etiologia e prevenção ainda são escassos e inconsistentes. O grande desafio a ser vencido continua sendo a diminuição da ocorrência das lesões, adotando metodologias que possam ser utilizadas pela indústria sem comprometer o desempenho zootécnico das aves, essencial para atender as demandar do mercado mundial.

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APÊNDICES

Apêndice 1: Normas para publicação de artigos no periódico Poultry Science

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Accepted manuscripts are forwarded by the editor-in- chief to the editorial office for technical editing and type- setting. At this point the technical editor may contact the authors for missing information or figure revisions. The manuscript is then typeset, figures reproduced, and au- thor proofs prepared.

Proofs

Author proofs of all manuscripts will be provided to the corresponding author. Author proofs should be read care- fully and checked against the typed manuscript, because the responsibility for proofreading is with the author(s). Corrections may be returned by fax (217-378-4083), mail, or e-mail. For faxed or mailed corrections, changes to the proof should be made neatly and clearly in the margins of the proof. If extensive editing is required, corrections should be provided on a separate sheet of paper with a symbol indicating location on the proof. Changes sent by e-mail to the technical editor must indicate page, column, and line numbers for each correction to be made on the proof. Corrections can also be marked using the note and highlight tools to indicate necessary changes. Author al- terations to copy exceeding 10% of the cost of composi- tion will be charged to the author.

Editor queries should be answered on the galley proofs; failure to do so may delay publication. Proof corrections should be made and returned to the technical editor within 48 hours of receipt. The publication charge form should be returned with proof corrections so as not to delay publication of the article.

Publication Charges and Offprints

Poultry Science has two options available for the pub- lication of articles: conventional page charges and Open Access (**OA**).

OA. For authors who wish to publish their papers OA (available to everyone when the issue is posted online), au- thors will pay the OA fee when proofs are returned to the editorial office. Charges for OA are $1,500 if at least one au- thor is a current professional member of PSA; the charge is $2,000 when no author is a professional member of PSA.

Conventional Page Charges. The current charge for publication is $100 per printed page (or fraction thereof) in the journal if at least one author is a professional mem- ber of PSA. If no author is a member of PSA, the publication charge is $170 per journal page.

Offprints. Offprints may be ordered at an additional charge. When the galley proof is sent, the author is askedto complete an offprint order requesting the number of offprints desired and the name of the institution, agency, or individual responsible for publication charges.

Color Charges. The cost to publish in color in the print journal is $600 per color image; a surcharge for off- prints will also be assessed. At the time of submission on ScholarOne Manuscripts, authors will be asked to ap- prove color charges for figures that they wish to have published in color in the print journal. Color versions of figures will be included in the online PDF and full-text article at no charge.

MANUSCRIPT PREPARATION: STYLE AND FORM

General

Papers must be written in English. The text and all sup- porting materials must use American spelling and usage as given in *The American Heritage Dictionary, Webster's Third New International Dictionary,* or the *Oxford Ameri- can English Dictionary.* Authors should follow the style and form recommended in *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers.* 2006. 7th ed. Style Manual Committee, Council of Science Editors, Reston, VA.

Authors should prepare their manuscripts with Microboldface and italic. Text that follows a first subheading should be in a new paragraph.

Second Subheadings. Second subheadings begin the first line of a paragraph. They are indented, boldface, italic, and followed by a period. The first letter of each important word should be capitalized. The text follows immediately after the final period of the subheading.

Title Page

The title page shall begin with a running head (short title) of not more than 45 characters. The running head is centered, is in all capital letters, and shall appear on the top of the title page. No abbreviations should be used.

The title of the paper must be in boldface; the first letter of the article title and proper names are capitalized, and the remainder of the title is lowercase. The title must not have abbreviations.

Under the title, names of authors should be typed (first name or initial, middle initial, last name). Affili- ations will be footnoted using the following symbols:

*, †, ‡, §, #, ǁ, and be placed below the author names. Do not give authors' titles, positions, or degrees. Num- bered footnotes may be used to provide supplementary information, such as present address, acknowledgment of grants, and experiment station or journal series num- ber. The corresponding author should be indicated with 1 soft Word and upload them using the fewest files pos a numbered footnote (e.g., Corresponding author: mysible to facilitate the review and editing process.

Authors whose primary language is not English are strongly encouraged to use an English-language service to facilitate the preparation of their manuscript. A partial list of services can be found in the *Poultry Science* Manuscript checklist.

Preparing the Manuscript File

Manuscripts should be typed double-spaced, with lines and pages numbered consecutively, using Times New Roman font at 12 points. All special characters (e.g., Greek, math, symbols) should be inserted using the sym- bols palette available in this font. Complex math should be entered using MathType from Design Science (http:// www.dessci.com). Tables and figures should be placed in separate sections at the end of the manuscript (not placed within the text). Failure to follow these instructions may result in an immediate rejection of the manuscript.

Headings

Major Headings. Major headings are centered (ex- cept ABSTRACT), all capitals, boldface, and consist of ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), ACKNOWLEDGMENTS (optional), AP- PENDIX (optional), and REFERENCES.

First Subheadings. First subheadings are placed on a separate line, begin at the left margin, the first letter of all important words is capitalized, and the headings are name@university.edu). Note that there is no period after the corresponding author's e-mail address.

The title page shall include the name and full address of the corresponding author. Telephone and FAX numbers and e-mail address must also be provided. The title page must indicate the appropriate scientific section for the paper (i.e., Education and Production; Environment, Well-Being, and Behavior; Genetics; Immunology, Health, and Disease; Metabolism and Nutrition; Molecular, Cellular, and Developmental Biology; Physiology, Endocrinology, and Reproduction; or Processing, Products, and Food Safety).

Authors may create a full title page as a one-page document, in a file separate from the rest of the paper. This file can be uploaded and marked "not for review." Authors who choose to upload manuscripts with a full title page at the beginning will have their papers forwarded to reviewers as is.

Abbreviations

Author-derived abbreviations should be defined at first use in the abstract and again in the body of the manuscript. The abbreviation will be shown in bold type at first use in the body of the manuscript. Refer to the Miscellaneous Usage Notes for more information on abbreviations.

Abstract

The Abstract disseminates scientific information through abstracting journals and through conveniencefor the readers. The Abstract, consisting of not more than 325 words, appears at the beginning of the manuscript with the word ABSTRACT without a following period. It must summarize the major objectives, methods, results, conclusions, and practical applications of the research. The Abstract must consist of complete sentences and use of abbreviations should be limited. References to other work and footnotes are not permitted. The Abstract and Key Words must be on a separate sheet of paper.

Key Words

The Abstract shall be followed by a maximum of five key words or phrases to be used for subject indexing. These should include important words from the title and the running head and should be singular, not plural, terms (e.g., broiler, not broilers). Key words should be formatted as follows: **Key words:** . . .

Introduction

The Introduction, while brief, should provide the read- er with information necessary for understanding research presented in the paper. Previous work on the topic should be summarized, and the objectives of the current research must be clearly stated.

Materials and Methods

All sources of products, equipment, and chemicals used in the experiments must be specified parenthetically at first mention in text, tables, and figures [i.e., (model 123, ABC Corp., Provo, UT)]. Model and catalog num- bers should be included. Information shall include the full corporate name (including division, branch, or other subordinate part of the corporation, if applicable), city, and state (country if outside the United States), or Web address. Street addresses need not be given unless the reader would not be able to determine the full address for mailing purposes easily by consulting standard refer- ences.

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.

Papers must contain analyzed values for those dietary ingredients that are crucial to the experiment. Papers deal- ing with the effects of feed additives or graded levels of a specific nutrient must give analyzed values for the rel- evant additive or nutrient in the diet(s). If products were used that contain different potentially active compounds, then analyzed values for these coupounds must be given for the diet(s). Exceptions can only be made if appropri- ate methods are not available. In other papers, authors should state whether experimental diets meet or exceed the National Research Council (1994) requirements as ap- propriate. If not, crude protein and metabolizable energy levels should be stated. For layer diets, calcium and phos- phorus contents should also be specified.

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 equiv- alents:

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 d-α-tocopherol

In the instance of vitamin D3, cholecalciferol is the ac- ceptable 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. Statisti- cal 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. Refer- ence to a statistical package without reporting the sourc- es 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 anal- ysis should justify the interpretations and conclusions. When possible, results of similar experiments should be pooled statistically. Do not report a number of similar ex- periments 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 chemi- cal analyses of the same sample usually do not consti- tute independent experimental units. Measurements on the same experimental unit over time also are not inde- pendent and must not be considered as independent ex- perimental 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 ex- ample, 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 pro- cedures are based on the assumption that experimental units have been assigned to treatments at random. If ani- mals are stratified by ancestry or weight or if some other initial measurement should be accounted for, the model should include a blocking factor, or the initial measure- ment 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 vari- able, 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 ar- rangement in 5 randomized complete blocks consisting of initial BW.” Note that **a factorial arrangement is not a de- sign**; 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 er- ror 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 ex- perimental 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 ob- servations 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 prob- lems. Accordingly, it is well to state how the computing was done and how the parameters were estimated. Approximations should be accompanied by cautions con- cerning possible biases.

