Universidade Estadual de Londrina

RAFAEL HUMBERTO DE CARVALHO

BEM-ESTAR ANIMAL E QUALIDADE DE CARNE DE AVES

Londrina
2016

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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal da Universidade Estadual de Londrina como requisito parcial para a obtenção do título de Doutor.

Orientador: Prof. Dr. Massami Shimokomaki.

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2016

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração
Automática do Sistema de Bibliotecas da UEL

Carvalho , Rafael Humberto de .
BEM-ESTAR ANIMAL E QUALIDADE DE CARNE DE AVES / Rafael Humberto de Carvalho . - Londrina, 2016.
275 f. : il.

Orientador: Massami Shimokomaki .
Tese (Doutorado em Ciência Animal) - Universidade Estadual de Londrina, Centro de Ciências Agrárias, Programa de Pós-Graduação em Ciência Animal, 2016.
Inclui bibliografia.

1. Bem-estar animal - Teses. 2. Qualidade de carne - Teses. 3. Avicultura - Teses. 4. Alimentos - Teses. I. Shimokomaki , Massami . II. Universidade Estadual de Londrina. Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência Animal. III. Título.

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Londrina, 23 de março de 2016.

DEDICO

Ao professor, orientador, amigo e "pai científico".
Massami Shimokomaki

AGRADECIMENTOS

À Deus que orienta meus passos.

Aos meus pais, Jesué Carvalho e Lúcia Carvalho pelo amor e incentivo constante na minha formação profissional e pessoal.

Ao meu orientador Prof. Dr. Massami Shimokomaki, por abrir as portas para meu futuro e me orientar em momentos de difíceis decisões, pela orientação durante toda a realização do trabalho e no qual tenho muito orgulho de ser seu orientado.

À Profa. Dra. Adriana Lourenço Soares pelo apoio e dedicação prestados, para que este trabalho pudesse ser desenvolvido, onde sua paciência e orientação foram fundamentais para o seu sucesso.

Ao meu supervisor na Espanha Prof. Dr. Mario Estévez García, que me proporcionou uma grande oportunidade de aprendizagem e crescimento, tanto profissional quanto pessoal.

As Professoras Dra. Elza Iouko Ida e Dra. Marta Madruga pelos conselhos e ajuda dispensados para o meu crescimento profissional.

Ao Programa de pós-graduação em Ciência Animal da UEL e a todos seus professores, pelos ensinamentos, apoio e orientação.

Aos professores do instituto IPROCAR de Cáceres Espanha pela colaboração e parceria, em especial ao Prof. David Morcuende.

À BRF pela oportunidade de execução desta pesquisa e a todos os funcionários que auxiliaram nas diversas etapas desenvolvidas em especial ao diretor Paulo Guarnieri, aos supervisores Hamilton Camargo, Juliane Valentini, Adriane Marangoni, Alice Sulchinski, Joice Dalforno e Juciléia Castro.

Aos estagiários, Paulo Barbetta e Ramom Grigio muito obrigado pela amizade e ajuda profissional.

Aos amigos de Chapecó, Vinicius Agostini, Bruno Abdalla, Erielcio Lopes e Jeferson Zucco muito obrigado pelo acolhimento e amizade.

Ao meu irmão Marcelo, que sempre me apoiou em todos os momentos, fossem eles maus ou bons.

À minha namorada, amiga e companheira: Gabriela Martini. Seu carinho, apoio e compreensão permaneceram comigo ao término deste trabalho e continuarão para as muitas outras etapas que atravessaremos juntos.

À toda minha família, que me apoiaram todo esse tempo, e tenho certeza, continuarão sempre ao meu lado.

Aos meus amigos e amigas que fiz durante o curso, pelas lições de vida e momentos que passamos. Em especial, aqueles que tive mais contato: Francisco Júnior, Talita Kato, Danielle Honorato, Cintia Handa, Sandra Luz, Ayda Lahmar, Tolga Akcan, Encarna Ginés e Estefanía Jiménez.

Aos integrantes dos grupos de carnes Brasil-Espanha, pelo convívio social e acadêmico.

À Universidade Estadual de Londrina instituição que me abriu as portas para o futuro desde a graduação.

À CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) e Fundação Araucária, pela concessão da bolsa e auxílio financeiro deste trabalho.

Ao CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) pelo auxílio financeiro destinado à esta pesquisa (Proc. 471609/2011-0) e a Bolsa Sanduiche (Proc. 314656/2014-6 e 03165/2015-2).

La inspiración existe, pero tiene que encontrarte trabajando

Pablo Picasso

CARVALHO, Rafael Humberto de. **Bem-estar animal e qualidade de carne de aves.** 2016. 275 d. Tese (Doutorado em Ciência Animal) – Universidade Estadual de Londrina, Londrina, 2016.

RESUMO

Brasil é um dos líderes mundiais de produção e exportação de carne de aves, ranqueando-se como o terceiro maior produtor de perus e o segundo de frangos. É o primeiro e terceiro exportador de carnes de frangos e perus, respectivamente. As aves são influenciadas negativamente quando o seu bem-estar animal não é atendido e a sua produção fica comprometida. O bem-estar animal é fortemente afetado nas etapas pré-abate, devendo a indústria avícola preocupar-se a fim de evitar problemas com a qualidade da carne, particularmente nas atividades do manejo durante o transporte da granja ao frigorifico e dependendo das condições do transporte as aves são propensas a desenvolver anormalidades na carne. A presente tese abrange em sua maioria os resultados de experimentos voltados ao manejo de perus não somente nas condições de pré-abate como também na sua carcaça e nas amostras de filé de peito (*Pectoralis major m.).* Sendo assim, tem como objetivo avaliar o bem-estar animal e qualidade de carne de aves desde o pré-abate até o produto final ie da granja ao garfo. Esses resultados originaram 8 artigos dos quais 2 foram publicados, outro encontra-se submetido e os demais estão sendo preparados para a suas submissões em revistas científicas de impacto. As condições desfavoráveis ao bem-estar foram avaliadas pela medida da incidência de carnes PSE (*Pale, Soft, Exudative*) provocadas principalmente pelo estresse térmico (experimento A), afetando a glicólise muscular (experimento B). Esse estresse pode ser provocado pela apanha da ave na granja (experimento C) e pelo transporte no verão e no inverno (experimentos D, E e F). Quantificou-se a variação na incidência das carnes PSE durante o verão e inverno com o surgimento de outra anormalidade, as carnes DFD (*Dark, Firm, Dry*) (experimento G). Finalmente, o fenômeno das carnes PSE em frangos foi avaliado em detalhes em relação à origem da formação das oxidações lipídica e proteica em amostras em que a anormalidade foi provocada *in vitro* e o experimento executado nos laboratórios da Universidade Extremadura, Espanha, e os resultados compõem o experimento H.

Palavras-chave: Avicultura. Estresse oxidativo. Frangos. Manejo. Microambiente térmico. Peru. Transporte de aves.

CARVALHO, Rafael Humberto de. **Animal welfare and quality of poultry meat.** 2016. 275 p. Thesis (Doctoral Degree in Animal Science) – Universidade Estadual de Londrina, Londrina, 2015.

ABSTRACT

Brazil is within the world leadership in relation to poultry meat production and export and it is ranked as the third major turkey and second broiler country producer. It is also ranked as the first and the third exporter country of broiler and turkey meat, respectively. Poultry meat production is negatively influenced while its animal welfare is not favorable. This animal welfare is strongly affected at pre slaughter phase and industries should take care in order to avoid meat qualities problems particularly on the management activities of transport from the farm to the slaughterhouse and depending on the in-transit conditions, birds are prone to produce meat abnormalities. This thesis covers mostly the results of experiments related to the turkeys' management not only in the pre - slaughter conditions as well as on their carcasses and on the breast file meat samples (*Pectoralis major m.*). The objective of this work was to evaluated the animal welfare and quality of poultry meat from the farm to fork. The experimental results originated 8 articles and 2 of them were already published, another one was submitted and the others are currently being prepared for their submission to scientific journals. The unfavorable conditions to animal welfare were assessed by measuring the incidence of PSE meat (Pale, Soft, Exudative) caused by heat stress (experiment A) thus affecting the muscle glycolysis (experiment B). This stress can be caused by bird`s harvesting at the farm (experiment C), and in transit by truck during summer and winter seasons (experiments D, E and F). The incidence of PSE meat was quantitatively evaluated during summer and winter seasons and other meat color abnormality was detected, the so called-DFD meat (Dark, Firm, Dry) (experiment G). Finally, the PSE meat phenomenon in broilers was evaluated in detail in relation to the origin of lipid and protein oxidation in samples where this meat abnormality was induced in vitro and the experiment was carried out in the laboratories of the University Extremadura, Spain, and the results constitute the experiment H.

Keywords: Aviculture. Broiler. Oxidative stress. Handling. Thermic microenvironment. Transport of poultry. Turkey.

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DIVISÃO DO TRABALHO

O presente trabalho foi desenvolvido para contribuir com conhecimento sobre a cadeia produtiva de aves no Brasil, enfatizando o seu bem-estar e qualidade da carne. O conteúdo do trabalho foi dividido em nove secções, sendo a primeira constituída de introdução e revisão de literatura, e as demais em forma de experimentos e redigidos em formato de artigos científicos para posterior publicação.

Experimento A – The incidence of pale, soft, and exudative (PSE) turkey meat at a Brazilian commercial plant and the functional properties in its meat product. (Publicado).

Experimento B - Glycolysis rate delay in turkey breast *pectoralis major m*. in a commercial air chilling processing line and meat qualities. (Publicado).

Experimento C - Effect of turkeys catching by hand and machine on injuries rates and meat quality. (Em preparação).

Experimento D - In-transit development of color abnormality in turkey breast meat during Brazilian winter season. (Submetido).

Experimento E- Relationship between turkey breast filet PSE-like and mortality in transit and the microclimate generated within the commercial truck container. (Em preparação).

Experimento F - Influence of distance on turkey transportation conditions and the occurrence of DOA, PSE-like (Pale, Soft, and Exudative) and DFD-like (dark, firm and dry) meat in different seasons. (Em preparação).

Experimento G - The Effect of Heat Stress and Wind Chill on Turkey Meat during Brazilian Commercial Truck Transportation. (Em preparação).

Experimento H - Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry. (Em preparação).

1 INTRODUÇÃO

Nas últimas décadas, a produção e o consumo de carne de aves têm aumentado significativamente em todo o mundo. América do Norte e Europa vêm perdendo espaço no mercado, enquanto China e Brasil tornaram-se novos centros de produção. De acordo com o USDA (2013), o Brasil classifica-se como terceiro maior produtor de carne de peru no mundo com 363 mil toneladas anuais, enquanto EUA com 2669 mil toneladas e Europa com 1910 mil toneladas, são primeiro e segundo colocados, respectivamente. Os números demonstram a potencial importância desse tipo de carne para a economia brasileira.

A moderna avicultura vem buscando a excelência em sua produção, aliada ao baixo custo e excelente qualidade dos produtos, reunindo os diversos conhecimentos fundamentados nas áreas de sanidade, genética, manejo e nutrição, atendendo as demandas e exigências dos mercados consumidores e visando uma maior inserção no mercado internacional. A capacidade de constante evolução tecnológica da cadeia produtiva de aves tornou-se uma característica peculiar cada vez mais pertinente na avicultura brasileira, concretizada nos últimos anos.

O bem-estar animal é um termo amplo que abrange tanto o desenvolvimento físico quanto mental. Pesquisadores desta área condicionam que as avaliações de bem-estar animal devem levar em conta provas científicas obtidas a partir da sua estrutura, funções e comportamento (BRAMBELL, 1965; DUNCAN, 2005), sendo assim, tanto o alojamento quanto o manejo devem garantir que o animal esteja fisicamente saudável, ou seja, não interferir no seu funcionamento biológico (FRASER et al., 1997).

Posterior a produção, inicia-se o processo de abate, inicialmente temos o período pré-abate que envolve a apanha das aves, carregamento, transporte e tempo de espera no frigorifico são práticas de manejo que, bem conduzidas, são fundamentais para atender o bem-estar, a sobrevivência e a qualidade da carne (LUDTKE et al., 2006).

As linhagens de peru atualmente estão ganhando mais peso e atingindo em torno de 23,5 kg, aos 140 dias de idade, dificultando a sua apanha (BARBUT, 2015). Recentemente surgiram máquinas comerciais para captura de aves; a captura automatizada está sendo cada vez mais utilizada na indústria avícola, principalmente devido aos altos custos trabalhistas e de mão de obra escassa (WEEKS; BUTTERWORTH, 2004; BARBUT, 2015).

Com relação ao transporte das aves e ao tempo de espera no abatedouro, as condições ambientais: temperatura, umidade relativa do ar e ventilação são de extrema importância. O controle do microambiente é relevante ao conforto, pois afeta diretamente o bem-estar do animal, além de determinar alterações nas propriedades funcionais das proteínas e, consequentemente nos atributos de qualidade da carne (GUARNIERI et al., 2004; SIMÕES et al., 2009; OBA et al., 2009; WILHELM et al., 2010; SPURIO et al., 2015; XING et al., 2015; JIANG et al., 2015).

O fenômeno *Pale, Soft, Exudative* (PSE) está intimamente correlacionado com o bem-estar animal e qualidade de carnes; sendo que sua mensuração tem sido utilizada como modelo para a medida da intensidade do estresse em diferentes situações (OLIVO; SHIMOKOMAKI, 2006; CARVALHO et al., 2014; SIMÕES et al., 2010). As carnes PSE possuem características de palidez, maciez e exsudação na sua superfície, cujas propriedades funcionais ficam comprometidas devido à desnaturação proteica em consequência da rápida glicólise *post-mortem* em temperatura relativamente alta (WISMER-PEDERSEN, 1959). As características da carne PSE refletem na recusa dos consumidores, devido à coloração pálida e excesso de exsudação em carcaças descongeladas tornando-se também inapropriada à indústria de processamento (KISSEL et al., 2009; DROVAL et al., 2012).

Algumas estratégias de minimização na incidência de PSE estão sendo observadas e estudadas com intuito de diminuir o estresse pré-abate, destacando-se as condições ambientais (CARVALHO et al., 2015), transporte (SIMÕES et al., 2009; LANGER et al., 2010; SPURIO et al., 2015), apanha (NIJDAM et al., 2005), efeito da luminosidade pré-abate (BARBOSA et al., 2013), além de modificações na dieta (OLIVO et al., 2001).

Estimou-se nos EUA que esta anormalidade representa 5-40% da carne que é produzida na indústria avícola, o que indica uma perda de mais de US$ 200 milhões por ano pela indústria (OWENS et al., 2000). No Brasil, a perda com frangos de corte foi estimada em US$ 55 milhões por ano (SPURIO et al., 2015) e a incidência de carnes PSE em perus ocasiona prejuízos de aproximadamente US$ 5 milhões anuais (CARVALHO et al., 2014). O presente trabalho tem como objetivo avaliar o bem-estar animal e qualidade de carne de aves desde o pré-abate até o produto final.

2 REVISÃO DE LITERATURA

2.1 PRODUÇÃO BRASILEIRA DE PERUS

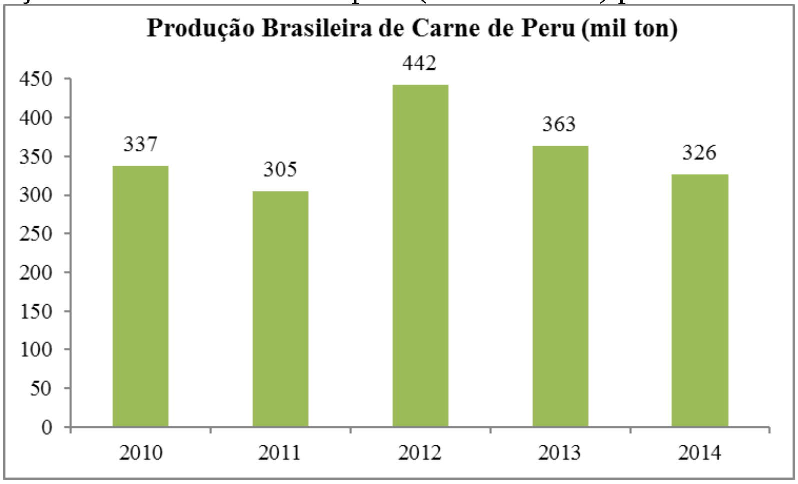
Nas últimas décadas, a avicultura avançou, partindo de uma produção baseada em pequenas propriedades para um modelo de economia de escala, ou seja, a criação e os processos obtiveram um alto grau de eficiência operacional. Neste cenário, a produção de perus seguiu o mesmo alinhamento à de frangos, pois também são utilizadas estruturas modernas com alto grau de tecnologia.

Peru é o nome dado às aves *Galliformes* do gênero *Meleagris* com variantes domésticas e selvagens. A espécie de produção utilizada para fim comercial no Brasil trata-se da *Meleagris gallopavo*. O Grupo Aviagen hoje é o maior fornecedor de material genético para a indústria brasileira de perus, comercializando as marcas *British United Turkeys* (BUT) e *Nicholas* (COSTA, 2006; AVIAGEN, 2015).

A produção de perus se caracteriza por duas fases distintas, pois ao contrário dos frangos de corte, os perus necessitam de duas instalações diferentes para completar o ciclo de produção. A primeira fase é denominada de iniciador ou fase inicial de criação, onde as aves são alojadas com um dia de vida e permanecem até completar aproximadamente 28 dias de idade (AVIAGEN, 2015). A segunda fase é denominada de terminador ou fase final de criação; nesta fase as aves são alojadas com 28 dias e permanecem até a idade de abate. O ciclo de produção no sistema terminador varia de acordo com a faixa de peso pretendida. Os perus inteiros geralmente consumidos no Natal, cuja faixa de peso oscila de três a seis quilos, são de maneira geral, fêmeas, e tem idade entre 56 e 70 dias. Outro grupo envolve animais que são processados em produtos industrializados, geralmente são machos com faixa de peso variando de 16 a 23 kg e idade entre 120 a 150 dias. Quanto às fêmeas abatidas para fim de industrialização seu peso gira em torno de 11 kg entre 95 e 120 dias de idade (KAIBER, 2005; AVIAGEN, 2015).

A produção brasileira de carne de peru no ano de 2014 foi de 326 mil toneladas (Figura 1) e deste total 79 % foram comercializados *in natura* e 21 % destinados à produção de processados.

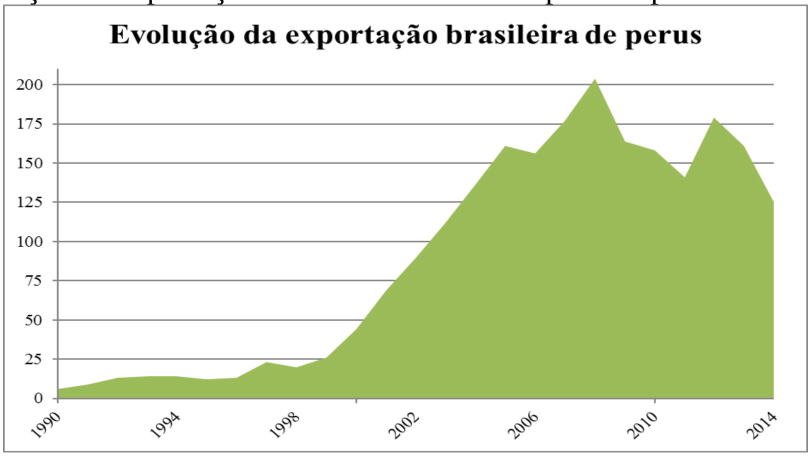
Figura 1 – Produção brasileira de carne de peru (mil toneladas) para os anos de 2010 a 2014.



Fonte: (UBABEF 2015).

Os estados do Paraná e Santa Catarina são os maiores produtores com 26,58% e 24,15%, respectivamente. De acordo com o USDA (2015), o Brasil classifica-se como terceiro maior produtor de carne de peru no mundo, enquanto os EUA e Europa são o primeiro e segundo, respectivamente. Houve nas duas últimas décadas um crescente aumento nas exportações brasileiras de carne de peru (Figura 2).

Figura 2 – Evolução da exportação brasileira de carne de peru no período de 1990-2014.



Fonte: (UBABEF 2015).

2.2 MANEJO

2.2.1 Manejo Pré-Abate

No período pré-abate das aves, o manejo de jejum, captura, carregamento, transporte e tempo de espera no matadouro, são atividades que devem ser bem conduzidas, pois são essenciais para não comprometer o bem-estar, a sobrevivência e a qualidade da carne. De acordo com Rocha et al. (2008), 90% das lesões nas condenações de carcaças observadas pelo serviço de inspeção sanitária ocorrem nesta fase.

2.2.1.1 Apanha (Captura)

A apanha é a primeira operação em contato direto com as aves gerando grande acometimento de estresse, que por sua vez influencia diretamente no bem-estar e, posteriormente nas perdas da qualidade das carcaças. A indústria avícola tem como meta minimizar as perdas econômicas geradas nesta etapa, que ocorrem principalmente em partes nobres da carcaça, como peito, coxas e asas (CASTILLO; RUIZ, 2010; LUDTKE et al., 2010; CARVALHO et al., 2015).

A captura e a manipulação na etapa de apanha estão entre os principais fatores determinantes da eficiência e rentabilidade das indústrias que produzem aves em grande escala (NILIPOUR, 1996).

De acordo com Barbut (2015), os perus estão ficando mais pesados (23,5 kg aos 140 dias), tornando mais difícil capturar, levantar e colocá-los em uma caixa. A manipulação de aves mais pesadas também é mais arriscada em termos de contusões, luxações asas/pernas, bem como para a segurança do trabalhador.

O trabalho de apanha, apesar de ser simples, exige grande treinamento da mão de obra e força física, além de ser considerada uma atividade desagradável. Tal procedimento deve ser realizado preferencialmente nas horas com temperaturas mais amenas do dia, ou seja, durante o período noturno ou ao amanhecer, uma vez que o ambiente fica mais calmo para manejar as aves. No Brasil, a apanha mais comum é realizada manualmente, sendo feita de diversas maneiras: pelo dorso, pelas duas pernas ou por uma. O melhor procedimento de apanha é o individual pelo dorso, devendo ser realizado com as duas mãos para prender as asas junto ao corpo, evitando possíveis contusões e/ou fraturas (LUDTKE et al., 2010).

Segundo o Protocolo de bem-estar para frangos e perus, as empresas (integradoras) e/ou produtores devem ter um programa de treinamento para garantir o bem-estar; os encarregados da apanha devem ter um líder para fazer o monitoramento da equipe, não sendo tolerados maus tratos e brutalidade no manejo durante o procedimento (UBA, 2008).

Na avicultura moderna praticamente todos os aspectos da produção de aves foram automatizados ao longo das últimas décadas. A apanha ou captura ainda em sua grande parte são realizadas manualmente e carregadas em gaiolas (LACY; CZARICK, 1998). A apanha também pode ser realizada por meio de sistema mecânico, porém com pouca frequência em razão do alto custo do investimento, da exigência de adaptações dos galpões e da dificuldade de higienização da máquina. Alguns países utilizam equipamentos para suspender as aves do chão e colocando-as nas caixas de transporte sem que exista o contato com humanos (LUDTKE et al., 2010).

Atualmente são várias as máquinas comerciais disponíveis para captura, pois a automatização está sendo cada vez mais utilizada pela indústria avícola, principalmente em razão dos altos custos trabalhistas e da escassez de mão de obra (WEEKS, BUTTERWORTH, 2004). As colheitadeiras mecânicas (Figura 3) reúnem os perus e conduzem para esteiras rolantes até a gaiola de transporte (DELEZIE et al., 2005). A automatização tem como intuito reduzir a quantidade de tempo que as aves são expostas ao contato físico com os homens e movendo-as suavemente, evitando maior acometimento de estresse.

Figura 3 – Máquina automatizada de captura de perus.

Fonte: (CIEMCALABRIA, 2015).

De acordo com Duncan (1986), as aves capturadas por máquinas sentem menos medo que aquelas capturadas manualmente. A imobilidade tônica (IT) (isto é, menos medo) das aves capturadas manualmente, ou seja, estratégias involuntárias de defesa (medo) duram aproximadamente o dobro de tempo, sugerindo que elas ficam mais assustadas ou amedrontadas. Estes resultados indicam que em curto prazo o estresse está associado com a captura, reduzido consideravelmente através do uso máquina ao invés de capturá-las manualmente. Entretanto, o tipo de colheitadeira mecânica pode ser importante para garantir estas condições, embora um estudo tenha constatado que a captura automatizada por uma máquina "*Chicken Cat*" não resultou em melhoria significativa nas medidas fisiológicas de estresse (NIJDAM et al., 2005).

De acordo com Knierim e Gocke (2003), comparando aves capturadas manualmente com aquelas capturadas com máquina "*Chicken Cat*", houve menor incidência de luxações e fraturas na perna (50%), asa (22%) e dorso (27%), e menor percentagem de aves com uma ou mais lesões (inferior a 30%) que aquelas capturadas manualmente.

Segundo Delezie et al. (2006), o uso de uma colheitadeira comercial de frangos relatou níveis mais baixos de tensão e mais curtas durações de IT. Estes resultados sugerem que a máquina pode ser mais eficaz na redução do estresse em certos pontos do processo. Vários autores, examinando diferentes modelos de colheitadeiras automatizadas, observaram significativamente menos hematomas proporcionados às aves quando capturadas pela máquina (LACY; CZARICK, 1998; KNIERIM; GOCKE, 2003; NIJDAM et al., 2005; DELEZIE et al., 2006).

Duas desvantagens potenciais são relatadas entre a colheita mecânica e colheita manual, ambas relacionadas com os operadores da máquina:

1 - Trabalhadores que operam colheitadeiras automatizadas podem não visualizar aves mortas e/ou não abater aquelas que estão doentes, sendo assim, aumenta a probabilidade de *dead on arrival* (DOA) acontecer. Vários estudos relataram taxas mais elevadas de DOA com a utilização de máquinas de capturas (KNIERIM; GOCKE, 2003; DELEZIE et al., 2005; DELEZIE et al., 2006), índices estes ocasionados devido à inclusão de aves que já estavam mortas ou doentes.

2 - Uso de colheitadeiras mecânicas requer treinamento e experiência, e o bem-estar animal poderia ser comprometido durante o período em que os operadores aprendem a operar eficazmente a máquinas (DELEZIE et al., 2005; DELEZIE et al., 2006).

No Reino Unido apurou-se que as principais causas de morte em aves durante a apanha são o deslocamento e a fratura da pelve, onde atingiram mais de 76% das condenações. Isto se deve ao fato das aves baterem as asas quando o apanhador as suspende por somente uma perna, podendo acarretar uma torção na articulação da pelve, causando deslocamento ou fratura do fêmur. Sendo assim, o osso é forçado para o interior da cavidade abdominal, podendo atingir e romper os sacos aéreos, promovendo a entrada de sangue nos pulmões (LUDTKE et al., 2008).

Lesões durante o processo de captura e encaixotamento são de grande preocupação para a indústria; hematomas de pernas, peito e asas podem comprometer até 25% dos frangos processados nos EUA (FARSAIE et al., 1983). De acordo com Kettlewell e Turner (1985), até 20% das aves são descartadas devido às lesões relacionadas à captura.

Empresas de frangos e perus têm contínua dificuldade para recrutar e reter trabalhadores dispostos a realizar este trabalho, por ser fisicamente exigente. Obviamente, a colheita mecânica não só reduziria significativamente o número de pessoas necessárias para capturar e carregar as aves, mas também melhoraria nas condições de trabalho dos manipuladores. Também a captura mecânica apresenta-se economicamente mais viável do que a captura manual (LACY; CZARICK, 1998).

2.2.1.2 Banho e transporte das aves

Posterior ao carregamento, as aves são banhadas na fazenda antes do transporte, este manejo ajuda a minimizar temperaturas altas no verão brasileiro. A aplicação do banho reduz a temperatura, entretanto aumenta a umidade relativa do ar no microclima do caminhão de transporte (SIMÕES et al., 2009; LANGER et al., 2010; SPURIO et al., 2015).

Simões et al. (2009) relataram que a aplicação do banho na fazenda antes do transporte de frangos de corte reduziu a ocorrência de carnes PSE. Entretanto, Langer et al. (2010) demonstraram que este manejo promoveu condições desfavoráveis para as aves em transportes de curta distância (3 km), mas para distâncias relativamente maiores (68 km) a aplicação é benéfica e reduziu a incidência de carnes PSE durante o verão.

O transporte de perus da fazenda para a planta de processamento baseia-se desde o fim do carregamento das aves na gaiola do caminhão até pendura na linha de abate. No passado, o processo era manual, com trabalhadores que capturavam as aves e colocavam-nas em caixas. Isto ainda é feito em algumas partes do mundo, principalmente no Brasil, embora a mecanização já seja vista em várias integradoras (BARBUT, 2015).

O transporte das aves é realizado sob diversas condições de distâncias e tempos, refletindo diretamente na qualidade da carne. Na maioria dos casos, são os principais responsáveis por perdas (mortes). Existe uma associação direta entre a qualidade da carne e o manejo pré-abate, seja na produção, no transporte de animais ou abatedouro (EC, 1999; SPURIO et al., 2015).

Na criação intensiva de aves é requerida que as aves sejam transportadas para o abatedouro em caminhões, muitas vezes por longas distâncias. Durante o transporte as aves ficam expostas a uma variedade de agentes estressores (aceleração, vibração, impactos, jejum alimentar e hídrico, mistura social, perturbações e ruídos) (NICOL; SCOTT, 1990), levando ao comprometimento do bem-estar e, consequentemente, aumento de mortalidade e redução da qualidade de carne (MITCHELL et al., 1992; MITCHELL; KETTLEWELL, 1998).

Os principais fatores de estresse durante o transporte de frangos de corte estão relacionados ao microclima da carroceria, os valores elevados de temperatura e umidade relativa são os principais responsáveis pelo DOA (HUNTER et al., 1997; MITCHELL; KETTLEWELL, 1998; SPURIO et al., 2015).

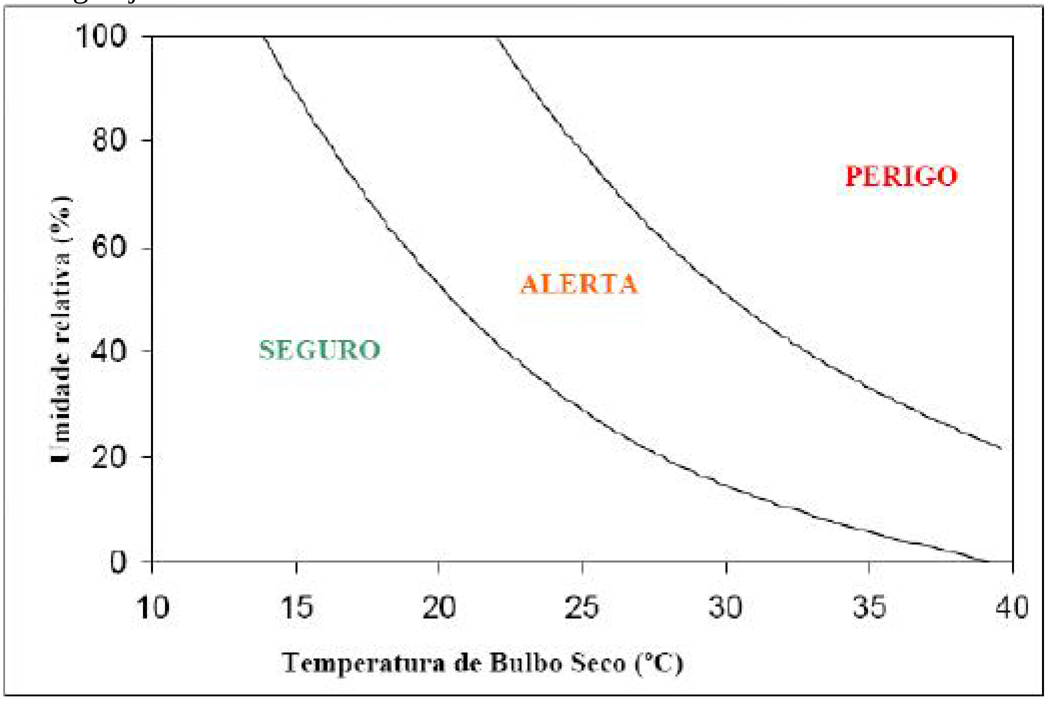
A distribuição das aves mortas na carga não é aleatória; o fenômeno reflete a variação de ventilação e conforto das regiões da carroceria. Um estudo envolvendo a caracterização tridimensional das condições ambientais dentro caminhões de transporte de frango, apontou grande heterogeneidade das variáveis ambientais ao longo do transporte (MITCHELL; KETTLEWELL, 1998).

Durante o transporte existe uma produção de calor metabólico pelas aves, criando gradientes térmicos entre caixas de transporte e o ambiente exterior, que é totalmente influenciado pela ação do vento, resultando em distribuição de temperatura heterogênea durante todo trajeto (MITCHELL; KETTLEWELL, 1994; SPURIO et al., 2015). Esta carga térmica deve ser dissipada pela presença de ventilação natural ou forçada, portanto, pouco fluxo de ar através da estrutura do caminhão resultará no acúmulo de calor e umidade, cuja combinação causará estresse térmico às aves (MITCHELL; KETTLEWELL, 2004; SPURIO et al., 2015).

Estudos apontaram a presença de um núcleo térmico em cargas de caminhão gerado por má ventilação, e também, relataram que em determinadas regiões da carroceria a temperatura e umidade são mais elevadas, no entanto, tais estudos foram desenvolvidos em regiões temperadas (MITCHELL et al., 1992; MITCHELL; KETTLEWELL, 1998). Faltam pesquisas que comprovem o perfil térmico das cargas nas condições tropicais, especialmente quando intensas flutuações térmicas diárias sazonais são consideradas. A formação de um microclima térmico no interior dos veículos afeta o bem-estar e promove o desenvolvimento de carnes PSE (SIMÕES et al., 2009; SPURIO et al., 2015).

Estudos realizados por Mitchell e Kettlewell (2004) estabeleceram um modelo para descrever o microambiente do caminhão de transporte, associando as condições de transporte às necessidades fisiológicas; sendo assim, o modelo permite estabelecer as zonas de conforto térmico para as aves durante o transporte (Figura 4); as quais foram denominadas como "segura", "alerta" e "perigo" mediante a combinação de temperatura e umidade relativa, com base no índice de carga térmica denominada Temperatura Equivalente Aparente (TEA).

Figura 4 – Zonas de conforto térmico para o transporte de frangos durante o transporte da granja ao abatedouro.



Fonte: Adaptado de Mitchell e Kettlewell (2004).

De acordo com Mitchell e Kettlewell (2004), a umidade relativa no caminhão de transporte raramente fica abaixo de 70% devido à perda de água pelas aves; os autores recomendam temperatura de 26 - 27°C como o máximo admissível no caminhão, sendo estas condições não comprometedoras do bem-estar das aves. De acordo com Mitchell et al. (1990), se a temperatura do microambiente do caminhão no transporte de frangos for de 28°C e a umidade relativa for de 80%, resultará em aumento de 0,42°C/h na temperatura corporal das aves.

A condição térmica durante o transporte é um fator de relevância, pois associada à elevada umidade relativa do ar, proporciona elevação da temperatura corporal e consequentemente provoca alcalose respiratória. Porém, pouca atenção é dada para o microambiente que se forma no caminhão durante o transporte da granja ao abatedouro, podendo ser a causa primária que compromete o bem-estar, resultando em mortalidade ou reduzindo a qualidade final da carne (MITCHELL; KETTLEWELL, 1998).

Trabalhos realizados em países de clima frio, relatam que além da influência do microambiente do caminhão que se forma entre as aves, as exigências térmicas representadas por episódios de calor ou frio intenso podem causar efeitos negativos na qualidade da carne e na taxa de mortalidade (MITCHELL; KETTLEWELL, 1994; WARRIS et al., 1993; MITCHELL; KETTLEWELL, 1998).

De acordo com Kettlewell et al. (2001) existem dois tipos de ventilação durante o movimento do veículo; passiva (ou natural) e ativa (ou mecânica). Ventilação passiva é a situação que existe na maioria dos veículos de transporte. A troca de ar dentro do caminhão é conduzida em virtude da flutuação térmica, movimento do próprio veículo e pelo vento predominante. Com a ventilação ativa, ventiladores são instalados no veículo para proporcionar a circulação do ar em todos os momentos.