Contrasts (preferably orthogonal) are used to answer specific questions for which the experiment was de- signed; they should form the basis for comparing treat- ment means. Nonorthogonal contrasts may be evalu- ated by Bonferroni *t* statistics. The exact contrasts tested should be described for the reader. Multiple-range tests are not appropriate when treatments are orthogonally ar- ranged. 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 ar- rangements, 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 impor- tant 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 tradition- ally have been reserved for $P < 0.05$ and $P < 0.01$, re- spectively; 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 deci- mal. 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" rela- tionship 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 con- tained 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 Re- sults 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 submit- ting. One of the hallmarks for experimental evidence is repeatability. Care should be taken to ensure that experi- ments are adequately replicated. The results of experi- ments 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 Dis- cussion section or Acknowledgments, if present. The Appendix may contain supplementary material, expla- nations, and elaborations that are not essential to other major sections but are helpful to the reader. Novel com- puter 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, re- fer 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 sec- tion, papers must be published or accepted for publica- tion. Manuscripts submitted for publication can be cited as "personal communication" or "unpublished data" in the text.

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

In the References section, references shall first be list- ed alphabetically by author(s)' last name(s), and then chronologically. The year of publication follows the au- thors' names. As with text citations, two or more publi- cations 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 abbrevia- tions given in journals database of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/que- ry.fcgi?db=journals). 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 chal- lenge 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. Reg- ist. 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 elec- tronic nose technologies for quality assessment of color and odor of shrimp and salmon. PhD Diss. Univ. Florida, Gaines- ville.
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 fea- ture 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 prob- lems). 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 place- ment 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 super- scripts 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 †$P \leq 0.10$. 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 identi- fied 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 publi- cation. 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. Sym- bols 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 de- scriptor by a comma or parentheses, and should be consistent within a manuscript.
• ***Shading and Fill Patterns.*** For bar charts, use dif- ferent 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 ap- parent only when the figure is reproduced in gray- scale.
• ***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 cap- tion or with a scale bar on the figure. Reduction for publication can make a magnification power desig- nation (e.g., 100×) inappropriate.
• ***Caption.*** The caption should provide sufficient in- formation 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 read- able at final publication size.

Color Figures. Submitted color images should be at least 300 ppi. The cost to publish each color figure is $600; 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.

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 scien- tific 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 ab- breviations 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 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 edi- tors 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 abbrevia- tion following in bold type in parentheses the first time they are mentioned in the main body of the text. Abbre- viations 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, ab- breviations shall be defined within each of these units of the manuscript.

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 immunoglobulin

IL interleukin

IU international units

kb kilobase pairs

kDa kilodalton

L liter*

L:D hours light:hours darkness in a photoperiod (e.g., 23L:1D)

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 2
R coefficient of determination, multiple

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 ab- breviated 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
RFLP restriction fragment length polymorphism
RH relative humidity
RIA radioimmunoassay
RNA ribonucleic acid

rpm revolutions per minute
s second
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., invitro, in vivo, in situ, a priori). However, genus and spe- cies of plants, animals, or bacteria and viruses should be italicized. Authors must indicate accent marks and other diacriticals on international names and institutions. Ger- man 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 writ- ten 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 se- quences be submitted to one of the sequence databases. Acceptance for publication is contingent on the submis- sion of sequence data to one of the databases. The fol- lowing statement should appear as a footnote to the title on the title page of the manuscript. "The nucleotide se- quence data reported in this paper have been submitted to GenBank Submission (Mail Stop K710, Los Alamos Na- tional Laboratories, Los Alamos, NM 87545) nucleotide sequence database and have been assigned the accession number XNNNNN."

Publication of the description of molecular clones is as- sumed 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 camera- ready figures no larger than 21.6 × 27.9 cm in standard (portrait) orientation. Abbreviations should follow *Poultry Science* guidelines.

Gene and Protein Nomenclature. Authors are re- quired to use only approved gene and protein names and symbols. For poultry, full gene names should not be itali- cized. Gene symbols should be in uppercase letters and should be in italics. A protein symbol should be in the same format as its gee except the protein symbol should not be in italics.

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 num- bered 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.

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Apêndice 2. Desempenho zootécnico dos frangos de corte no período de 8 a 49 dias

Consumo alimentar, %	Tratamento	GP, g	CA, g:g	CR, g
100	1	3602.5	1.388	6183.5
100	1	3646.3	1.341	5955.3
100	1	3722.9	1.409	6204.6
100	1	3818.1	1.410	6227.8
100	1	3575.2	1.395	6184.8
100	1	3533.7	1.243	5896.7
100	1	3587.7	1.262	5884.4
100	1	3730.4	1.324	6077.7
100	1	3735.1	1.363	6030.5
100	1	3457.7	1.442	5766.4
100	1	3528.5	1.263	6096.9
100	1	3640.5	1.518	6184.1
90	2	3130.0	1.303	5242.8
90	2	3129.9	1.292	5203.5
90	2	3277.7	1.297	5245.1
90	2	3163.2	1.354	5374.4
90	2	3184.1	1.167	5211.2
90	2	3142.7	1.373	5330.0
90	2	3226.8	1.389	5365.4
90	2	3200.3	1.369	5243.0
90	2	3141.8	1.375	5223.3
90	2	3189.0	1.395	5377.3
90	2	3191.3	1.383	5309.5
90	2	3395.9	1.332	5661.4
80	3	2722.6	1.263	4619.2
80	3	2846.7	1.375	4729.8
80	3	2835.9	1.484	4794.6
80	3	2829.3	1.566	4728.8
80	3	2869.7	1.410	4633.1
80	3	2825.6	1.483	4785.6
80	3	3071.0	1.470	5145.2
80	3	2797.6	1.373	4620.4
80	3	2749.7	1.359	4691.7
80	3	2829.7	1.398	4728.4
80	3	2795.9	1.358	4715.5
80	3	2855.3	1.384	4814.2
70	4	2432.8	1.736	4347.0
70	4	2464.8	1.427	4358.4
70	4	2483.8	1.397	4244.6
70	4	2618.6	1.395	4251.5
70	4	2405.3	1.267	4031.4
70	4	2383.5	1.456	4199.6
70	4	2414.5	1.412	4126.0
70	4	2451.0	1.417	4157.6

70	4	2449.1	1.409	4125.0
70	4	2567.3	1.381	4103.8
70	4	2448.9	1.400	4154.9
70	4	2534.8	1.431	4259.8
60	5	2083.0	1.686	3800.6
60	5	2199.3	1.516	3776.5
60	5	2063.7	1.261	3578.0
60	5	2123.8	1.670	3688.7
60	5	2133.1	1.483	3614.1
60	5	2066.4	1.619	3736.9
60	5	2073.6	1.436	3638.5
60	5	2135.4	1.398	3667.6
60	5	2066.8	1.406	3662.4
60	5	2146.2	1.443	3694.6
60	5	2090.7	1.430	3650.1
60	5	2072.8	1.453	3655.0
50	6	1739.8	1.957	3271.0
50	6	1748.1	1.563	3134.1
50	6	1740.5	1.556	3036.7
50	6	1793.0	1.753	3228.4
50	6	1765.3	1.600	3146.4
50	6	1684.3	1.549	3057.2
50	6	1713.3	1.506	3155.3
50	6	1769.8	1.401	3125.7
50	6	1760.6	1.477	3159.8
50	6	1780.9	1.413	3208.8
50	6	1751.1	1.493	3043.1
50	6	1730.7	1.489	3039.7

Apêndice 3. Ecogenicidade das amostras de peito de acordo com o consumo alimentar em diferentes idades.

Consumo alimentar, %	7 d	14 d	21 d	28 d	35 d	42 d	49 d
100	68.06	84.34	67.02	45.09	93.14	57.96	91.72
100	64.68	77.01	32.06	97.09	82.5	102.5	68.49
100	63.76	92.25	85.04	99.02	73.94	96.96	82.62
100	64.11	78.42	73.81	84.36	66.19	111.76	101.97
100	65.49	80.74	95.37	84.18	93.83	94.9	92.88
100	60.71	69.98	83.68	95.47	84.51	110.06	100.96
100	75.59	67.07	85.4	84.74	89.83	123.54	88.52
100	68.92	78.23	101.88	86.79	94.52	99.95	103.93
100	63.99	72.01	45.63	92.43	84.35	98.8	97.86
100	54.6	79.26	59.13	72.58	71.21	77.4	101.27
100	65.92	75.71	88.15	73.94	96.08	87.18	88.49