De acordo com Hoxey et al. (1996), o padrão de fluxo de ventilação resultante do movimento do veículo é principalmente da traseira para frente do caminhão, sendo a parte frontal a menos ventilada em comparação com a traseira. O movimento do fluxo de ar deve-se principalmente ao design da carroceria do caminhão (Figura 5), que na Inglaterra tem a parte frontal fechada. Entretanto, no Brasil, estudos realizados por Simões et al. (2009) e Spurio et al. (2015) relataram um fluxo de ar da parte frontal do caminhão para a traseira, tal hipótese do fluxo deve-se ao diferencial no design da carroceria com a parte frontal aberta (Figura 6).

Figura 5 – Caminhão utilizado para transporte de aves em países de clima temperado.

Fonte: (HOXEY et al., 1996).

Figura 6 - Caminhão utilizado para transporte de aves em países de clima tropical.

Fonte: (SIMÕES 2009)

2.2.1.3 Descanso pré-abate

O descanso pré-abate é considerado o tempo entre a chegada do caminhão no abatedouro até o momento de pendura na linha de abate. Esta etapa de descanso é uma prática rotineira e muito importante para obter uma carne de qualidade. De acordo com Guarnieri et al. (2004) a utilização do período de espera está se tornando uma prática comum nas plantas comerciais de abate de aves no Brasil, o uso de um ambiente ventilado e temperatura amena ajuda a manter os animais confortáveis diminuindo o estresse térmico. Devido às diversas formas de estresse geradas no transporte, as aves podem chegar com o sistema cardiorrespiratório alterado e a temperatura corporal superior à normalidade, sendo assim o local de espera deve proporcionar as melhores condições para minimizar os efeitos provocados pelo estresse, devendo ser sombreado, possuir uma temperatura amena e circulação de ar (GUARNIERI et al., 2004; LUDTKE et al., 2006).

De acordo com Ludtke et al. (2006), quando o ambiente associa ventilação e aspersão de água, esse processo reduz o excesso de calor e diminui a agitação das aves. A aspersão com água durante o tempo de descanso é recomendada quando a temperatura ambiental varia de 10 a 30°C e a umidade relativa está abaixo de 80%. Todavia não é recomendada quando as temperaturas ambientais são inferiores a 10°C, pois pode causar tremor muscular, depleção de glicogênio e problemas com a qualidade de carne.

Segundo Guarnieri et al. (2004), o banho de aspersão de água associado à ventilação antes do abate de frangos contribuiu para a recuperação da homeostase fisiológica e repercutiram positivamente nos processos bioquímicos, evitando a instalação do fenômeno PSE em filés de peito.

De acordo com Kannan et al. (1997), frangos abatidos imediatamente após 3h de transporte apresentaram nível de corticosterona maior do que aqueles que permaneceram por 4h de descanso antes de serem abatidos. O elevado nível de corticosterona no plasma resultou em carnes de frango mais pálidas.

2.2.2 Manejo de Abate

O processo de abate possui as seguintes etapas: pendura, insensibilização, sangria, escaldagem, depenagem, evisceração, resfriamento e/ou congelamento (LANGER et al., 2010). A fase de pendura consiste na retirada das gaiolas do caminhão na plataforma de recepção sendo realizada manualmente (LUDTKE et al., 2006). Para o abate de aves recomenda-se o uso luz azul (luz violeta) no ambiente, e o uso de uniformes de cor azul, objetivando tranquilidade das aves (LUDTKE et al., 2006; BARBOSA et al., 2013).

A etapa de insensibilização tem como objetivo de evitar a dor e sofrimento dos animais e o Ministério da Agricultura Pecuária e Abastecimento (MAPA) elaborou a Instrução Normativa n°3, que regulamenta todos os métodos permitidos para a insensibilização no abate humanitário, sendo obrigatória para todos os estabelecimentos destinados ao abate a aplicação da insensibilização imediata como etapa prévia à sangria. A inconsciência gerada pela insensibilização tem por objetivo impedir que a ave sofra durante a sangria, devendo se manter inconsciente até ocorrer a morte (BRASIL, 2000).

Fase posterior, a sangria das aves deve ser realizada imediatamente após a insensibilização, objetivando o rápido escoamento do sangue que ocasiona a isquemia, levando à morte cerebral (BRASIL, 2000). A morte da ave deve ocorrer antes da recuperação da sensibilidade. A incisão/corte deve ser realizada nos grandes vasos do pescoço, podendo ser manual ou automática, ambos os métodos são eficientes (LUDTKE et al., 2006).

A etapa de escaldagem é executada logo após o término da sangria e morte da ave, o processo alia condições definidas de temperatura e tempo, ajustados às características das aves em processamento (espécie, idade, tamanho etc...). Os métodos mais comuns são por pulverização de água quente e vapor ou por imersão em tanque com água aquecida através de vapor (BRASIL, 1998).

A depenagem é regulamentada pela Portaria nº. 210 (BRASIL, 1998), o processo é mecanizado, executado com as aves suspensas em noréas. A remoção total das penas é devida à ação de depenadores. Logo em seguida a evisceração deverá ser executada, isolada da área de escaldagem e depenagem, compreendendo, desde a operação de corte da pele do pescoço, até a limpeza total das carcaças. Nessa seção poderão ser efetuadas as fases de pré-resfriamento, gotejamento, embalagem primária e classificação, desde que a área permita a perfeita acomodação dos equipamentos (BRASIL, 1998).

2.2.2.1 Sistemas de resfriamento de carcaças

Na indústria avícola moderna, houve impressionantes aumentos na velocidade da linha de processamento de carne de peru, possibilitados através de avanços em pesquisa, engenharia, informática e outras áreas afins. Em geral, estes desenvolvimentos contribuíram para um aumento na velocidade de linha, sendo o mais importante o aumento significativo da capacidade de processamento por linha, isto é, de 12.000 kg por hora em 1970 para 72.000 kg atualmente (BARBUT, 2015).

A velocidade da linha de processo passa fundamentalmente pelo resfriamento das carcaças. Os processos *post-mortem* sob refrigeração de carcaças são essencialmente utilizados para garantir a segurança dos alimentos e maximizar a vida útil, além da manutenção da textura, cor entre outros fatores. Após o abate da ave, a carcaça tem que ser refrigerada reduzindo a temperatura, em seguida, manter a temperatura da carne inferior a um valor que irá assegurar a qualidade e segurança do produto (SAVELL et al., 2005).

De acordo com Lawrie (2005), inicialmente a dissipação de calor era realizada através de meios naturais, de modo que os animais eram abatidos em estações mais frias do ano, sendo o produto armazenado em cavernas como forma de prolongar a vida útil e manter a qualidade. Hoje, os sistemas de refrigeração devem cumprir a tarefa de refrigerar as carcaças durante o período crítico após o abate, no qual também ocorre o desenvolvimento do *rigor mortis*.

A área de resfriamento, localizada após a área de evisceração, tem por objetivo promover o resfriamento de carcaças e miúdos de aves, devendo possuir um *chiller* com sistema de entrada e saída de água, além de sistema de resfriamento separado para fígado, coração e moela (BRASIL, 1998).

De acordo com Klassen et al. (2009), são várias as técnicas utilizadas na operação de resfriamento, desde o uso de tanques com gelo à pulverização com água gelada, tanques com água e gelo ou resfriadores contínuos. Entre os métodos de resfriamento industrial disponíveis, destacam-se: *air chiller* (túneis de ar seco refrigerado) e *chiller* (tanques de imersão em água).

Segundo James et al. (2006), são três os métodos mais comuns de refrigeração utilizados para aves: imersão em água (*chiller* de água + gelo); pulverização (spray com água fria); com ar (câmaras-frias, *air chiller*). Um número limitado de estudos analisou o resfriamento com criogênicos (ARAFA, 1978), mas em 1982, foi relatado que houve pouca adoção comercial (LILLARD, 1982).

O resfriamento por imersão é realizado da seguinte forma: as carcaças são movidas através de um tanque ou uma série de reservatórios que contêm a água ou uma mistura gelada de gelo e água. Brant (1974) afirma que o ganho de peso durante a imersão é uma possível desvantagem deste método. Atualmente, a refrigeração por imersão é utilizada com temperatura máxima de entrada de água de 4 °C sob constante agitação, a fim de diminuir a absorção de água pelas carcaças. As taxas de refrigeração, ou seja, a velocidade com que a carcaça resfria-se por método de imersão é elevada comparada com métodos de ar e spray (JAMES et al., 2006).

O método de spray de refrigeração é caracterizado pela pulverização de água sobre as carcaças suspensas em ambiente de ar refrigerado. O termo spray abrange uma série de definições na literatura, e às vezes é nomeado diferentemente ocasionando confusão (JAMES et al., 2006).

O terceiro método *air chiller*, o ar refrigerado é soprado sobre as carcaças. Devido à grande escala de abate diário nos frigoríficos, as carcaças são normalmente suspensas em ganchos e colocadas continuamente nos trilhos. O tempo de refrigeração e perda de peso das carcaças são relacionados com as condições no interior do túnel e espaçamento entre carcaças (JAMES et al., 2006).

De acordo com Thomson et al. (1974), houve diminuição do tempo de resfriamento entre 10 e 15% quando foi aumentada a velocidade do ar de 3,5 para 7,0 m/s. Os autores relataram uma queda de cerca de 10 °C em 20 min para as carcaças não embaladas penduradas com ar a 2 °C e uma velocidade de 2,5 m/s. Vacinek e Toledo (1973) observaram que carcaças penduradas pelo jarrete em posição vertical, geralmente são resfriadas mais rapidamente do que carcaças em uma posição horizontal. A suspensão expõe uma maior área de superfície para a refrigeração.

A refrigeração de carcaças resulta diretamente no declínio do pH *post-mortem* (CARVALHO et al., 2015). O pH diminui desde o início da refrigeração, com a conversão de glicogênio em lactato em condições de refrigeração. Quanto menor a temperatura de refrigeração menor será a velocidade do declínio do pH (JACOB et al., 2012, PEDRÃO et al., 2015).

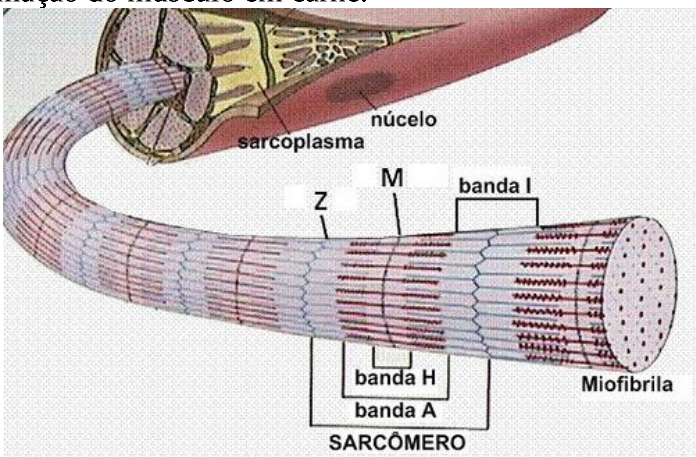
Carvalho et al. (2015) relataram em sistemas de resfriamento por ar frio um retardamento na velocidade de queda do pH em filés de peito de peru, amostras resfriadas por ar frio apresentou velocidade de queda de 0.031 unidades/hora, enquanto amostras controles sob temperatura ambiente a velocidade foi 0,151 unidades/hora.

2.3 TRANSFORMAÇÃO DO MÚSCULO EM CARNE

No abate com a interrupção do fluxo sanguíneo do músculo e a consequente paralização do suprimento de oxigênio, ocorrem intensas modificações químicas e físicas (estruturais) que levam à transformação do músculo em carne ou à mudança *post mortem* do músculo (KUBOTA et al., 1993).

Os principais componentes da carne são água (65 a 80%), proteína (16 a 22%), gordura (3 a 13%) e cinzas. As proteínas da carne são similares em todos os animais de abate, podendo ser classificadas segundo a solubilidade, em três grandes grupos: proteínas sarcoplasmáticas, miofibrilares e insolúveis. A unidade de organização estrutural do músculo esquelético é a fibra muscular que consiste em elementos proteicos na sua composição, que são as miofibrilas, entre as quais está a solução de sarcoplasma, uma fina rede de túbulos e o retículo sarcoplasmático (Figura 7) (LAWRIE, 2005).

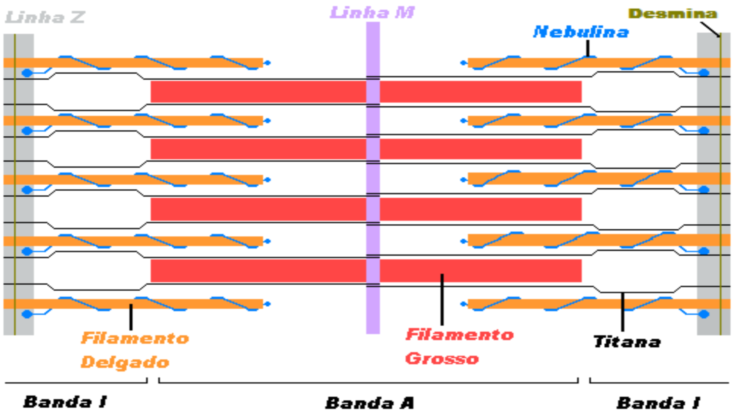
Figura 7 – Representação do músculo esquelético, indicando os elementos envolvidos na transformação do músculo em carne.



Fonte: (LAWRIE 2005)

As proteínas miofibrilares são estruturas da fibra muscular formadas por um agrupamento ordenado de filamentos grossos e delgados do tecido muscular, destacando-se em abundância a miosina e actina, respectivamente. Os filamentos grossos contêm ainda proteínas como as proteínas C e M, enquanto que os filamentos delgados são compostos por tropomiosina, troponina e α – actinina (ORDÓÑEZ, 2005). Nas linhas Z estão localizadas outras proteínas como α-actinina, desmina, filamina, vimentina e sinemina. A nebulina é encontrada principalmente na região da banda I nos filamentos delgados, enquanto que a titana ou titina está distribuída ao longo dos filamentos grossos e delgados (Figura 8) (SHIMOKOMAKI et al., 2006).

Figura 8 – Esquema representativo do sarcômero e algumas de suas proteínas estruturais (nebulina, titana e desmina).



Fonte: (SHIMOKOMAKI et al., 2006).

2.4 ALTERAÇÕES *POST-MORTEM* E GLICÓLISE

Após o abate, a interrupção da circulação sanguínea provocada pela sangria cessa o fornecimento de oxigênio e de nutrientes (ORDÓÑEZ, 2005). Entretanto, o animal morre em poucos minutos pós-sangria, mas suas células continuam a metabolizar e a responder por horas após a paralisação da respiração. Durante este intervalo, as células musculares continuam a utilizar a respiração aeróbica para produção e consumo de adenosina trifosfato (ATP). Quando cessa o oxigênio celular a célula passa a depender apenas do metabolismo anaeróbico para o suprimento de suas necessidades de ATP, utilizando as reservas de glicogênio muscular, mantendo a capacidade de contrair e relaxar. O glicogênio é então convertido em ácido lático, produto final do metabolismo anaeróbico, que se acumula no músculo devido à falta de circulação sanguínea (SAMS, 1999).

Após este processo ocorrem ligações entre os filamentos de actina e miosina, de forma que o músculo começa uma nova fase de contração contínua até que as reservas de ATP se esgotem. Com o esgotamento do ATP, as ligações actina-miosina se completam e o músculo entra em contração irreversível, perdendo extensibilidade, denominado *rigor mortis* (ORDÓÑEZ, 2005). Nesse estado, a musculatura atinge o *rigor mortis* ou rigidez cadavérica, ou seja, os músculos transformam-se em carnes.

Dransfield e Sosnicki (1999) concluíram que a instalação do *rigor mortis* em aves ocorre em aproximadamente uma hora, entretanto, a velocidade de queda de pH é influenciada por diversos fatores, como a espécie animal, taxa de glicólise, aspectos genéticos, níveis iniciais de glicogênio, o tipo de músculo, a temperatura em que ocorre o processo *post-mortem* e fatores de estresse. Tipicamente em frangos de corte os valores de pH variam de 6,6 a 6,2 em 15 minutos após o abate (PEARSON, 1994), e entre 6,4 a 6,2 para perus (LESIAK et al., 1996).

A resolução do *rigor mortis* (maturação) compreende as mudanças posteriores ao desenvolvimento da rigidez cadavérica que determinam o relaxamento lento do músculo, provocando amolecimento da carne (ROÇA, 1997; SHIMOKOMAKI et al., 2006; WILHELM et al., 2010).

De acordo com Alvarado e Sams (2002), o declínio do pH *post-mortem* (PM) e a temperatura são importantes na determinação da desnaturação das proteínas cárnicas. Existe uma relação estreita entre a temperatura e o pH muscular do peito de peru desossado. A temperatura do peito entre 25 e 26 °C por 60 min PM está associado com o pH reduzido.

Carvalho et al. (2015) reportaram que a taxa de diminuição do pH do músculo é influenciada pela temperatura de refrigeração e o completo *rigor mortis* foi alcançado 24 h PM sob condições comerciais utilizando sistemas de refrigeração por ar, sugerindo que filés de perus devem ser manipulados pelos processadores após este período de tempo, a fim de manter a funcionalidade das proteínas miofibrilares.

Estudos realizados por Lee et al. (1976) relataram que a oscilação de temperatura ambiental pré-abate impacta diretamente na velocidade de queda do pH *post-mortem* das aves. Segundo os autores, quando os animais são estressados pelo calor (38°C), o pH apresentou-se mais baixo comparado a aves mantidas sob a temperatura de conforto térmico (20°C), frio (4°C) e frio extremo (-20°C).

Segundo Alvarado e Sams (2002), a refrigeração PM insuficiente ou baixa pode contribuir para o desenvolvimento de carnes PSE, sugerindo que a indústria processadora de peru deve atentar-se para que o *Pectoralis major* chegue à temperatura de 25 °C ou menos em 60 minutos PM, reduzindo o risco de ocorrer o desenvolvimento da carne PSE. Os autores relataram que se o peito não for refrigerado em 105 min PM, a qualidade da carne vai alterar no que tange a coloração, capacidade de retenção de água, textura do produto e sua integridade.

2.5 Bem-Estar Animal e suas Mensurações

O conhecimento atual de bem-estar como uma medida da qualidade de vida, caminha no contexto da história social e cultural do cuidado com os animais e seu uso para produção de alimentos, bem como uma base de conhecimento relacionada com a expansão da sua fisiologia e etologia. Cientificamente, o bem-estar animal é um termo amplo que abrange tanto o desenvolvimento físico quanto mental. Pesquisadores desta área condicionam que as avaliações de bem-estar animal devem levar em conta provas científicas obtidas a partir da sua estrutura, funções e comportamento (BRAMBELL, 1965; DUNCAN, 2005).

Alguns critérios são adotados para estudar a capacidade de sentir prazer e dor dos animais (BENTHAM, 2005) ou as suas capacidades cognitivas superiores (NCB, 2005), com isso, alguns autores consideram a habitação e condições do ambiente da sua criação. Tanto o alojamento e quanto o manejo devem garantir que o animal esteja fisicamente saudável, ou seja, não interferir com o seu funcionamento biológico, viver naturalmente, se comportarem normalmente, e ser livre de dor e de outras circunstâncias negativas que induzem aos estados afetivos negativos (FRASER et al., 1997). Outros autores, tais como Fraser et al. (1997), sugerem que bem-estar animal implica na ausência de dor, medo e fome, permitindo um alto nível de funcionamento biológico, ou seja, o crescimento normal, livre de doenças.

O estresse é um dos principais parâmetros utilizado para avaliar o bem-estar animal. Grandin (1998) descreve que em condições estressantes, os animais desenvolvem mecanismos de respostas quando sua homeostasia está ameaçada, necessitando de ajustes fisiológicos ou comportamentais para adaptar-se aos aspectos adversos do manejo ou ambiente. Existem pelo menos dois sistemas para avaliar o estresse: um por meio do comportamento e o outro pela avaliação dos parâmetros biológicos (respostas endócrinas e/ou enzimáticas) nos fluídos ou músculos dos animais. No caso dos animais de produção com finalidade de produção de proteína de origem animal, as informações do estresse *antemortem*, podem ser avaliadas em suas carcaças (SHAW; TUME, 1992).

Segundo Bomholt et al. (2005), um dos sistemas primários endocrinológicos envolvidos na resposta ao estresse denomina-se eixo hipotálamo pituitária adrenal (HPA), no qual em condições de estresse atua liberando glicocorticóides. Tais substâncias podem ser usadas como indicadores para presença e a intensidade de um estressor, com duas ressalvas:

1) não informam quanto ao tipo de agente estressante (positivo ou negativo) que estimula o sistema hipotálamo-pituitária-adrenal (HPA);

2) a maioria dos procedimentos de amostragem são eles próprios estressantes para os animais, confundindo as medições.

Portanto, a avaliação do estresse com base nos níveis de glicocorticóides tem limitações. Além disso, estresse ou sofrimento pode existir sem a concomitante ativação do eixo HPA.

Algumas pesquisas têm demostrado uma relação direta entre o nível de estresse dos animais com a qualidade da carne resultante (SAMS, 1999; PETRACCI et al., 2004; GUARNIERI et al., 2004, BIANCHI et al., 2007; SIMÖES et al., 2009), grande parte dos experimentos demostram que o estresse pré-abate pode ser medido na carcaça dos animais PM; os pesquisadores que utilizaram a metodologia da mensuração em carcaças verificaram bioquimicamente a influência do estresse provocado nas etapas pré-abate e pós-abate, afetando o bem-estar animal e qualidade do produto final (GUARNIERI et al., 2004; PEDRÃO et al., 2015).

Fatores relacionados com o manejo pré-abate, que envolvem estresse nas ações de embarque, transporte até o abatedouro, desembarque, densidade de alojamento, além de período de descanso dos animais após o transporte, são comumente estressantes e exercem grande influência na qualidade da carne (GUARNIERI et al., 2002; 2004; DEVINE et al., 2006).

A melhoria do bem-estar animal pode afetar positivamente vários aspectos da qualidade do produto, como por exemplo, na redução da ocorrência de carnes PSE e *dark firm and dry* (DFD) (SAMS, 1999; OLIVO et al., 2001; GUARNIERI et al., 2004; PETRACCI et al., 2004; BIANCHI et al., 2007; SIMÖES et al., 2009), bem como na diminuição da incidência de quebra de ossos, contusões ósseas, redução do potencial e resistência a doenças, diminuindo o efeito imunossupressor do estresse crônico e a necessidade de antibióticos, claramente ligando a qualidade do bem-estar para outros aspectos da qualidade dos alimentos e segurança alimentar (HUGHES; CURTIS 1997; JONES 1997, 2001; FAURE et al., 2003).

O desejo dos consumidores em adquirir produtos cujo respeito aos animais é preconizado vem aumentando durante as últimas décadas (BOIVIN et al., 1998ab, RUSHEN et al., 1999), estes esperam que os animais, especialmente com fins alimentícios, passem a ser produzidos e processados com maior respeito ao seu bem-estar (HARPER; HENSON, 2001).

2.6 *PALE, SOFT AND EXUDATIVE* (PSE)

O termo PSE tem como significado de suas iniciais do inglês, *Pale, Soft e Exudative*, que em tradução, significam carnes com características pálidas ou amareladas, flácidas ou moles, exsudativas ou molhadas (OLIVO; SHIMOKOMAKI; FUKUSHIMA, 1998; SHIMOKOMAKI et al., 2006). Este fenômeno preocupa a indústria cárnea e, sobretudo, aquela que se dedica ao abate e ao processamento de carne de aves (DIRINCK et al., 1996). As carnes PSE têm se tornado um dos grandes problemas enfrentados pela indústria avícola, cujo cálculo dos prejuízos financeiros decorrentes da perda como consequência do PSE é difícil, devido a sua origem multifatorial. Porém, Owens (2009) estimou ser de US$ 200 milhões ao ano para as indústrias das aves nos Estados Unidos. Já no Brasil, estimou-se o prejuízo de US$ 50 a 55 milhões somente para frangos levando-se em consideração a perda de 1,0 a 1,50 % de água por carcaça (SPURIO et al., 2016).

As características de carnes PSE, foram primeiramente caracterizadas em suínos provenientes da manifestação da síndrome de *Porcine Stress Syndrome* (PSS) (CHEAH et al., 1984) ou Hipertermia Maligna (HM) (FUJII et al., 1991). A PSS é desencadeada por fatores de estresse ambientais e/ou fisiológicos, como mudanças na temperatura ambiente, excitação, transporte e exercícios, que podem ocasionar à morte inesperada dos animais (CHEAH et al., 1984).

Esta síndrome é uma miopatia hereditária e pode ser desencadeada por indução anestésica com halotano (HALL et al., 1966) e clorofórmio (HARRISON et al., 1969), ou com relaxantes musculares como succinilcolina. (MITCHELL; HEFFRON, 1982; FUJII et al., 1991). Os sintomas são manifestados por meio de rigidez do músculo, aumento progressivo e rápido da temperatura corpórea, taquicardia, taquipnéia, hiperventilação, acidose lática e elevados níveis de metabólitos no soro sanguíneo (SYBESMA; EIKELENBOON, 1969; JONES et al., 1972). Em suínos, nas carnes PSE durante a contração muscular, foi observada uma excessiva liberação de íons Ca^{2+} nas células, ocasionando um rápido metabolismo anaeróbico e rigidez do músculo (MITCHELL; HEFFRON, 1982; BERTOL, 2005). Essa relação nos suínos deve-se a uma mutação no gene que codifica a proteína rianodina (RYR1 - canal de passagem de cálcio). O receptor encontrado possui uma mutação, devido a substituição de um único aminoácido, Cisteína por Arginina (FUJII et al., 1991; MACLENNAN; PHILLIPS, 1992).

No tecido muscular o defeito no receptor rianodina causa a manutenção do canal aberto, permitindo um poderoso fluxo de cálcio ao sarcoplasma (MICKELSON; LOUIS, 1996). Devido o canal manter-se aberto, dificulta-se que o cálcio seja removido do sarcoplasma conduzindo assim, a contratura muscular, hipermetabolismo e hipertermia.

Em frangos de corte, devido a uma grande concentração de cálcio durante a formação de carne PSE, ocorre um aumento da atividade das proteases, que afeta a integridade da estrutura muscular, prejudicando a funcionalidade da proteína da carne de frango (WILHELM et al., 2010).

De acordo com Olivo et al. (2001), o desenvolvimento de carnes PSE é caracterizado por uma glicólise PM rápida, que acarreta um acelerado declínio do pH quando a temperatura do músculo ainda é relativamente alta, provocando a precipitação das proteínas sarcoplasmáticas e menor capacidade de retenção de água, em razão da desnaturação das proteínas miofibrilares.

Estudos realizados por Pedrão et al. (2014) mostraram certo retardamento da taxa de glicólise em peitos de frango, promovido por meio do tratamento de refrigeração (*chiller*). Os autores relataram que a formação de carne PSE pode ser induzida através do sistema de refrigeração do frigorífico.

Kijowski e Niewiarowicz (1978) e Olivo et al. (2001) sugeriram que o pH indicativo de PSE na carne de frango seria de 5,7 em15 minutos *post-mortem*, indicando que o *rigor mortis* é mais acelerado comparado aos de suínos, cujo pH final é atingido após 45 minutos.

O desenvolvimento de carnes PSE em aves apresenta várias semelhanças com a de suínos (SOLOMON et al., 1998; SOSNICKI et al., 1998), porém, ainda não está esclarecido se existe uma relação entre as linhagens de frango e a condição PSE.

De acordo com Le Bihan-Duval et al. (2003), os mecanismos fundamentais deste fenômeno ainda não foram bem elucidados em frangos. Estudos demonstraram que o teste do halotano em perus e frangos não foi eficiente para classificar aves como normais e susceptíveis ao PSE (MCKEE et al., 1998; WHEELER et al., 1999). Entretanto estudos realizados por Marchi et al. (2010) demostraram que o anestésico halotano mostrou ser um agente estressor, desencadeando alterações bioquímicas nos músculos dos frangos semelhantes às desencadeadas pelo estresse térmico pré-abate.

Carvalho et al. (2015) estudando diferentes tipos de instalações de frangos de corte, relataram que aves criadas em aviários *dark house* apresentam maior incidência de carne PSE, indicando que a criação das aves sob melhores condições climáticas, menor temperatura, tornam-nas mais susceptíveis ao estresse promovido pelo manejo pré-abate.

Os fatores que conduzem ao estresse e influenciam na qualidade da carne com aumento da incidência do fenômeno PSE, foram descritos como condições de manejo pré-abate a que são submetidas às aves, como jejum alimentar, apanha, transporte, temperatura e umidade relativa do ambiente (SAMS, 1999; OLIVO et al., 2001; GUARNIERI et al., 2004; OBA, et al., 2009; PETRACCI et al., 2009; SIMÕES et al., 2009; LANGER et al., 2010; BARBOSA et al., 2013; SPURIO et al., 2015).

Marchi et al. (2009) sugeriram que além de fatores genéticos, fatores ambientais podem predispor as aves ao desenvolvimento do PSE; destacando o estresse por calor durante o final da fase de crescimento ou período pré-abate (PETRACCI et al., 2004; BIANCHI et al., 2007; SIMÖES et al., 2009). Simões et al. (2009) concluíram que a aplicação do banho de água sobre os frangos após o carregamento no verão reduziu a ocorrência de filés PSE em cerca de 10%, devido à diminuição de temperatura no microambiente do caminhão.

Ferket e Qureshi (1992) afirmaram que aves susceptíveis ao estresse térmico apresentaram diminuição do peso corpóreo e dos níveis de anticorpos como tentativa de prevenção ao estresse no desenvolvimento de PSE. O desenvolvimento de PSE pôde ser controlada através do banho de aspersão de água, imediatamente antes das aves serem abatidas (GUARNIERI et al., 2004), por meio da adição de vitamina E (α-tocoferol) na dieta (OLIVO et al., 2001), e finalmente devido à inibição da atividade da enzima fosfolipase A_2 (PLA_2) (SOARES et al., 2003).

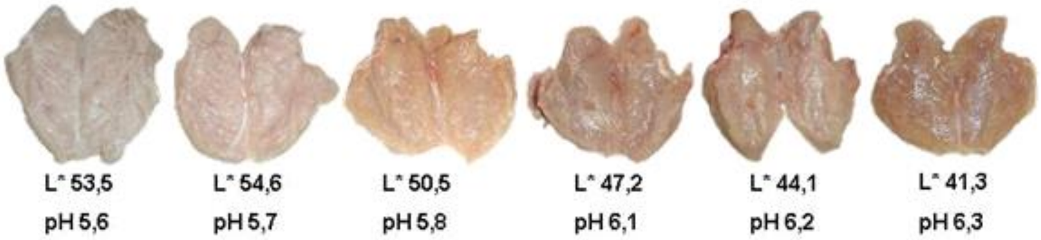
Segundo Soares et al. (2003), a ativação da PLA_2 em situação de estresse ocorreu em frangos, sugerindo que o aumento relativo dessa atividade no ambiente sarcoplasmático maior é devido a concentração de Ca^{2+} e seria o gatilho iniciador dos sintomas do PSE, sugerindo a possibilidade de que tal como nos suínos exista o Avian Stress Sindrome semelhantemente ao PSS.

De acordo com Barbut (1997), McCurdy et al. (1996) e Kissel et al. (2009), carnes PSE apresentaram comprometimento das suas propriedades funcionais, resultando em produtos industrializados defeituosos e em problemas tecnológicos como pouca emulsificação, força do gel enfraquecida, diminuição do rendimento, baixa coesividade e textura inadequada, que influenciaram diretamente na qualidade final do produto e em perdas econômicas industriais, além de obterem uma baixa aceitação pelos consumidores (LARA et al., 2002, DROVAL et al., 2012). Entretanto, Kissel et al. (2009) verificaram a possibilidade de utilizar a carne de frango PSE como uma fonte de matéria-prima na produção de mortadelas com aditivos; estas apresentaram valores de dureza e mastigabilidade significativamente maior do que os da mortadela processada com carne normal.

O desenvolvimento de carnes PSE foi prognosticado pela combinação de análises de pH, cor e capacidade de retenção de água no *Pectoralis major* de frangos (SWATLAND, 1995). A água extracelular e a estrutura proteica extremamente fechada provocam a reflexão da luz incidente; uma dispersão desta luz em uma superfície muscular é diretamente proporcional à sua quantidade de desnaturação proteica causada pelo baixo pH (ANADON, 2002). Le Bihan-Duval et al. (2003) constataram que desnaturação proteica interfere na aparência física da carne, que influencia na quantidade de luz que lhe é refletida.

Segundo Olivo et al. (2001), quanto maior o grau de desnaturação proteica, menor luminosidade é refletida nas fibras, consequentemente mais luz é dispersa, o que ocasiona palidez à carne (Figura 9).

Figura 9 – A coloração na superfície da carne indica que o aumento de palidez está diretamente relacionado com a desnaturação das proteínas, ocasionada pela redução do pH.



Fonte: (ODA et al., 2003).

Pesquisadores têm proposto a utilização de valores de Luminosidade (L*) (sistema CIELAB ou Hunter) para classificação de carnes de aves em PSE e Normal. Barbut (1997) sugeriu para carnes de frango PSE valor de L* ≥ 49,00, enquanto Soares et al. (2002) classificaram como PSE, valor de L* ≥ 53,00 e Normal, valores de 44,00 ≤ L * ≤ 53,00.

Lara et al. (2002) estabeleceram que o fenômeno PSE em frangos possa ser detectado pela combinação dos valores de pH (abaixo de 5,80) e cor (valor L* > 52,00) mensurados 24 horas após o abate. Esta classificação pode ser muito útil e aplicada com facilidade na planta de abate pelos frigoríficos como um indicador das propriedades funcionais da carne que possibilita o adequado emprego carnes nas linhas de processamento. A metodologia para detecção de carnes PSE em perus é objeto de grande debate na comunidade científica; somente uma simples mensuração de cor (L*) não é uma forma de detecção aceitável de predição (CHAN et al., 2011; MOLETTE et al., 2008). Por outro lado, vários autores evidenciaram que o sistema de cor é uma importante ferramenta e indicadora de carnes PSE de frangos (BARBUT, 1993, 1996; OWENS et al., 2000; ZIOBER et al., 2010; CARVALHO et al., 2014). Outros autores também relatam que cada planta de processamento deve apresentar um valor de corte (L*) para carnes PSE (BARBUT, 1996).

Owens et al. (2000) em seu estudo sobre incidência de carnes PSE em perus, usando os paramentos L*, a*, b*, pH e CRA (capacidade de retenção de água), encontraram um valor de L* entre 41 e 63, e indicaram que o valor de L* é o melhor indicativo de carnes PSE, sugerindo que carnes PSE possui L* > 53,00.

De acordo com Barbut (1998), o parâmetro L* ≥ 50/51 identifica corretamente a carne de peru PSE, cuja classificação de cor está correlacionada significativamente com CRA. A classificação colorimétrica mostrou ser um método não destrutivo e não invasivo, quando comparado com a medição de valores de pH. Fraqueza et al. (2006) estudaram carnes PSE em carne de peru e relataram uma associação entre os valores de pH e L* 24 *post-mortem*. Tais autores propõem como classificação os valores: 44,00 ≤ L* ≤ 50,00 para carnes normais e L* > 50,00 para carnes PSE. A Tabela 1 mostra os valores comparativos de PSE de peru obtidos em diferentes países.

Tabela 1 – Valores de L* para classificação de carnes PSE em perus e incidência em diferentes países.

	Barbut (1998)	Owens et al. (2000)	Fraqueza et al. (2006)	Carvalho et al. (2014)
País	Canadá	USA	Portugal	Brasil
Frequência (n°. de aves)	4000	2995	977	2610
Variação de L*	38 até 57	41 até 63	35 até 55	42 até 66
L* de corte	50/51	53	50	53
Incidência %	12	40	8	41

Fonte: (CARVALHO et al., 2014)

A incidência de carnes PSE em perus relatada por Carvalho et al. (2014) é superior às encontradas no Canadá (BARBUT, 1998) e Portugal (FRAQUEZA et al., 2006), entretanto são semelhantes à obtida nos EUA por Owens et al. (2000). A elevada incidência de carnes PSE observada no Brasil é proporcionada pelos efeitos do clima tropical de verão, associando alta temperatura e umidade relativa do ar (SIMÕES et al., 2009, SPURIO et al., 2015).

A carne PSE começou a ganhar certo interesse para a pesquisa industrial nas últimas décadas, em que vários trabalhos indicaram que a ocorrência do problema pode variar de 5 a 30%, dependendo do lote, época do ano e fatores associados com o transporte (LESIÓW; KIJOWSKI, 2003; OWENS et al., 2000; SIMÕES et al., 2009).