100	54.3	68.17	85.15	79.63	88	100.66	97
90	60.91	76.64	68.06	97.12	99.84	111.83	113.39
90	63.37	83.12	66.87	74.15	77.09	88	86.62
90	57.45	85.89	70.6	79.9	61.88	93.66	85.27
90	58.83	78.39	79.01	87.48	43.24	89.76	93.02
90	69.84	81.43	81.31	87.88	85.53	106.64	109.58
90	54.54	67.08	40.12	61.63	77.19	93.51	103.03
90	75.05	77.26	89.09	92.28	99.73	101.74	97.27
90	66.89	82.51	79.23	70.2	88.76	96.96	94.23
90	59.91	69.43	69.71	91.96	93.13	105.18	102.13
90	73.71	71.71	61.64	87.67	86.25	90.03	94.31
90	67.94	68.5	82.11	63.82	78.34	100.34	99.76
90	65.43	76.22	80.41	88.16	87.95	97.44	99.11
80	65.43	79.22	76.08	88.79	89.62	85.56	99.75
80	59.69	87.34	55.72	61.33	72.49	84.24	83.43
80	61.71	93.23	87.51	50.13	96.91	94.24	87.68
80	62.41	88.66	75.6	83.95	70.53	95.69	89.5
80	62.98	77.65	71.95	87.58	89.91	90.01	87.76
80	62.48	66.74	76.44	70.3	65.38	87.39	94.61
80	68.15	77.08	74.85	81.97	84.26	87.81	92.96
80	64.52	78.01	75.11	81.32	79.49	83.57	88.1
80	59.81	66.58	72.54	81.42	69.84	83.11	85.79
80	66.63	73.19	73.66	91.77	89.73	104.28	97.83
80	63.27	70.62	62.53	80.36	90.36	87.98	94.25
80	72.83	67.81	99.99	70.64	81.01	93.25	98.34
70	54.17	73.89	65.54	87.77	87.9	76.93	78.52
70	68.28	85.48	84.15	95.7	82.47	103.16	91.14
70	58.32	91.42	83.98	81.37	77.38	90.31	96.42
70	75.92	85.19	67.18	86.24	64.59	80.99	102.44
70	63.7	72.48	84.3	78.74	84.02	86.31	95.44
70	64.19	63.02	67.15	70.75	63.12	82.88	75.53
70	79.66	70.41	76.52	89.08	89.53	87.76	95.66
70	62.33	75.14	78.00	89.89	87.87	96.23	89.39
70	66.38	69.17	66.17	85.66	79.96	72.93	96.25
70	64.49	77.12	85.67	81.43	74.69	98.48	90.75
70	64.34	67.67	59.17	72.72	70.53	86.26	85.89
70	59.92	62.7	69.36	75.68	77.15	88.37	97.92
60	65.35	69.67	75.59	72.38	73.95	83.98	85.4

60	58.8	79.04	84.99	86.44	80.39	80.43	75.97
60	61.44	83.82	74.05	74.59	85.04	75.42	79.4
60	63.43	77.31	69.47	83.3	81.01	91	78.25
60	61.45	76.48	76.03	83.83	78.5	93.01	94.78
60	61.42	68.03	64.65	75.96	77.68	77.59	88.62
60	70.14	70.91	79.13	86.32	79.42	84.86	88.75
60	67.9	74.26	72.5	84.92	84.57	76.11	78.86
60	62.21	70.04	74.55	86.59	83.22	94.6	91.57
60	60.88	67.5	59.6	71.32	69.06	79.36	78.91
60	68.53	65.73	71.39	73.43	78.62	85.41	66.83
60	62.97	71.01	59.55	83.9	85.1	91.66	81.48
50	61.62	73.8	66.21	86.28	90.2	88.9	99.56
50	64.89	94.13	74.58	78.28	83.78	89.45	90.11
50	59.71	73.95	81.34	72.85	72.89	91.94	84.17
50	70.2	73.34	67.01	76.07	75.74	78.47	80.77
50	63.6	74.07	68.5	54.74	45.52	84.93	82.72
50	61.31	70.05	67.64	71.38	66.32	84.59	85.01
50	70.13	56.95	80.47	76.32	79.32	77.91	83.22
50	60.06	70.96	72.04	90.77	91.09	93.51	100.71
50	59.95	67.74	82.66	95.41	80.94	94.33	101.13
50	67.27	73.32	63.27	74.13	72.49	86.53	77.63
50	66.98	77.24	66.98	69.54	66.59	86.52	84.19
50	69.56	78.06	62.22	76.27	83.7	95.5	74.78

Apêndice 4. Ecogenicidade do peito de acordo com escore de WB de 7 a 49 d

Escore WB	7 d	14 d	21 d	28 d	35 d	42 d	49 d
3	68.06	84.34	67.02	45.09	93.14	57.96	91.72
4	64.68	77.01	32.06	97.09	82.5	102.5	68.49
4	63.76	92.25	85.04	99.02	73.94	96.96	82.62
4	64.11	78.42	73.81	84.36	66.19	111.76	101.97
4	65.49	80.74	95.37	84.18	93.83	94.9	92.88
3	60.71	69.98	83.68	95.47	84.51	110.06	100.96
4	75.59	67.07	85.40	84.74	89.83	123.54	88.52
3	68.92	78.23	101.88	86.79	94.52	99.95	103.93
2	63.99	72.01	45.63	92.43	84.35	98.80	97.86
4	54.6	79.26	59.13	72.58	71.21	77.40	101.27
4	65.92	75.71	88.15	73.94	96.08	87.18	88.49
4	54.3	68.17	85.15	79.63	88.00	100.66	97.00
4	60.91	76.64	68.06	97.12	99.84	111.83	113.39

3	63.37	83.12	66.87	74.15	77.09	88.00	86.62
2	57.45	85.89	70.6	79.90	61.88	93.66	85.27
4	58.83	78.39	79.01	87.48	43.24	89.76	93.02
3	69.84	81.43	81.31	87.88	85.53	106.64	109.58
4	54.54	67.08	40.12	61.63	77.19	93.51	103.03
2	75.05	77.26	89.09	92.28	99.73	101.74	97.27
3	66.89	82.51	79.23	70.2	88.76	96.96	94.23
3	59.91	69.43	69.71	91.96	93.13	105.18	102.13
4	73.71	71.71	61.64	87.67	86.25	90.03	94.31
4	67.94	68.5	82.11	63.82	78.34	100.34	99.76
4	65.43	76.22	80.41	88.16	87.95	97.44	99.11
3	65.43	79.22	76.08	88.79	89.62	85.56	99.75
2	59.69	87.34	55.72	61.33	72.49	84.24	83.43
3	61.71	93.23	87.51	50.13	96.91	94.24	87.68
2	62.41	88.66	75.6	83.95	70.53	95.69	89.5
2	62.98	77.65	71.95	87.58	89.91	90.01	87.76
2	62.48	66.74	76.44	70.3	65.38	87.39	94.61
2	68.15	77.08	74.85	81.97	84.26	87.81	92.96
2	64.52	78.01	75.11	81.32	79.49	83.57	880
2	59.81	66.58	72.54	81.42	69.84	83.11	85.79
2	66.63	73.19	73.66	91.77	89.73	104.28	97.83
2	63.27	70.62	62.53	80.36	90.36	87.98	94.25
4	72.83	67.81	99.99	70.64	81.01	93.25	98.34
2	54.17	73.89	65.54	87.77	87.9	76.93	78.52
2	68.28	85.48	84.15	95.7	82.47	103.16	91.14
1	58.32	91.42	83.98	81.37	77.38	90.31	96.42
2	75.92	85.19	67.18	86.24	64.59	80.99	102.44
1	63.7	72.48	84.3	78.74	84.02	86.31	95.44
1	64.19	63.02	67.15	70.75	63.12	82.88	75.53
2	79.66	70.41	76.52	89.08	89.53	87.76	95.66
2	62.33	75.14	78.00	89.89	87.87	96.23	89.39
2	66.38	69.17	66.17	85.66	79.96	72.93	96.25
1	64.49	77.12	85.67	81.43	74.69	98.48	90.75
2	64.34	67.67	59.17	72.72	70.53	86.26	85.89
3	59.92	62.7	69.36	75.68	77.15	88.37	97.92
2	65.35	69.67	75.59	72.38	73.95	83.98	85.4
1	58.8	79.04	84.99	86.44	80.39	80.43	75.97
2	61.44	83.82	74.05	74.59	85.04	75.42	79.4

2	63.43	77.31	69.47	83.3	81.01	91.00	78.25
1	61.45	76.48	76.03	83.83	78.50	93.01	94.78
1	61.42	68.03	64.65	75.96	77.68	77.59	88.62
2	70.14	70.91	79.13	86.32	79.42	84.86	88.75
1	67.9	74.26	72.5	84.92	84.57	76.11	78.86
1	62.21	70.04	74.55	86.59	83.22	94.6	91.57
1	60.88	67.5	59.6	71.32	69.06	79.36	78.91
1	68.53	65.73	71.39	73.43	78.62	85.41	66.83
1	62.97	71.01	59.55	83.9	85.1	91.66	81.48
1	61.62	73.8	66.21	86.28	90.2	88.9	99.56
1	64.89	94.13	74.58	78.28	83.78	89.45	90.11
1	59.71	73.95	81.34	72.85	72.89	91.94	84.17
1	70.2	73.34	67.01	76.07	75.74	78.47	80.77
2	63.6	74.07	68.5	54.74	45.52	84.93	82.72
1	61.31	70.05	67.64	71.38	66.32	84.59	85.01
1	70.13	56.95	80.47	76.32	79.32	77.91	83.22
2	60.06	70.96	72.04	90.77	91.09	93.51	100.71
1	59.95	67.74	82.66	95.41	80.94	94.33	101.13
1	67.27	73.32	63.27	74.13	72.49	86.53	77.63
1	66.98	77.24	66.98	69.54	66.59	86.52	84.19
1	69.56	78.06	62.22	76.27	83.7	95.5	74.78