Estudos realizados com perus no verão brasileiro relataram incidência de 41% de carnes PSE. Os autores propuseram valor de corte ($L^* \geq 53,00$) para carnes PSE na determinada planta de processamento, em comparação com peito de peru normal, o peito PSE *in natura* apresentou maior valor de L* (4,74 un), menor pH (0,12 un) e menor capacidade de retenção de água (5,47 %). Em relação ao produto final (peito de peru defumado), produzidos com carnes PSE e normal, o peito de peru defumado PSE apresentou menor rendimento (2,12 %), menor pH (0,11 unidades), maior valor de L* (5,93 unidades), maior força de cisalhamento (3,86 N) e menor umidade (0,55 %) (CARVALHO et al., 2014).

De acordo com Carvalho et al. (2014), o prejuízo da indústria brasileira com carnes PSE de peru pode chegar em aproximadamente U$ 5.1 milhões anuais, sendo assim, necessárias a introdução de práticas para melhorar o bem-estar animal e evitar condições estressantes para as aves.

2.7 *DARK*, *FIRM AND DRY* (DFD)

Outra anomalia com grande importância para a indústria avícola é denominada carne DFD, tal carne possui coloração escura, textura firme e aparência seca, características que são relacionadas com alto valor final do pH e maior funcionalidade das proteínas, resultando em maior capacidade de retenção de água (CRA), sendo assim proporciona uma textura firme e superfície aparentemente seca (OWENS; SAMS, 2000; BARBUT et al., 2005).

A alta CRA e pH da carne DFD traz como aspecto negativo a susceptibilidade à contaminação microbiana e acarreta em menor vida útil (ALLEN et al., 1998). Além disso, a maior CRA resultante das carnes DFD promove menor reflexão de luz da superfície, e a cor da carne é substancialmente mais escura (BARBUT et al., 2005; SWATLAND, 1994).

Carne DFD em peito de aves foi investigada para frangos de corte (BARBUT, 1997, QIAO et al., 2001; WOELFEL et al., 2002) e perus (ZHANG; BARBUT, 2005). Do ponto de vista morfológico a carne DFD apresenta fibras musculares dispostas de maneira adensada e compacta em comparação com carne normal (BARBUT et al., 2005). Em relação ao ponto de corte ou "*cut off*" para pH final de carne com características DFD, vários autores sugerem valores de 6,1 ou superior (BARBUT, 1997, 1998; BERRI et al., 2001, QIAO et al., 2001; WOELFEL et al., 2002; PETRACCI et al., 2004; BARBUT et al., 2005).

No entanto, ponto de corte para luminosidade (L*) utilizado para a classificação DFD foi divergente nos diferentes estudos acima mencionados, Petrucci et al.

(2004) para frangos de corte sugeriu L* < 50, já outros autores recomendam L* < 46 (BARBUT 1997; QIAO et al., 2001; WOELFEL et al., 2002; BARBUT et al., 2005). Barbut (1998) e Petracci et al. (2004) relataram que os valores de corte devem ser estabelecidos para cada indústria processadora e baseados nas características da carne encontrada.

Estudos sugerem que a carne DFD está relacionada com estresse em longo prazo antes do abate, tal efeito faz com que a depleção do glicogênio muscular proporcione maior pH *post-mortem* (OWENS; SAMS, 2000; DADGAR et al., 2012). Muitos fatores afetam a incidência de carnes DFD, incluindo estresse durante o transporte (LESIOW et al., 2007; WARRIS et al., 1999), jejum (KOTULA; WANG, 1994), estresse climático, especialmente o estresse pelo frio (NICOL E SAVILLE-WEEKS, 1993; WEBSTER et al., 1993), descanso pré-abate (tempo de espera) (WARRIS et al., 1999), e comportamento agressivo poderiam contribuir para a depleção de glicogênio muscular e resultar em menor quantidade de lactato e maior pH *post-mortem.*

A incidência de carne DFD em aves tem sido estudada por Lesiow et al. (2007) e Petracci et al. (2004). De acordo com Petracci et al. (2004), carne de peito com coloração mais escura são encontradas no inverno em comparação com o verão. Lesiow et al. (2007) confirmaram que o transporte durante o inverno aumentou significativamente a incidência de carne DFD em frango de corte.

Dadgar et al. (2011) relataram que frangos de corte expostos a condições de frio antes do abate apresentaram menor temperatura corporal e menores níveis de glicose sanguínea. A incidência de carne DFD em peito de frango aumentou mais de 50% quando as aves foram expostas as condições de frio (< -11 °C) comparado com aves expostas a 20 °C.

Dadgar et al. 2010 relataram em peito de frango que o maior pH final, coloração mais escura, maior CRA e maior rendimento pós processamento são encontradas em carne de aves transportadas em temperaturas abaixo de 0 °C em comparação com aves transportadas a temperaturas mais amenas (acima de 0 °C).

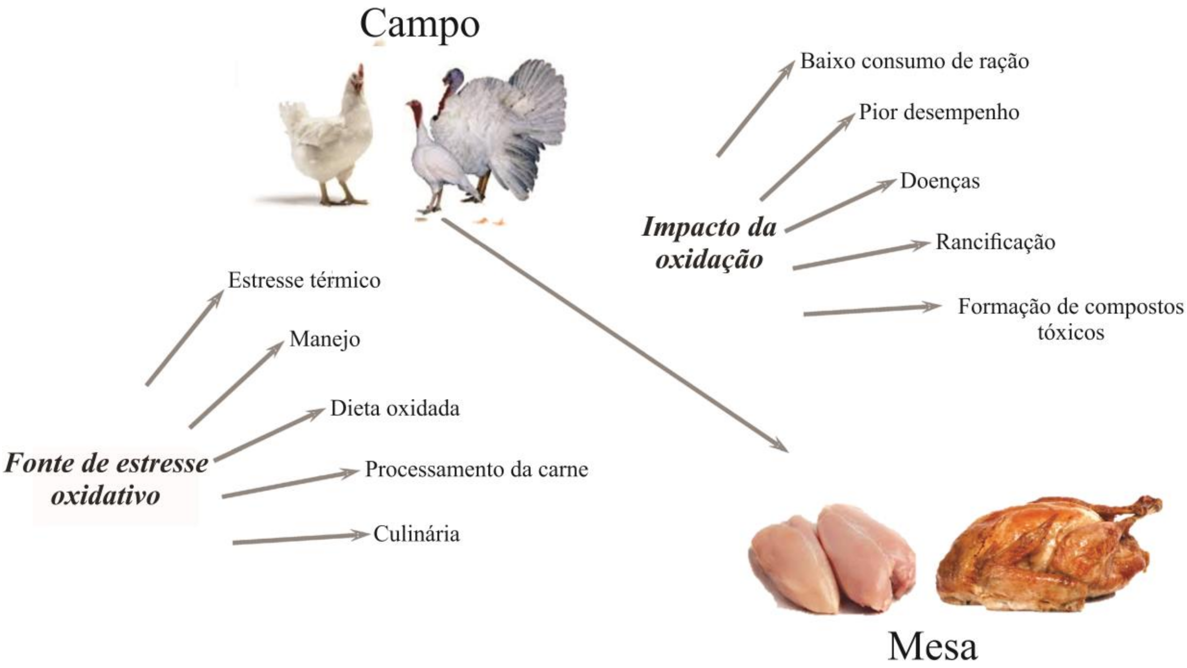
As características da carne DFD em peito já são estabelecidas, no entanto, as causas da anomalia na indústria avícola não são claras, pois o processo é multifatorial. É importante compreender os desafios para diminuir a incidência de DFD na indústria avícola, especialmente na temporada de inverno e encontrar soluções para uso correto da carne (DADGAR et al., 2010).

2.8 Estresse Oxidativo em Carne de Aves

Reações oxidativas são conhecidas por acontecer bioquimicamente em diversos processos e funções celulares. O desequilíbrio homeostásico endógeno entre pró-oxidantes e antioxidantes nos tecidos *in vivo* acarreta o dano oxidativo celular, conhecido como estresse oxidativo (KOHEN; NYSKA, 2002).

Segundo Estevez (2015) as aves estão susceptíveis a reações oxidativas durante todas as fases da cadeia, desde a produção até o consumo da carne. A natureza e extensão das consequências oxidativas dependerá: 1) fase ou estágio entre o "campo e a mesa" em que o dano oxidativo é causado e 2) os componentes alimentares que são afetados pelas reações de oxidação (Figura 10).

Figura 10 – Danos do estresse oxidativo em aves: fontes de estresse oxidativo e impacto da oxidação.



Fonte: adaptado de Estevez (2015).

A oxidação é tipicamente iniciada por espécies reativas de oxigênio do "*reactive oxygen species'* (ROS), que são produzidas durante o metabolismo celular (CADENAS; DAVIES, 2000). As aves domésticas tais como, frango de corte e peru são suscetíveis ao estresse oxidativo, provavelmente devido ao resultado da massiva seleção genética para maiores músculos de peito, aumento da massa corporal e rápidas taxas de crescimento (FELLENBERG; SPEISKY, 2006; SIHVO et al., 2013).

A formação de ROS é um resultado esperado devido ao metabolismo aeróbico ocasiona danos oxidativos aos lipídios e proteínas musculares. A principal fonte de ROS em músculos de frango de corte é a troca de elétrons da cadeia respiratória nas mitocôndrias durante a redução do oxigênio molecular à água (MUJAHID et al., 2007).

O estresse oxidativo traz consequências negativas às aves *in vivo* e posteriormente em sua carne. Recentes trabalhos indicam que o calor pré-abate e dieta são os meios mais relevantes de estresse oxidativo e que podem levar a danos biológicos, distúrbios graves de saúde, taxas de crescimento mais baixas e perdas econômicas (FELLENBERG; SPEISKY, 2006; ZHANG et al., 2011; SIHVO et al., 2013).

Altan et al. (2003); Wang et al. (2009) e Ismail et al. (2013), relataram que a indução de estresse pré-abate em frangos de corte por exposição ao calor induz os músculos a oxidação de lipídios (LOX), oxidação de proteínas (PROTOX) e diversos distúrbios fisiológicos. Além das enfermidades, a oxidação tem sido associada com vários distúrbios metabólicos e fisiológicos *in vivo* comprometendo seriamente a vida da ave, tais como insuficiência cardíaca (FATHI et al., 2011), e também ao impacto na qualidade de carne como: defeito de coloração rósea (HOLOWNIA et al., 2003), peito amadeirado "*wooden breast*" (SIHVO et al., 2013), e peito estriado "*white striping*" (KUTTAPPAN et al., 2013).

Após o abate, os mecanismos antioxidantes gradativamente entram em colapso, enquanto as alterações bioquímicas que ocorrem durante a conversão do músculo em carne favorecem a oxidação (MIN; AHN, 2005; ESTEVEZ, 2011). O declínio do pH facilita a oxidação de componentes musculares, pois substâncias como H^+ favorecem o ciclo redox de mioglobina e também sua ação pró-oxidante (ESTEVEZ, 2011), também atuando na liberação de ferro livre-catalítico de oxidação e no sistema de enzimas antioxidantes (catalase, glutationa peroxidase e sódio dismutase). A ocorrência LOX e PROTOX *post-mortem* também foi encontrada durante armazenamento carne de frango (ZHANG et al., 2011), peru (CHAN et al., 2011), ema (FILGUEIRAS et al., 2010) e avestruz (LEYGONIE et al., 2012).

As consequências de LOX e PROTOX *postmortem são* altamente dependentes da origem da carne, tipo do musculo, espécies e as condições de armazenamento (ESTEVEZ, 2011). O elevado grau de instauração dos lipídios em carne de aves tem sido associado à alta susceptibilidade desta carne em sofrer intensa LOX, mas outros fatores tais como enzimas endógenas e teor de ferro também podem ser influentes (MIN et al., 2008; UTRERA; ESTEVEZ, 2013).

Utrera et al. (2014) relataram que quando animais são expostos a um intenso ambiente pró-oxidante, tal como ao estresse pré-abate pelo calor, a carne mostra-se maior susceptibilidade à LOX. Elevadas taxas de LOX afetam significativamente as proteínas e que por sua vez apresentam maior carbonilação e formação de ligações cruzadas. Estas alterações bioquímicas foram responsáveis pela deterioração de várias características de qualidade, incluindo perda da capacidade de retenção de água (CRA) e aumento de dureza (ESTEVEZ et al., 2015).

Os efeitos da LOX sobre a qualidade da carne fresca têm sido amplamente estudados nas últimas décadas, entretanto PROTOX tem sido gradualmente reconhecida como um importante fator de deterioração da qualidade em carne fresca de aves (ESTEVEZ, 2015). PROTOX durante a maturação e armazenagem de carne de aves tem sido ligado à perda de funcionalidades, incluindo CRA (ZHANG et al., 2011; CHAN et al., 2011; FILGUEIRAS et al., 2010; LEYGONIE et al., 2012). Além disso, o impacto de PROTOX na funcionalidade das proteínas afeta a aptidão da carne para ser submetida a processos tecnológicos de processamento, onde tais propriedades funcionais como: emulsificação, CRA, formação de gel, entre outros são prejudicadas (XIONG, 2000; ESTEVEZ, 2011).

Mais recentemente, tem sido estudado o envolvimento de estresse por calor como um indutor de estresse oxidativo (ALTAN et al., 2003; SAHIN et al., 2003; MUJAHID et al., 2005; LIN et al., 2006). O estresse oxidativo é caracterizado pela presença de espécies reativas a oxidação em excesso comparada com a capacidade antioxidante disponível nas células animais (HALLIWELL; WHITEMAN, 2004). Estas substâncias são altamente reativas e podem modificar biologicamente macromoléculas, tais como proteínas, lipídios e ácidos nucléicos (DAVIES, 1995).

Moléculas oxidadas são reativas com outras moléculas resultando em reação de cadeia e quando não controlada, pode gerar grandes danos aos tecidos. Ademais, o estresse oxidativo pode alterar o equilíbrio redox celular como as enzimas catalase (CAT), glutationa peroxidase (GSH) e sódio dismutase (SOD) acarretando alterações na defesa antioxidante, transições de células, respostas inflamatórias dos organismos (ESTEVEZ, 2015).

A inativação enzimática de ROS no tecido muscular é alcançada principalmente pela atuação das SOD, CAT e GSH. A SOD e CAT são enzimas antioxidantes reagindo diretamente com espécies de radicais, enquanto GSH regenera antioxidantes oxidados (LIU et al., 2014).

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3 EXPERIMENTO A (Publicado)

LWT - Food Science and Technology 59 (2014) 883–888

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

The incidence of pale, soft, and exudative (PSE) turkey meat at a Brazilian commercial plant and the functional properties in its meat product

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The incidence of pale, soft, and exudative (PSE) turkey meat at a Brazilian commercial plant and the functional properties in its meat product

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ABSTRACT

The objective of this study was to report the incidence of pale, soft, and exudative (PSE) turkey breast meat at a commercial plant in southern Brazil and the consequences on the quality of meat processing. This study was subdivided into 2 experiments: I) incidence of PSE meat and II) effects on meat processing. Experiment I: A total of 2610 breast filets were collected, and pH and color were tested. A value of 53.0 was determined using a Minolta lightness test (L*) as the cutoff value for PSE meat characterization. The incidence of PSE meat was 41.7%. Experiment II: A total of 16 filets *in natura*, 8 PSE and 8 normal samples, were analyzed for pH, color, and water holding capacity (WHC); they were processed into smoked products and yield, color, pH, texture, and moisture were evaluated. Functional properties were compared between PSE and normal meat samples; a 5.5% lower WHC was identified as the consequence of color abnormality. Finally, these PSE meat samples were processed into smoked meat, and the product was 31.3% harder than processed normal meat. The results showed approximately 5%–10% weight loss in PSE meat, with a risk of serious some economic losses for the poultry industry.

Key words: Functional meat properties, Meat quality, Turkey, Smoked meat product

Abbreviations

PSE: pale, soft, and exudative;

RH: relative humidity;

WHC: water holding capacity; .

W_i: initial weight;

W_f: final weight;

SF: shear force.

1. Introduction

In recent decades, the production and consumption of poultry meat have rapidly increased worldwide. North America and Europe have gradually lost their market share, while China and Brazil have become new centers of production (Windhorst, 2006). Brazil is the third leading producer of turkey meat in the world, with a total production of 363 million tons (MT); it follows the USA and EU, with 2,623 and 1,985 MT, respectively (USDA, 2014). These figures demonstrate the potential importance of this type of meat for the Brazilian economy. Color abnormalities can occur during processing, with meat appearing pale in color, soft, and wet on the surface because of poor water holding capacity (WHC) (Barbut et al., 2008). This functional defect results from protein denaturation caused by rapid postmortem muscle glycolysis, which lowers pH while the carcass is still warm (Barbut et al., 2008; Olivo, Soares, Ida, & Shimokomaki, 2001). Most frequently used in reference to pork, the incidence of PSE meat is being observed with increasing attention at turkey and broiler processing plants. It has been estimated that this problem affects approximately 5%–40% of the total turkey meat that is produced by the poultry industry, representing a loss in excess of US$200 million per annum (Owens, Hirschler, McKee, Martinez-Dawson, & Sams, 2000). Nevertheless, the incidence of PSE turkey meat in Brazil has not been extensively evaluated till date. Thus, the objective of this study was to describe the incidence of PSE turkey breast meat under summer season conditions at a commercial processing plant and its consequences on the meat products.

2. Materials and methods

2.1. Experiment I

2.1.1. Incidence of PSE meat

A total of 2610 (50 ± 10 per day of sampling) breast meat samples were obtained (turkeys of BUT-9 lineage, 140 days of age) from a commercial processing plant in the Brazilian southern region during the summer in 2012 and 2013. The weather conditions in this region are characterized by a minimum and maximum temperature of 24 and 36 °C, respectively. The relative humidity (RH) value was 46% to 68% throughout the 45 sample collection days as measured using a Kestrel 4000 instrument (Nielsen-Kellerman, Boothwyn, PA, USA). The birds were grown on 45 turkey farms with a distance of 20 ± 5 km away from the slaughter plant in a cooperative integrated system, with lairage time of 80 ± 10 min. Feed withdrawal took place 9:00 ± 0:30 h before slaughter, according to the standard industry practice, consisting of the sequence of hanging, electrically stunning, bleeding, scalding, defeathering, evisceration, cooling the carcass through a tunnel of cold air (mean–6 C for 6 h), deboning, and taking off the breast samples *(pectoralis major).* The samples were collected randomly and refrigerated at 4 C for 24 h for subsequent color and pH analyses.

2.1.2. Color and pH measurements in fresh breast meat

We measured pH by inserting electrodes into the ventral cranial part of the filet using a contact pH meter system (Testo 205, Lenzkirch, Germany). Analyses were performed in triplicate 24 h postmortem, as reported previously by Olivo, Soares, Ida, and Shimokomaki (2001) for chicken. A Minolta CR400 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) was used to evaluate the color and lightness (L*) at the dorsal surface of the intact skinless breast muscles. The L* values were measured at 3 sites on the same sample: the proximal extremity of the muscle, the distal extremity, and the medial side halfway between the proximal and the distal extremity (Soares et al., 2003).

2.2. Experiment II

2.2.1. Meat processing

A total of 16 breast filet meat samples (2700 ± 100 g) were divided into 2 treatment groups (n = 8 each) and classified as PSE (L* > 53.0) and normal (L* ≤ 53.0), as reported by Owens, Hirschler, McKee, Martinez-Dawson, and Sams (2000). Color, pH, and WHC values were determined in fresh samples before the processing of smoked meat. Each filet sample was processed separately by adding ingredients, following the steps of grinding, tumbling with the ingredients listed in Table 1, stuffing, smoking, vacuum packing, and pasteurizing for the production of smoked products (Fig. 1).

2.2.2. Color and pH measurements in the smoked product

These assays were similar to the ones described in section 2.1.2. Analysis of pH values was performed in triplicate, whereas for L* measurement, 5 pieces (3 cm thick) from the same sample were analyzed at 5 sites, 25 measurements total.

2.2.3. WHC of fresh breast meat

This measurement was carried out based on the technique of Hamm (1960), as described in Wilhelm, Maganhini, Hernández-Blazquez, Ida, and Shimokomaki (2010). After 24 h postmortem, samples were collected from the cranial side of the breast filets and cut into cubes 2.0 ± 0.10 g. A total of 16 samples were analyzed in triplicate. They were first carefully placed between 2 pieces of filter paper on acrylic plates and then left under a 10-kg weight for 5 min. The samples were weighed and WHC was determined using the weight of exudate water and the following equation: $100 - [(W_i - W_f/W_i) \times 100]$, where W_i and W_f are the initial and final sample weight, respectively.

2.2.4. Texture measurement in the smoked product

The smoked meat products were cut into rectangular pieces (*n* = 5) measuring 1.50 cm high × 1.00 cm wide × 2.00 cm long. Shear force (SF) was measured using the texturometer Mycro Stable Systems TA-XT2i (Stable Micro Systems, Godalming, Surrey, United Kingdom) coupled with Warner Bratzler lamina, and the results were expressed in Newtons (N). The test conditions were as follows: pretest speed 5.0 mm/s, test speed 10.0 mm/s, posttest speed 10.0 mm/s, and distance 20 mm as described previously (Wilhelm, Maganhini, Hernández-Blazquez, Ida, & Shimokomaki, 2010).

2.2.5. Moisture measurement in the smoked product

The gravimetric method was used in a forced-circulation oven at the temperature of

105 C for 6 h until stabilization of weight, and the results were calculated using the mass difference according to AOAC (1997).

2.2.6. Statistical analysis

The Statistica software for Windows 7.0 (StatSoft, Tulsa, USA) was used. Student's *t* test at 5% probability ($P < 0.05$) was used for comparing the differences between the 2 treatments of PSE and normal meat samples, and Pearson's correlation coefficient to assess the correlation of the pH and L* values.

3. Results

3.1. Experiment I: PSE meat incidence

Color variation of fresh turkey breast meat is shown in Fig. 2. The L* values varied from 42.28 (dark) to 65.73 (pale), with the average value being 52.56. The pH variation is shown in Fig. 3: pH varied from 5.38 to 6.07, with an average value of 5.72. There was a significant ($P < 0.05$) negative Pearson correlation (Fig. 4) between the pH and L* values, with a moderate value of the coefficient (–0.47).

3.2. Experiment II: Meat processing

Table 2 shows that fresh samples classified as PSE meat had lower pH (5.61) and a higher L* value (55.18) compared to normal fillets: pH of 5.73 and L* 50.44. The PSE meat samples had 5.5% lower WHC values.

Table 3 shows characteristics of the smoked PSE meat samples. The final yield was

2.1% lower compared to the normal samples, and the pH and L* values were 6.30 and 73.97, respectively, which were significantly different (P < 0.05) from the normal sample values of 6.41 and 68.04, respectively. As for SF and moisture, smoked meat prepared from the PSE meat samples showed average values of 12.33 N and 75.61%, respectively. These values differed (P < 0.05) from those of the normal samples: 8.47 N and 76.16%, respectively.

4. Discussion

4.1. Experiment I

Incidence of PSE meat

The methodology for detection of PSE meat has been a subject of debate because a single measurement of color, according to some reports, is not sufficient for accurate detection of this condition (Chan, Omana, & Betti, 2011; Molette, Remignon, Babile, & Fernandez, 2008). On the other hand, several authors have provided evidence that a system of color measurement tests an important attribute of meat quality, and it is often used as an indicator of PSE meat (Barbut, 1993; Barbut, 1996; Kauffman et al., 1993, Ziober et al., 2010). Some of those authors have also suggested that for more reliable confirmation of PSE meat, the cutoff value should be determined by each research lab or commercial plant (Barbut, 1996).

Owens, Hirschler, McKee, Martinez-Dawson, and Sams (2000) in their study on the incidence of PSE turkey meat using parameters such as L*, a*, b*, pH, and WHC, found that L* values range from 41 to 63. Moreover, the values of L* were the best predictor of the PSE status.

According to Barbut (1998), the L* parameter should be 50/51 to properly identify

PSE turkey meat; this classification by color correlates significantly with WHC. This colorimetric classification is claimed to be a nondestructive and noninvasive method compared with measurement of pH values. Such color evaluation provides an opportunity to quickly detect PSE meat after the cutoff level is established. Fraqueza, Cardoso, Ferreira, and Barreto (2006) studied PSE status in turkey meat and reported an association between the $pH_{24\ h}$ and L* values. Those authors propose, as a classification criterion, values of $44.00 \leq L^* \leq 50.00$ for normal meat and $L^* > 50.00$ for PSE meat.

Table 4 shows comparative values of PSE and normal turkey meat obtained in different countries in relation to the results of the present work. The data on L* values reported herein are higher than those reported in Canada (Barbut, 1998) and Portugal (Fraqueza, Cardoso, Ferreira, & Barreto, 2006), as shown in Table 4. These results, however, are similar to those obtained in the USA by Owens, Hirschler, McKee, Martinez-Dawson, and Sams (2000). Therefore, using Fig. 2 and Table 4, we determined a convenient cutoff value for detection of PSE meat in turkey breast filets under the conditions of our experiment: $L^* \geq 53.0$.

The high incidence of PSE meat is most likely the result of combined effects of a high temperature and RH, as reported previously for broilers (Langer et al., 2010; Oba et al., 2009; Simões et al., 2009). These conditions are common for the summer season in Brazil's southern region. McCurdy, Barbut, and Quinton (1996) showed that during the Canadian summer months, the incidence of PSE meat is higher than in the winter season. Heat stress in poultry has been studied extensively, and in general, studies have shown that heavier birds are more susceptible to the deleterious effects of higher temperatures and RH (Lu, Wen, & Zhang, 2007; Mills, Mitchell, & Mahon, 1999; Simões et al., 2009). At high temperatures, evaporative cooling is the primary mechanism for the birds to alleviate thermal stress. On the other hand, with the combination of high RH and heat, evaporative cooling is impaired; this situation makes it harder for the birds' bodies to dissipate heat (Yahav, Goldfield, Plavnik, & Hurwitz, 1995). McCurdy, Barbut, and Quinton (1996) evaluated seasonal effects on the incidence of PSE meat in turkey processing and reported highest L* values in the summer season and lowest values in the winter. Furthermore, paleness of turkey meat is associated with lower muscle pH and lower WHC (Barbut, 1993, Barbut, 1996; McCurdy, Barbut, & Quinton, 1996; McKee, & Sams, 1997; McKee, & Sams, 1998; Rathgeber, Boles, & Shand, 1999). Nevertheless, Barbut (1993); McCurdy, Barbut, and Quinton (1996); and Owens, Hirschler, McKee, Martinez-Dawson, and Sams (2000) used only CIELAB color measurement to classify turkey meat as PSE with a cutoff system. All those authors reported that the L* value is significantly affected by muscle pH. Barbut (1993) reported the pH range of 5.68 to 6.03 and observed a significant negative correlation between these values and the values of L*, thereby showing that turkey meat with higher L* shows lower pH values. These results corroborate those reported herein, but the negative correlation observed (–0.47) is insufficient and does not allow technicians to use pH for classifying meat samples as PSE. Besides, WHC of turkey breasts classified as PSE is 5.5% lower (Table 2) than that in samples classified as normal, in line with the report by Froning, Babji, and Mather (1978) who reported that meat with lower pH shows a lowered WHC value. Meat samples with lower WHC tend to exhibit exuding properties.

The turkey breast samples classified as PSE using the L* > 53 cutoff show pH and WHC values lower than those in normal samples, in agreement with existent literature on PSE meat classification (Barbut, 1998; Fraqueza, Cardoso, Ferreira, & Barreto, 2006; Owens, Hirschler, McKee, Martinez-Dawson, & Sams, 2000).

4.2. Experiment II

Meat processing

The results shown in Table 3 demonstrate that functional meat properties are affected under the fresh PSE meat development condition because the WHC content values (Table 2) are lower in these samples. In addition, the ingredients (Table 1) added to raw materials (Table 2) are different in absolute values, but maintain their relative proportion in relation to pH, L*, and WHC values in comparison to the smoked product (Table 3). Daigle et al. (2005) reported that the addition of ingredients, in particular, turkey collagen, soy protein concentrate, or carrageenan, improves some of the functional properties of turkey chunks and formed deli rolls. Nonetheless, the ingredients in our present experiments are not capable of changing functional properties of the product; although a larger amount of vegetable protein was added in relation to the previous report by Daigle et al. (2005), we did not observe a lower product yield using PSE meat (Table 3). This result is probably the consequence of lower values of pH and moisture (Table 3) due to lower pH and WHC of raw materials (Table 2).

Obviously, there was a difference between the raw material and the processed product because the smoked product prepared from PSE meat shows lower pH and significantly higher L* compared to the product prepared from the control meat samples. These differences suggest that the product's color is determined directly by the conditions of the raw material, whereas additional ingredients do not contribute to improvement of these parameters.

Finally, PSE status also influences SF because the processed product made of PSE meat shows 31.3% higher toughness compared to the normal samples. This effect is probably the consequence of more shrinkage and less moisture in the PSE meat samples. Not surprisingly, its yield is 2.1% lower because functional properties of the meat proteins are affected.

One of the consequences of thermal stress during the poultry-related industrial procedures is the economic losses due to the increase in percentage of PSE meat. The results reported herein lead us to hypothesize high costs of this problem, although further testing is needed. Brazilian production of turkey meat in 2013 was 363 MT (USDA, 2014). With PSE meat claiming the 41% share in the summer season and the average loss of 5.5% in WHC of PSE meat (Table 2) and taking into consideration the average price of US$2.50 per kilogram, a rough estimate of the economic loss is approximately US$5.1 million.

5. Conclusion

We analyzed the incidence of PSE turkey meat in a southern region of Brazil. The incidence is high in the summer, at approximately 41%, and this meat has lower quality before and after processing. The results show an approximate 5–10% weight loss in PSE turkey meat samples, raising the prospect of economic losses for the poultry meat industry. The first priority is to introduce management tools to maintain animal welfare and thus prevent stressful conditions for the birds.

6. Acknowledgements

This project was funded by CNPq Proc. 471609/2011-0. FGP holds a postdoctoral scholarship from CAPES Proc. 23038007638201119 MRP. EII and MS are CNPq Research Fellows, and MS is also a Brazilian Senior Visiting Professor Scholar from CAPES.

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Figure captions

Fig. 1. A flowchart of production of smoked turkey breasts.

Fig. 2. A histogram showing the distribution of L* values in turkey breast filets (*n* = 2610).

Fig. 3. A histogram showing the distribution of pH in turkey breast filets (*n* = 2610).

Fig. 4. The relationship between L* and pH values in turkey breast filets (*n* = 2610) (Regression line: pH=6.392 – 0.0127 * L*, $p<0.05$).

Table 1. Ingredients used for preparing smoked turkey breast from PSE and normal meat products.

Ingredients	%
Water	17.50
Preservative	0.08
Flavoring	0.13
Spices	0.60
Sodium Nitrite	0.02
Colorants	0.01
Starch	6.14
Either PSE or normal ground turkey fillet meat	72.07
Vegetable protein	1.63
Salt	1.82

Table 2. pH, L* and WHC determination of turkey breast fillet meat from PSE and normal meat from a commercial processing plant 24 h *post-mortem*.

	Fresh meat samples	
	Normal (n=8)	PSE (n=8)
pH	$5.73^{a} \pm 0.02$	$5.61^{b} \pm 0.01$
L*	$50.44^{b} \pm 1.49$	$55.18^{a} \pm 0.82$
WHC %	$79.73^{a} \pm 1.14$	$74.16^{b} \pm 1.16$

Means followed by different letters on the same row differ by a t-test at the 5% significance level ($P<0.05$).

WHC-Water Holding Capacity

Table 3. Values of pH, L*, WHC, yield, SF and moisture of smoked turkey processed with either PSE or normal meat.

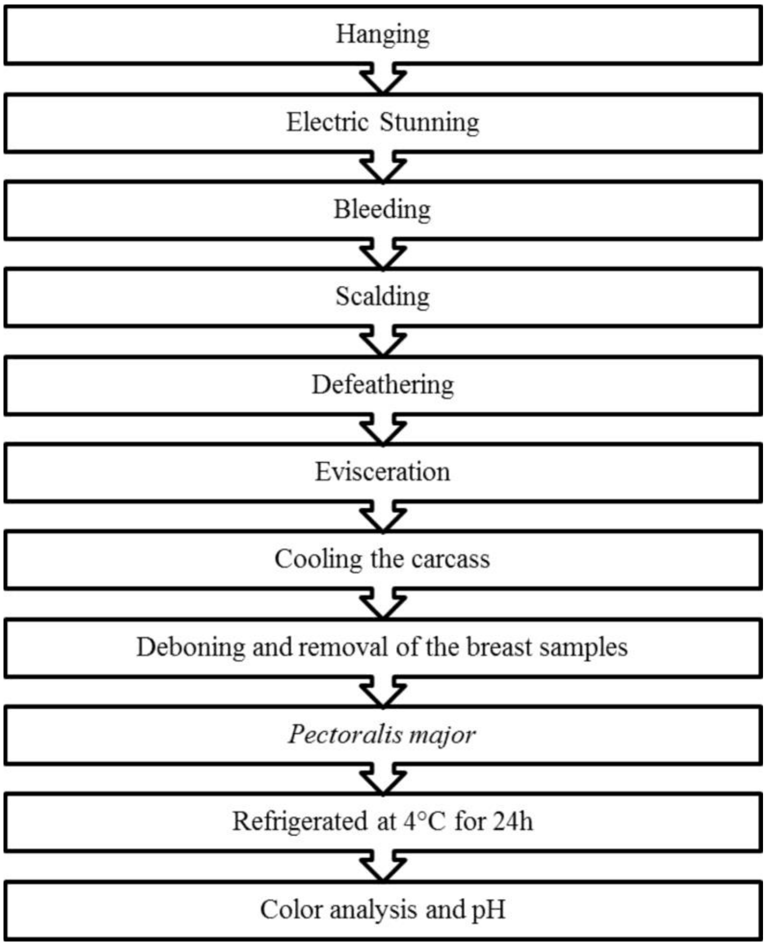
	Smoked product	
	Normal (n=8)	PSE (n=8)
Yield (%)	$87.59^{a} \pm 0.45$	$85.47^{b} \pm 0.39$
pH	$6.41^{a} \pm 0.01$	$6.30^{b} \pm 0.02$
L*	$68.04^{b} \pm 0.95$	$73.97^{a} \pm 0.51$
SF (N)	$8.47^{b} \pm 0.50$	$12.33^{a} \pm 1.09$
Moisture (%)	$76.16^{a} \pm 0.46$	$75.61^{b} \pm 0.45$

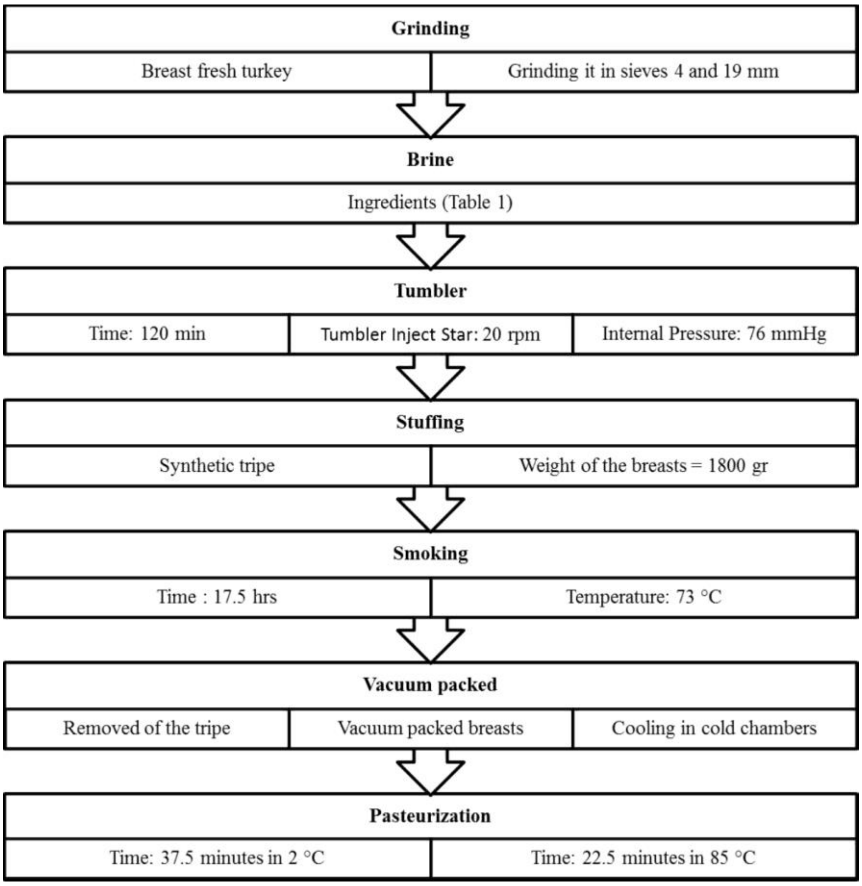
Means followed by different letters on the same row differ by a t-test at the 5% significance level ($P<0.05$).