Apêndice 5. Profundidade do peito de acordo com o escore de WB de 7 a 49 d

Escore WB	7 d	14 d	21 d	28 d	35 d	42 d	49 d
3	8.03	15.76	18.79	17.42	23.64	23.33	27.42
4	10.61	14.70	23.03	20.45	25.15	20.30	25.15
4	8.64	12.12	19.55	18.33	22.73	27.12	26.97
4	8.94	12.73	17.88	21.36	22.73	31.67	23.33
4	10.76	13.48	16.52	21.67	23.18	27.12	27.58
3	8.94	15.76	16.97	22.27	20.15	22.73	23.64
4	11.21	19.24	17.73	21.67	26.82	23.94	30.45
3	9.55	15.76	19.09	18.64	21.52	26.52	27.58
2	11.82	16.06	22.27	23.79	26.36	26.97	26.21
4	7.27	14.70	16.97	18.33	24.24	29.24	20.61
4	8.33	12.73	16.82	21.67	26.06	29.39	29.55
4	8.33	15.15	17.42	20.61	24.55	27.88	29.09
4	8.79	15.15	23.64	25.15	24.55	20.45	21.97
3	8.94	11.52	21.21	16.06	24.70	28.94	29.70
2	10.00	12.73	20.30	18.48	19.70	25.30	26.67

4	8.33	12.12	20.15	21.52	22.88	14.85	22.27
3	8.03	11.21	18.94	16.97	20.61	24.85	26.67
4	8.79	12.73	19.85	19.70	24.55	27.42	20.00
2	10.76	12.12	18.48	17.12	20.61	23.79	24.85
3	9.85	12.88	19.55	18.64	21.06	23.64	24.39
3	8.03	13.18	21.52	18.94	21.82	23.79	28.18
4	9.09	18.64	24.70	25.00	27.27	29.55	33.79
4	8.79	15.15	22.27	20.45	23.64	26.36	27.73
4	7.73	13.03	17.88	16.82	20.30	25.76	26.21
3	9.55	13.33	19.09	20.30	18.48	27.58	28.94
2	8.94	11.52	18.03	19.24	21.06	24.85	26.36
3	8.94	12.42	21.97	18.18	22.12	25.45	28.03
2	9.70	10.30	17.42	17.73	19.70	21.82	24.24
2	8.18	9.24	16.36	16.52	17.73	20.00	23.18
2	9.39	11.36	19.85	17.12	18.79	25.15	25.61
2	11.52	15.61	21.21	15.91	20.76	23.18	25.45
2	8.48	14.70	20.00	17.88	23.48	27.12	28.18
2	8.79	11.67	20.45	20.91	24.85	25.61	28.94
2	8.79	14.09	21.06	19.39	23.33	26.06	27.58
2	9.70	13.33	15.61	16.82	16.97	21.82	22.27
4	9.70	13.64	20.76	18.79	22.12	26.67	25.15
2	8.94	12.42	17.27	17.27	18.94	22.73	25.30
2	10.61	11.52	17.73	16.97	18.64	22.12	22.73
1	10.30	11.67	18.03	18.03	18.18	23.33	22.73
2	10.00	12.88	16.97	17.88	17.42	22.58	24.85
1	8.33	11.36	16.36	16.06	15.45	20.00	19.09
1	8.64	13.18	16.82	17.73	20.76	21.82	23.64
2	10.76	11.82	15.30	14.55	17.27	19.85	20.76
2	9.70	12.12	17.58	18.03	21.06	23.64	25.45
2	9.24	13.03	19.39	17.27	20.30	25.91	24.70
1	8.94	12.73	17.27	16.06	18.18	22.12	21.82
2	10.15	12.58	17.27	18.33	19.85	23.94	26.21
3	10.45	14.24	20.00	17.73	21.21	25.30	26.21
2	10.30	11.82	15.91	15.76	17.73	20.45	22.58
1	11.06	10.00	17.27	15.15	17.42	21.36	20.45
2	8.79	11.97	18.79	17.42	19.39	24.39	24.09
2	9.55	9.39	13.79	15.00	18.03	19.70	22.88
1	9.55	10.76	16.21	14.85	18.03	18.79	22.73

1	8.48	12.12	16.82	15.76	16.36	19.24	21.21
2	9.09	11.82	16.67	16.21	17.83	20.00	21.52
1	9.09	11.82	15.76	15.15	17.27	19.24	21.67
1	8.79	11.52	0.00	14.85	16.52	19.24	18.64
1	7.12	12.42	15.45	15.00	16.52	17.73	20.15
1	10.61	12.58	16.36	15.61	17.88	20.61	21.52
1	6.97	10.76	13.79	14.24	21.82	19.39	19.39
1	7.88	11.67	16.06	14.70	13.03	19.70	18.18
1	8.18	9.24	17.27	16.67	18.48	20.15	21.52
1	7.88	9.55	15.76	14.39	17.27	19.55	20.00
1	10.15	11.36	16.21	14.72	18.03	18.94	23.18
2	10.91	11.06	18.94	15.45	16.67	21.36	24.85
1	9.70	11.36	14.85	14.09	15.15	18.03	19.70
1	10.76	12.12	17.27	14.70	16.97	18.48	20.15
2	8.48	12.12	16.52	15.45	17.27	20.91	21.06
1	9.70	11.97	15.30	13.33	14.85	17.73	16.36
1	8.03	11.52	14.70	14.85	15.15	19.09	19.55
1	10.30	12.27	15.45	16.21	18.64	18.48	19.09
1	8.48	12.27	13.33	13.64	15.76	17.27	18.03

Apêndice 6. Peso vivo, peso do peito e medidas do peito aos 49 d.

Escore WB	Peso vivo, g	Peso peito, g	Comprimento (mm)	Largura (mm)	Altura (cm)
3	3609	671	200	75	2.5
4	3563	606	185	60	2.5
4	3495	715	185	70	3
4	3615	902	200	70	3.5
4	3397	647	210	80	2.5
3	3210	781	195	74	3
4	3690	642	205	78	3
3	3660	721	210	80	3
2	3398	636	215	75	3
4	3265	709	195	70	3.5
4	3615	704	200	78	3
4	3650	600	205	70	2.5
4	3278	827	200	70	2.5
3	3098	630	195	72	2.5
2	3166	601	205	70	2.5
4	3360	749	200	75	3
3	3363	652	200	80	3
4	3250	697	190	79	3
2	3210	601	210	75	2
3	3270	595	215	73	3

3	3248	603	205	70	2.5
4	3250	828	190	73	3.5
4	3220	632	200	73	2
4	3610	705	205	68	3.5
3	3072	690	190	73	2.5
2	3028	524	190	65	2
3	3008	593	200	75	2
2	3001	476	195	63	2.5
2	3068	510	200	75	2
2	3030	543	190	75	2
2	3075	559	200	74	2
2	2810	541	200	70	2
2	2806	572	200	70	2.5
2	3025	603	195	70	2
2	2940	451	195	63	2
4	3085	577	190	65	2
2	2494	394	180	55	2
2	2524	427	190	67	1.5
1	2609	401	195	66	1.5
2	2593	421	190	68	1.5
1	2460	391	195	63	1.5
1	2860	447	200	68	1.5
2	2425	388	195	65	2
2	2531	461	195	70	2.5
2	2501	456	195	68	1.5
1	2800	480	205	59	1.5
2	2650	476	180	65	2
3	2810	530	190	66	3
2	2165	364	175	65	2
1	2255	340	180	65	1
2	2359	419	180	70	1.5
2	2339	381	185	65	2.5
1	2246	316	195	61	1.5
1	2205	356	185	58	1.5
2	2115	352	175	65	2
1	2210	380	195	70	1.5
1	2106	315	185	70	1.5
1	2300	328	185	62	1.5
1	2210	343	190	65	2
1	2275	329	195	57	1.5
1	1848	277	175	60	1.5
1	2516	385	195	60	1.5
1	1888	301	180	60	1.5
1	1906	302	180	60	1.5
2	1925	363	170	70	1.5
1	1783	262	175	55	1.5
1	1895	287	175	55	1

2	1893	282	175	53	2
1	1862	285	185	58	1
1	1931	286	170	58	1.5
1	1889	261	170	60	1.5
1	1837	267	175	60	1

Apêndice 7. Medidas de pH e cor[1] das amostras de peito aos 49 d.