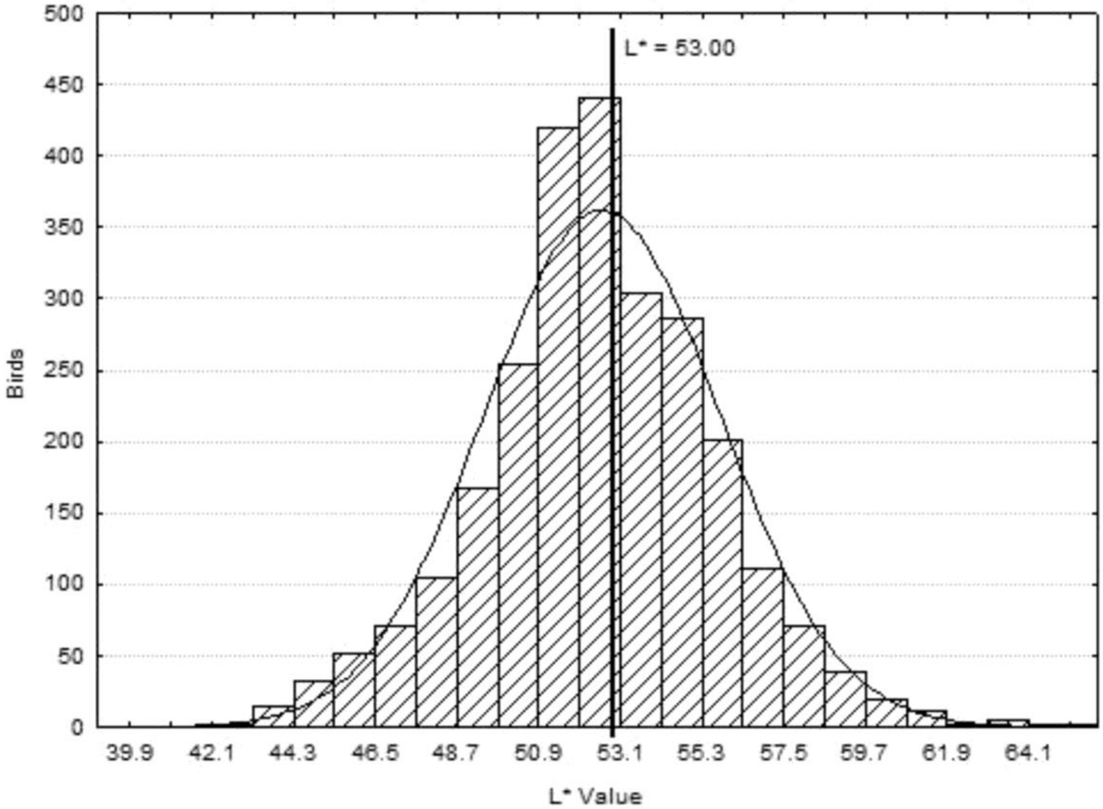
SF-Shear Force

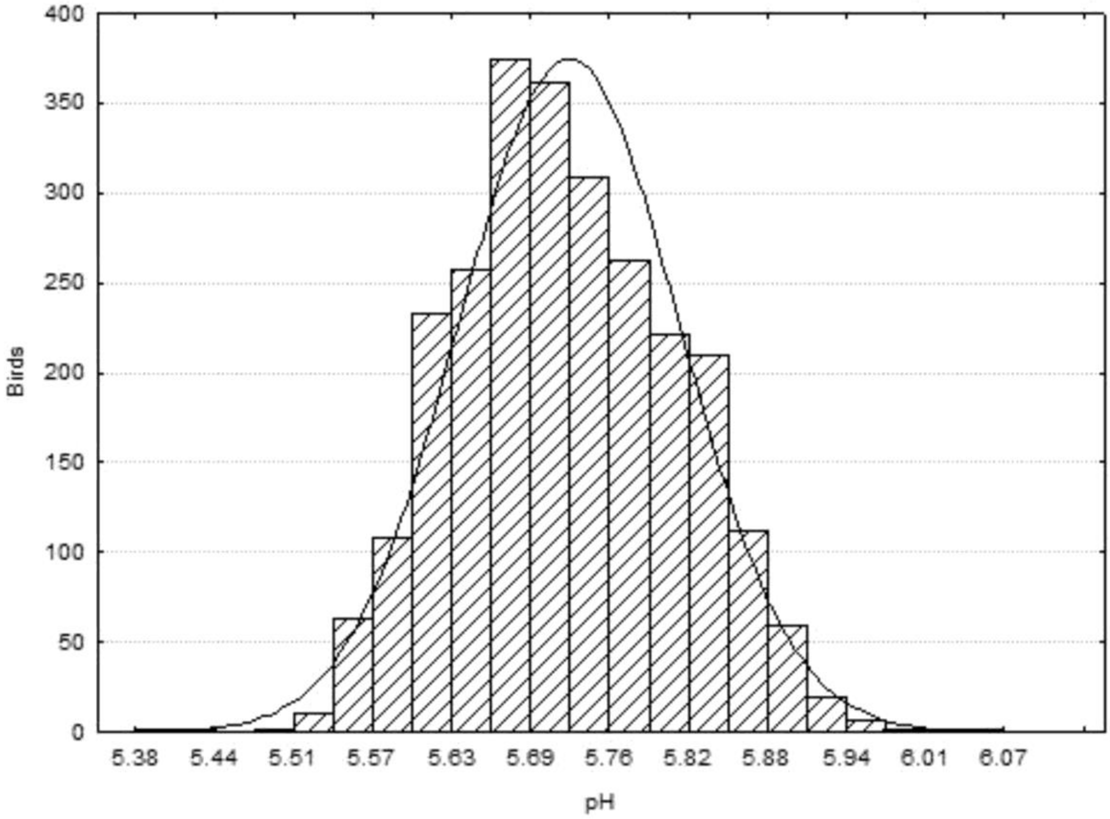
Table 4. Comparative values of PSE and normal turkey meat obtained in different countries in relation to the results described in this work.

	Barbut (1998)	Owens et al. (2000)	Fraqueza et al. (2006)	Present work
Country	Canada	USA	Portugal	Brazil
Frequency	4000	2995	977	2610
L* range	38 to 57	41 to 63	35 to 55	42 to 66
L* cutoff	50/51	53	50	53
Incidence %	12	40	8	41.7

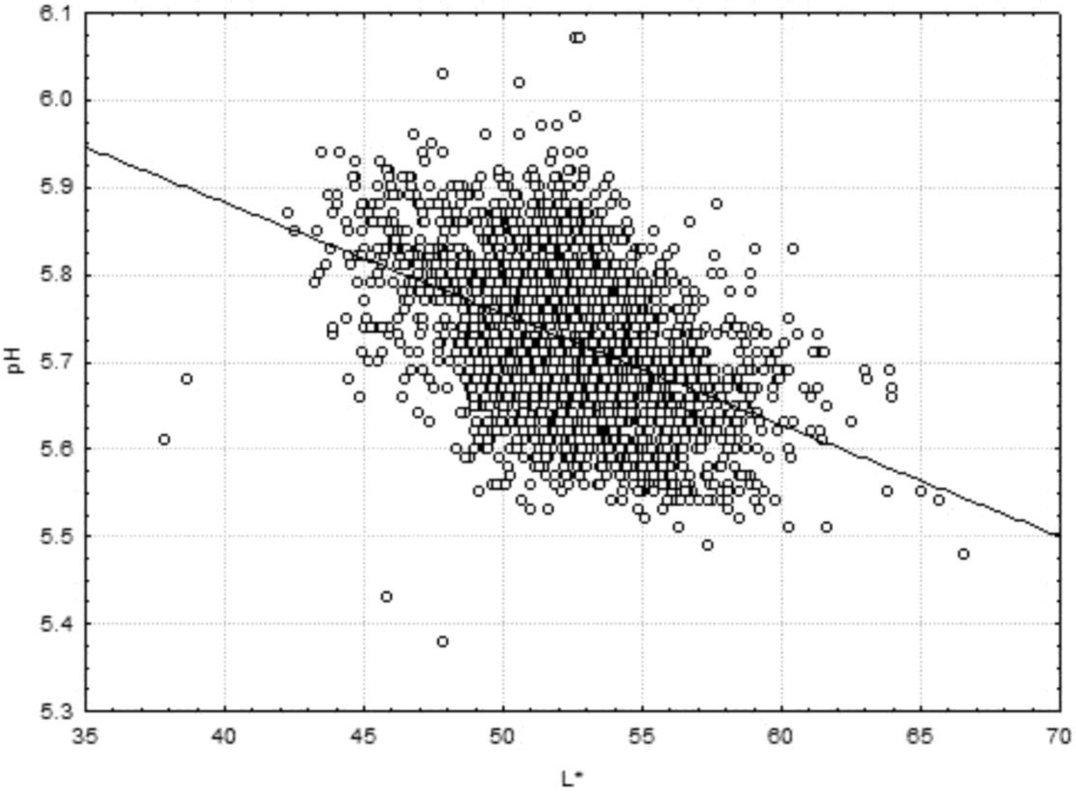

Hanging
Electric Stunning
Bleeding
Scalding
Defeathering
Evisceration
Cooling the carcass
Deboning and removal of the breast samples
Pectoralis major
Refrigerated at 4°C for 24h
Color analysis and pH


Grinding
Breast fresh turkey
Grinding it in sieves 4 and 19 mm
Brine
Ingredients (Table 1)
Tumbler
Time: 120 min
Tumbler Inject Star: 20 rpm
Internal Pressure: 76 mmHg
Stuffing
Synthetic tripe
Weight of the breasts = 1800 gr
Smoking
Time : 17.5 hrs
Temperature: 73 °C
Vacuum packed
Removed of the tripe
Vacuum packed breasts
Cooling in cold chambers
Pasteurization
Time: 37.5 minutes in 2 °C
Time: 22.5 minutes in 85 °C


L* = 53.00
Birds
500
450
400
350
300
250
200
150
100
50
0
39.9
42.1
44.3
46.5
48.7
50.9
53.1
55.3
57.5
59.7
61.9
64.1
L* Value



400
350
300
250
200
150
100
50
0
Birds
5.38
5.44
5.51
5.57
5.63
5.69
5.76
5.82
5.88
5.94
6.01
6.07
pH



6.1
6.0
5.9
5.8
5.7
5.6
5.5
5.4
5.3
pH
35
40
45
50
55
60
65
70
L*

4 EXPERIMENTO B (Publicado)

International Journal of Poultry Science 14 (9): 516-520, 2015
ISSN 1682-8356

Glycolysis Rate Delay in Turkey Breast *Pectoralis major m.* in a Commercial Air Chilling Processing Line and Meat Qualities

Glycolysis rate delay in turkey breast *pectoralis major m*. in a commercial air chilling processing line and meat qualities

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ABSTRACT

The development of *rigor mortis* (RM) in turkey *pectoralis major* under routine commercial plant conditions was described. Carcass samples (n=40) were refrigerated by air chill (AC) in a processing line and the final temperature (T) of 4°C was reached after 7h *postmortem* (PM) and the ultimate pH (pHu) of 5.68 was achieved after 24h PM. For results comparison, carcasses were kept at ambient temperature (AT), averaging 20 ± 2°C and the pHu of 5.67 was reached after 5h PM. The AC samples presented darker and higher water holding capacity values compared to AT samples after 10h PM while at 24h PM these results did not differ between delay and fast glycolysis. Finally, the rate of glycolysis was directly affected by air refrigeration and the fillet samples should be processed at 24h PM to ensure complete RM onset thus preventing the decrease of meat qualities.

Key words: Meat color, pH curve, Temperature, Water Holding Capacity.

Short running title: Chilling postmortem glycolysis in turkey breast

INTRODUCTION

Cooling is a critical step in animal carcass processing at commercial slaughterhouses, with important effects on meat quality and cost (Jacob *et al.*, 2012). In refrigeration systems, the carcasses are cooled rapidly to reduce microbiological proliferation and food safety risks (Honikel, 1998; Mor-Mur and Yuste, 2010). The Brazilian Ministry of Agriculture requires that poultry carcasses be chilled to 4º C or lower for subsequent commercialization of poultry products and meat derived products (Brasil, 1998).

Three technologies are currently commercially available for chilling: immersion chill (IC), air chill (AC), and combi in-line air chill (CIAC) (Demirok *et al.*, 2013). The cooling rate affects the biochemical processes within the muscle and influences the meat visual appearance (Ledward, 1985; Rosenvold and Wiklund, 2011), flavor characteristics (Thompson *et al.*, 2005), tenderness, water holding capacity (WHC) and color (Zamora *et al.*, 1996; Savell *et al.*, 2005). Several studies have compared the meat quality obtained under these three refrigeration systems (Davey and Garnett, 1980; Jaime *et al.*, 1992; Aalhus *et al.*, 2002; James *et al.*, 2006; Jacob *et al.*, 2012; Demirok *et al.*, 2013). A chilling rate either too slow or too fast can result in a lower quality of meat (Joseph, 1996).

Thus, the diverse technologies applied to chill carcasses during postmortem processes can promote different pH values during *rigor mortis* onset (Jacob *et al.*, 2012). Few suggestions have been implied that under AC system there was a production of meat at the quality level similar to that obtained by the other chilling methods (Bowling *et al.*, 1987; Van Moeseke *et al.*, 2001; Jacob *et al.*, 2012; Demirok *et al.*, 2013). Therefore, this work aimed to evaluate the development of *rigor mortis* in turkey *pectoralis major* obtained directly from commercial processing lines refrigerated by AC systems by measuring the pH, color and water holding capacity of the resulting meat.

MATERIALS AND METHODS

Sampling and refrigeration systems

The experiment was conducted in a commercial turkey slaughterhouse in Santa Catarina State, Brazil, in the summer of 2013. Eighty carcass samples of BUT-9 lineage turkeys, with a weight of 18.00 ± 0.5 kg were divided equally into two treatments: 1) treatment by Air Chill (AC) and 2) treatment at ambient temperature (AT). Both treatments followed the routine process of the commercial slaughter line, which consisted in sequence of hanging, electrical stunning, bleeding, scalding, defeathering and evisceration (45 ± 3 minutes) (Carvalho *et al.*, 2014).

Air Chilling (AC) treatment

Carcasses were held by the hocks on shackles in the commercial AC room for approximately 360 minutes. The air velocity in the room was 1.5 m/s with a temperature of -6 ±2°C and an RH of 75%, as measured by a Kestrel 4000 Instrument (Nielsen-Kellerman, Boothwyn, PA, USA). After cooling, the carcasses were deboned, and samples from the AC treatment were stored at 4°C according to Brazilian legal requirements (Brasil, 1998) for up to 26 h for further analysis.

Ambient Temperature (AT) treatment

Carcasses were randomly collected after deboning and stored at 20 ± 2°C for 26 hours and the pH changes were monitored throughout the experiment. This treatment was set for comparative purpose in relation to AC samples.

Temperature and pH determination

pH and temperature were measured (in duplicate) by inserting electrodes into the *pectoralis major* (pH meter system, Testo 205, Lenzkirch, Germany) as described in Olivo *et al.* (2001). The measurements were performed on samples at 0.06, 1, 4, 5, 6, 7, 9, 10, 22, 24 and 26 h *postmortem* (PM).

Color determination

This evaluation was carried out using a Minolta CR-400 colorimeter, taking five different reading points per sample for color determination (L*, a*, b*), as described by Soares *et al.* (2003) for broiler breast meat. Analyzes were performed at 10 and 24 h PM.

Water-holding capacity (WHC)

The WHC was determined based on the technique reported originally by Hamm (1960) and described in Wilhelm *et al.* (2010). A total of 80 samples divided into two treatment (n=40) was collected from the cranial side of the breast fillets, cut into 2.0-g (±0.10) cubes, and analyzed in triplicate. They were first carefully placed between two filter papers placed in acrylic plates and then left under a 10 kg weight for 5 min. The samples were weighed, and the WHC was determined by the exudate water weight through the following formula: 100 - [(Wi -Wf / Wi) x 100], where Wi and Wf are the initial and final sample weights, respectively. Analyzes were performed at 10 and 24h PM, similarly to the previous item.

Statistical analysis

The results were analyzed by the program Statistica for Windows 7.0. The Student t-test at 5 % probability ($P \leq 0.05$) was used to determine significant difference between the two treatments AT and AC at the same PM time. The Tukey's test at 5 % probability ($P \leq 0.05$)

was used to determine significant difference among PM time for temperature and pH of AT or AC meat samples.

RESULTS AND DISCUSSION

The effect of carcasses chilling on the *pectoralis major m.* temperature decline is shown in Fig. 1. At 0.06 h PM and there was no temperature difference relative to both treatments. At 1.0 h PM, the carcasses temperature in the AT samples conditions was 39 °C, while that of the carcasses kept under AC conditions was not significantly different. However, a dramatic change was observed at 4.0 h PM as the temperature of the carcasses in AC was significantly lower than that of observed in samples kept in the AT conditions. The final temperatures of 24.7 °C and 2.3 °C were reached after 22 h and 9 h PM, respectively, in the AC and AT treatments, with chilling rates of 0.69 °C/h and 4.19 °C/h, respectively. The results reported by McKee and Sams (1998) and Khan (1971) indicated that high temperature PM (10 °C - 40 °C) in poultry accelerated depletion (exhaustion) of ATP within the muscle samples influencing the final meat characteristics. Femery and Pool (1960) also observed high temperatures PM provided the increase in the rate of glycogen degradation in broiler muscle. In turkey breast under higher temperatures there was an accelerated PM metabolism as reported by Rathgeber *et al.* (1999).

----INSERT FIGURE 1----

In relation to the pH values, no difference within the treatments at 0.06 and 1.0 h PM was observed (Fig. 2). However, after 5 h PM, the carcasses from AT conditions reached their ultimate pH (pHu) value of 5.67, while those from AC reached a pHu value of 5.67 after 24 h PM and the chilling rates were 0.151 and 0.031 un/h, respectively. These results corroborated that there was a close relationship between both temperature and pH as at higher T values a quicker glycolysis onset occurred thus the pHu was reached approximately 5-fold faster than in the samples stored under chilling conditions. This fact indicated that a fast muscle metabolism occurred under higher temperatures as reported previously (Alvarado and Sams, 2002, 2004; Zhu *et al.*, 2013).

----INSERT FIGURE 2----

The pH and temperature parameters influenced the meat qualities, in particular the color parameter (Alvarado and Sams, 2002). Color is an important attribute for customer satisfaction at the time of the purchase (Fletcher, 1999; Droval *et al.*, 2012). The L* value of the meat depends on the amount of light that is scattered. Swatland (1993, 2008) reported that an increased scattering of light due to denaturation of the sarcoplasmic proteins and an increase in extracellular water were responsible for paler meat. The effects of chilling after 10 and 24 h PM on the turkey breast meat color and WHC are shown in Table 1.

----INSERT TABLE 1----

In fillets at 10 h PM, the samples chilled (AC) had significantly lower L* values than those from AT, probably as the consequence of lower T (Fig.1) and higher pH (Fig. 2) observed for AC fillets. Several researches have reported color changes in meat turkey breast that were submitted to accelerated rate of glycolysis PM (Froning *et al.*, 1978; Pietrzak *et al.*, 1997; McKee and Sams, 1998). McKee and Sams (1998) also observed high values of L * 4h PM in turkey breasts submitted between 20 - 40 °C when compared with those submitted at 0 °C, immediately after evisceration. The same authors also suggested that carcasses stored at higher temperatures resulted in acceleration of *rigor mortis* as the consequence of quicker biochemical changes in muscle. The incidence of pale soft and exudative (PSE) meat was not detected in this particular experiment probably because of amount sampling as its number was not sufficient although as reported elsewhere this phenomenon occurred (Carvalho *et al.*, 2014).

In relation to the WHC, samples from the AT treatment after 10 h PM were significantly lower in comparison to those from the AC treatment, although at 24 h PM they were not different from each other as the pH values were quite similar and finally the a*, b* values were not significantly different in any treatment (Table 1).

In conclusion, the rate of muscle pH decline is directly affected by the chilling temperature. Complete *rigor mortis* was reached up to 24 h PM under the air chilling commercial conditions, indicating that turkey fillets should be handled by the processors after this length of time in order to keep its myofibrillar proteins functionalities.

ACKNOWLEDGMENTS

The authors acknowledge funding for this project by CNPq Proc. 471609/2011-0. FGP holds a post-doctoral scholarship from CAPES Proc 23038007638201119. MRP, EII and MS are CNPq Research Fellows and MS was also a Brazilian Senior Visiting Professor Scholar from CAPES.

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LEGENDS TO FIGURES

Figure 1. **Temperatures (T) values of turkey carcass measured at postmortem times of 0.06, 1 h, 4 h, 5 h, 6 h, 7 h, 9 h, 10 h, 22 h, 24 h and 26 h for the following treatments: Ambient temperature (AT) and Air Chill System (AC).**

Standard deviation bars are indicated (n=40 per treatment group).

Means followed by different uppercase letters in the same treatment differ by Tukey test at 5% significance ($P \leq 0.05$) among postmortem time.

Means followed by different lowercase letters in the same time differ by Student's t test at 5% significance ($P \leq 0.05$) between treatments.

Figure 2**. pH values of turkey carcass measured at postmortem times of 0.06, 1 h, 4 h, 5 h, 6 h, 7 h, 9 h, 10 h, 22 h, 24 h and 26 h for the following treatments:**

Ambient temperature (AT) and Air Chill System (AC).

Standard deviation bars are indicated (n=40 per treatment group).

Means followed by different uppercase letters in the same treatment differ by Tukey test at 5% significance ($P \leq 0.05$) among postmortem time.

Means followed by different lowercase letters in the same time differ by Student's t test at 5% significance ($P \leq 0.05$) between treatments.

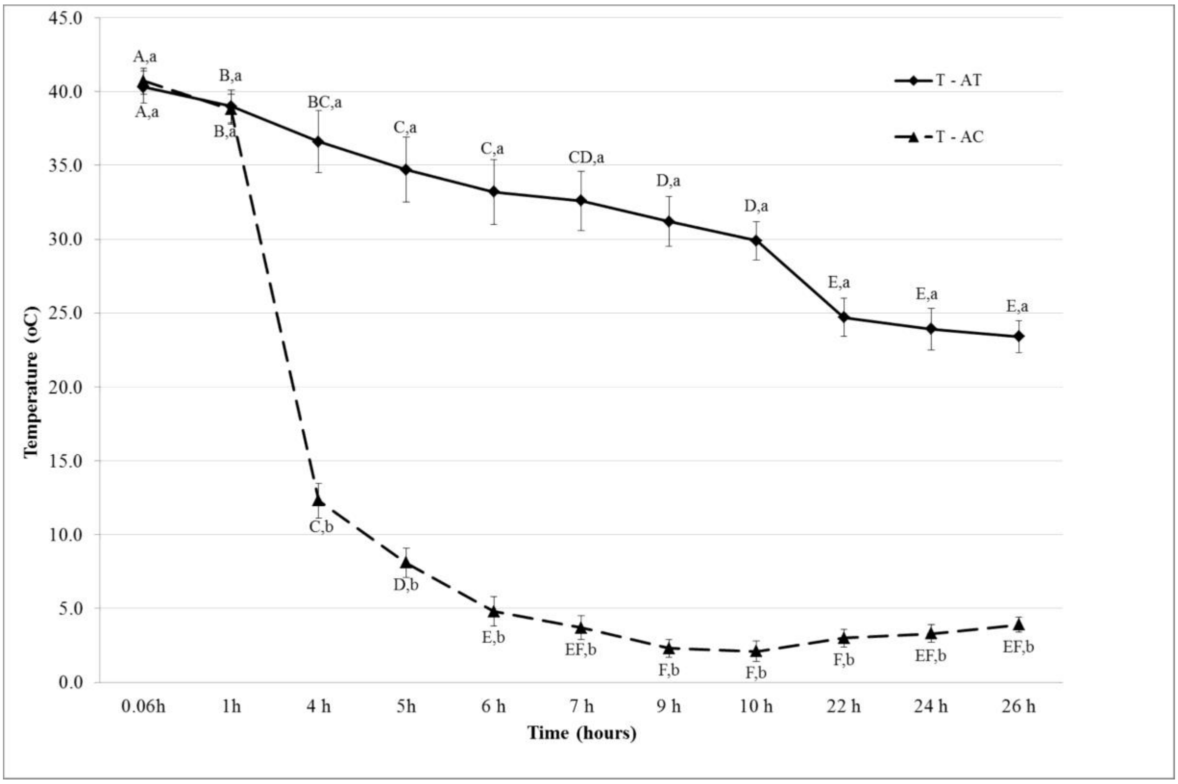
Table 1**. Values of L *, a *, b * and the water holding capacity (WHC) of turkey fillet measured at 10 h and 24 h postmortem under the following treatments: Ambient temperature (AT) and Air Chill System (AC)**

Time (PM)	AT	AC
L* 10 h	$52.26^{A,a} \pm 2.55$	$49.01^{B,b} \pm 2.49$
L* 24 h	$52.89^{A,a} \pm 2.79$	$52.72^{A,a} \pm 2.30$
a* 10 h	$4.89^{A,a} \pm 1.45$	$4.74^{A,a} \pm 1.37$
a* 24 h	$5.26^{A,a} \pm 1.51$	$4.20^{A,a} \pm 1.58$
b* 10 h	$3.78^{A,a} \pm 1.31$	$4.09^{A,a} \pm 1.53$
b* 24 h	$4.08^{A,a} \pm 1.40$	$4.61^{A,a} \pm 1.76$
WHC (%) 10 h	$74.72^{A,b} \pm 1.77$	$77.89^{A,a} \pm 1.91$
WHC (%) 24 h	$74.37^{A,b} \pm 1.70$	$74.57^{B,b} \pm 1.63$

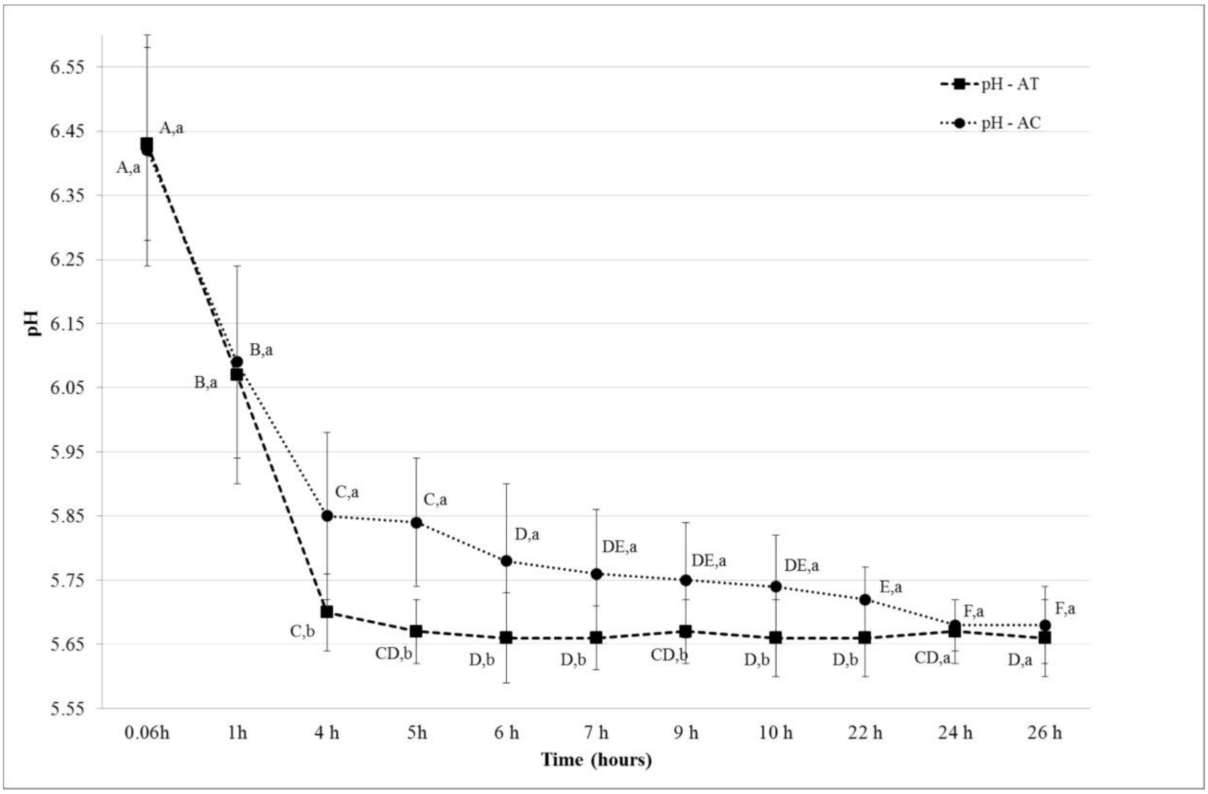
Means followed by different uppercase letters in the same column for each parameter differ by Student's t-test at a 5% significance level (P<0.05).

Means followed by different lowercase letters in the same row differ by Student's t test at the 5% significance level (P<0.05).

PM: *postmortem*



Temperature (oC)
Time (hours)
T - AT
T - AC
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0.06h
1h
4 h
5h
6 h
7 h
9 h
10 h
22 h
24 h
26 h
A,a
B,a
BC,a
C,a
C,a
CD,a
D,a
D,a
E,a
E,a
E,a
A,a
B,a
C,b
D,b
E,b
EF,b
F,b
F,b
F,b
EF,b
EF,b



pH
6.55
6.45
6.35
6.25
6.15
6.05
5.95
5.85
5.75
5.65
5.55
pH - AT
pH - AC
A,a
A,a
B,a
B,a
C,a
C,b
C,a
CD,b
D,a
D,b
DE,a
D,b
DE,a
CD,b
DE,a
D,b
E,a
D,b
F,a
CD,a
F,a
D,a
0.06h
1h
4 h
5h
6 h
7 h
9 h
10 h
22 h
24 h
26 h
Time (hours)

5 EXPERIMENTO C (Em preparação)

THE JOURNAL OF
APPLIED POULTRY RESEARCH

Effect of turkeys harvesting by hand and machine on injuries rates and meat quality

O artigo encontra-se nas normas do periódico Journal Applied Poultry Research.

Endereço: http://japr.oxfordjournals.org/

Effect of turkeys catching by manual and machine on injuries rates and meat quality

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Running title: Mechanical Turkey Harvesting

Primary Audience: Food Scientists, Researchers, Poultry Meat Processors, Poultry Farmers

SUMMARY

A study trial was conducted to compare manual catching of turkeys with a mechanical catching method under commercial conditions. Both methods were compared with respect to the incidence of bruises and dead on arrival (DOA), meat quality and incidence PSE-like meat. The turkeys originated from 5 commercial turkey farms during summer of 2013. Turkeys (Nicholas lineage, male, 140d of age, 18 kg) were utilized. Postmortem measurements of bruises, L*, a*, b*, pH_{45min}, pH_{24h} and water-holding capacity were performed. The results indicated that there was no significant difference in bruises and DOA values, which suggested that mechanically catching turkey did not promote more damage than those harvested manually. Catching method influence the meat quality, pH_{45min}, pH_{24h} and water holding capacity indicated that manual methods induced more stress. Manual harvesting was associated with higher incidence of PSE-like (9%) meat than machine catching. Our findings indicated that both catching methods have effectiveness in relation to the time of collect. The mechanical machine appears to be a good alternative to manual catching. However, some aspects of mechanical catching require further improvements.

Key words: Animal welfare, Commercial slaughterhouse, DOA, Nicholas 700, PSE-like.

DESCRIPTION OF PROBLEM

The catching is the first step of pre-slaughter management, wherein birds at first are exposed to stress. Stress directly influences the animal's welfare and carcass quality [1]. The capture and handling in the catching stage are among the main factors determining the efficiency and profitability of industries that produce poultry on large scale [2].

In the past, birds' catching was performed manually and workers caught turkeys placing them into a transport truck [3]. This is carried out normally in some parts of the world, especially in Brazil [1], but mechanization can be seen in many countries, because currently the birds are relatively heavier [3].

Currently there are various commercial machines available for capture; automation is increasingly being used by the poultry industry, largely because of high labor costs and scarcity [4]. Mechanical harvesters gather turkeys and lead at conveyor belts to transport cage [3,5]. Automation has the intention to reduce the amount of time that birds are exposed to physical contact with people and moving them smoothly, avoiding higher incidence of stress [6].

The type of mechanical harvester can be important to ensuring the best possible comfortable conditions [7] and authors evaluating different models of automated harvesters, observed difference between the amount of hematomas provided to the birds when captured by machine [5,7,8,9]. Injuries during the capture process and caging are extremely concern to the industry, up to 20% of the birds are discarded because of the capture-related injuries [10], stress promoted by the pre-slaughter steps causes abnormalities in meat, one of the main consequences of stress is known as PSE meat (pale, soft and exudative)[1]. Losses of PSE poultry meat were estimated to cause an annual monetary loss of over US$ 200 million in the USA [11] and US$ 5.1 million in Brazil to the turkey industry [1].

The aim of this work was therefore to evaluate the effect of catching machine on turkey welfare by measuring quantitatively the hematomas and incidence of PSE-like meat.

MATERIALS AND METHODS

Samples Collection

This study was conducted in a commercial plant in city of Chapecó (Latitude: 27º 05' 47" S; Longitude: 52º 37' 06" W; Altitude: 674m) Santa Catarina, State, Brazil, January 2013 and February 2013. From a total of 4,800 birds (species: *Meleagris gallopavo*; lineage: Nicholas-700; male; 140 days of age and 18 ± 2kg). The conditions activities from the farm to the turkey commercial processing plant are illustrated in the flow chart shown in Carvalho et al. [1]. The feed was removed 9 to 12 hrs before slaughter, and water was provided *ad libitum*. Birds were handled in accordance with the principles and procedures outlined by the Londrina State University Animal Care and Use Ethical Committee (167/2015).

Manual Catching (MA)

A total of 2400 turkeys were captured manually, divided into 5 replicates (n=480) at different 5 farms. Professional catching teams carried out the manual catching: eight catchers and one leader (general assistant). The process of catching was performed gently holding the turkey by the neck and propelling the animal to the transport truck. The catching and loading average time was 21± 3 min.

Mechanical Catching (MC)

A total of 2400 turkeys were captured mechanical, divided into 5 replicates (n=480) at different 5 farms. The catching machine (turkey) used was positioned at the entrance of the shed where is performed the pre-loading, the animals are led to the sliding door and slowly were carried to the front towards the caging belt. The boarding automatically drops the turkeys inside the cages with no operator contact. Six operators (2 in the cab and 4 in the shed) performed the harvesting. The catching and loading average time was 23± 3 min.

Transport and Slaughter

The animals were reared on 5 turkey farms under a cooperative system and subsequently transported over a distance of 24 ± 5 km for a journey that takes approximately 40 ± 10 min. Upon arrival at the slaughterhouse facilities, the birds were placed in a holding area under water spray and ventilation for 40 ± 20 min before slaughtering. The animals were sacrificed according to standard industry practices, which consisted of hanging, electrically stunning, bleeding, scalding, defeathering, evisceration, cooling the carcass through a tunnel of cold air (6°C for 6 h) and deboning [1]. Subsequently, the breast meat samples (*Pectoralis major*) were collected and refrigerated at 4°C until completing 24 h to measure the color (L* value), pH and WHC thus evaluating the occurrence of PSE-like meat [1]. The postmortem inspection procedure was performed by the Regulation of Industrial Inspection Sanitary of Animal Products – (RIISPOA) by macroscopic visual inspection of carcasses [12]. Total and partial bruises were analyzed in carcasses of the turkey.

PSE-like Meat Evaluation

Samples of breast fillets (n=501) (*Pectoralis major*) were collected (50 animals per transport load). The meat samples were classified as PSE-like according to their L* values [13], with L* ≥ 53 and pH < 5.6, considered typical for PSE-like turkey meat, as thoroughly discussed in Carvalho et al. [1]. This evaluation was performed using a Minolta CR-400 colorimeter, taking five different reading points per sample for color determination (L*, a*, b*), as described by Carvalho et al. [14]. The pH_{45min} and pH_{24h} were measured (in duplicate) by inserting electrodes into the *pectoralis major m* [15] as described in Carvalho et al. [14].

Water Holding Capacity (WHC)

This measurement was carried out based on the technique of Hamm [16], as described in Wilhelm et al. [17]. After 24 h postmortem, samples were collected from the cranial side of the breast filets and cut into cubes 2.0 ± 0.10 g. A total of 15 samples were analyzed in triplicate per replicate of harvesting. They were first carefully placed between 2 pieces of filter paper on acrylic plates and then left under a 10-kg weight for 5 min. The samples were weighed and WHC was determined using the weight of exudate water and the following equation: 100 - [(Wi - Wf / Wi) X 100], where Wi and Wf are the initial and final sample weight, respectively**.**

Statistical analysis

The data were analyzed using Statistica for Windows (Statsoft). Meaningful comparisons were generated using Student´s t test (5 % level). For the incidence of PSE-like and normal meat, the binary variation (1 and 0) was used, where 1 denotes PSE meat and 0 denotes normal meat.

RESULTS

Both systems (MC and MA) were shown to be effective in relation to the time spent for loading of turkeys, approximately 25 minutes per load. Table 1 shows the results of percentages of DOAs, partial bruises, partial and total dermatosis, not differed significantly between the two catching methods ($P \geq 0.05$).

Table 2 shows comparative values pH_{45min}, pH_{24h}, L*, a*, b* and WHC of MC and MA catching. The MA meat samples pH_{45min} was 0.14 units lower ($P<0.05$) compared to the MC, and also for MC the pH_{24h} and L* values were 5.75 and 51.88, respectively, which were significantly different ($P<0.05$) from the MA meat samples values of 5.71 and 53.57, respectively. As for WHC it showed average values of 20.33 %. These values differed ($P<0.05$) in 2.45 % from those of the MA meat samples. The mean percentage of PSE-like and normal meat is shown in Figure 2. Incidence of PSE meat was lower ($P<0.05$) in 9% for MH method.

DISCUSSION

Our results indicated that by using the MC difference ($P\geq0.05$) could not be verified among the DOA index, bruises and dermatosis, suggesting that both can be used without losses by poultry industries. It is important to observe that use of machine did not increase the number of injuries in birds compared to the current systems MA [10].

Nijdam et al. [7] compared mechanical and manual catching in broilers found no difference between the decline and ultimate pH in both methods. Our result shows that at 45 minutes and 24 hours *postmortem* the birds manually harvested presented lower pH values. Factors that can influence pH are transport, shackling, stunning, processing of meat [14,19,20]. However, the *pectoralis* muscle of mechanically harvesting turkey lost less fluid compared with the *pectoralis* muscle of manually harvesting turkeys. These results can be explained because different declines in *postmortem* pH influence the degree of protein denaturation WHC (2.45 %, in our studies) lower for MA. Training and introduction of technology on the process of catching significantly contribute to improving the welfare of birds and reduce injury/bruises [18].

Knierim and Gocke [8] reported that the incidence of different types of injuries can be affected by the method of capture and also by other factors such as transport systems, bruises on wing is main prevalent type of injury ah the both mechanical and manual catching in broilers. Nijdam et al. [7] reported that significantly differ between manual and machine for percentages of DOAs in chicken, higher losses mechanical catching, oppositely [8] found no difference in DOAs at the methods of manual and mechanical catching, the model and system used to chickens is different to that used for turkey, therefore can increase incidence of DOAs, our experiments birds were taken carefully to the boarding platform and gently transported by treadmill, dead or debilitated birds were identified and not loaded, showed not difference in the number of DOAs. Mitchell and Ketewell [4] reported that in slaughterhouses mortality and losses carcass records should be examined, evaluated accurately the level of injuries, fractures and bruises.

The turkey industry has been faced with the increasing problem of PSE meat, this PSE turkey meat is pale in color, has lower water-holding capacity (WHC), and forms softer gels [1,11], and this meat showed 10% weight loss in PSE turkey meat samples. One of the consequences of PSE-meat related for the turkey industry in Brazil is the economic losses approximately US$5.1 million per annum [1], also the poor functional properties of the PSE meat reflected in purchasers' behavior preferred normal meat [21].

Mitchell and Ketewell [4] reported several ideas on models patented machines, for improvement of collected of live poultry. There is a tendency at the process to be mechanized because catching birds is not an enjoyable task for workers due to the requirement for physical strength and repetitive movements, besides nearly always performed during the first morning hours and in all weather conditions [3,10].

A main obstacle in the development and acceptance of commercial catchers it has been the requirement for them to operate in different models of poultry houses [4]. In our study, most of the farms belonging to the process industry did not possess the ideal conditions at the use of machine, making it difficult to access because most of the poultry houses owned more than 20 years old and reflect the standard designs that were prevalent at the time were installed. The use both methods catching has considerable scope for improvement with regard to physical damage for turkeys during load, this can be reduced by applying guidelines for farmers, supervisors and processors.

CONCLUSIONS AND APPLICATIONS

1. The mechanical catching has effectiveness in relation to the time of collect and can be used for capture.

2. We observed that mechanical catching with this type of machine does not cause higher mortality rates (DOA) and bruising.

3. For the meat quality, differences were found between turkeys mechanically and manually catching as the manual harvesting presented higher PSE-like meat incidence.

4. Design of the birds' houses should be improved or adapted for the catching machine operation.

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Acknowledgements

This project was funded by CNPq Proc. 471609/2011-0. RHC and DCBH are under CAPES graduate scholarships. EII and MS are CNPq Research Fellows, and MS was also a Brazilian Senior Visiting Professor Scholar from CAPES.

Figure and Legends

Figure 1.

PSE-like meat incidence (mean ± SEM) of both catching methods, manual (n = 250) and mechanical (n = 250). Values with different superscripts are significantly different during the harvesting process at $P<0.05$.

Table 1 Percentages of turkeys (mean ± SEM) with different types of dermatosis, bruises and percentage of dead on arrival (DOA) for both catching methods.

	Machine Catching	Manual Catching	P value
DOA (%)	$0.022^{a} \pm 0.007$	$0.020^{a} \pm 0.008$	0.6938
Partial bruises (%)	$19.8^{a} \pm 6.22$	$12.2^{a} \pm 4.84$	0.0817
Total dermatosis (%)	$1.80^{a} \pm 0.83$	$1.00^{a} \pm 0.70$	0.1411
Partial dermatosis (%)	$3.80^{a} \pm 1.64$	$3.40^{a} \pm 1.14$	0.6665

Means followed by different letters on the same row differ by a t-test at the 5% significance level (P<0.05). n = 5 transport with 480 birds each per treatment.

Table 2 Values of meat pH_{45min}, pH_{24h}, L*, a*, b* and WHC from samples taken from birds under machine and manual catching's.

	Machine Catching	Manual Catching	P value
$pH_{45\ min}$	$6.26^{a} \pm 0.18$	$6.12^{b} \pm 0.13$	0.000
$pH_{24\ h}$	$5.75^{a} \pm 0.08$	$5.71^{b} \pm 0.10$	0.009
L*	$51.88^{b} \pm 3.05$	$53.57^{a} \pm 2.24$	0.000
a*	$4.65^{a} \pm 1.17$	$4.47^{a} \pm 1.06$	0.077
b*	$4.01^{a} \pm 1.24$	$4.73^{a} \pm 0.95$	0.862
WHC (%)	$20.33^{b} \pm 1.03$	$22.78^{a} \pm 1.16$	0.000

Means followed by different letters on the same row differ by a t-test at the 5% significance level ($P<0.05$). pH, L*, a* and b* n = 250 per treatment and 50 per replicate. WHC: n = 75 per treatment and 15 per replicate.

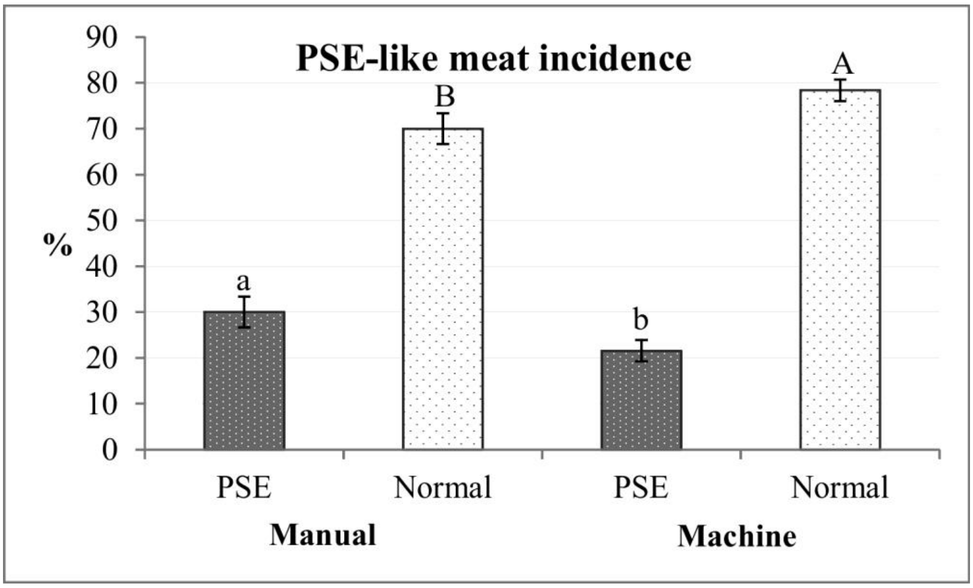

Figure 1 PSE-like meat incidence (mean ± SEM) of both catching methods, manual (n = 250) and mechanical (n = 250). Values with different superscripts are significantly different during the harvesting process at $P<0.05$.

6 EXPERIMENTO D (Submetido)

Animal Science Journal

In-transit development of color abnormality in turkey breast meat during Brazilian winter season

Journal:	*Animal Science Journal*
Manuscript ID	Draft
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Carvalho, Rafael ; Universidade Estadual de Londrina, Department of Veterinary Preventive Medicine

In-transit development of color abnormality in turkey breast meat during Brazilian winter season

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Short running title: Turkey transport in winter conditions

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ABSTRACT

This work evaluated the effects of open vehicle container microclimate, throughout the 38 ± 10 km journey from the farm to the slaughterhouse, on turkey transported commercially during the Brazilian winter season. The journey was initiated immediately after water bath (WiB) or without water bath (WoB) application (n=16, each). The meat from animals located at the inferior compartments of the middle and rear truck regions and subjected to WiB treatment had higher dark, firm and dry (DFD-like) and had lower pale, soft and exudative (PSE-like) meat incidence than those from animals located at inferior compartments in the front of the container. Lower incidence of pale meat and dead on arrival (DOA) index were observed in WoB group in relation to the WiB group. Assessment of turkeys transported under Brazilian southern winter conditions revealed that breast meat quality can be affected by relative humidity, air ventilation, temperature, and transport under sub-optimal conditions can cause color abnormalities and the formation of either PSE-like or DFD-like meat.

Key words: Animal welfare, commercial slaughterhouse, DFD-like, PSE-like, winter climate.

INTRODUCTION

The poultry industry in Brazil is subjected to several detrimental factors that impact meat quality. A variety of unfavorable weather conditions exist in Brazil, which is located in tropical and subtropical zones (Carvalho *et al.* 2014). During transport from the farm to the slaughter plant, birds are subjected to stressors that compromise their welfare, affecting muscle metabolism that results in deterioration of meat quality (Mitchell *et al.* 1992; Mitchell & Kettlewell 1998; Carvalho *et al.* 2015a). Primary in-transit stress factors are related to variability of transport container microclimates (Hunter *et al.* 1997; Mitchell & Kettlewell, 1998, Carvalho *et al.* 2014).

Previous research has established the existence of air ventilation deviations that impact animal welfare (Simões *et al*. 2009; Langer *et al.* 2010; Spurio *et al.* 2015). Kettewell and Mitchell (1993) conducted a three-dimensional characterization of the environmental conditions inside the cargo hold of commercial trucks loaded with chickens and reported great regional disparities of temperature, humidity, and ventilation within loads.

Several reports indicated that there is a thermal core in which thermal load and relative humidity are higher and that this core corresponds to low-ventilation regions within the loaded truck container (Hoxey *et al.* 1996; Simões *et al.* 2009; Spurio *et al.* 2015). However, these studies were carried out within temperate regions. Experimental research is scarce within tropical climate zones that experience intense daily temperature fluctuations (Mitchell *et al.* 1992; Kettlewell & Mitchell 1992; Simões *et al.* 2009; Langer *et al.* 2010; Vieira *et al.* 2011; Spurio *et al.* 2015).

Previous reports have described the correlation of bird management practices, both at the farm and in transit to commercial slaughterhouses, on the prevalence of pale, soft and exudative (PSE-like) meat and dead on arrival (DOA) index under summer conditions (Guarnieri *et al.* 2004; Carvalho *et al.* 2014; Spurio *et al.* 2015). The stress promoted by longer transit times caused depletion of muscle glycogen resulting in higher postmortem muscle pH (Dadgar *et al.* 2010; Dadgar *et al.* 2012), which correlated with lower lightness (L*) value (Carvalho *et al.* 2015).

The aim of this work was to evaluate the effect of current farm-to-slaughterhouse cargo transport practices during the Brazilian winter season on turkey welfare and meat quality.

MATERIALS AND METHODS

Experimental designed and statistical analysis

The experimental design was randomized into a 6 × 2 factorial arrangement (truck position × water bath treatment) with 6 positions: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR) and two water bath treatments (WiB or WoB), providing for 12 treatments, with 8 replicates for each treatment. WiB or WoB were carried out for 5 min just before leaving the farm. The data were analyzed using ANOVA and factorial analysis methods in the SISVAR statistical package (Ferreira 2008). Meaningful comparisons were generated using Tukey´s test (1% level). For incidences of PSE-like meat, the binary variation (1 and 0) was used, where 1 denoted PSE-like meat and 0 denoted normal meat. For incidences of dark, firm and dry (DFD-like) meat, the binary variation (1 and 0) was used, where 1 denoted DFD-like meat and 0 denoted normal meat.

Animals

This study was conducted during Brazilian winter months between May 2014 and August 2014 in a commercial plant in Chapecó city area (Latitude: 27º 05' 47" S; Longitude: 52º 37' 06" W; Altitude: 674m), Santa Catarina State, within the southern region of Brazil. All sampling days had similar weather conditions. The transportation conditions and activities from the farm to the turkey commercial processing plant are illustrated in the flow chart shown in Fig 1.

-----Insert Figure 1-----

The weather conditions in this region were characterized by the minimum and maximum temperatures of -5 and 26°C, respectively, with relative humidity (RH) variations from 39 to 65% throughout the 73 days of sampling, as measured using a Kestrel 4000 instrument (Nielsen-Kellerman, Boothwyn, PA, USA). The birds were males of Nicholas 700 lineage grown under regular acclimatized aviaries to an age of 140 days and an average live weight of 18 ± 2 kg. Feed was removed 9 to 12 h before slaughter, and water was provided *ad libitum*. The animals were manually placed into crates at a density of 8 birds (98 ± 2 kg/m^2) each and installed within the truck open container. The average catching and loading time was 24 min and after that the birds were subject or not to the water bath treatment for approximately 5 min. Birds were handled in accordance with the principles and procedures outlined by the Londrina State University Animal Care and Use Ethical Committee (167/2015).

Truck Container Microenvironment Assessment

Six portable weather meter devices with bidirectional Kestrel anemometers and data logging capability were set to take measurements at 30-second intervals during each journey. Instruments were fixed and oriented in the direction of highest velocity, all devices were calibrated for relative humidity, temperature and ventilation before the experiment. The devices were installed (suspended in cage, among the birds) at the SF, IF, SM, IM, SR and IR of the truck container compartments and at positions between columns 1 and 2, 3 and 4, and 5 and 6, as shown by the gray rectangles in Figure 2. Ambient temperature and relative humidity were measured at three different points: at the beginning of transport, arrival at the slaughterhouse plant and during holding time.

-----Insert Figure 2-----

The relative humidity (RH), air ventilation (AV), and temperature (T) values were determined as in Spurio *et al.* (2015), and wind chill (WC) values were simultaneously measured allowing for a representative analysis of the heterogeneous distribution of the thermal microenvironment within the loaded vehicle. WC, or apparent temperature, is an index that combines air temperature and air ventilation to measure the perceived equivalent corporal temperature (NOAA, 2014). RH, AV, T, and WC were automatically obtained from the 6 data loggers and downloaded to a computer.

Transport and Slaughter

A total of 32 transport journeys were evaluated, either with bath (WiB) (n=16) application or without bath (WoB) (n=16). These processes of birds harvesting and loading were only started when the ambient temperature has reached the temperature of 5 °C. The animals were grown on 16 turkey farms under a cooperative system and subsequently transported over a distance of 38 ± 10 km for a journey that took approximately 95 ± 20 min. Upon arrival at the slaughterhouse facilities, all birds were placed in a holding area under water mist and ventilation for approximately 70 min before slaughtering. The animals were sacrificed according to standard industry practices, which consisted of hanging, electrically stunning, bleeding, scalding, defeathering, evisceration, cooling the carcass through a tunnel of cold air (6°C for 6 h) and deboning (Carvalho *et al.* 2014). Subsequently, the breast meat samples (*Pectoralis major*) were collected and refrigerated at 4°C for 24 h prior to analysis. Classification of meat as normal, PSE-like (Carvalho *et al.* 2014), and DFD-like (Carvalho *et al*. in preparation) was performed by measurement of color and pH values.

PSE-like and DFD-like Meat Measurement

Samples of breast fillets (n=1,344) (*Pectoralis major*) were collected (42 animals per load). The meat samples were classified as PSE-like, normal, and DFD-like meat (Table 1) by the pH value and lightness (L*), as thoroughly discussed in Carvalho *et al.* (2014) and Carvalho *et al.* (in preparation).

-----Insert Table 1-----

Color determinations were performed using a Minolta CR-400 colorimeter using five different reading points per sample (L*, a*, b*), as described in Carvalho *et al.* (2014). The pH was measured in duplicate by inserting electrodes into the breast meat (Testo 205, Testo AG, Lenzkirch, Germany) as described in Carvalho *et al.* (2015b).

Dead on Arrival (DOA)

The percentage of DOA birds per loaded truck was calculated by counting each dead bird during the hanging step at the slaughtering plant, taking care to observe the truck region from which the birds were collected (Spurio *et al.* 2015).

DOA = (Number of birds dead / Number of birds transported) x 100

RESULTS AND DISCUSSION

Truck Container Microenvironment Assessment

Table 2 lists the results of T and RH. No significant interactions between bath treatment and truck container compartment position for T ($P \geq 0.01$) were observed. However, by analyzing separately first the bath and subsequently the birds' container positions, they indeed were significantly different ($P<0.01$). Pre-transport water baths caused a temperature drop within the every truck compartment. WoB group temperatures were an average of 7.64 °C higher than WiB treatment. Positionally, the temperatures of all superior regions of the truck (SF, SM and SR) and the IF position were lower than those in the IM and IR compartments (Table 2). RH strongly correlated with truck compartment position and bath treatment ($P<0.01$). The highest RH values were observed for IM and IR in the WiB groups, and the lowest RH values occurred for WoB groups regardless the truck compartment. Figure 3 shows detailed step-by-step variations throughout the journey from the farm to the processing plant and subsequent the holding time for T (A) and RH (B).

-----Insert Table 2-----

-----Insert Figure 3-----

In Brazil, currently, there is a pattern for truck container for transport of live turkeys from farms to the commercial slaughterhouses irrespective of the specific season. However, its design does not meet the wide range of tropical and subtropical climates found in this continental size country. Watts *et al.* (2011) reported experiments at temperature of up to 20 °C, when broilers showed low production of heat and relative humidity. Conversely, they found that low temperatures resulted in increased heat production by birds in-transit. In our experiments, the exposure of poultry to a temperature of 5 °C in-transit led to a temperature increase in every container compartment at the end of the journey, suggesting heat production by the birds (Fig. 3B). The application of a pre-transportation bath decreased temperature as reported in previous studies (Simões *et al.* 2009; Langer *et al.* 2010; Spurio *et al.* 2015). In addition to producing heat in cold temperatures, these birds also retained more moisture (Xin *et al.* 2001; Watts *et al*. 2011).

Watts *et al.* (2011) suggested maintenance of environmental conditions during transport in which the birds produce as little as heat possible. Ideally, the temperature should remain within the birds' thermo neutral zone, *i.e.,* between 23 and 29 °C (Meltzer, 1983) or 18 and 30 °C (Pereira & Nääs, 2008). Results herein show the formation of a thermal core at the inferior middle and rear regions of the truck, likely developed due to heat production by the birds (Watts *et al.* 2011) and poorer ventilation within IM and IR compartments (Fig. 3A, Table 3). Therefore, it is concluded that there was no heat dissipation from these regions of the container environment. The IM/IR thermal core also showed higher RH, which is harmful to the welfare of the birds. Watts *et al.* (2011) also stated that effective management of poultry transport must to take into consideration high environment humidity levels that exacerbate the stressful effects on birds. These findings corroborate other reports on bird welfare that stated birds would be able to withstand the unfavorable effects of low temperatures by maintaining a dry environment in-transit (Hunter *et al.* 1999). Furthermore, the thermal core found in our study is different in relation to the experiments carried out in other countries (Hoxey *et al.* 1996; Mitchell & Kettlewell 2004), because of the truck container design. Hoxey *et al.* (1996) and Mitchell and Kettlewell (2004) reported thermal core formation at the front of containers that had an air impermeable barrier at the front of the truck. Conversely, truck containers commonly used in Brazil are fully ventilated, allowing air to enter during vehicle motion, thus promoting more air flow in the front compartments SF and IF (Table 3 and Fig. 4) as previously shown by Spurio *et al.* (2015). Figure 4 shows in detail step-by-step variations throughout the journey from the farm to the slaughterhouse and during the holding time for AV (A), and WC (B).

-----Insert Table 3-----

-----Insert Figure 4-----

Table 3 and Figure 4 present the values obtained for AV (m/s) and WC (°C), which were determined by using the association of air temperature and air ventilation to measure the perceived equivalent temperature felt by the birds (NOAA, 2014). For AV, no interaction ($P \geq 0.01$) was found between the truck compartment and bath treatment. However, by separately analyzing firstly the AV and subsequently the WC felt by the birds, they indeed were significantly different ($P<0.01$). Lower average values of AV were observed at the IR and IM positions, regardless of whether the birds were under WiB or WoB treatment. The highest AV value was recorded at the SF position, exhibiting a difference of 3.05 m/s relative to the lowest values at the IM and IR positions.

Similarly to AV, no interaction was observed between the truck compartment and bath treatment for WC ($P\geq0.01$), although separately analyzing firstly the bath and subsequently the birds container position, they were significantly different ($P<0.01$). The WiB treatment group showed lower WC values. The animals in the SF presented lower WC values because of the lower T (Table 2) and highest AV (Table 3 and Fig. 4) values. The WC values varied from 3 to 18 °C. The 15 °C WC variation within the truck containers, which incorporated the AV measurements, was smaller than the temperature variation measured outside of the truck containers, which include an AV value in their determination (NOAA, 2014).

Meat Quality and meat color abnormalities

Watts *et al.* (2011) reported that birds exposed to negative temperatures had depleted energy reserves (glycogen), resulting in serious damage to animal welfare, an increase in DOA, and lower meat quality. These transport outcomes cause a great deal of financial loss for the poultry industry (Strawford *et al.* 2011).

The data in Table 4 indicate that for PSE-like meat incidences, no interaction was found for PSE-like variable ($P \geq 0.01$) between factors (truck compartment and bath treatment). However, when analyzing factors separately, the effect of truck compartment was significant ($P > 0.01$). Lower PSE-like values were found in meat samples taken equally from animals located at IR and IM, but the highest PSE-like meat incidences were found at the front of the truck compartment independent use of bath.

In testing for DFD-like meat, there was interaction ($P < 0.01$) between factors (truck compartment and bath treatment). The highest DFD-like value was found in meat samples taken equally from animals at IR and IM compartments under WiB treatment.–The lowest DFD-like meat incidence was found in birds located at every compartment of WoB group.

The highest incidence of DFD-like meat was found at the IM and IR compartments. Associating these results with the microclimate data (T, RH and AV) suggests that WC directly affected animal welfare; low temperature values combined with high wind velocity caused thermal discomfort (NOOA, 2014). Such hypothermal conditions cause depletion of muscle glycogen in order to keep the body warm. Basal metabolic activities are amplified in response to temperatures below the comfort zone in order to maintain body temperature in homoeothermic species (Julian *et al.* 1989; Dadgar *et al.* 2012). In addition, our results indicate high RH at the rear truck container leading to higher incidence of DFD-like meat, showing the relationship between RH, animal welfare, and thus meat quality.

-----Insert Table 4-----

The data in Table 5 indicate that for the pH and L* values, significant interactions (P<0.01) occurred between compartments and bath treatment. The highest pH values observed were for IM and IR compartments under WiB. Measured pH values did not differ under WoB regardless of truck compartment. For the L* value, there was an interaction (P<0.01) between truck compartments and bath treatment (Table 5), with lower values observed for IM and IR under WiB treatment and the highest values observed in WoB, regardless of truck compartment.

Temperatures inside poultry transport vehicles have been intensely studied and related to physiological signs for poor animal welfare and meat quality (Freeman *et al.* 1984; Hunter *et al.* 1999; Nijdam *et al.* 2005). The ambient temperatures for birds in-transit affect meat quality parameters (Dadgar *et al.* 2010; Spurio *et al.* 2015). Indeed in this experiment, we found differences in meat quality parameters due to the location of truck container compartments (Tables 4 and 5). Various authors have reported that exposure of birds to low temperatures (4, 5, and 7 °C) before slaughter resulted in breast meat with better functional properties because of higher *postmortem* pH values (Froning *et al.* 1978; Babji *et al.* 1982; Holm & Fletcher, 1997; Dadgar *et al.* 2012). In our case, the highest pH values were observed in IR and IM positions, indicating the importance of higher relative humidity in cold weather conditions affecting the WC while in-transit (Watts *et al.* 2011).

-----Insert Table 5-----

Our results showed that samples taken from IM and IR positions had darker breast meat. Color variation is an important attributed for consumer acceptance, especially if multiple color fillets were packed with noticeable color differences (Droval *et al.* 2012). According to

Dadgar *et al.* (2010), cold conditions during transport adversely affect the breast color, and at temperatures below 0 °C the breast meat darkened significantly. Bianchi *et al.* (2006) reported that chicken breast meat exposed to temperatures below 12 °C was significantly darker compared with those exposed to temperatures between 12 °C and 18 °C and above 18 °C. Babji *et al.* (1982) reported that turkeys subjected to cold weather (4 °C for 4 hours) and control (21 °C for 4 h) exhibited significantly higher myoglobin content than did turkeys subjected to heat treatment (38 °C) for 4 h.

Turkey breast meat harvested during the winter in Brazil had higher pH values and lower L * values than those for meat harvested during the summer (Carvalho *et al.* 2014). In our experiments, the environmental temperature of 5 °C and high relative humidity (Table 2) at IR and IM positions promoted the formation of DFD-like meat, while in the SF, IF and SM regions, there was a higher incidence of PSE-like meat. These findings led us to believe that two phenomena that impact animal welfare occurred simultaneously: hypothermia and hyperthermia. DFD-like meat was associated with hypothermia as the birds exposed to a wet environment with excessive RH and relatively low T, formed an unfavorable thermal core at the IR and IM compartments. Consequently, the birds used their reserved energy (glycogen) to maintain thermal homeostasis (Julian et al. 1989; Julian, 2005; Dadgar *et al.* 2010; Dadgar *et al.* 2012) resulting in DFD meat (McCurdy *et al.* 1996; Dadgar *et al.* 2010; Dadgar *et al.* 2012). The physiological stress leading to PSE-like meat formation can be closely associated to mammal malignant hyperthermia (MH), which is triggered by genetic factors (Fujii *et al.* 1991). The MH is responsible for the series of biochemical reactions resulting in uncontrollable glycolysis with increased lactic acid production because of the excessive Ca^{2+} within the sarcoplasm milieu (Cheah & Cheah, 1981). In our study, the frontal compartments showed a high-ventilation microenvironment in cold weather, resulting in homeostatic imbalance in the poultry for a short period, inducing the formation of PSE-like meat. The values of DOA (dead on arrival) were evaluated but not exposed, because the small number of deaths during transport (25 deaths in 21760 birds transported) did not generate sufficient data for statistical analysis.

In conclusion, the WC and RH were important factors on the bird's welfare in winter season. The results demonstrate that for turkeys transported under these conditions the breast meat quality was affected, bringing about variations in meat color abnormalities. Thus, a better truck container design is necessary to maintain an adequate microenvironment throughout the vehicle.

ACKNOWLEDGMENTS

The authors acknowledge funding for this project by CNPq Proc. 471609/2011-0. FGP holds a post-doctoral scholarship from CAPES Proc 23038007638201119. RHC is under CAPES and Fundação Araucária graduate scholarships. MRP, EII, and MS are CNPq Research Fellows. MS was also a Brazilian Senior Visiting Professor Scholar from CAPES.

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Figures and Legends

Figure 1 Turkey handling sequence for transportation from the farm to the processing plant.

Figure 2 A) Photograph of the truck container side loaded with turkeys showing the strategic locations of the data-loggers: SF: Superior Front, IF: Inferior Front, SM: Superior Middle, IM: Inferior Middle (IM), SR: Superior Rear and IR: Inferior Rear. B) Photograph of the truck container front-view showing the positions of the columns and the data-loggers (dark gray). B) Photograph of the truck container rear-view showing the positions of the columns and the data-loggers (dark gray). D) Photograph showing the positions of the data-loggers in the transport cage.

Figure 3 Variations in (A) temperature and (B) relative humidity (RH) in the container truck with elapsed time for the different turkey transport treatments superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR) and ambient temperature (AT) and ambient relative humidity (ARH) during a typical winter journey of 95 min and a holding time of 70 min at the slaughterhouse. n = 4 per treatment.

Figure 4 Variations in (A) air ventilation and (B) wind chill (WC) in the container truck with elapsed time for the different turkey transport treatments during a typical winter journey of 95 min and a holding time of 70 min at the slaughterhouse. n = 4 per treatment.

Table 1 Classification of PSE-like, normal, and DFD-like meat.

	L* values	pH values
[≠]PSE-like	L* > 53.00	pH < 5.60
[#]Normal	44.00 ≤ L * ≤ 53.00	5.60 ≤ pH ≤5.90
[#]DFD-like	L * < 44.00	pH > 5.90

[≠]Carvalho *et al.* (2014) [#]Carvalho *et al.* (in preparation).

Table 2 Mean values of the Temperature (T) and the Relative Humidity (RH) determined with (WiB) or without (WoB) bath treatments immediately before leaving the farm. Birds were located in one of 6 different container compartments: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR).

	Vehicle compartments	WiB	WoB	Average
#T (°C)	SF	6.28	13.93	10.10^{b}
	IF	7.06	14.38	10.72^{ab}
	SM	6.47	13.97	10.22^{b}
	IM	7.91	15.89	11.90^{a}
	SR	6.71	14.27	10.49^{ab}
	IR	7.99	15.87	11.93^{a}
CV= 15.62 %	Average	7.07^{B}	14.71^{A}	
$RH (%)	SF	61.77^{A,c}	50.07^{B,a}	55.92
	IF	66.60^{A,c}	49.38^{B,a}	57.99
	SM	65.93^{A,c}	50.22^{B,a}	58.07
	IM	74.39^{A,ab}	50.34^{B,a}	62.36
	SR	67.77^{A,bc}	50.73^{B,a}	59.25
	IR	75.25^{A,a}	50.66^{B,a}	62.95
CV= 11.74 %	Average	68.62	50.23	

$^{a-b}$ Different letters on the same column indicate significant differences, as measured by the Tukey test (P<0.01). $^{A-B}$ Different letters on the same line indicate significant differences, as measured by the Tukey test (P<0.01). $ The analyzed variables exhibited interaction to each other (P<0.01). # The analyzed variables did not exhibit interactions with each other (P≥0.01). n = 32 transport journeys.

Table 3 Mean values of the Air Ventilation (AV) and the Wind Chill (WC) measured with (WiB) or without (WoB) bath treatments immediately before leaving the farm. Birds were located in one of 6 different container compartments: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR).

	Vehicle compartments	WiB	WoB	Average
	SF	3.27	3.15	3.21[a]
	IF	1.61	1.60	1.61[b]
#AV	SM	0.83	0.81	0.82[c]
	IM	0.17	0.15	0.16[d]
	SR	0.67	0.65	0.66[c]
	IR	0.17	0.13	0.15[d]
CV= 24.13 %	Average	1.12[A]	1.08[A]	
	SF	3.86	13.16	8.51[c]
	IF	6.16	14.56	10.36[ab]
#WC	SM	6.66	14.89	10.77[ab]
	IM	10.43	18.41	14.42[a]
	SR	7.27	15.45	11.36[ab]
	IR	10.51	18.49	14.50[a]
CV= 23.21 %	Average	7.48[B]	15.82[A]	

[a-b] Different letters on the same column indicate significant differences, as measured by the Tukey test ($P<0.01$). [A-B] Different letters on the same line indicate significant differences, as measured by the Tukey test ($P<0.01$). # The analyzed variables did not exhibit interactions with each other ($P\geq0.01$). n = 32 transport journeys.

Table 4 Mean values of the PSE-like and the DFD-like incidence throughout the turkey transportation period with (WiB) or without (WoB) water bath treatments immediately before leaving the farm. Birds were located in one of 6 different truck container compartments: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR).

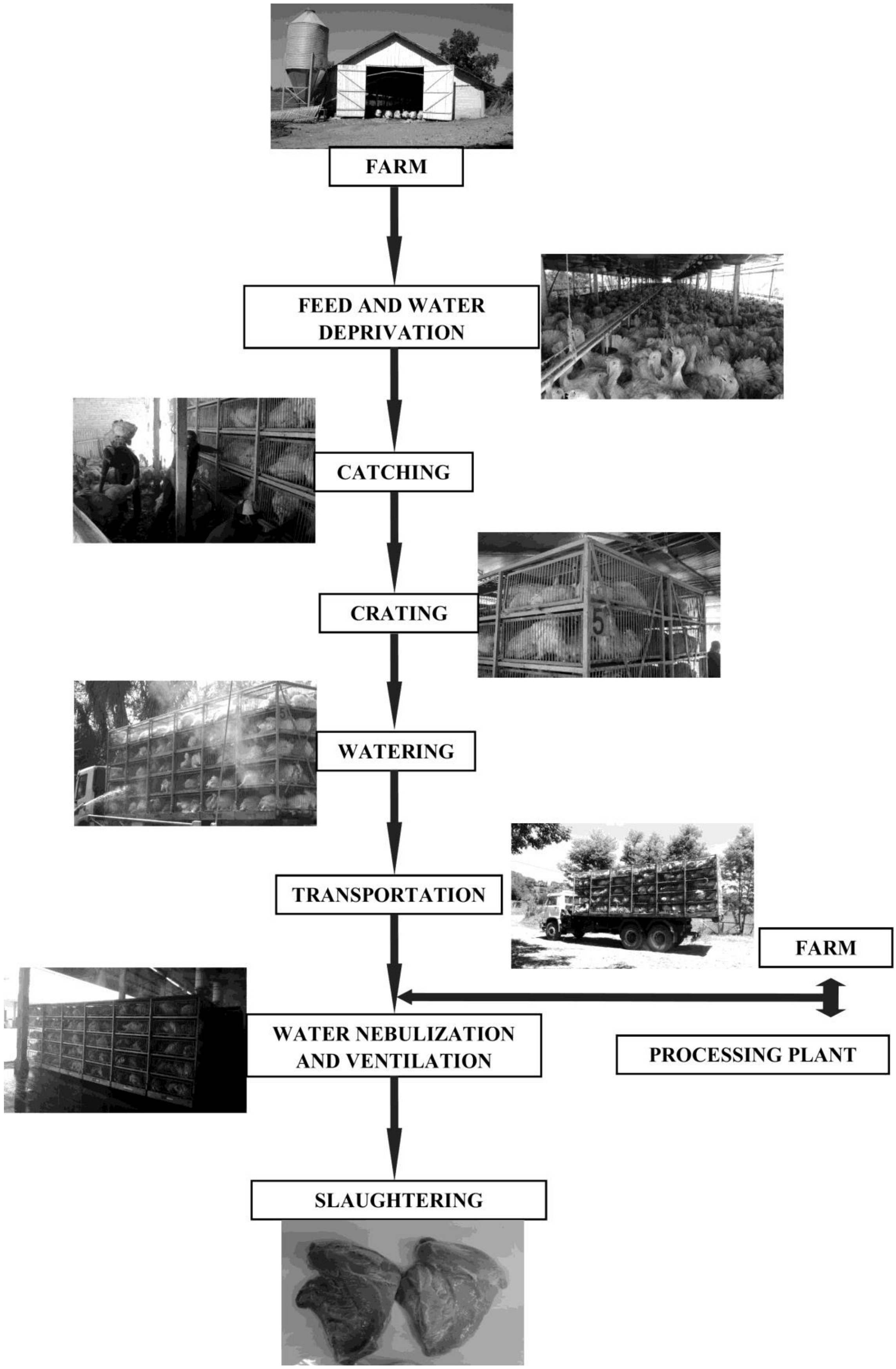
	Vehicle compartments	WiB	WoB	Average
#PSE-like	SF	0.12	0.07	0.09^{a}
	IF	0.09	0.06	0.07^{ab}
	SM	0.02	0.06	0.04^{abc}
	IM	0.01	0.01	0.01^{c}
	SR	0.05	0.02	0.04^{bc}
	IR	0.01	0.01	0.01^{c}
CV= 26.51 %	Average	0.05^{A}	0.04^{A}	
$DFD-like	SF	$0.04^{A,c}$	$0.03^{A,a}$	0.04
	IF	$0.04^{A,c}$	$0.03^{A,a}$	0.04
	SM	$0.13^{A,b}$	$0.05^{B,a}$	0.10
	IM	$0.28^{A,a}$	$0.06^{B,a}$	0.17
	SR	$0.17^{A,b}$	$0.04^{B,a}$	0.11
	IR	$0.25^{A,a}$	$0.06^{B,a}$	0.16
CV= 32.07 %	Average	0.15	0.04	

$^{a-b}$ Different letters on the same column indicate significant differences, as measured by the Tukey test (P<0.01). $^{A-B}$ Different letters on the same line indicate significant differences, as measured by the Tukey test (P<0.01). $The analyzed variables exhibited interactions with each other (P<0.01). #The analyzed variables did not exhibit interactions with each other (P≥0.01). n =1,344 breast meat samples were shared into transport journeys.