Escore WB	pH	L*	a*	b*
3	5.42	52.49	11.80	15.75
4	5.56	64.10	8.22	15.44
4	5.56	55.76	13.38	17.61
4	5.63	62.76	10.81	16.00
4	5.71	56.96	9.72	17.28
3	5.65	61.28	10.43	17.17
4	5.74	59.50	14.42	13.32
3	5.75	61.64	11.54	19.07
2	6.09	55.14	14.15	14.75
4	5.84	59.72	10.07	14.37
4	5.87	59.33	9.17	12.55
4	5.85	55.87	12.12	18.03
4	5.85	59.35	12.41	16.30
3	5.44	60.76	8.19	17.20
2	5.76	52.52	10.20	14.60
4	5.7	64.01	7.28	19.89
3	5.57	52.74	11.68	16.31
4	5.72	57.39	12.01	15.02
2	5.76	51.93	13.56	14.56
3	5.92	53.00	12.46	13.49
3	5.56	55.65	10.23	18.86
4	5.6	61.57	9.57	18.05
4	5.7	54.51	9.66	17.44
4	5.93	53.64	11.59	17.05
3	5.79	59.02	10.18	18.44
2	5.45	53.83	11.35	13.25
3	5.43	56.68	8.83	19.26
2	5.37	54.47	9.13	13.72
2	5.4	56.25	9.94	18.62
2	5.51	53.73	11.13	15.48
2	5.5	54.26	9.16	18.13
2	5.45	57.64	8.39	18.00
2	5.59	55.42	9.32	19.02
2	5.64	57.80	8.89	18.95
2	5.57	53.55	8.58	15.03
4	5.61	53.84	9.20	16.31
2	5.38	51.46	10.15	15.22
2	5.35	54.66	9.60	16.70
1	5.39	52.37	10.59	11.48

2	5.57	54.20	9.11	14.54
1	5.28	54.52	10.93	16.31
1	5.38	49.48	11.94	13.45
2	5.35	57.86	8.60	18.53
2	5.52	50.86	12.88	13.63
2	5.45	53.75	10.10	15.63
1	5.41	54.46	10.28	17.71
2	5.58	54.05	9.40	16.61
3	5.64	53.99	10.75	14.41
2	5.41	49.09	10.01	10.25
1	5.26	53.45	10.48	14.10
2	5.52	51.30	12.68	15.74
2	5.5	51.37	9.48	12.87
1	5.42	48.62	10.16	10.69
1	5.34	54.66	8.60	16.80
2	5.55	52.33	11.88	14.19
1	5.3	51.40	9.91	15.60
1	5.24	52.17	12.28	11.99
1	5.43	53.08	9.11	15.19
1	5.37	54.40	8.36	14.02
1	5.4	53.02	8.66	14.92
1	5.44	52.58	9.61	12.31
1	5.41	54.58	9.59	17.53
1	5.44	51.98	10.22	14.07
1	5.39	55.15	8.09	15.17
2	5.38	52.54	10.55	15.22
1	5.38	51.37	13.36	12.04
1	5.34	52.72	11.41	12.03
2	5.32	51.55	10.87	13.13
1	5.42	56.81	9.03	18.15
1	5.35	52.28	11.87	14.61
1	5.65	52.92	10.44	14.46
1	5.28	50.19	9.57	13.34

[1] Sistema tri cromático de Minolta, onde luminosidade = L; coordenada vermelho = a, coordenada amarelado = b.

Apêndice 8. Medidas de qualidade de carne de acordo com o grau de miopatia aos 49 d.

Escore WB	PPC, %[1]	CRA, %[2]	Deformação cranial	Deformação medial	Força cranial	Força medial	Cisalha-mento	Firmeza
3	6.5	90.4	86.6	82.3	12.8	10.9	27.0	3.38
4	8.0	89.2	95.6	159.8	15.8	24.0	14.9	2.20
4	11.0	89.7	89.0	75.9	10.8	10.8	24.9	3.42
4	10.5	84.5	136.1	104.3	19.1	18.1	7.7	1.20
4	4.5	93.9	85.4	93.8	11.5	13.3	34.4	4.11
3	8.5	89.1	103.8	168.5	13.9	25.8	9.4	1.19

4	10.5	86.7	141.8	173.6	18.9	23.6	14.8	1.67
3	9.0	92.3	81.0	101.4	11.0	13.6	8.5	1.25
2	5.0	94.3	97.0	100.4	12.6	14.7	12.8	1.94
4	9.0	91.6	83.6	99.3	10.7	14.3	9.9	1.39
4	7.5	94.4	104.1	104.8	14.8	15.1	12.0	1.80
4	8.5	93.1	102.6	112.3	15.9	15.1	21.8	3.01
4	10.0	88.9	153.2	131.7	19.5	17.6	7.7	1.11
3	3.5	90.2	72.7	96.3	13.4	14.7	13.8	1.62
2	5.5	89.1	76.3	81.7	11.7	12.0	9.8	1.25
4	9.0	88.6	85.2	105.1	13.3	18.7	7.9	0.89
3	5.5	92.1	70.2	100.7	9.8	14.1	21.2	2.60
4	4.0	90.9	55.5	97.1	9.8	14.6	9.5	1.30
2	4.0	92.1	76.7	96.9	10.0	15.4	20.8	2.95
3	6.5	93.3	85.1	67.7	11.4	10.2	8.6	1.18
3	6.0	92.1	107.0	101.7	16.4	15.5	10.4	1.53
4	5.0	94.5	83.6	102.6	11.0	15.4	5.6	0.75
4	7.0	92.0	103.7	102.1	14.6	15.3	13.1	1.80
4	2.5	90.5	101.1	81.8	15.2	14.4	9.7	1.37
3	7.5	85.6	95.4	109.9	13.5	14.7	15.0	1.99
2	3.5	93.2	127.4	143.2	15.4	22.9	7.6	1.06
3	5.0	91.5	108.1	112.9	14.5	15.0	13.1	1.66
2	4.0	93.4	105.1	116.7	14.8	15.3	11.1	1.56
2	4.5	93.3	58.9	95.2	10.2	16.6	18.1	2.28
2	4.5	92.6	85.9	79.1	12.6	12.3	7.6	1.06
2	4.5	89.7	96.2	90.4	14.6	14.3	22.7	2.76
2	3.5	94.0	57.5	68.7	8.7	10.4	11.6	1.50
2	6.5	91.5	78.1	124.9	11.9	16.9	18.3	2.31
2	4.5	93.4	99.3	63.6	14.5	10.0	15.7	2.36
2	4.0	84.5	43.3	92.4	5.1	13.6	20.6	2.28
4	6.5	88.0	104.1	105.1	17.0	14.2	13.1	1.27
2	2.0	91.7	101.7	97.3	15.7	16.4	9.4	1.26
2	2.0	93.0	94.5	78.6	13.7	11.6	15.0	2.18
1	4.0	92.4	119.2	124.1	20.3	22.2	23.7	2.87
2	4.0	91.9	102.4	75.4	14.8	11.3	25.3	3.41
1	3.5	93.0	114.7	117.2	17.2	19.7	34.5	4.19
1	2.5	93.3	81.5	99.1	10.9	13.8	31.6	3.89
2	3.0	90.8	99.6	136.5	15.9	22.9	18.4	1.74
2	4.0	90.9	95.5	89.0	15.3	12.9	13.8	1.93

2	2.5	77.3	75.8	72.6	10.2	12.6	18.6	2.91
1	4.5	88.4	81.0	60.2	12.1	11.5	42.0	5.39
2	2.5	92.9	117.2	122.0	18.2	20.8	17.9	2.52
3	6.0	91.9	112.9	107.1	14.2	13.4	17.3	2.69
2	3.0	94.0	89.0	88.9	12.6	13.6	13.5	1.61
1	4.0	92.3	90.4	97.3	12.4	15.5	20.5	2.60
2	2.5	88.4	100.3	119.9	15.6	19.5	11.3	1.60
2	3.5	88.2	79.6	81.5	11.4	13.2	13.2	1.79
1	2.0	89.9	141.5	127.2	20.0	17.8	28.2	3.25
1	2.5	91.5	125.0	134.2	17.2	18.6	28.1	3.80
2	3.5	88.8	92.2	84.6	12.8	11.0	13.9	1.80
1	3.5	88.5	77.6	70.1	12.0	12.1	26.3	3.53
1	2.5	93.7	97.2	101.2	12.9	13.3	15.9	2.14
1	4.5	91.0	80.8	71.7	11.5	13.0	24.3	3.07
1	2.5	91.9	115.6	141.1	17.1	18.1	18.6	2.36
1	2.5	91.3	102.4	133.4	15.9	19.3	21.9	2.54
1	3.0	94.2	89.5	96.1	12.1	14.4	8.2	1.25
1	1.5	92.0	89.3	98.0	13.6	17.8	12.5	1.76
1	3.0	92.4	79.6	102.0	11.5	15.6	9.6	1.29
1	2.5	90.9	83.9	106.1	14.3	18.7	17.3	2.33
2	3.5	95.3	112.1	117.8	15.9	16.5	38.2	4.99
1	2.5	92.0	88.8	76.1	12.9	11.9	29.6	3.68
1	0.5	93.4	88.9	92.1	14.4	14.8	24.3	2.55
2	0.5	93.1	62.3	51.3	7.9	8.2	15.6	2.33
1	2.0	93.5	126.3	148.3	19.0	21.6	28.7	3.17
1	1.0	89.7	102.4	109.0	14.3	16.5	10.1	1.22
1	1.0	87.2	109.6	96.9	14.0	15.0	13.4	1.75
1	4.0	91.3	90.3	84.1	12.9	15.6	25.7	3.53

[1] Perda por cocção
[2] Capacidade de retenção de água

Apêndice 9. Enzimas do soro de acordo com o grau de miopatia aos 49 d.

Escore WB	ALT[1]	AST[2]	CK[3]	LDH[4]
3	14	472	8650	3277
4	23	2483	37406	7642
4	18	4363	39232	9585
4	5	1711	58087	.
4	0	614	16643	6109
3	17	1843	47029	12122
4	16	813	15049	3725
3	3	661	13983	3775

2	6	309	10543	1564
4	1	1040	27803	4227
4	0	1056	36219	6113
4	0	954	26436	5042
4	15	1633	45028	9196
3	23	739	20505	4472
2	0	643	15751	4487
4	5	1068	40872	9174
3	0	1497	9618	4903
4	0	1438	53376	.
2	0	2445	9504	5311
3	0	630	22103	5443
3	0	851	22356	6853
4	0	433	8387	3193
4	0	966	30256	7139
4	0	888	27798	5308
3	4	1004	34248	8305
2	6	411	11614	3877
3	9	430	10397	3452
2	0	364	9022	3384
2	2	384	11252	5648
2	2	413	11050	5993
2	3	368	9707	1459
2	4	687	.	5008
2	0	551	13394	3685
2	0	646	11840	1424
2	0	479	9045	4189
4	3	569	15983	5776
2	1	340	9154	5758
2	7	338	7327	3073
1	0	338	5411	3390
2	5	327	9088	3587
1	0	281	6645	3852
1	7	438	10175	5159
2	0	326	9728	5673
2	0	316	9167	1717
2	8	427	11894	5230
1	2	441	11881	1933
2	13	687	18934	6010
3	0	440	10725	4414
2	15	338	9916	5519
1	0	288	5227	2486
2	0	346	10800	6015
2	2	331	7538	3431
1	0	345	6646	5546
1	1	359	9477	5178
2	0	329	7387	3384

1	0	346	8524	2681
1	2	215	8460	2166
1	5	336	9415	3371
1	0	332	8311	3867
1	0	416	9823	2810
1	1	280	4084	3965
1	0	295	4667	3440
1	10	265	3973	3706
1	22	337	3106	2336
2	0	315	4887	2705
1	0	273	2254	3331
1	0	276	2523	3018
2	5	292	3379	2320
1	0	290	2296	3541
1	0	301	2931	3684
1	0	258	3710	1878
1	0	285	2628	3056

[1] Alanina aminotrasnferase
[2] Aspartato transaminase
[3] Creatina quinase
[4] Lactato desidrogenase

Apêndice 10. Dados utilizados para análise de correlação aos 21 d.

Escore WB	Profundidade	Ecogenicidade	CK	a	PPC, %	Deformação cranial
0	11.08	65.57	4763	9.95	0.50	13.75
0	11.33	73.67	.	13.22	0.60	9.96
0	11.57	53.40	.	11.41	1.20	5.14
0	11.57	64.14	.	13.02	1.00	14.89
0	11.57	67.08	9596	10.67	0.50	11.43
0	11.69	75.73	.	11.99	0.80	7.62
0	12.17	74.04	11019	10.10	0.50	13.93
0	12.29	61.64	19007	11.35	0.60	12.97
0	12.41	65.45	.	11.26	2.00	11.00
0	12.41	72.03	9871	10.59	0.40	11.24
0	12.41	74.74	10270	12.22	0.60	4.45
0	12.41	77.36	8595	10.68	0.80	10.99
0	12.53	81.60	8863	11.14	0.70	13.44
0	12.77	78.00	.	10.01	0.90	16.42
0	12.89	59.87	.	14.08	0.90	17.67
0	12.89	62.13	5311	12.24	0.90	11.38
0	12.89	62.18	9730	11.89	0.70	13.53
0	12.89	73.82	6765	11.17	1.00	12.34
0	12.89	75.02	11224	11.38	0.80	13.61
0	13.13	63.67	.	11.36	1.00	11.85
0	13.13	68.12	.	10.67	1.10	14.10
0	13.25	73.61	7193	12.01	1.40	12.98
0	13.25	87.00	.	11.82	2.20	10.67

0	13.37	60.20	4892	9.93	0.80	11.63
0	13.37	64.80	.	10.73	0.90	9.00
0	13.73	66.79	.	11.70	0.50	12.61
0	13.73	78.90	.	11.15	0.90	5.59
0	13.86	69.39	.	11.03	0.90	15.48
0	14.10	79.91	.	11.40	1.10	6.79
0	14.22	70.67	.	11.73	1.00	5.31
0	14.22	73.28	.	11.27	0.80	12.47
0	14.22	77.76	.	11.61	1.20	16.10
0	14.34	54.23	6567	11.82	0.80	8.60
0	14.34	66.76	.	13.10	1.40	11.09
0	14.34	73.70	10598	10.89	0.90	19.36
0	14.70	61.90	10846	10.08	1.10	6.19
0	14.70	65.51	.	11.34	1.60	13.59
0	14.82	75.56	.	11.85	1.50	8.18
0	14.94	65.56	4959	12.08	0.80	10.25
0	14.94	66.41	7799	12.38	0.60	13.35
0	15.18	68.00	9359	10.18	0.80	12.37
0	15.30	65.13	9965	11.16	1.10	12.82
0	15.66	67.06	7998	11.66	0.70	12.29
0	15.90	73.34	10642	11.16	1.20	8.40
1	10.36	77.67	.	11.70	1.30	9.57
1	10.60	72.76	.	11.70	1.00	14.05
1	12.29	73.57	.	14.90	0.80	8.05
1	13.13	72.20	.	12.04	0.70	16.12
1	13.13	77.33	5702	11.45	0.90	6.36
1	13.37	61.65	.	12.84	1.20	8.28
1	13.37	68.20	5939	14.96	0.70	7.54
1	14.34	75.77	6257	15.75	0.70	6.08
1	15.18	57.99	11042	12.35	0.70	6.16
1	15.18	65.46	6867	11.16	0.70	13.83
1	15.18	74.27	7161	10.75	1.00	10.98
1	15.30	73.22	13284	12.28	0.90	6.86
1	15.42	68.65	.	12.69	1.60	10.44
1	15.54	82.82	.	11.21	1.30	13.80
1	15.90	75.91	.	13.16	1.60	7.97
1	16.02	62.47	.	13.09	1.40	8.10
1	16.75	80.45	.	12.40	1.40	9.57
1	16.87	84.94	9477	12.85	0.90	8.09
1	17.59	61.14	10870	12.05	1.90	8.98
2	13.37	93.30	10397	13.03	0.70	10.72
2	13.73	93.70	10703	12.54	1.80	7.83
2	14.58	74.38	.	14.88	1.70	6.87
2	14.58	79.98	.	12.39	1.10	12.23
3	14.94	95.04	10062	12.22	1.70	9.12
3	15.63	81.56	.	13.08	1.90	8.54
3	16.39	80.59	.	16.57	2.30	7.02

3	17.47	83.33	.	16.65	1.70	8.82
3	20.36	76.49	14400	13.55	2.20	10.22

*A ausência de alguns resultados (.) é devida a ausência dos escores na idade avaliada.

Apêndice 11. Dados utilizados para análise de correlação aos 28 d.

Escore WB	Profundidade	Ecogenicidade	CK	a	PPC, %	Deformação cranial
0	10.84	58.18	14016	13.69	1.50	47.01
0	13.61	62.08	7424	10.52	1.00	59.23
0	12.41	63.54	6197	13.27	1.50	64.48
0	10.84	64.63	4641	13.75	1.00	57.55
0	12.65	65.10	5866	10.49	1.00	52.71
0	10.00	65.31	4450	11.22	1.50	65.99
0	12.05	66.44	6989	13.75	1.00	66.94
0	12.17	66.63	5933	10.30	0.50	53.11
0	11.57	67.49	10369	10.61	1.00	56.84
0	12.53	68.02	6242	11.53	1.50	57.29
0	10.36	68.51	9121	11.43	1.00	78.08
0	10.72	68.99	8408	9.95	1.00	59.58
0	11.20	69.08	7388	12.61	0.50	84.33
0	13.37	69.15	7802	11.09	2.00	55.64
0	11.57	69.38	5430	10.58	1.00	65.80
0	12.53	69.55	8359	11.51	1.50	62.98
0	14.10	70.08	20051	11.69	1.00	55.95
0	10.36	70.58	6130	9.52	1.50	73.13
0	11.33	71.86	5136	12.70	.	49.95
0	13.61	72.25	4984	11.48	1.00	60.98
0	12.77	72.40	7465	11.40	2.50	51.74
0	14.22	72.78	6925	13.95	.	25.13
0	11.08	73.02	6047	12.83	0.50	76.11
0	12.29	73.19	5891	10.81	1.50	55.40
0	10.98	73.49	5443	13.03	1.00	52.34
0	13.37	74.27	8365	13.47	1.50	56.28
0	12.17	75.14	6655	11.28	1.00	39.67
0	13.37	75.52	6802	12.68	1.50	40.80
0	11.20	76.99	5851	11.38	1.00	45.56
0	11.33	77.33	5582	11.34	1.50	73.94
0	12.65	78.25	5815	10.60	2.00	63.11
0	11.33	79.67	6024	12.31	1.50	53.68
0	11.69	82.70	4735	11.06	1.00	59.01
0	11.81	83.30	5794	11.56	1.00	63.16
0	11.57	83.60	3770	12.45	1.00	62.47
0	12.29	89.31	6691	14.52	1.50	29.61
1	13.01	65.31	7896	12.90	1.50	36.97
1	13.86	68.88	4288	12.97	2.00	71.96
1	12.05	69.01	9616	12.94	2.50	78.13
1	10.96	69.90	7488	9.85	0.50	65.19