Table 5 Mean values of pH and L* determined with (WiB) or without (WoB) bath treatments immediately before leaving the farm. Birds were located in one of 6 different container compartments: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR).

	Vehicle compartments	WiB	WoB	Average
	SF	$5.79^{A,c}$	$5.81^{A,a}$	5.80
	IF	$5.82^{A,c}$	$5.84^{A,a}$	5.83
$^{\$}$pH	SM	$5.80^{A,c}$	$5.82^{A,a}$	5.81
	IM	$5.89^{A,ab}$	$5.85^{B,a}$	5.87
	SR	$5.84^{A,bc}$	$5.83^{A,a}$	5.84
	IR	$5.92^{A,a}$	$5.85^{B,a}$	5.89
CV= 35.98 %	Average	5.85	5.83	
	SF	$50.27^{A,a}$	$48.99^{A,a}$	49.63
	IF	$48.52^{A,ab}$	$48.24^{A,a}$	48.38
$^{\$}$L	SM	$48.15^{A,b}$	$48.46^{A,a}$	48.30
	IM	$45.67^{B,c}$	$47.37^{A,a}$	46.52
	SR	$47.81^{A,b}$	$47.49^{A,a}$	47.65
	IR	$44.27^{B,c}$	$47.07^{A,a}$	45.67
CV= 11.74 %	Average	47.45	47.93	

$^{a\text{-}b}$ Different letters on the same column indicate significant differences, as measured by the Tukey test (P<0.01). $^{A\text{-}B}$ Different letters on the same line indicate significant differences, as measured by the Tukey test (P<0.01). $^{\$}$The analyzed variables exhibited interactions with each other (P<0.01). n =1,344 breast meat samples were shared into transport journeys.


FARM
FEED AND WATER DEPRIVATION
CATCHING
CRATING
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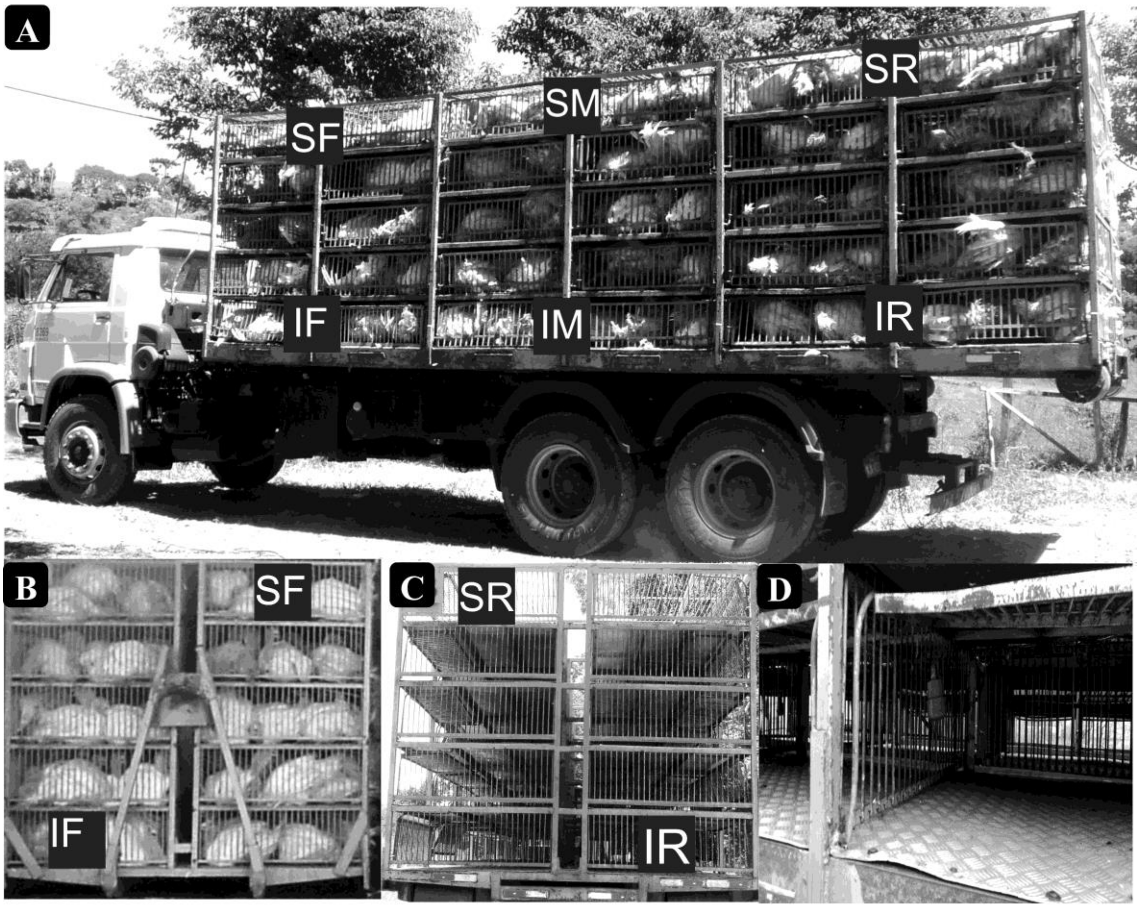
TRANSPORTATION
FARM
PROCESSING PLANT
WATER NEBULIZATION AND VENTILATION
SLAUGHTERING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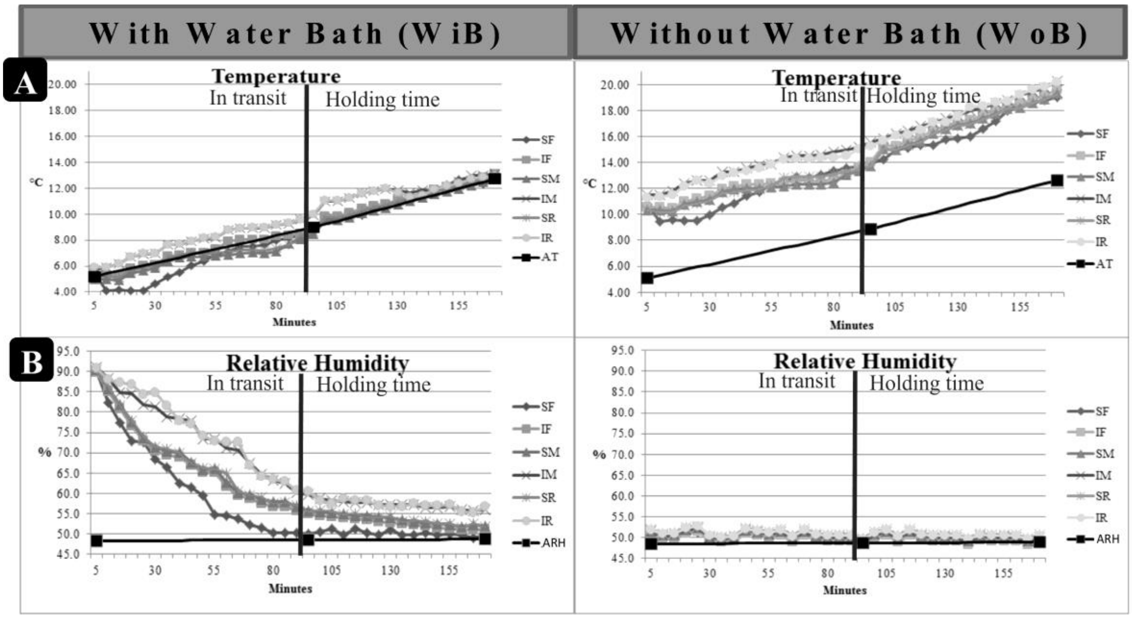
With Water Bath (WiB)
Without Water Bath (WoB)
A
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In transit
Holding time
°C
Minutes
SF
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SM
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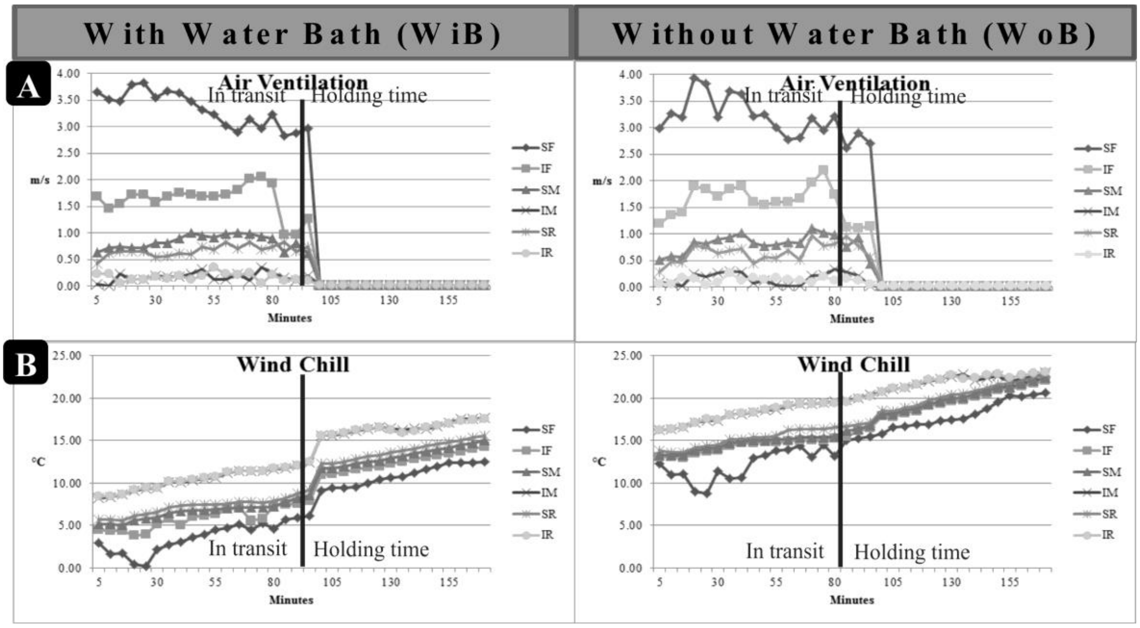
AT
B
Relative Humidity
%
ARH


With Water Bath (WiB)
Without Water Bath (WoB)
A
B
Air Ventilation
Wind Chill
In transit
Holding time
m/s
°C
Minutes
SF
IF
SM
IM
SR
IR

7 EXPERIMENTO E (Em preparação)

Animal Science Journal

Relationship between turkey breast filet PSE-like and mortality in transit and the microclimate generated within the commercial truck container.

O artigo encontra-se nas normas do periódico Animal Science Journal.

Endereço: http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1740-0929.

Relationship between turkey breast filet PSE-like and mortality in transit and the microclimate generated within the commercial truck container

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Short running title: Turkey transport and DOA index

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ABSTRACT

This study evaluated the effects of commercial transport practices on the welfare of turkeys by measurement of Dead on Arrivals (DOA) and Pale, Soft and Exudative (PSE-like) meat assessment. The experimental design was entirely randomized in a 6 × 2 factorial arrangement (truck container compartments × bath treatments) with birds positioned: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR) and two bath treatments: with bath (WiB) and without bath (WoB) with 8 replications for each treatment. The animals were transported for a time of 95 minutes journey from the farm to the slaughterhouse during the Brazilian summer. Results herein show the formation of a thermal core at the inferior middle and rear regions of the truck, likely developed due to heat production by the birds and influence of microclimate (temperature, relative humidity and air ventilation). The IM and IR container compartments under WoB treatment presented the highest ($P<0.01$) numbers of PSE-like meat incidence and DOA index values compared with those located at the front under WiB treatment. The microclimate affects the welfare and consequently the quality of turkey meat.

Key words: Animal welfare, commercial slaughterhouse, Nicholas 700, tropical climate, turkey meat quality.

INTRODUCTION

Although the country presents optimum performance either quanti- or qualitatively, the poultry industry in Brazil possesses some detrimental factors for maintaining meat quality particularly by locating within the tropical and subtropical regions suffering obviously warm climate by the average summer temperatures. Under uncomfortable transportation conditions, poultry can undergo unfavorable consequences, such as the dead on arrival (DOA) and breast pale, soft, exudative-like (PSE-like) meat.

Previous reports have described the effect of the management practices for poultry at the farm and during their transportation to the commercial slaughterhouse on the occurrence of PSE-like in breast fillet meat under both summer and winter conditions (Guarnieri *et al*. 2004; Carvalho *et al*. 2014). Similar experiments related to the transporting broilers have indicated that the animals suffered thermal stress, depending on their location within the open truck container (Simões *et al*. 2009; Langer *et al*. 2010). It was found out that the relative humidity (RH), air ventilation (AV), temperature (T) and also the formed heat index (HI) were factors that simultaneously influenced the stress level (Mitchell & Kettlewell 2004). These facts motivated recently the development of a truck container design in order to reduce broiler chicken PSE-like meat (Spurio *et al*. 2015).

Other research groups reported that pre-slaughter maneuvers were important to maintain the animal welfare and the meat quality. These activities consisted in water shower spray with resting after transport under high temperatures, relieving the stress condition recovering energy homeostasis indicating that the adenosine monophosphate-activated protein kinase (AMPK) was the key factor to regulate the muscle glycolysis (Jiang *et al*. 2015; Xing *et al*. 2015).

The discomfort due to thermal stress leads to physiological stress, which results in a faster post-mortem pH decline while the carcass temperature is still warm, resulting in the development of PSE-like meat (Guarnieri *et al*. 2004; Mitchell & Kettlewell 2004; Barbut *et al*. 2008). This phenomenon is promoted by the denaturation of myofibrillar and sarcoplasmic proteins, thereby compromising their functional properties, such as their texture, flavor, and juiciness, as reported by the consumers (Droval *et al*. 2012), and also in the meat products (Kissel *et al*. 2009; Carvalho *et al*. 2014). Losses of PSE-like poultry meat were estimated to cause an annual monetary loss of over US$ 200 million in the USA (Owens *et al*. 2009) and US$ 5.1 million in Brazil to the turkey industry (Carvalho *et al*. 2014).

DOA is a bird that died in the period between catching at the farm to the moment before slaughtering promoted particularly by inappropriate management maneuvers during transport consequently generating thermal stress leading to the increased mortality rate (Mitchell & Kettlewell 2004; Ritz *et al*. 2005).

The aim of this work was therefore to evaluate the effect of routine practices truck transportation from the farm to the commercial slaughterhouse during the Brazilian summer season on turkey welfare by measuring quantitatively the incidence of DOA and PSE-like meat.

MATERIALS AND METHODS

Experimental design and statistical analysis

The experimental design was randomized into a 6 × 2 factorial arrangement (truck position × water bath treatment) with 6 positions: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR) and two water bath treatments: with bath (WiB) and without bath (WoB), providing for 12 treatments, with 8 replicates for each treatment. WiB or WoB were carried out for 5 min just before leaving the farm. The data were analyzed using ANOVA and factorial analysis methods in the SISVAR statistical package (Ferreira 2008). Meaningful comparisons were generated using Tukey´s test (1% level). For incidences of PSE-like meat, the binary variation (1 and 0) was used, where 1 denoted PSE-like meat and 0 denoted normal meat.

Animals

This study was conducted during the summer on days that were selected for similar weather conditions from December 2012 to February 2013, in a commercial plant in Chapecó city (Latitude: 27º 05' 47" S; Longitude: 52º 37' 06" W; Altitude: 674m) Santa Catarina State, Brazil. The transportation conditions activities from the farm to the turkey commercial processing plant was as described in Carvalho *et al*. (2014).

The weather conditions in this region were characterized by the minimum and maximum temperatures of 24 and 36°C, respectively, and the relative humidity (RH) values varied from 43 to 67% throughout the 56 days of sampling, as measured using a Kestrel 4000 instrument (Nielsen-Kellerman, Boothwyn, PA, USA). The birds were of Nicholas 700 lineage, male gender, 140 days old, with an average live weight of 18 ± 2 kg and grown under regular acclimatized aviaries. The feed was removed 9 to 12 h before slaughter, and water was provided *ad libitum*. The animals were manually placed into crates at a density of 8 birds (98 ± 2 kg/m^2) each and installed within the truck open container. The average catching and loading time was 24 min and after that the birds were subject or not to the water bath treatment for approximately 5 min. Birds were handled in accordance with the principles and procedures outlined by the Londrina State University Animal Care and Use Ethical Committee (167/2015).

Truck Container Microenvironment Assessment

Six portable weather meter devices with bidirectional Kestrel anemometers and data logging capability were set to take measurements at 30-second intervals during each journey. Instruments were fixed and oriented in the direction of highest velocity, all devices were calibrated for relative humidity, temperature and ventilation before the experiment. The devices were installed (suspended in cage, among the birds) at the SF, IF, SM, IM, SR and IR of the truck container compartments and at positions between columns 1 and 2, 3 and 4, and 5 and 6, as shown by the gray rectangles in Figure 1. Ambient temperature and relative humidity were measured at three different points: at the beginning of transport, arrival at the slaughterhouse plant and during holding time.

-----Insert Figure 1-----

The relative humidity (RH), air ventilation (AV), and temperature (T) values were determined as in Spurio *et al*. (2015), and heat index (HI) values were simultaneously measured allowing for a representative analysis of the heterogeneous distribution of the thermal microenvironment within the loaded vehicle. HI, or apparent temperature, is an index that combines air temperature and relative humidity to measure the perceived equivalent corporal temperature (NOAA, 2014). RH, AV, T, and HI were automatically obtained from the 6 data loggers and downloaded to a computer.

Transport and Slaughter

A total of 32 transport journeys were evaluated, either with bath (WiB) (n=16) for 5 minutes or without bath (WoB) (n=16). These processes of birds harvesting and loading were only started when the ambient temperature has reached the temperature of 33 °C. The animals were grown on 16 turkey farms under a cooperative system and subsequently transported over a distance of 38 ± 10 km for a journey that takes approximately 95 ± 20 min. Upon arrival at the slaughterhouse facilities, the birds were placed in a holding area under water mist and ventilation for 70 min before slaughtering. The animals were sacrificed according to standard industry practices, which consisted of hanging, electrically stunning, bleeding, scalding, defeathering, evisceration, cooling the carcass through a tunnel of cold air (-6°C for 6 h) and deboning (Carvalho *et al*. 2014). Subsequently, the breast meat samples (*Pectoralis major*) were collected and refrigerated at 4°C until completing 24 h to measure the color (L* value) and pH and thus evaluating the occurrence of PSE-like meat (Carvalho *et al*. 2014).

PSE-like Meat Measurement

Samples of breast fillets (n=1,344) (*Pectoralis major*) were collected (42 animals per transport load). The meat samples were classified as PSE-like according to their L* and pH values (Testo AG, Lenzkirch, Germany), with L* > 53 and pH < 5.60, considered typical for PSE-like turkey meat, as thoroughly discussed in Carvalho *et al*. (2014).

Color determinations were performed using a Minolta CR-400 colorimeter using five different reading points per sample (L*, a*, b*), as described in Carvalho *et al.* (2014). The pH was measured in duplicate by inserting electrodes into the breast meat (Testo 205, Testo AG, Lenzkirch, Germany) as described in Carvalho *et al.* (2015b).

Dead on Arrival (DOA)

The percentage of DOA birds per loaded truck was calculated by counting each dead bird during the hanging step at the slaughtering plant, taking care to observe the truck region from which the birds were collected (Spurio *et al.* 2015).

DOA = (Number of birds dead / Number of birds transported) x 100

RESULTS AND DISCUSSION

Meat Color Abnormalities, PSE-like Incidence and DOA Index

Table 1 lists the results of pH and L* values. No significant interactions between bath treatment and truck container compartment position for pH and L* ($P \geq 0.01$) were observed. However, by analyzing separately first the bath treatments and subsequently the birds' container positions, they indeed were significantly different ($P<0.01$). The lowest pH values observed were for IM and IR compartments and WoB treatment. Oppositely, for the lightness (L*), highest values observed for IM and IR compartments and the highest values observed in WoB.

-----Insert Table 1-----

The data in Table 2 indicate that for PSE-like meat incidences, no interaction was found for PSE-like variable ($P \geq 0.01$) between factors (truck compartment and bath treatment). However, when analyzing factors separately, the effect of truck compartment was significant ($P<0.01$). Highest PSE-like values were found in meat samples taken equally from animals located at IR and IM, but the lower PSE-like meat incidences were found at the SF, IF, SM and SR of the truck compartment. In testing for DOA index, there was interaction ($P<0.01$) between factors (truck compartment and bath treatment). The highest DOA index was found in meat samples taken equally from animals at IR and IM compartments under WoB treatment.

-----Insert Table 2-----

Values of T, RH, AV and HI in transit

Table 3 lists the results of T and RH. No significant interactions between bath treatment and truck container compartment position for T ($P \geq 0.01$) were observed. However, by analyzing separately first the bath and subsequently the birds' container positions, they indeed were significantly different ($P<0.01$). Pre-transport water baths caused a temperature drop within the every truck compartment. WoB group temperatures were an average of 5.37 °C higher than WiB treatment. Positionally, the temperatures of inferior regions of the truck IM (34.87 °C) and IR (34.89 °C) position were highest than those in the SF (33.05 °C) compartments (Table 3).

-----Insert Table 3-----

RH strongly correlated with truck compartment position and bath treatment ($P<0.01$). The highest RH values were observed for IM and IR in the WiB groups, and the lowest RH values occurred for WoB groups regardless the truck compartment. Figure 2 shows detailed step-by-step variations throughout the journey from the farm to the processing plant and subsequent the holding time for T (A) and RH (B).

-----Figure 2----

Table 4 and Figure 3 present the values obtained for AV (m/s) and HI (°C), which were determined by using the association of air temperature and relative humidity to measure the perceived equivalent temperature (NOAA 2014). For AV, no interaction ($P \geq 0.01$) was found between the truck compartment and bath treatment. However, by separately analyzing firstly the truck compartment and subsequently the bath treatment, the truck compartment was significantly different ($P<0.01$). Lower average values of AV were observed at the IR and IM positions, regardless of whether the birds were under WiB or WoB treatment. The highest AV value was recorded at the SF position, exhibiting a difference of 3.18 m/s relative to the lowest values at the IM and IR positions. Similarly, to AV, no interaction was observed between the truck compartment and bath treatment for HI ($P\geq0.01$), although separately analyzing firstly the bath and subsequently the birds container position, they were significantly different ($P<0.01$). The WiB treatment group showed lower HI values. The SF and SM compartments presented lower HI values because of the lower T (Table 3 and Figure 2A) and lower RH (Table 3 and Figure 2B) values. Figure 4 shows detailed step-by-step variations throughout the journey from the farm to the processing plant and subsequent the holding time for AV (A) and HI (B).

-----Insert Table 4-----

-----Insert Figure 3-----

Relationship of microlimate, PSE-like Meat Incidence and DOA Index

Tables 2 and 3 indicate that IR and IM compartments had the highest value for the variables T and RH. In addition, as expected, AV presented the lowest value at the IR or IM positions consequently the HI index presented the highest value (Table 4), formed an unfavorable thermal core at the IR and IM compartments. Therefore, the highest value for the PSE-like meat incidence and for the DOA index (Table 2) was expected at these same compartments, suggesting that those locations were where the birds faced the poor welfare conditions and thus underwent thermal stress. As shown in Figure 3B, the HI pattern of the birds throughout the journey was different, depending on whether the water bath was applied before leaving the farm. Under WiB treatment, the HI values remained between 42 °C at the beginning to its maximum 46 °C after 120 min of the journey at a holding time at the IR or IM positions, whereas under WoB treatment, the HI values increased gradually from 43 to 55 °C under similar conditions, indicating that the water bath alleviated the uncomfortable animals conditions. In fact, the DOA index at the IR position was approximately 80 % higher under WoB treatment compared with WiB treatment (Table 2).

In conclusion, the thermal core was formed primarily at the rear and middle inferior compartments of the vehicle container, and the birds located at these zones produced higher amounts of DOA index and a higher PSE-like meat incidence values. A water bath treatment applied to the birds while at the farm reduced the occurrence of DOA index and PSE-like meat incidence values. A better truck container design is necessary to obtain adequate ventilation throughout the vehicle to improve the animal welfare in transit.

ACKNOWLEDGMENTS

The authors acknowledge funding for this project by CNPq Proc. 471609/2011-0. FGP holds a post-doctoral scholarship from CAPES Proc 23038007638201119. RHC is under CAPES and Fundação Araucária graduate scholarships. MRP, EII, and MS are CNPq Research Fellows. MS was also a Brazilian Senior Visiting Professor Scholar from CAPES.

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Figures and Legends

Figure 1 Turkey handling sequence for transportation from the farm to the processing plant.

Figure 2 A) Photograph of the truck container side loaded with turkeys showing the strategic locations of the data-loggers: SF: Superior Front, IF: Inferior Front, SM: Superior Middle, IM: Inferior Middle (IM), SR: Superior Rear and IR: Inferior Rear. B) Photograph of the truck container front-view showing the positions of the columns and the data-loggers (dark gray). B) Photograph of the truck container rear-view showing the positions of the columns and the data-loggers (dark gray). D) Photograph showing the positions of the data-loggers in the transport cage.

Figure 3 Variations in (A) temperature and (B) relative humidity (RH) in the container truck with elapsed time for the different turkey transport treatments superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR) and ambient temperature (AT) and ambient relative humidity (ARH) during a typical winter journey of 95 min and a holding time of 70 min at the slaughterhouse. n = 4 per treatment.

Figure 4 Variations in (A) air ventilation and (B) heat index (HI) in the container truck with elapsed time for the different turkey transport treatments during a typical winter journey of 95 min and a holding time of 70 min at the slaughterhouse. n = 4 per treatment.

Table 1. Mean values of the pH and the lightness (L*) measurement throughout the turkey transportation period either with water bath (WiB) or without water bath (WoB) treatments before leaving the farm. The birds were located at 6 different truck container regions: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR)

	Vehicle regions	WiB	WoB	Average
	SF	5.76	5.74	5.75^{a}
	IF	5.74	5.72	5.73^{a}
#pH	SM	5.75	5.73	5.74^{a}
	IM	5.70	5.68	5.70^{b}
	SR	5.73	5.70	5.71^{ab}
	IR	5.70	5.67	5.69^{b}
CV= 3.74 %	Average	5.73^{A}	5.71^{B}	
	SF	49.20	50.83	49.08^{d}
	IF	48.35	49.81	50.01^{c}
#L*	SM	49.64	50.79	50.22^{bc}
	IM	51.80	53.25	52.53^{a}
	SR	50.29	51.09	50.69^{b}
	IR	52.27	53.20	52.73^{a}
CV= 4.65 %	Average	50.26^{B}	51.50^{A}	

$^{a\text{-}b\text{-}c}$ Different letters on the same column indicate significant differences, as measured by the Tukey test ($P<0.01$). $^{A\text{-}B}$Different letters on the same line indicate significant differences, as measured by the Tukey test ($P<0.01$). #The analyzed variables did not exhibit interactions with each other ($P\geq0.01$). n =1,344 divided into transport journeys

Table 2. Mean values of the PSE-like meat incidence and the DOA index values throughout the turkey transportation period either with water bath treatments (WiB) or without water bath (WoB) treatments before leaving the farm. The birds were located at 6 different truck container regions: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR)

	Vehicle regions	WiB	WoB	Average
	SF	4.5	6.6	5.5^{b}
	IF	5.3	8.5	6.9^{b}
# PSE-like (%)	SM	4.8	6.4	5.6^{b}
	IM	8.9	21.1	15.0^{a}
	SR	5.1	10.2	7.6^{b}
	IR	11.8	22.6	17.2^{a}
CV= 21.52 %	Average	6.7^{A}	12.6^{A}	
	SF	$0.01^{A,a}$	$0.12^{A,bc}$	0.06
	IF	$0.06^{A,a}$	$0.12^{A,bc}$	0.09
$ DOA (%)	SM	$0.01^{A,a}$	$0.01^{A,c}$	0.01
	IM	$0.12^{B,a}$	$0.43^{A,b}$	0.28
	SR	$0.01^{A,a}$	$0.06^{A,bc}$	0.03
	IR	$0.25^{B,a}$	$1.31^{A,a}$	0.78
CV= 26.01 %	Average	0.07	0.34	

[a-b] Different letters on the same column indicate significant differences, as measured by the Tukey test (P<0.01). [A-B] Different letters on the same line indicate significant differences, as measured by the Tukey test (P<0.01). [#]The analyzed variables did not exhibit interaction with each other (P≥0.01). [$]The analyzed variables exhibited interaction with each other (P<0.01).

n = 1,344 divided into transport journeys (PSE-like meat). n = 32 transport journeys (DOA)

Table 3. Mean values of the Temperature (T) and the Relative Humidity (RH) determined with birds with (WiB) and without bath (WoB) treatments just before leaving the farm. They were located at 6 different container regions: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR)

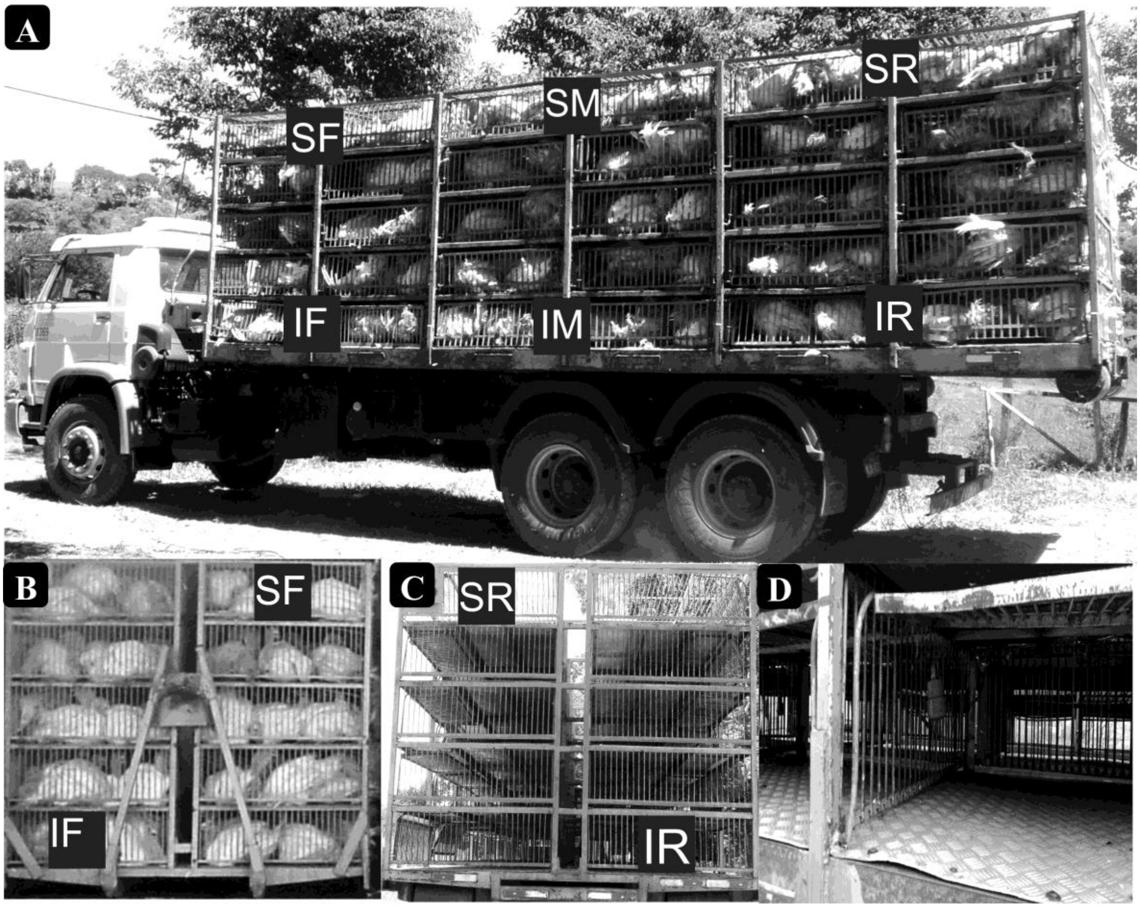
	Vehicle regions	WiB	WoB	Average
	SF	30.37	35.73	33.05^{c}
	IF	31.18	36.18	33.68^{b}
$^{\#}$T (°C)	SM	30.60	35.92	33.26^{bc}
	IM	32.04	37.70	34.87^{a}
	SR	30.83	36.06	33.45^{bc}
	IR	32.08	37.71	34.89^{a}
CV= 10.50 %	Average	31.18^{B}	36.55^{A}	
	SF	$62.28^{A,c}$	$50.47^{B,a}$	56.37
	IF	$67.00^{A,c}$	$49.75^{B,a}$	58.38
$^{\$}$RH (%)	SM	$66.30^{A,c}$	$50.62^{B,a}$	58.46
	IM	$74.79^{A,ab}$	$50.71^{B,a}$	62.75
	SR	$68.13^{A,bc}$	$51.25^{B,a}$	59.69
	IR	$75.76^{A,a}$	$51.06^{B,a}$	63.41
CV= 11.56 %	Average	69.04	50.64	

$^{a\text{-}b\text{-}c}$ Different letters on the same column indicate significant differences, as measured by the Tukey test ($P<0.01$). $^{A\text{-}B}$Different letters on the same line indicate significant differences, as measured by the Tukey test ($P<0.01$). $^{\#}$The analyzed variables did not exhibit interactions with each other ($P\geq0.01$). $^{\$}$The analyzed variables exhibited interactions with each other ($P<0.01$). n = 32 transport journeys

Table 4. Mean values of the Air Ventilation (AV) and the Heat Index (HI) measured under 2 treatments, i.e., with (WiB) and without a bath (WoB) before leaving the farm, at the 6 different container regions throughout turkey transportation: superior front (SF), inferior front (IF), superior middle (SM), inferior middle (IM), superior rear (SR) and inferior rear (IR)

	Vehicle regions	WiB	WoB	Average
	SF	3.52	3.40	3.46^{a}
	IF	1.87	1.85	1.86^{b}
#AV (m/s)	SM	1.08	1.06	1.07^{c}
	IM	0.27	0.29	0.28^{d}
	SR	0.91	0.90	0.91^{c}
	IR	0.25	0.28	0.26^{d}
CV= 29.08 %	Average	1.32^{A}	1.30^{A}	
	SF	34.06	42.65	38.35^{c}
	IF	37.17	43.48	40.33^{b}
# HI (°C)	SM	35.55	43.21	39.38^{bc}
	IM	42.35	48.12	45.24^{a}
	SR	36.61	43.86	40.23^{b}
	IR	42.88	48.35	45.62^{a}
CV= 13.21 %	Average	38.10^{B}	44.95^{A}	

$^{a-b-c-d}$ Different letters on the same column indicate significant differences, as measured by the Tukey test (P<0.01). $^{A-B}$ Different letters on the same line indicate significant differences, as measured by the Tukey test (P<0.01). #The analyzed variables did not exhibit interactions with each other (P≥0.01). n = 32 transport journeys.