1	15.06	71.07	7783	12.17	1.00	46.32
1	14.10	71.76	5434	14.66	2.00	52.01
1	12.77	73.63	9961	11.42	2.00	69.39
1	13.64	76.04	5972	14.77	2.50	58.24
1	13.25	76.09	6703	14.13	1.50	64.47
1	13.61	77.17	5063	12.18	.	67.27
1	16.27	77.36	10745	14.51	1.00	39.93
1	15.30	80.84	6270	13.67	1.00	54.18
1	16.75	83.43	8353	10.99	2.00	37.54
1	13.73	84.85	10958	14.36	2.50	72.66
1	14.22	85.37	4906	13.05	1.00	44.40
1	13.73	91.57	7158	11.88	1.00	38.70
1	16.27	102.03	11950	14.97	2.00	56.57
2	14.34	60.77	5479	12.09	2.00	47.37
2	14.70	68.58	11694	12.46	1.00	69.29
2	13.13	68.68	8019	12.03	2.50	38.48
2	13.49	68.88	10539	11.90	1.00	61.12
2	15.66	69.23	10468	13.40	2.00	34.08
2	15.54	73.53	8878	14.99	3.50	53.72
2	14.34	73.54	10526	12.45	2.00	45.84
2	14.70	83.44	12532	14.99	2.50	47.74
2	15.78	84.98	7695	12.80	1.50	41.62
2	15.06	88.19	11372	13.61	1.00	52.40
3	16.87	35.92	9565	13.89	1.50	59.92
3	18.19	44.04	5772	15.28	4.50	55.50
3	18.55	55.08	24197	14.50	2.00	29.70
3	17.71	76.23	10651	16.85	3.00	55.17
3	16.99	77.02	15018	13.64	2.00	47.68
3	15.42	78.56	10948	12.51	1.00	45.76
3	17.23	81.83	14179	13.56	.	43.65
3	17.23	85.60	12020	16.12	1.00	50.73
3	17.59	88.15	33031	10.18	2.00	40.20

*A ausência de alguns resultados (.) é devida a ausência dos escores na idade avaliada.

Apêndice 12. Dados utilizados para análise de correlação aos 35 d.

Escore WB	Profundidade	Ecogenicidade	CK	a	PPC, %	Deformação cranial
0	13.79	78.45	4417	10.71	1.00	195.79
0	14.55	80.41	4856	11.47	1.00	133.22
0	15.00	93.94	4073	10.13	2.00	77.75
0	15.15	73.18	5495	9.52	2.00	73.94
0	15.15	83.03	5950	11.97	1.00	105.05
0	15.76	64.61	5191	11.94	2.00	90.37
0	15.76	68.27	7945	11.19	1.00	95.37
0	15.91	72.34	9744	12.48	1.00	96.22
0	16.06	79.14	5568	9.59	1.00	151.39

0	16.52	75.66	6016	10.51	1.00	83.91
0	16.52	87.50	4080	10.94	1.50	84.63
0	16.52	.	7976	11.85	2.50	104.37
0	17.27	76.11	5598	10.55	1.00	96.73
0	17.58	88.81	6902	10.78	1.80	.
0	18.03	52.34	8715	12.43	1.00	.
0	18.33	81.75	5179	14.08	2.50	118.75
0	18.33	85.10	8088	12.05	1.50	93.77
0	18.48	.	6460	10.54	1.00	88.62
0	18.64	71.86	5637	12.32	2.50	71.73
0	18.64	.	4544	13.21	2.00	83.48
0	18.79	72.41	5909	11.49	0.00	93.02
0	18.79	85.75	5113	13.76	2.50	125.14
0	19.09	79.06	7940	10.83	2.50	112.14
1	15.30	74.28	7564	11.93	2.00	94.39
1	17.88	62.66	5246	10.55	1.50	110.51
1	18.03	80.01	4926	11.93	2.00	108.26
1	18.03	92.05	9668	12.21	1.50	95.52
1	18.18	81.76	4928	10.30	1.00	102.77
1	18.33	77.99	4479	10.85	2.00	.
1	18.64	61.68	10993	9.88	3.00	95.39
1	19.24	75.76	8041	11.80	2.00	117.44
1	19.39	69.01	6008	12.29	2.00	75.88
1	19.39	93.62	7879	12.94	2.00	109.69
1	20.45	87.23	7576	12.28	2.00	89.02
1	20.45	90.69	7570	13.60	2.00	105.70
1	21.82	66.61	5692	11.75	2.50	81.73
1	21.97	81.15	9236	12.53	2.00	98.58
1	22.12	89.58	9341	11.79	3.50	105.31
1	22.27	91.47	14137	12.05	1.50	97.17
1	22.58	82.92	10288	12.14	3.50	98.74
2	16.82	64.48	6311	11.90	1.50	79.51
2	19.09	82.17	8343	12.67	2.50	77.29
2	19.24	85.71	4600	11.84	2.00	94.24
2	19.85	63.75	7028	11.34	2.00	111.64
2	20.91	86.56	12054	12.34	2.50	75.65
2	21.06	76.10	8086	10.38	3.00	88.53
2	21.82	91.53	9554	12.89	2.00	98.14
2	22.42	.	9490	12.27	3.00	89.11
2	22.73	88.56	10116	11.13	3.00	106.12
2	22.73	94.79	11261	12.00	3.50	92.52
2	22.73	95.19	8331	9.67	2.50	94.88
2	23.79	.	10740	11.47	3.50	98.04
2	24.39	86.74	16298	11.01	4.00	106.20
3	20.00	85.89	10422	13.50	3.00	89.46
3	20.15	80.00	9181	12.46	2.50	99.82
3	21.67	84.71	12091	12.87	3.50	89.51

3	24.85	81.07	10401	13.09	2.50	84.71
3	25.45	91.06	32413	14.15	5.50	77.96
3	26.21	.	14307	12.10	4.00	.
3	26.52	90.23	10973	12.62	2.50	92.51
3	28.48	93.67	23928	12.81	6.00	100.53
4	22.73	89.52	35685	13.21	3.50	77.97
4	24.09	84.21	29968	14.10	2.50	93.26
4	24.24	91.88	11376	14.67	3.50	87.91
4	24.39	96.10	10503	13.42	4.00	94.31
4	25.91	.	38898	9.08	4.50	82.79
4	26.06	96.64	40337	10.56	5.00	78.37
4	26.36	76.10	19979	17.32	6.50	80.93
4	26.36	98.83	26665	12.56	5.00	.
4	26.52	99.17	27111	18.24	5.00	95.50
4	27.42	81.43	22478	17.02	5.50	.
4	29.24	101.30	59708	13.56	8.50	77.83

*A ausência de alguns resultados (.) é devida a ausência dos escores na idade avaliada.

Apêndice 13. Dados utilizados para análise de correlação aos 42 d.

Escore WB	Profundidade	Ecogenicidade	CK	a	PPC, %	Deformação cranial
0	16.06	88.73	6998	10.74	2.50	102.91
0	16.52	86.23	7368	11.37	1.00	103.23
0	16.82	.	6514	13.27	2.00	462.24
0	17.73	84.2	4773	11.42	1.50	104.83
0	18.64	.	8505	12.10	1.50	141.18
0	19.24	80.9	8727	12.51	1.50	111.34
1	16.52	89.54	8521	12.62	1.50	135.12
1	17.27	86.71	8533	13.19	3.00	89.19
1	17.73	83.05	8084	12.53	1.50	114.11
1	18.33	94.07	9202	11.94	2.00	99.89
1	18.79	79.81	9832	11.04	1.50	130.44
1	19.39	85.33	7851	12.66	2.50	88.18
1	19.39	88.84	7139	14.07	1.00	86.61
1	19.42	.	4985	11.41	2.50	102.09
1	19.70	81	8753	12.17	2.00	143.74
1	20.00	72.25	11326	10.65	2.50	100.24
1	20.00	92.98	10895	11.46	3.50	95.65
1	20.30	69.6	7518	10.87	3.50	93.46
1	20.61	94.47	7334	13.49	2.50	83.68
1	21.36	90.7	11096	12.33	4.00	118.86
1	21.82	87.23	7669	11.73	4.00	147.00
1	23.31	88.9	9015	10.37	4.00	97.78
2	18.48	85.32	7500	11.06	2.00	134.98
2	19.24	80.16	7937	12.31	2.00	99.47
2	19.24	92.27	6067	12.62	1.50	100.15
2	19.50	87.71	12884	12.03	3.00	95.35

2	20.15	85.05	5460	12.21	3.00	122.37
2	20.30	85.54	13168	11.80	2.00	113.90
2	20.30	91.95	10615	12.06	3.00	110.46
2	21.21	.	11214	10.96	2.50	112.88
2	21.36	93.44	7539	10.89	4.00	140.03
2	21.52	87.67	8810	11.42	3.50	97.28
2	21.82	94.22	17098	12.64	5.50	110.70
2	22.27	.	13559	12.74	4.00	89.39
2	22.27	.	16168	11.62	2.50	104.04
2	22.88	78.9	11090	12.08	2.00	91.34
2	23.03	73.15	11145	12.74	3.00	98.98
2	23.18	85.76	13003	12.86	3.00	107.18
2	23.42	90.63	9782	13.30	2.50	122.39
2	23.94	70.12	11336	10.21	3.50	100.90
2	24.39	82.81	19151	13.66	1.50	99.33
2	24.39	92.6	9971	12.76	5.50	103.35
2	24.70	88.9	12768	11.44	6.00	93.45
2	25.45	88.9	13559	12.31	3.00	97.07
2	25.91	95.89	11986	12.59	3.50	84.11
3	21.67	99.2	19466	12.33	4.50	95.40
3	22.88	91.32	23381	13.85	2.00	110.18
3	23.48	73.52	22214	13.60	3.00	85.83
3	23.94	83.11	14013	11.81	3.50	126.02
3	24.24	88.62	10567	13.25	2.50	105.58
3	24.85	87.76	15781	12.04	3.50	92.20
3	25.74	88.23	9165	11.82	6.50	103.91
3	26.52	.	12333	12.87	3.50	120.64
3	28.18	101.58	42415	9.52	5.50	73.28
3	30.61	87.07	42847	14.07	6.00	71.24
3	30.61	97.24	35198	14.56	4.50	80.52
4	17.42	108.7	34625	16.62	8.50	80.52
4	23.94	82.97	11081	12.57	3.00	87.23
4	24.09	104.94	16216	13.36	3.00	90.41
4	24.24	91.12	20792	11.04	5.50	113.69
4	24.55	100.3	11775	13.46	2.50	115.77
4	25.76	92.09	39530	12.52	4.00	94.48
4	26.21	103.56	30582	12.64	3.50	100.06
4	26.72	95.6	23391	14.22	3.50	91.21
4	27.31	97.76	35611	16.82	4.00	85.24
4	27.73	86.97	49775	17.95	6.00	136.74
4	27.88	93.54	30398	12.09	4.50	86.04
4	28.33	103.4	41937	13.80	5.00	100.74
4	28.48	100.78	17684	13.14	4.50	124.49
4	29.09	93.25	24365	17.64	3.00	107.60
4	29.24	78.62	50596	13.28	3.00	81.98
4	32.27	84.58	45029	10.59	6.00	95.30

*A ausência de alguns resultados (.) é devida a ausência dos escores na idade avaliada.

Apêndice 14. Dados utilizados para análise de correlação aos 49 d.

Escore WB	Profundidade	Ecogenicidade	CK	a	PPC, %	Deformação cranial
1	16.36	.	2296	9.03	2.00	126.3
1	18.03	74.78	2628	9.57	4.00	90.335
1	18.18	99.56	4084	9.61	3.00	89.49
1	18.64	91.57	8460	12.28	2.50	97.18
1	19.09	84.19	3710	10.44	1.00	109.59
1	19.09	95.44	6645	10.93	3.50	114.655
1	19.39	81.48	9823	8.66	2.50	102.4
1	19.55	77.63	2931	11.87	1.00	102.355
1	19.70	85.01	2254	13.36	2.50	88.845
1	20.00	84.17	3973	10.22	3.00	.
1	20.15	78.91	9415	9.11	4.50	80.835
1	20.15	83.22	2523	11.41	0.50	88.925
1	20.45	75.97	5227	10.48	4.00	90.385
1	21.21	88.62	9477	8.60	2.50	124.985
1	21.52	66.83	8311	8.36	2.50	115.575
1	21.52	90.11	4667	9.59	1.50	89.255
1	21.67	78.86	8524	9.91	3.50	.
1	21.82	90.75	11881	10.28	4.50	80.95
1	22.73	94.78	6646	10.16	2.00	141.545
1	22.73	96.42	5411	10.59	4.00	119.245
1	23.18	80.77	3106	8.09	2.50	.
1	23.64	75.53	10175	11.94	2.50	.
2	20.76	95.66	9728	8.60	3.00	99.56
2	21.06	100.71	3379	10.87	0.50	62.25
2	21.52	88.75	7387	11.88	3.50	92.175
2	22.27	94.25	9045	8.58	4.00	.
2	22.58	85.4	9916	10.01	3.00	89.035
2	22.73	91.14	7327	9.60	2.00	94.54
2	22.88	78.25	7538	9.48	3.50	79.625
2	23.18	87.76	11252	9.94	4.50	58.92
2	24.09	79.4	10800	12.68	2.50	100.345
2	24.24	89.5	9022	9.13	4.00	105.075
2	24.70	96.25	11894	10.10	2.50	75.825
2	24.85	82.72	4887	10.55	3.50	.
2	24.85	97.27	9504	13.56	4.00	76.7
2	24.85	.	9088	9.11	4.00	102.435
2	25.30	78.52	9154	10.15	2.00	101.74
2	25.45	89.39	9167	12.88	4.00	95.515
2	25.45	92.96	9707	9.16	4.50	96.205
2	25.61	94.61	11050	11.13	4.50	85.855
2	26.21	85.89	18934	9.40	2.50	.
2	26.21	97.86	10543	14.15	5.00	97.025
2	26.36	83.43	11614	11.35	3.50	.
2	26.67	85.27	15751	10.20	5.50	76.32

2	27.58	97.83	11840	8.89	4.50	99.27
2	28.18	88.1	24350	8.39	3.50	57.49
2	28.94	85.79	13394	9.32	6.50	78.08
3	23.64	100.96	47029	10.43	8.50	103.82
3	24.39	94.23	22103	12.46	6.50	85.08
3	26.21	97.92	10725	10.75	6.00	112.93
3	26.67	109.58	9618	11.68	5.50	70.18
3	27.42	91.72	8650	11.80	6.50	86.645
3	27.58	103.93	13983	11.54	9.00	81.025
3	28.03	87.68	10397	8.83	5.00	108.12
3	28.18	102.13	22356	10.23	6.00	107.02
3	28.94	99.75	34248	10.18	7.50	95.405
3	29.70	86.62	20505	8.19	3.50	72.695
4	20.00	103.03	53376	12.01	4.00	55.485
4	20.61	101.27	27803	10.07	9.00	83.575
4	21.97	113.39	45028	12.41	10.00	.
4	22.27	93.02	40872	7.28	9.00	85.23
4	23.33	101.97	58087	10.81	10.50	.
4	25.15	.	37406	8.22	8.00	95.62
4	25.15	98.34	15983	9.20	6.50	104.145
4	26.21	99.11	27798	11.59	2.50	101.085
4	26.97	82.62	39232	13.38	11.00	88.98
4	27.58	92.88	16643	9.72	4.50	85.385
4	27.73	99.76	30256	9.66	7.00	103.68
4	29.09	97	26436	12.12	8.50	102.58
4	29.55	88.49	36219	9.17	7.50	104.05
4	30.45	88.52	15049	14.42	10.50	.
4	33.79	94.31	8387	9.57	5.00	83.575

*A ausência de alguns resultados (.) é devida a ausência dos escores na idade avaliada.

VITA

Cristina Tonial Simões, filha de Carlos Alberto Bitencourt Simões e Ester Salete Tonial, nascida em 18 de fevereiro de 1991, em Porto Alegre – RS. Completou o ensino fundamental no colégio São Judas Tadeu e o ensino médio no Colégio Dom Bosco, ambos localizados na cidade de Porto Alegre – RS concluindo os estudos em dezembro de 2007. Em 2009 ingressou no curso de Medicina Veterinária na Universidade Federal do Rio Grande do Sul. No último semestre da faculdade foi Estagiária Nível Superior na empresa BRF S.A., na unidade de Videira – SC, na área extensão agropecuária com foco em avicultura, sob supervisão de Robison Biesek. Formou-se Médica Veterinária em dezembro de 2015. No primeiro semestre de 2016 ingressou como aluna de mestrado com dedicação exclusiva no Programa de Pós Graduação em Zootecnia da UFRGS, sob orientação do professor PhD. Sergio Luiz Vieira. Além de ter se envolvido em diversos projetos de pesquisa ao longo do seu mestrado, teve a oportunidade de participar em três eventos científicos internacionais, onde em ambos realizou apresentações orais em inglês sobre trabalhos desenvolvidos no Aviário de Ensino e Pesquisa. Foi submetida à banca de defesa de Dissertação em Março de 2018.