A
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C
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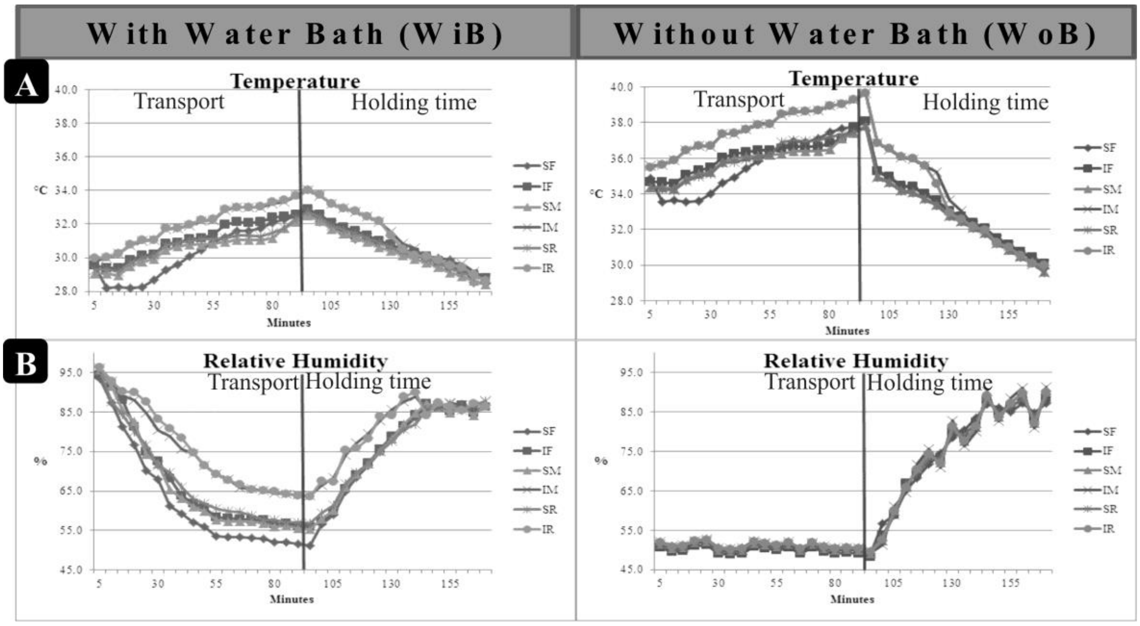
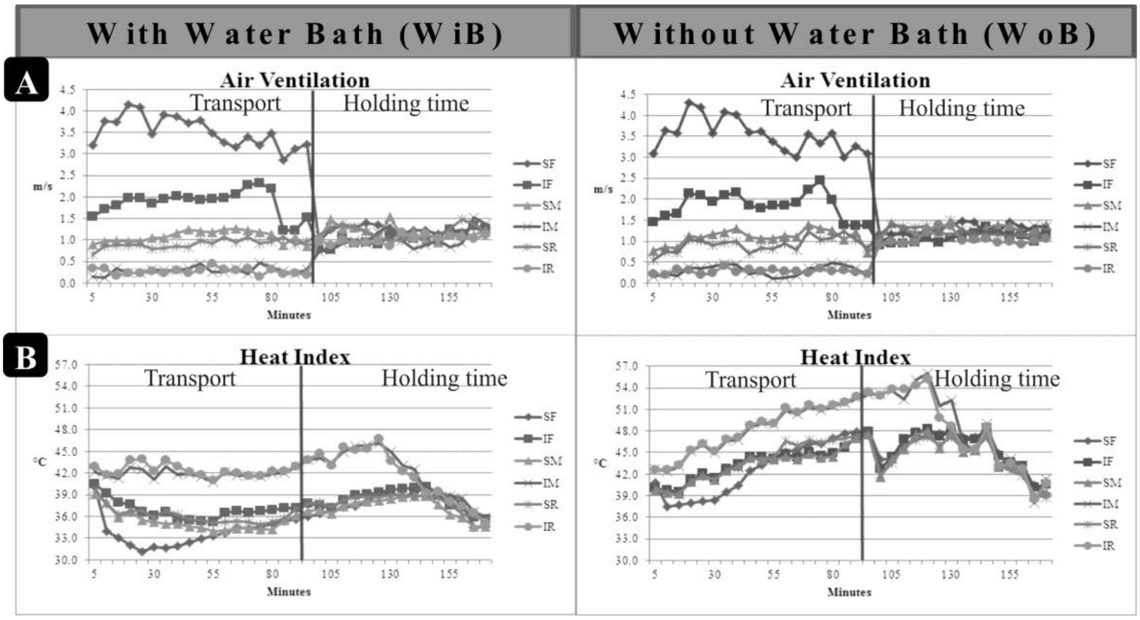
D



With Water Bath (WiB)
Without Water Bath (WoB)
A
Temperature
Transport
Holding time
°C
Minutes
SF
IF
SM
IM
SR
IR
B
Relative Humidity
%



With Water Bath (WiB)
Without Water Bath (WoB)
A
Air Ventilation
Transport
Holding time
m/s
Minutes
SF
IF
SM
IM
SR
IR
B
Heat Index
°C

8 EXPERIMENTO F (Em preparação)

Animal Science Journal

Influence of distance on turkey transportation conditions and the occurrence of DOA, PSE-like (Pale, Soft, and Exudative) and DFD-like (dark, firm and dry) meat in different seasons

O artigo encontra-se nas normas do periódico Animal Science Journal.

Endereço: http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1740-0929.

Influence of distance on turkey transportation conditions and the occurrence of dead on arrival (DOA), PSE-like (Pale, Soft, and Exudative) and DFD-like (dark, firm and dry) meat in different seasons

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Running title: Turkey transport in different seasons

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ABSTRACT

Poor meat quality and living conditions are the cause of high losses to poultry processing plants. In Brazil, the formation of a thermal microclimate within the vehicle during the transport of turkey from farm to slaughterhouse affects the birds' welfare and potentially promotes abnormalities in meat and dead on arrival (DOA). Thus, the aim of this work was to evaluate the effects of routine practices on the vehicle microclimate formed during transportation of turkey in both summer and winter seasons. Nicholas male turkeys (140d of age) were divided into 4 groups and were transported by truck. Treatments: 1- long distance (LD-48km) under bath pre-transport (WiB); 2-short distance (SD-22km) under bath pre-transport (WiB); 3-LD under without bath (WoB) and 4-SD under WoB. The experiments were performed during winter and summer seasons. The microclimate inside the truck (temperature, relative humidity and ventilation) was analyzed. PSE-like, DFD-like and DOA indexes were evaluated. Turkey transportation in summer showed higher temperature increase consequently higher PSE-like meat development of and DOA index, whereas in winter season, the lower temperature caused meat darkness (L*) and higher incidence of DFD-like meat. The water bath at the farm reduced the occurrence of PSE-like meat in the summer but it increased during the winter. Long distances/times during summer were deleterious to the birds' welfare, producing a higher incidence of PSE-like meat and DOA index.

Key words: Abnormalities of meat, Animal welfare, commercial slaughterhouse, meat quality, poultry.

INTRODUCTION

Previous works described for the first time the effect of turkey management practices at the farm and during transportation from the farm to the slaughterhouse on the occurrence of PSE-like and DFD-like meat in breast fillet meat in the Brazilian summer and winter seasons. The pre-slaughter management from farm to the slaughterhouse under different stresses conditions such as higher temperature and relative humidity, ventilation, acceleration, vibration, movement, impact, food and water deprivation, social disruption, and noise affects the bird´s welfare (Nicol and Scott 1990; Mitchell & Kettlewell 1998; Simões *et al.* 2009; Spurio *et al.* 2015).

These combinations reflect directly the quality of the final product (meat) and most often they are primarily responsible for higher losses to the industries. In transit, birds are subjected to many stressing situations that compromise their welfare, and cause a high mortality rate and meat lower quality (Mitchell *et al.* 1992; Guarnieri *et al.* 2004; Simões *et al.* 2009; Langer *et al.* 2010; Spurio *et al.* 2015).

The poultry industry in Brazil has several detrimental characteristics for maintaining meat quality, due to a tropical-subtropical climate and strong seasonal thermal oscillations (Carvalho *et al.* 2014; 2015a).

Previous studies reported that pre-transport water bath applied at the farm reduced the occurrence of PSE syndrome in meat. Long distances and higher times and long transit times are harmful to the birds’ welfare, resulting in more PSE meat (Simões *et al.* 2009; Spurio *et al.* 2015; Jiang *et al.* 2015). It is known that the microclimate in the truck rear it is not homogeneous, with variations in ventilation rate and other living parameters (Hoxey *et al.* 1996; Simões *et al.* 2009; Langer *et al.* 2010). Therefore, the objective of this study was to evaluate the effects of pre-slaughter water bath and distance/time transportation on the quality of turkey meat during summer and winter seasons.

MATERIALS AND METHODS

Experimental Designed and Statistical Analysis

The experimental design was entirely randomized in a 2 × 2 factorial arrangement (distance × shower treatment) with 2 distance: short distance (SD) and long distance (LD) and two bath treatments: with bath (WiB) and without bath (WoB), for a total of 4 treatments, with 8 replications for each treatment, and assays were performed in the summer and winter seasons, separately. The data were analyzed using ANOVA and factorial analysis methods with the SISVAR statistical package (Ferreira, 2008). Meaningful comparisons were generated using Tukey´s test (1% level). For the incidence of PSE-like meat, the binary variation (1 and 0) was used, where 1 denotes PSE-like meat and 0 denotes normal meat. For the incidence of DFD-like meat, the same binary variation (1 and 0) was used, where 1 denotes DFD-like meat and 0 denotes normal meat.

Animals

This study was conducted in the summer and winter seasons. The chosen transport days were carefully selected for similar weather conditions from December 2012 to August 2013 (Table 1), in a commercial plant in the city of Chapecó (Latitude: 27º 05' 47" S; Longitude: 52º 37' 06" W; Altitude: 674m) Santa Catarina State, Brazil. The transportation activities from the farm to the turkey commercial processing plant are illustrated in Carvalho *et al.* (2014).

-----Insert Table 1**-----**

The weather conditions (Table 1) in the region were characterized by the minimum and maximum temperatures. The temperature and relative humidity (RH) were measured using a Kestrel 4000 instrument. The feed was removed 9 to 12 hrs before slaughter, and water was provided *ad libitum*. The animals were manually placed into crates at a density of 8 birds (98 ± 2 kg/m^2) each and installed within the truck open container. The average catching and loading time was 24 min. Birds were handled in accordance with the principles and procedures outlined by the Londrina State University Animal Care and Use Ethical Committee (Process #167/2015).

Truck Container Microenvironment Assessment

Six portable weather meter devices with bidirectional Kestrel anemometers and data logging capability were set to take measurements at an interval of 30 seconds during the journeys (Spurio *et al.* 2015). The temperature (T), relative humidity (RH), air ventilation (AV), heat index (HI) and wind chill (WC) values were simultaneously measured as in Spurio *et al.* (2015), allowing for a representative analysis of the heterogeneous distribution of the thermal microenvironment within the loaded vehicle. HI or apparent temperature is an index that combines air temperature and RH to measure the observed equivalent temperature, and WC or apparent temperature is an index that combines air temperature and air ventilation to measure the perceived equivalent temperature (NOAA, 2014ab)

Transport and Slaughter

A total of 64 transport journeys were evaluated, divided in summer (n=32) and winter (n=32) either with bath (WiB, n=16 per season) for 5 minutes or without bath (WoB, n=16 per season) application before leaving the farm. The animals were grown on 32 turkey farms under a cooperative system and subsequently transported over a distance as shown in Table 2.

-----Insert Table 2**-----**

The birds were placed in holding area under water mist and ventilation for 70 min before slaughtering. The animals were sacrificed according to standard industry practices, which consist of hanging, electrically stunning, bleeding, scalding, defeathering, evisceration, cooling the carcass through a tunnel of cold air (6°C for 6 h) and deboning. Subsequently, the breast meat samples (*Pectoralis major*) were collected and refrigerated at 4°C for 24 h to measure the color and pH and thereby determine the occurrence of PSE meat (Carvalho *et al.* 2014).

Color and pH measurements in fresh breast meat

The meat samples (n = 2,676) were classified as PSE-like, normal and DFD-like according to their L* values and pH as thoroughly discussed in Carvalho *et al.* (2014) and Carvalho *et al*. (in preparation), respectively.

- ✓ L* > 53.00 and pH < 5.60 = PSE-like meat.
- ✓ Normal: L* ≤ 53.00 and L* ≥ 44.00; and pH ≤ 5.90.
- ✓ L* < 44.00 and pH > 5.90 = DFD-like meat

This evaluation was performed using a Minolta CR-400 colorimeter, taking five readings at different points per sample for color determination (L*, a*, b*), as described by Carvalho *et al.* (2015b). The pH was measured (in duplicate) by inserting electrodes into the *pectoralis major m* as described in Carvalho *et al.* (2015b).

Mortality evaluated by Dead on Arrival (DOA)

A total 30,720 birds were transported, divided in summer (n = 15,360) and winter (n=15,360). The percentage of DOA per loaded truck was calculated by counting each dead bird during the hanging step at the slaughtering plant, taking care to observe the truck region from which the birds were collected (Spurio *et al.* 2015).

DOA = (Number of birds dead / Number of birds transported) * 100

RESULTS AND DISCUSSION

The temperature in transit as expected was higher in the summer season referring to the days of sampling (Table 1). For parameters such as RH and AV the behavior of variables was similar in the two seasons over the same distances (Table 3 and 4). HI values and WC were calculated respectively for summer and winter according to considerations guidelines by NOOA (2014ab). Table 3 shows results of temperature (T) and relative humidity (RH). For T no interactions ($P \geq 0.01$) between shower treatment and distance in summer were observed. However, by analyzing separately first the bath and subsequently the distance, they indeed were significantly different (P<0.01). Applying the bath pre-transportation lowers the temperature in of the truck were verified, for WoB groups the temperature was 5.4 °C higher than that observed at the WiB groups. In relation to distance, the temperatures at the SD treatment was lower (0.8 °C) compared with LD treatment.

-----Insert Table 3-----

In winter, there was an interaction between distance and shower treatment (P<0.01). The highest values were observed at the LD in WoB groups, whereas the lowest value occurred at the SD in WiB groups. Applying the bath pre-transportation temperature was 7.8 °C lowest at the SD in WiB groups than that observed at the LD in WoB groups. In general, with the application of pre-transport bath a decrease was observed in T and increased RH. Similar results were found by Simões *et al.* (2009), Langer *et al.* (2010) and Spurio *et al.* (2015). Both seasons at the LD in WoB groups higher temperature values were found in relation to SD, this phenomenon provided by transport time and heat produced by the birds (Simões *et al.* 2009; Strawford *et al.* 2011; Watts *et al.* 2011). According to Watts *et al.* (2011) birds in temperatures close to 20 °C produce less heat compared to birds transported at low temperatures (below 0 °C) resulting in increased heat during transport.In our study, the same phenomenon was observed, in fact in winter seasons evidently the birds produced more heat (Xin *et al*. 2001; Watts *et al.* 2011). Figure 1 shows in detail step-by-step variations throughout the journey from the farm to the slaughterhouse and during the holding time for T (A), and RH (B).

-----Insert Figure 1-----

For the RH in summer and winter seasons, there was an interaction (P<0.01) between the distance and the shower treatment (Table 3). In both seasons the highest values were observed at the SD in WiB groups, 27% approximately, whereas the lowest value occurred for WoB groups, independently of distance analyzed. Table 4 presents the determined values of AV, HI (summer) and WC (winter) which were determined by using the combination of the air temperature, relative humidity and the air ventilation to measure the perceived equivalent temperature (NOAA, 2014a,b). Figure 2 shows in detail step-by-step variations throughout the journey from the farm to the slaughterhouse and during the holding time for AV (A), and HI (B) and WC (C).

-----Insert Table 4-----

-----Insert Figure 2-----

For AV in summer and winter seasons, no interaction (P>0.01) and significant difference was found between the distance and the shower treatment. In transit the ventilation was unstable. In the summer season spray baths were applied during the holding period in conjunction with air ventilation and exhaust to improve the comfort of the birds. Jiang *et al.* (2015) indicated that bath after transport with forced ventilation decreased stress caused by transport under high temperature during summer. In winter there was no activation of air ventilation and spray bath, only a lower exhaust rate for the renewal of gases among rears. This phenomenon can be seen in detail in Figure 2. Guarnieri *et al.* (2004) reported the importance of holding time for animal welfare and meat quality.

No interaction (P≥0.01) between distance and shower treatment was observed for HI in summer season. However, by analyzing separately first the bath and subsequently the distance, they indeed were significantly different (P<0.01). The WiB treatment presented the lower HI temperature (7.7 °C) compared WoB groups. The SD presented lower HI (1.1 °C) values as a consequence of the lower T (Table 3). In relation WC in winter there was an interaction (P<0.01) between the distance and the shower treatment, with the lowest values observed for SD in WiB groups, using the bath pre-transportation temperature was 12.8 °C lower than that observed for the LD in WoB groups. Reports in the literature showed that the climatic condition during the transport of turkeys and chickens together with the variables of time (minutes) and distance (km) affect negatively or positively meat quality and animal welfare.

The data in Table 5 indicate that for the PSE-like and DFD-like meat incidence, there was interaction between factors (distance and shower treatment). In summer, a highest PSE-like meat value was found in meat samples taken equally from animals transported for a LD and WoB groups, oppositely in winter the highest PSE meat value was found in animals transported for a SD and WiB groups, our results showed a higher incidence of PSE meat in the summer compared to winter, such results corroborate McKee and Sams (1997); Owens and Sams (2000) in turkey and Voslarova *et al.* (2007) and Langer *et al.* (2010) in broiler, wherein the summer season with higher incidence of PSE meat.

In relation DFD-like meat in summer did not present abnormality of incidence for possible statistical analysis, in winter highest DFD-like meat value was found in meat samples taken equally from animals transported for a LD and WiB treatment, SD in WoB groups showed lowest incidence. The climatic conditions of tropical countries are critical to the development of these anomalies, where climate it has large oscillation.

DOA during the summer the highest values for DOA (Table 6), there was an interaction ($P<0.01$) between distance and bath treatment, and the highest value was observed for WoB group at the LD. The values of DOA in winter showed small number of deaths in transit and did not generate sufficient dates for statistical analysis. Petracci *et al.* (2006) showed DOA prevalence higher in turkeys in the summer (0.52%) than in autumn (0.29%), winter (0.29%), and spring (0.32%).

-----Insert Table 5-----

-----Insert Table 6-----

Factors such as temperature (McKee & Sams 1997; Bianchi *et al*. 2007; Voslarova *et al.* 2007; Zhu *et al.* 2013), relative humidity (Simões *et al.* 2009; Spurio *et al.* 2015) air ventilation (Xing *et al.* 2015; Spurio *et al.* 2015) and HI (Spurio *et al.* 2015) affect the animal welfare and quality of meat. Our results confirm that heat stress due to high environmental temperature, RH and less ventilation can be a key in the death and poor meat quality of turkey during pre-slaughter time, however low ambient temperatures (around 5 °C) also appear to be important role.

The data in Table 7 shows results for the pH and L* values, no interactions ($P \geq 0.01$) were verified for pH in summer. However, by analyzing separately first the bath and subsequently the distance, they indeed were significantly different ($P<0.01$). Highest pH values were observed WiB groups during summer. In winter there was an interaction ($P>0.01$) with the lowest values observed at the SD in WiB group and highest value LD in WiB groups. For the L* value, in summer no interaction ($P<0.01$) between distance and shower treatment (Table 7), summer shows the lowest values for SD in WiB groups and highest value occurred for LD in WoB group. For the L* value, in winter there was an interaction ($P<0.01$) between distance and shower treatment (Table 7), the lower values observed for LD and highest for SD, both in shower treatment (WiB).

-----Insert Table 7-----

The pH and L * values were also affected by transportation microclimate, during summer. The color (L*) is an important quality attribute measurement meat due be an indicator of PSE-like and DFD-like meat (Carvalho *et. al.*2014; Mckee & Sams 1997). McKee and Sams (1997) reported that with the increase of the temperature also occurs an increase in L* values in turkey *pectoralis major*, our findings also demonstrate this occurrence. The L * values is highly correlated with pH and water holding capacity (Owens & Sams 2000; Carvalho *et al.* 2014) our study showed higher L * values when the birds were transported at the LD and WoB treatment in summer; winter seasons lower values were showed occurring darker fillets in the LD in WiB groups, similar results were presented by Bianchi *et al.* (2007) whose variables L* and pH showed differences between seasons, in the summer the birds had higher L * values and lower pH values.

In conclusion, for turkey transportation during the summer and winter season in Brazil, several factors influence the development of PSE-like and DFD-like meat as well as DOA index. A water bath while at the farm reduced the occurrence of PSE-like meat in summer under short distance, but increased in winter at the short distance, in winter the bath treatment increases the occurrence of DFD-like at the long distance. Long distances/times in summer were deleterious to the birds' welfare, producing more PSE-like and DOA.

ACKNOWLEDGMENTS

The authors acknowledge funding for this project by CNPq Proc. 471609/2011-0. RHC is under CAPES and Fundação Araucária graduate scholarships. MS is CNPq Research Fellows and was also a Brazilian Senior Visiting Professor Scholar from CAPES.

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Figures and Legends

Figure 1. Variations in Temperature (A) and Relative Humidity (B) in the container truck with elapsed time for the different turkey transport treatments during a typical summer and winter of a journey of 30 and 110 min, and a holding time of 70 min at the slaughterhouse. n = 4 per treatment.

Figure 2. Variations in Air Ventilation (A), Heat Index (B) and Wind Chill (C) in the container truck with elapsed time for the different turkey transport treatments during a typical summer and winter of a journey of 30 and 110 min, and a holding time of 70 min at the slaughterhouse. n = 4 per treatment.

Table 1. Characterization of sampling days

	Summer (2012/2013)	Winter (2013)
Mouths	December and January	June, July and August
Lineage and gender	Nicholas-700, male	Nicholas-700, male
Weight	18 ± 2 kg	18 ± 2 kg
Number of fillets analyzed	1337	1339
Total days of sampling	32	32
Temperature range	24 to 36 °C	-2 to 24°C
Relative humidity range	43% to 67%	42% to 65 %

Table 2. Parameters of transport

	Short distance (SD)	Long distance (LD)
Distance (Km)	22 ± 3	48 ± 4
Time (minutes)	25 ± 10	60 ± 15

Table 3. Mean values of Temperature (T) and relative humidity (RH) determined under the case of turkeys with (WiB) and without bath (WoB) treatments just before leaving the farm. The turkeys were transported at 2 different distances: short distance (SD) and long distance (LD)

Summer	Distance	With Water bath	Without Water bath	Average
#T (°C)	SD	30.27	35.65	32.96b
	LD	31.10	36.45	33.77a
CV= 3.72 %	Average	30.68B	36.05A	
Winter				
$T (°C)	SD	5.25B,b	10.30A,b	7.78
	LD	8.09B,a	13.12A,a	10.06
CV= 4.60 %	Average	6.68	11.71	
Summer	Distance	With Water bath	Without Water bath	Average
$RH (%)	SD	76.16A,a	49.64B,a	62.90
	LD	57.93A,b	49.77B,a	53.85
CV= 8.01 %	Average	67.05	49.70	
Winter		With Water bath	Without Water bath	
$RH (%)	SD	77.74A,a	50.21B,a	63.98
	LD	59.50A,b	50.50B,a	55.00
CV= 7.21 %	Average	68.21	50.36	

a-b Different letters on the same column indicate significant differences, as measured by the Tukey test ($P<0.01$). A-B Different letters on the same line indicate significant differences, as measured by the Tukey test ($P<0.01$). #The analyzed variables did not exhibit interaction with each other ($P\geq0.01$). $The analyzed variables exhibited interaction with each other ($P<0.01$).

Table 4. Mean values of air ventilation (AV), heat index (HI) and wind chill (WC) determined under the case of turkeys with (WiB) and without bath (WoB) treatments just before leaving the farm. The turkeys were transported at 2 different distances: short distance (SD) and long distance (LD)

Summer	Distance	With Water bath	Without Water bath	Average
#AV(m/s)	SD	1.21	1.17	1.19
	LD	1.22	1.22	1.22
CV= 21.03 %	Average	1.22	1.20	
Winter				
#AV(m/s)	SD	1.00	1.03	1.01
	LD	1.01	1.06	1.04
CV= 14.23 %	Average	1.00	1.05	
Summer	Distance	With Water bath	Without Water bath	Average
$HI (°C)	SD	36.13	43.81	39.97a
	LD	38.26	46.03	41.14B
CV= 3.76 %	Average	37.19B	44.92A	
Winter		With Water bath	Without Water bath	
$WC (°C)	SD	5.23B,b	12.68A,b	8.95
	LD	8.41B,a	18.09A,a	13.25
CV= 8.65 %	Average	6.81	15.39	

[a-b] Different letters on the same column indicate significant differences, as measured by the Tukey test ($P<0.01$). [A-B] Different letters on the same line indicate significant differences, as measured by the Tukey test ($P<0.01$). #The analyzed variables did not exhibit interaction with each other ($P\geq0.01$). $The analyzed variables exhibited interaction with each other ($P<0.01$).

Table 5. Mean values of PSE-like and DFD-like meat incidence determined under the case of turkeys with (WiB) and without bath (WoB) treatments just before leaving the farm. The turkeys were transported at 2 different distances: short distance (SD) and long distance (LD)

Summer	Distance	With Water bath	Without Water bath	Average
$PSE meat (%)	SD	20.0A,b	21.0A,b	20.0
	LD	30.0B,a	37.0A,a	34.0
CV= 13.15 %	Average	25.0	29.0	
Winter				
$PSE meat	SD	11.0A,a	5.0 B,a	8.1
	LD	4.0A,b	7.0 A,a	5.2
CV= 5.04 %	Average	8.0	7.0	
Summer	Distance	With Water bath	Without Water bath	Average
DFD-like	SD	0.0	0.0	0.0
	LD	0.0	1.0	0.0
	Average	0.0	0.0	
Winter		With Water bath	Without Water bath	
$DFD-like	SD	11.0A,b	5.0B,a	8.0
	LD	25.0A,a	7.0B,a	16.0
CV= 34.45 %	Average	18.0	6.0	

a-b Different letters on the same column indicate significant differences, as measured by the Tukey test (P<0.01). A-B Different letters on the same line indicate significant differences, as measured by the Tukey test (P<0.01). $The analyzed variables exhibited interaction with each other (P<0.01).

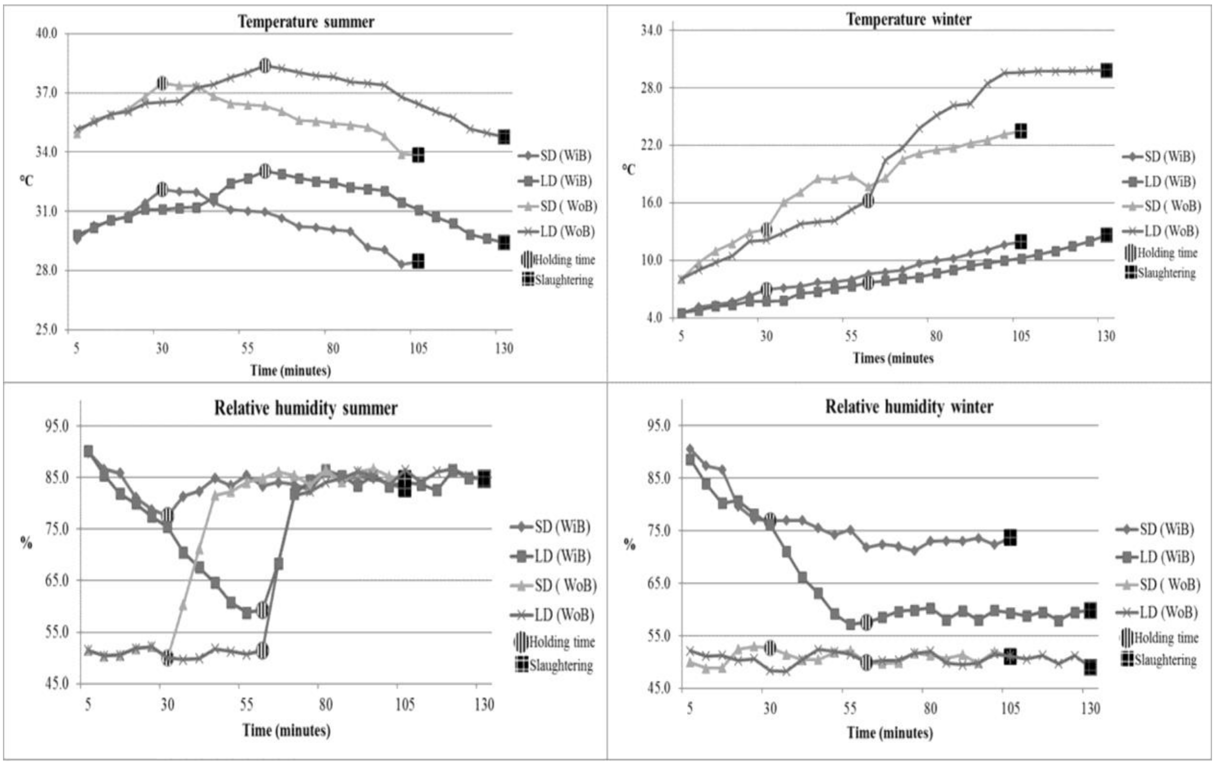
Table 6. Mean values of DOA index determined under the case of turkeys with and without bath treatments just before leaving the farm. The turkeys were transported at 2 different distances: short distance (SD) and long distance (LD)

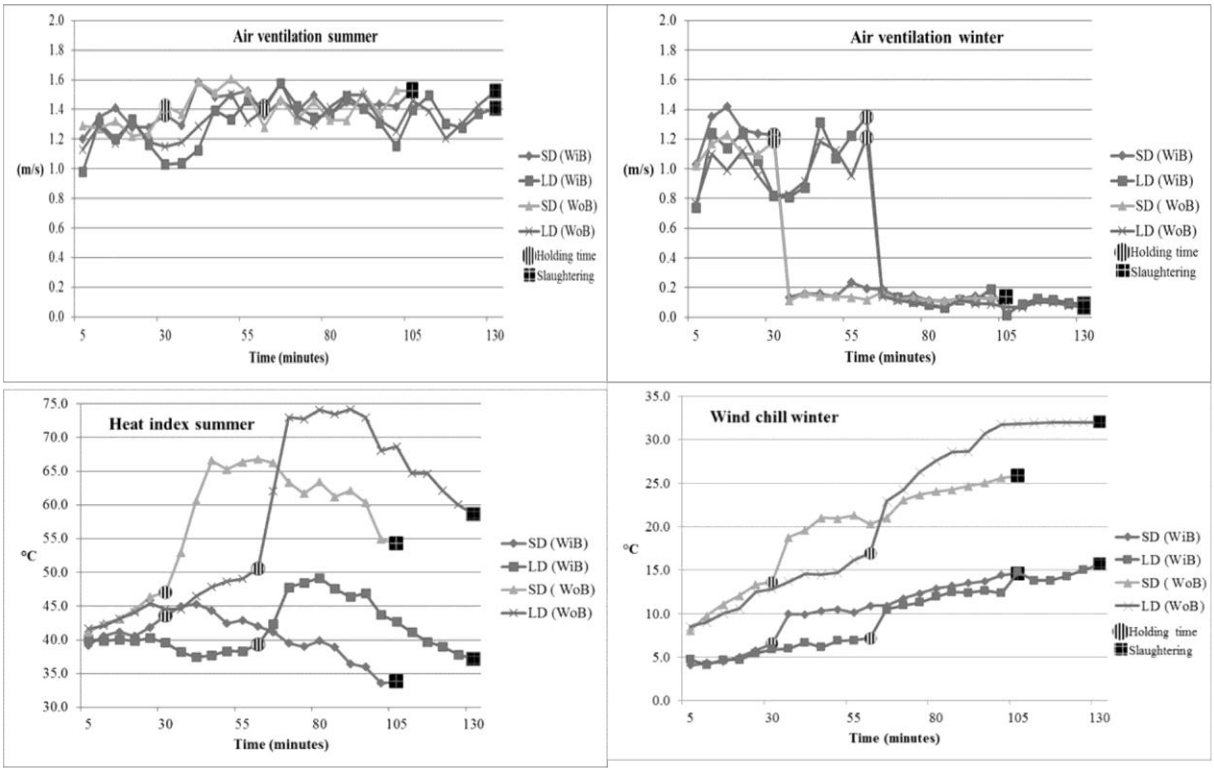
Summer	Distance	With Water bath	Without Water bath	Average
[$]DOA	SD	0.05B,a	0.20A,b	0.12
	LD	0.08B,a	0.50A,a	0.29
CV= 31.24 %	Average	0.06	0.35	
Winter		With Water bath	Without Water bath	
DOA	SD	0.00	0.00	0.00
	LD	0.01	0.00	0.01
CV= 12.01 %	Average	0.01	0.00	

Table 7. Mean values of pH and L* value determined under the case of birds with (WiB) and without bath (WoB) treatments just before leaving the farm. The birds were transported at 2 different distances: short distance (SD) and long distance (LD)

Summer	Distance	With Water bath	Without Water bath	Average
#pH	SD	5.75	5.73	5.74a
	LD	5.72	5.66	5.69b
CV= 7.30 %	Average	5.74A	5.69B	
Winter				
$pH	SD	5.79B,b	5.83A,a	5.81
	LD	5.90A,a	5.85B,a	5.88
CV= 9.34 %	Average	5.85	5.84	
Summer	Distance	With Water bath	Without Water bath	Average
#L*	SD	49.40	50.61	50.00a
	LD	50.06	51.28	50.97b
CV= 5.64 %	Average	49.73 B	50.94A	
Winter		With Water bath	Without Water bath	
$L*	SD	47.80A,a	46.77B,a	46.09
	LD	45.09B,b	47.10A,a	47.29
CV= 2.84 %	Average	46.44	46.92	

[a-b] Different letters on the same column indicate significant differences, as measured by the Tukey test ($P<0.01$). [A-B] Different letters on the same line indicate significant differences, as measured by the Tukey test ($P<0.01$). [$]The analyzed variables exhibited interaction with each other ($P<0.01$). [#]The analyzed variables did not exhibit interaction with each other ($P\geq0.01$).


Temperature summer
°C
Time (minutes)
Temperature winter
Times (minutes
Relative humidity summer
%
Time (minutes)
Relative humidity winter
SD (WiB)
LD (WiB)
SD (WoB)
LD (WoB)
Holding time
Slaughtering


Air ventilation summer
(m/s)
Time (minutes)
SD (WiB)
LD (WiB)
SD (WoB)
LD (WoB)
Holding time
Slaughtering
Air ventilation winter
(m/s)
Time (minutes)
SD (WiB)
LD (WiB)
SD (WoB)
LD (WoB)
Holding time
Slaughtering
Heat index summer
°C
Time (minutes)
SD (WiB)
LD (WiB)
SD (WoB)
LD (WoB)
Wind chill winter
°C
Time (minutes)
SD (WiB)
LD (WiB)
SD (WoB)
LD (WoB)
Holding time
Slaughtering

9 EXPERIMENTO G (Em preparação)

Animal Science Journal

The Effect of Heat Stress and Wind Chill on Turkey Meat during Brazilian Commercial Truck Transportation

O artigo encontra-se nas normas do periódico Animal Science Journal.

Endereço: http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1740-0929.

The Effect of Heat Stress and Wind Chill on Turkey Meat during Brazilian Commercial Truck Transportation

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Running title: DFD-like turkey meat

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ABSTRACT

The inferior quality and economic risk of pale, soft and exudative (PSE) or dark firm and dry (DFD) poultry meat warrant continuing research. The objective of this study was to verify the incidence of PSE-like meat and to characterize the DFD-like meat in a commercial turkey slaughterhouse in southern Brazil during summer and winter seasons. Color (L*) and pH distribution of turkey breast (n=5,352) meat was evaluated in different farms (n=64) during the 2012-2013 years to assess the frequency of the problem PSE-like and DFD-like meat. The pH, water-holding capacity, color (L*, a*, b*) were used for correlation (P<0.01) the variables and to establish cutoff values of DFD-like meat. We proposed values for DFD-like meat classification based on pH and L * values [DFD-like = L* < 44.00 and pH > 5.90]. The ambient and transportation microclimate were monitored through of measurements of temperature, relative humidity, ventilation, heat index and wind chill. Overall, mean L* value was the highest during the summer (P<0.01), indicating highest incidence of PSE meat (28.35 %), and lowest during winter, indicating a highest incidence of DFD-like meat (10.3 %). The thermal sensation affects the turkey welfare measured for PSE-like and DFD-like meat, and DOA (dead on arrival) index was at higher value during summer compared to winter season.

Key words: Animal Welfare, Color Meat, DOA index, Turkey Meat Quality.

INTRODUCTION

The production and consumption of poultry meat has increased rapidly around the world (Barbut, 2015). The Brazilian poultry industry has various critical factors to keep the meat qualities throughout the year, because Brazil is located within tropical and subtropical zones the birds are frequently exposed to different weather conditions (Carvalho *et al.* 2014). Problems with the quality of meat are generally caused by biochemical and morphological changes of the muscles (*antemortem*) and by processing (*postmortem*) (Solomon *et al.* 1998; Wilhelm *et al.* 2010; Pedrão *et al.* 2015).Two conditions are known: pale, soft and exudative (PSE), and dark, firm and dry (DFD) that are developed as the result of stress in the short term and long term, respectively (Dadgar *et al*. 2010).

In summer, the climatic effect can induce PSE problem in birds (Jiang *et al.* 2015; Spurio *et al.* 2015), as well as processing after slaughtering (Alvarado and Sams, 2004; Pedrão *et al.* 2015). Owens *et al.* (2000) and Barbut *et al.* (2008) suggested that PSE poultry breasts can be classified by color in the processing plant due to its high correlation with the functional properties. During the summer season, the poultry industry reports substantial losses in meat quality (Owens & Sams, 2000; Carvalho *et al.,* 2014).

Long stress conditions before slaughter influences the level of glycogen and final pH of breast muscle (Savenije *et al.* 2002; Carvalho *et al.,* 2014) resulting in high water holding capacity (WHC) and reduced shear force (SF) (Ngoka *et al.* 1982), these characteristics are typical of DFD meat. Another interesting aspect is that the birds are sensitive to the seasons by being homoeothermic animals (McCurdy *et al.* 1996; Carvalho *et al.* 2015b)

The objective of this work was to evaluate the incidence of PSE-like and DFD-like in turkey breast meat in a Brazilian commercial processing plant during summer and winter seasons.

MATERIAL AND METHODS

Animals

This study was conducted during the summer and winter seasons from December 2013 to September 2014 in a commercial plant in Chapecó city region (Latitude: 27º 05' 47" S; Longitude: 52º 37' 06" W; Altitude: 674m) Santa Catarina State, Brazil. The transportation activity conditions from the farm to the turkey commercial processing plant were described in details in Carvalho *et al.* (2014). The weather conditions in this region were characterized by the minimum and maximum temperatures (AT), and the relative humidity (AH) as measured using a Kestrel 4000 instrument (Nielsen-Kellerman, Boothwyn, PA, USA) as seen in Table 1. The feed was removed 9 to 12 h before slaughter, and water was provided *ad libitum*. The animals were manually placed into crates at a density of 8 birds each and installed within the truck open container. The average catching and loading time was 24 min. Birds were handled in accordance with the principles and procedures outlined by the Londrina State University Animal Care and Use Ethical Committee (Process #167/2015).

----Insert Table 1----

The animals were reared on 32 turkey farms under a cooperative system and subsequently transported over a distance of 38 ± 20 km for a journey that takes approximately 95 ± 20 min. Upon arrival at the slaughterhouse facilities, the birds were placed in a holding area under water mist and ventilation for 70 min before slaughtering. The animals were sacrificed according to standard industry practices, which consisted of hanging, electrically stunning, bleeding, scalding, defeathering, evisceration, cooling the carcass through a tunnel of cold air (-6°C for 6 h) and deboning (Carvalho *et al.* 2015a). Subsequently, the breast meat samples (*Pectoralis major*) were collected and refrigerated at 4°C until completing 24 h to measure the color (L*, a* and b* values), pH and water holding capacity (WHC) and thus evaluating the occurrence of PSE-like (Carvalho *et al.* 2014) and DFD-like meat.

Transport of Truck Container Microenvironment Assessment

Six portable weather meter devices with bidirectional Kestrel anemometers and data logging capability were set to take measurements at an interval of 30 seconds during the journeys (Spurio *et al.* 2015).

The transport temperature (TT), transport relative humidity (TH), air ventilation (AV), ambient temperature (AT), ambient relative humidity (TH), heat index (HI) and wind chill (WC) values were simultaneously measured as in Spurio *et al.* (2015), allowing for a representative analysis of the heterogeneous distribution of the thermal microenvironment within the loaded vehicle. The weather conditions in moment of transport were characterized by temperatures (AT), and the relative humidity (AH). HI or apparent temperature is an index that combines air temperature and RH to measure the observed equivalent temperature (NOAA, 2014a). HI is usually higher than the temperature evaluated by the dry bulb temperature (Mitchell and Kettlewell, 2004). WC or apparent temperature is an index that combines air temperature and air ventilation to measure the perceived equivalent temperature (NOAA, 2014b).

Color and pH measurements in fresh breast meat

This evaluation was performed using a Minolta CR-400 colorimeter, taking five different reading points per sample for color determination (L*, a* and b*), as described by Carvalho *et al.* (2014). The pH was measured (in duplicate) by inserting electrodes into the *pectoralis major m* as described in Carvalho *et al.* (2015). The meat samples (n = 5,352) were classified as PSE-like according to their L* values, with L* > 53 and pH < 5.60, considered typical for PSE-like turkey meat, as thoroughly discussed in Carvalho *et al.* (2014).

Water Holding Capacity (WHC)

This measurement was carried out based on the technique of Hamm (1960), as described in Wilhelm *et al.* (2010). After 24 h postmortem, samples were collected from the cranial side of the breast filets and cut into cubes 2.0 ± 0.10 g. A total of 52 samples were analyzed in triplicate. They were first carefully placed between 2 pieces of filter paper on acrylic plates and then left under a 10-kg weight for 5 min. The samples were weighed and WHC was determined using the weight of exudate water and the following equation: 100 - [(W_i - W_f / W_i) X 100], where W_i and W_f are the initial and final sample weight, respectively.

Mortality evaluated by Dead on Arrival (DOA)

A total 30,720 turkeys were transported from 64 different farms. The percentage of DOA per loaded truck was calculated by counting each dead bird during the hanging step at the slaughtering plant, taking care to observe the truck region from which the birds were collected (Spurio *et al.* 2015).

DOA = Number of birds dead / Number of birds transported

Statistical analysis

The Statistica software for Windows 12.0 (StatSoft, Tulsa, USA) was used. Student's t test at 1% probability (P<0.01) was used for comparing the differences between the 2 treatments of summer and winter, and Pearson's correlation (P < 0.01 and P <0.05) coefficient to assess the correlation of the pH, a*, b*, L* values and WHC.

RESULTS AND DISCUSSION

Weather conditions in the farm region

Table 2 shows the AT, TT, AH and TH during summer and winter seasons. The ambient temperature in the summer season showed approximately 5.5 times higher (P<0.01) compared to the winter. Although AH was not significantly different (P≥0.01). TT values in the summer season showed 3.1 times higher (P<0.01) compared to winter. The transport humidity (TH) was not significantly different (P≥0.01) as well as the ventilation (V) values. HI in the summer season showed a high value of 30.04 °C and obviously the WC in winter showed a lower value (10.6 °C). WC in summer was not measured as the temperatures need to be above -50°C and at or below 10°C (NOAA, 2014b).

----Insert Table 2----

Meat Characteristic in the summer season

Color variation of fresh turkey breast meat in summer is shown in Figure 1A. The L* values varied from 40.28 (dark) to 63.73 (pale), and the average value was 50.88. The pH variation is shown in Figure 1B, and its values varied from 5.49 to 6.03, and the average value was 5.72. There was a significant (P<0.01) negative Pearson correlation (Figure 2) between the pH and L* values, with value of the coefficient of −0.55.

----Insert Figure 1----

----Insert Figure 2----

During the summer poultry breast meat showed lightness, i.e. high L* values and also low pH values. In Brazilian summer high L* values were found to poultry (Simões *et al.* 2009; Spurio *et al.* 2015), and this phenomenon is related to the climate of the season (Table 1) and the warm weather is located outside the comfort zone (18 to 30 °C), affecting animal welfare and originated the birds stress (Spurio *et al.* 2015). During the pre-slaughter phases, the microclimate of transport container is also an important factor that influences the meat (see table 2). Researchers reported that during the summer season and unfavorable transport microclimate (temperature, relative humidity and ventilation) L* higher values and lower pH of meat were found (Simões *et al.* 2009; Spurio *et al*. 2015).

Meat Characteristic in the winter season and DFD-like characterization

Color variation of fresh turkey breast meat during winter season is shown in Figure 3A. The L* values varied from 36.38 (dark) to 60.69 (pale), and the average value was 47.69 units.

The pH variation is shown in Figure 3B and it varied from 5.59 to 6.20, and the average value was 5.84. There was a significant (P<0.01) negative Pearson correlation (Figure 4) between the pH and L* values, with a value of the coefficient of -0.61.

Correlations between the different measurements, such as L*, a*, b*, pH and WHC were evaluated (Table 3). A significant negative Pearson correlation is observed between L* and pH (-0.62, P < 0.01) and WHC (-0.47, P<0.01). pH values showed correlations between WHC (0.31, P <0.05), a* (-0.43, P < 0.01), b* (-0.31, P < 0.05).

The methodology for detection or prediction of anomalies in meat (PSE and DFD) has been a matter of debate, because a single measurement of color it is not sufficient for accurate detection of any of these conditions (Chan *et al.* 2011). Of the other hand, several authors reported evidence that a color measuring system is an important meat quality tool and is often used as an indicator of PSE meat (Kauffman *et al.* 1993; Barbut, 1993, 1996; Carvalho *et al.* 2014).

Owens *et al.* (2000) in their study of the incidence of PSE meat in turkeys using parameters such as L *, a *, b *, pH and WHC, reported that L * values range from 41 to 63. In addition, the values of L* was the best predictor of PSE meat condition. According to Barbut (1998), the parameter L * value of 50/51 correctly identifies turkey meat PSE as this classification is significantly correlated with WHC. Several researches indicated that the parameter (L*) can be used for identification of abnormalities in poultry meat, since that correlated with other attributes such as pH, WHC and drip loss (Barbut 1998; Owens *et al.,* 2000). According to McCurdy et al. (1996) obtained correlations between pH and L* (-0.62), and they suggested an L* cut off > 50/51 for detection PSE meat. Therefore, using Figures 3A, 3B, 4 and Table 3 we proposed a convenient cutoff value for detection of DFD-like meat in turkey breast filets under the conditions of our experiment: DFD-like meat = L* < 44 and pH > 5.90. Thus the results of this experiment associated to the previous (Carvalho *et al.* 2014) one encourages us to propose the following meat color abnormalities classification:

- ✓ L* > 53.00 and pH < 5.60 = PSE meat (Carvalho *et al*. 2014).
- ✓ Normal: 53.00 ≤ L* ≥ 44.00; and 5.60 ≤ pH ≤ 5.90.
- ✓ L* < 44.00 and pH > 5.90 = DFD-like meat.

Comparison summer vs winter

In Table 4 is shown the pH, L*, a*, b* values and the formed PSE-like and DFD-like meat and the occurrence of DOA index. The birds in the summer season showed average pH value 0.13 lower units, L* value 3.19 units higher, a* value lower 0.63 units and b* value lower 0.21 units compared to winter season samples (P≥0.01). In relation the classification of turkey breasts in summer there were 28.35% incidence of PSE meat and 1.2 % of DFD-like meat. During the winter season the incidence of PSE and DFD meat were 6.7% and 10.3%, respectively. The DOA index in summer was relatively high by 0.23% while in the winter season the DOA index was extremely low.

According to the results reported in this work L * values in the winter season were lower than those values found in the summer season. Corroborating our studies McCurdy *et al.* (1996) in Canada also showed lower L* values in winter and high values of L * in the summer. These authors reported higher incidence of PSE meat in the summer and similar results were found in our study, where the incidence of PSE was 21.7% higher (Table 4) compared with the winter. In general, there are reports showing that heavier birds are more susceptible to the deleterious effects of higher temperatures (Mills *et al.* 1999; Lu *et al.* 2007; Simões *et al.* 2009).

The high incidence of DFD-like meat (10.3 %) in the winter months is probably the result of the combined effects of low ambient temperature and transport temperature, as shown in Table 2. These conditions are common in the winter season in southern Brazil.

The DOA values were different between treatments (Table 4), suggesting that those values were related to thermal stress in the summer months, as the PSE meat incidence indicated. The commercial company where this study was continually conducted has a relatively effective practice regarding animal welfare compared to other reports; Petracci *et al.* (2006) reported an average of 0.48 % DOA (turkey) in the Italy. The season of slaughtering influenced the pre-slaughter mortality rate in turkeys; DOA prevalence is higher in turkeys in the summer (0.52%) compared whit autumn (0.29 %), winter (0.29%), and spring (0.32%) (Petracci *et al.* 2006). Vieira *et al.* (2011) reported that the mortality incidence in the summer was 0.42%, followed by spring (0.39%), winter (0.28%), and autumn (0.23%). The thermal sensation of the seasons calculated by heat (HI) and cold (WC) (see Table 2) affects the turkey meat characteristics such as pH, L*, a*, b* and occurrence of PSE-like and DFD-like meat, well as animal welfare.

CONCLUSION

We analyzed the incidence of DFD-like turkey meat in a southern region of Brazil. The incidence DFD-like meat is higher in winter and incidence PSE-like meat in summer, as well as DOA index in summer. The first priority is to introduce management tools to maintain animal welfare and thus prevent stressful conditions for the birds in seasons of the year.

ACKNOWLEDGEMENTS

This project was funded by CNPq Proc. 471609/2011-0. RHC is under CAPES and Fundação Araucária graduate scholarship. EII and MS are CNPq Research Fellows, and MS was also a Brazilian Senior Visiting Professor Scholar from CAPES/UTFPR.

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Table 1. Samples, period, animals and climatic characteristics of the region.

	Summer (2012/2013)	Winter (2013)
Lineage and gender	Nicholas-700, male	Nicholas-700, male
Weight	18 ± 2 kg	18 ± 2 kg
Number of fillets analyzed	2674	2678
Days of sampling	56	73
Temperature range	24 to 36 °C	-2 to 24°C
Relative humidity range	43 to 67%	42 to 65%

Table 2. Mean values of ambient temperature (AT), transport temperature (TT), ambient humidity (AH), transport humidity (TH),air ventilation (AV), heat index (HI) and wind chill (WC) performed under two treatments: summer and winter during turkey transportation.

	Summer	Winter
AT (°C)	$33.02^a \pm 0.51$	$5.97^b \pm 0.87$
#TT (°C)	$33.87^a \pm 2.84$	$10.89^b \pm 4.47$
AH (%)	$48.99^a \pm 4.57$	$44.92^a \pm 5.67$
#TH (%)	$59.84^a \pm 11.90$	$59.42^a \pm 12.93$
#V (m/s)	$1.31^a \pm 1.16$	$1.10^a \pm 1.12$
#HI (°C)	41.0 ± 4.32	-
#WC (°C)	-	10.6 ± 3.65

a-b Means ± standard deviation in the same line with no common superscripts are significantly different by Student t-test ($P < 0.01$).

Parameters analyzed during the transport of turkeys.

Table 3. Correlation coefficients among physical measurements of turkey breast meat samples.

Parameter	L*	a*	b*	pH	WHC %	Means	Stander
L*	1	-	-	-	-	48.90	2.72
a*	0.37*	1	-	-	-	3.86	0.95
b*	0.58*	0.60*	1	-	-	3.56	1.27
pH	-0.62*	-0.53*	-0.71*	1	-	5.97	0.10
WHC %	-0.47*	-0.43*	-0.31**	0.31**	1	77.16	1.88

*Values significant at $P < 0.01$ unless otherwise stated. n = 52.

**Values significant at $P < 0.05$ unless otherwise stated. n = 52

Table 4. Mean values of pH, L*, a*, b*, PSE and DFD meat in turkey breast meat performed under two treatments: summer and winter.

	Summer	Winter
pH	$5.71^{b} \pm 0.05$	$5.84^{a} \pm 0.06$
L*	$50.88^{a} \pm 1.68$	$47.69^{b} \pm 2.40$
a*	$4.45^{b} \pm 0.50$	$5.08^{a} \pm 0.67$
b*	$3.69^{b} \pm 0.65$	$3.90^{a} \pm 0.71$
PSE-like (%)	28.35	6.7
DFD-like (%)	1.2	10.3
DOA (%)	0.23^{a}	0.001^{b}

$^{a\text{-}b}$ Means ± standard deviation in the same row with no common superscripts are significantly different by Student t-test (P<0.01).

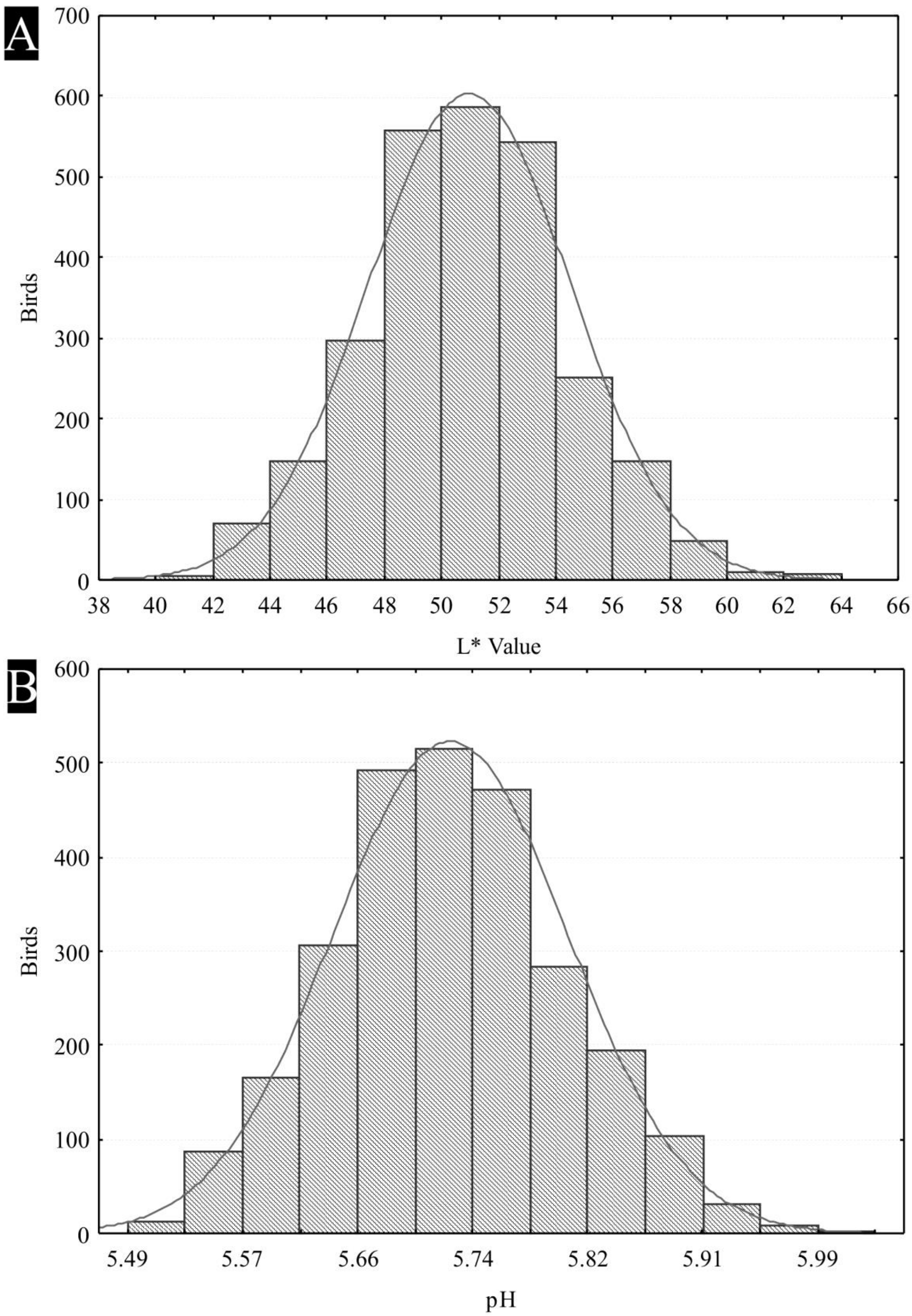

Figure 1. A) Histogram showing the distribution of L* values in turkey breast filets. **B)** Histogram showing the distribution of pH in turkey breast filets in summer season (*n* = 2674).

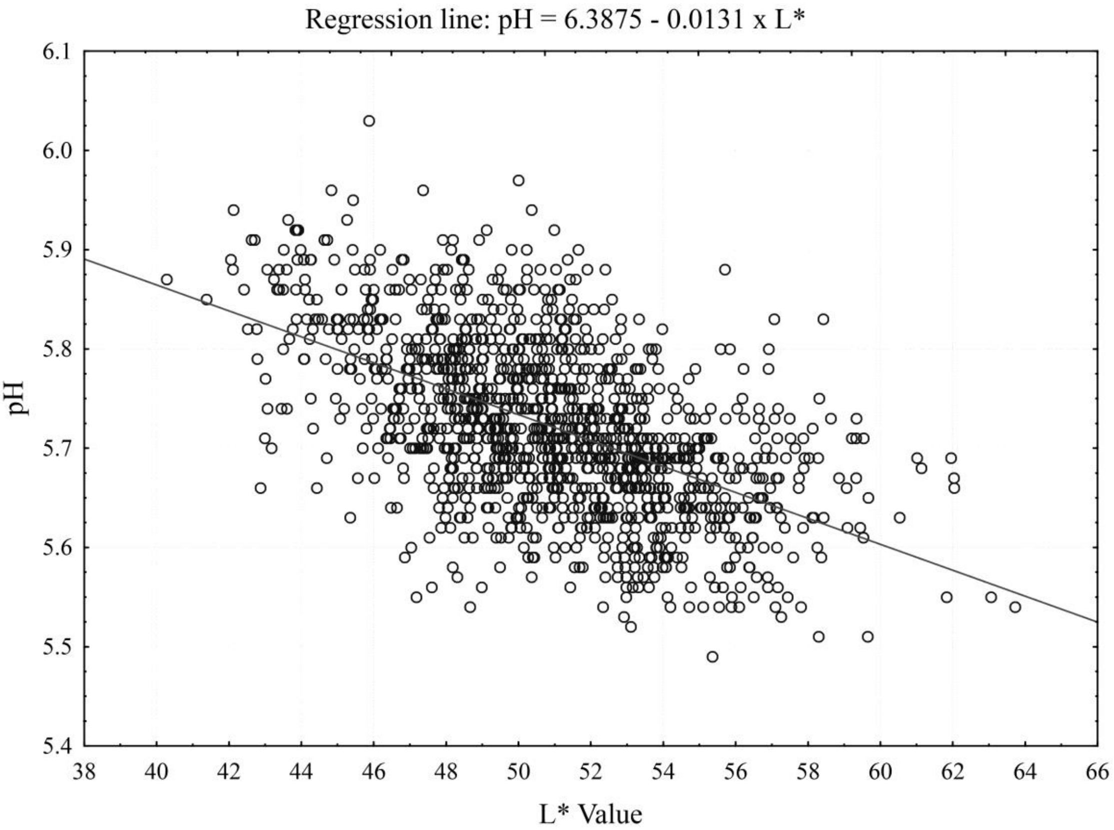

Figure 2. The relationship between L* and pH values in turkey breast filets in summer season (*n* = 2674).

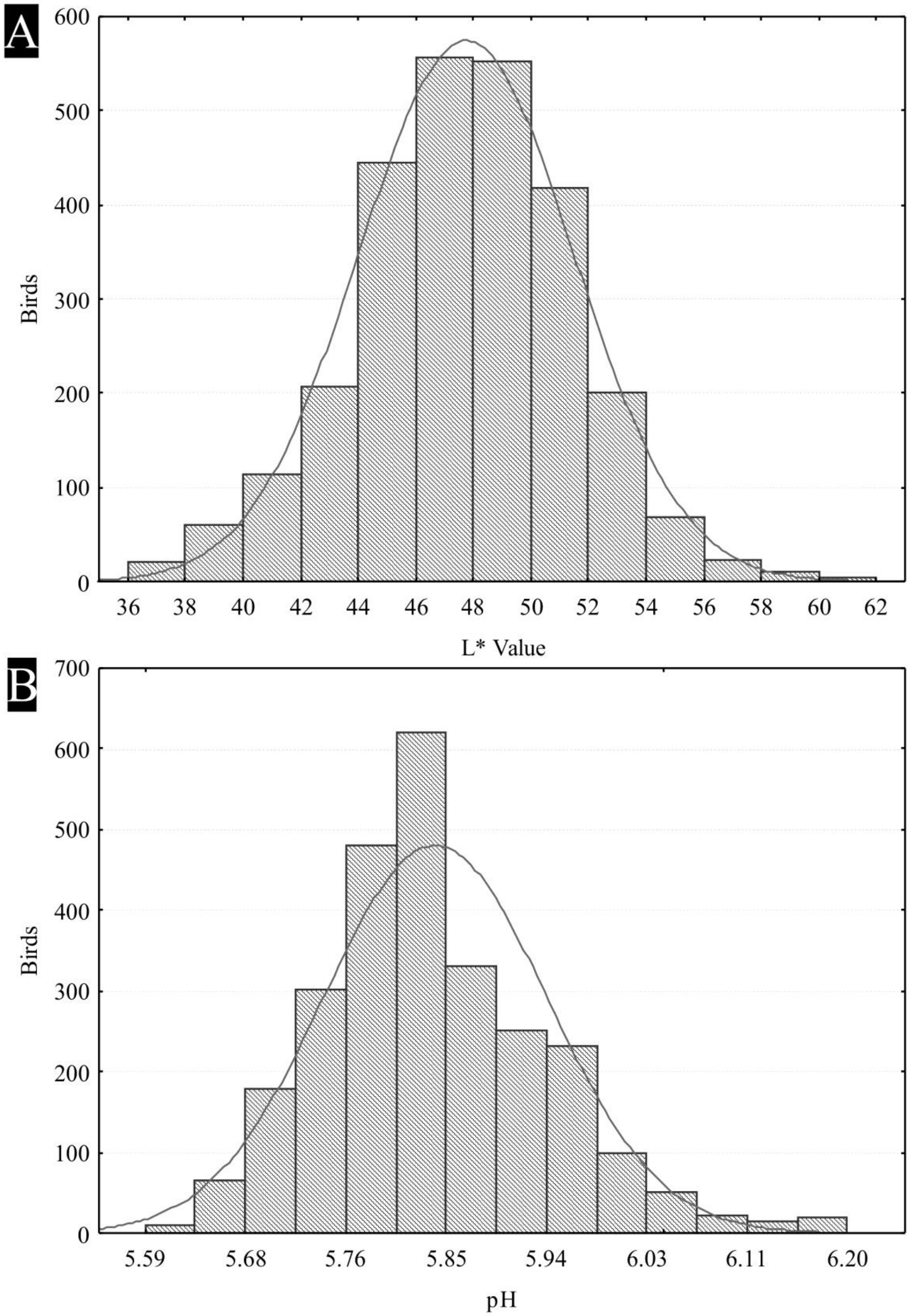

Figure 3. A) Histogram showing the distribution of L* values in turkey breast filets. **B)** Histogram showing the distribution of pH in turkey breast filets in winter season (*n* = 2678).

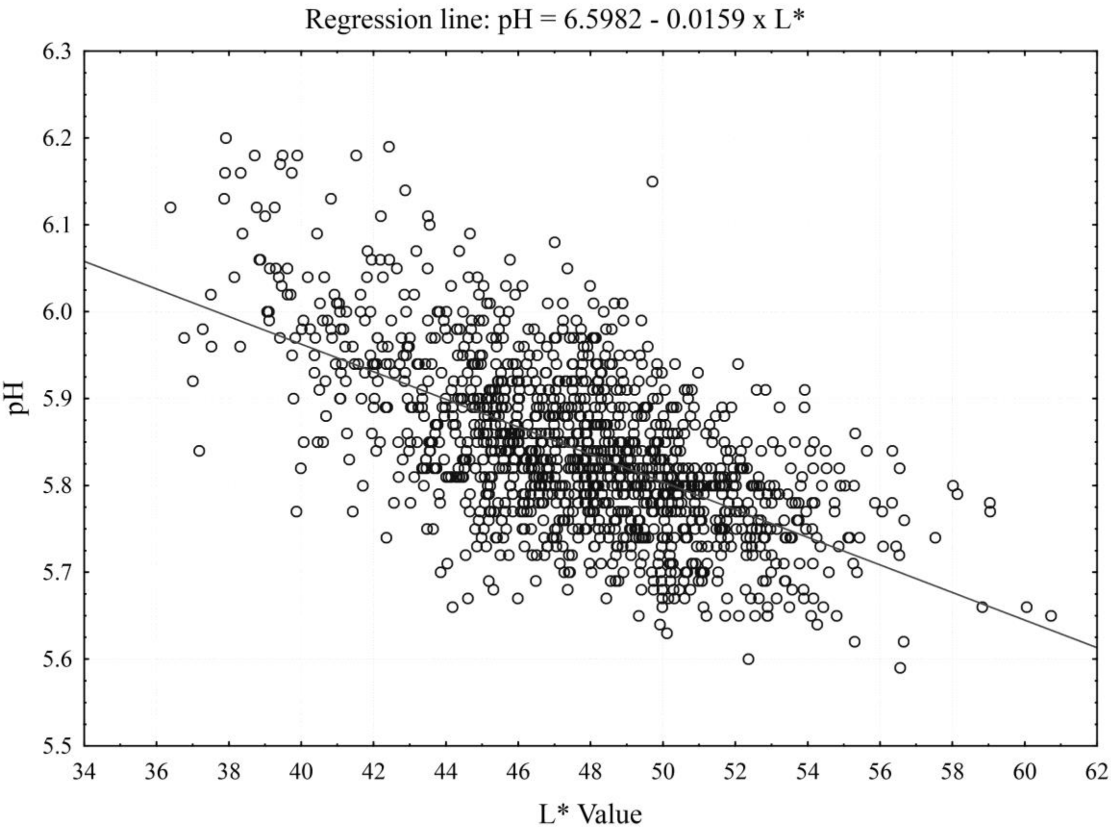

Figure 4. The relationship between L* and pH values in turkey breast filets in winter season (n = 2678).

10 EXPERIMENTO H (Em preparação)

Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry

O artigo encontra-se nas normas do periódico Food Chemistry.

Endereço: http://www.journals.elsevier.com/food-chemistry/

Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry

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ABSTRACT

This study examined the relationship between the redox balance in *post-mortem* muscle, the occurrence of protein oxidation and the onset of pale, soft and exudative (PSE-like) in chicken meat. A PSE condition was induced by incubation of chicken carcasses after 20-min *post-mortem* at 37 °C for 200 min followed by chilling to 4 °C. Compared to NORMAL meat, PSE-induced muscle consistently had a faster pH decline and lower pH values at 200 min (5.84 vs. 6.59) and 24h (5.69 vs. 5.96), a higher L* value (54.4 vs. 57.3), and reduced water holding capacity (WHC). Antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase activities were significantly lower in PSE-induced samples than in the NORMAL counterparts. PSE was more susceptible to protein oxidation than NORMAL during succeeding chilled storage with more intense tryptophan and thiols depletion, higher protein carbonylation and more intense formation of protein cross-links. Plausible explanations are provided to support the role of protein oxidation in the impaired quality PSE chicken meat.

Keywords: chicken; antioxidant enzymes; lipid oxidation; protein oxidation; PSE meat.

INTRODUCTION

The poultry industry is confronting a major challenge due to an increasing occurrence of pale, soft, and exudative (PSE)-like meat (Chan, Omana, & Betti, 2011). PSE meat occurs in swine by two causes, i) a genetic mutation on the ryanodine receptor (Ca^{++} metabolism) in muscle tissue, and ii) severe stress conditions prior to slaughter (Fujii et al., 1991). In chicken, however, only the latter has been confirmed as a driving cause for PSE occurrence (Carvalho et al., 2015; Wilhelm, Maganhini, Hernández-Blazquez, Ida, & Shimokomaki, 2010). There are also other factors which influence the development of PSE-like chicken meat, such as season (Langer et al., 2010), environment (Carvalho et al., 2015), heat stress (Spurio et al., 2015), and pre- and post-slaughter handling practices (Guarnieri et al., 2004). The consequences of broiler breast PSE meat have recently been the subject of experimental studies by several research groups (Barbut et al., 2008; Chan, et al., 2011; Owens, Alvarado, & Sams, 2009; Swatland, 2008; Carvalho et al., 2015).

PSE meat is characterized by low water holding capacity (WHC), soft texture, and light appearance. This functional defect is believed to take place as a result of protein denaturation caused by rapid *post-mortem* muscle glycolysis, which lowers pH while the carcass is still warm (Barbut et al., 2008; Lesiow & Xiong, 2013). Elevated *post-mortem* temperatures around 40 °C facilitates protein denaturation and the onset of PSE meat characteristics in turkey (McKee & Sams, 1998) and swine (Lesiow & Xiong, 2013) by accelerating the development of *rigor mortis* and a fast pH decline. Along with denaturation and proteolysis, muscle proteins also suffer oxidative damage after slaughter and during the subsequent meat aging (Martinaud et al., 1997; Rowe et al., 2004). Pre-slaughter stress is linked to an increased susceptibility to lipid and protein oxidation in broiler muscles (Estevez, 2015) and protein oxidation has been recurrently associated to an impaired functionality of meat proteins (Xiong, 2000; Utrera & Estévez, 2012). It is hence, reasonable to hypothesize that protein oxidation may play a role in the onset of PSE-like meat, but this extent has never been confirmed.

Muscle proteins undergo oxidative damage in the presence of reactive-oxygen species (ROS), transition metals and reducing sugars (Estevez, 2015). Among the assorted chemical manifestations of this damage, the depletion of tryptophan residues and free thiols and the formation of carbonyl residues and crosslinks have been already reported in raw and processed meat and poultry (Estévez, 2011; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). In *post-mortem* muscle, the collapse of the endogenous antioxidant system and the biochemical changes occurred during the conversion of muscle to meat promote the occurrence of *post-mortem* oxidative stress in poultry muscles (Estevez, 2015). The pH-decline, in particular, is responsible of the alteration of cellular compartmentalization and the release of pro-oxidant enzymes. Enzymes such as glutathione peroxidase, superoxide dismutase and catalase are known for contributing to counteract pro-oxidant factors and protect against lipid and protein oxidation in post-rigor meat (Liu et al., 2014; Zhang, Xiao, Lee, & Ahn, 2011). The connection between the redox balance in *post-mortem* muscle, the occurrence of protein oxidation and the onset of PSE-like meat is awaiting to be unveiled. A better understanding of the biochemical mechanisms behind the role of oxidative stress in PSE occurrence was the objective of this study.

MATERIAL AND METHODS

Materials and experimental design

A total of 16 Ross broilers (308 lineage, female, 34 days of age and 1.8 kg) were obtained from a commercial processing plant in the Caceres region, Spain. Feed was removed 10 hrs before slaughter and water was provided *ad libitum* to catching. Typical commercial cornsoybean meal diet was utilized and the birds were transported (155 km) to the slaughterhouse. The animals were slaughtered according to standard industry practices, which consisted of hanging, carbon dioxide stunning, bleeding, scalding, defeathering and evisceration. At that point (5 min. after slaughter), sixteen carcasses (T^{a} ~37.5ºC) were collected and randomly divided into two groups. One group of carcasses (n=8) was subjected to induction of PSE as described by Lesiow and Xiong (2013), with minor modifications. The carcasses were placed in separate plastic bags (Ziploc bags), and stored at 37 °C in a oven (JP Selecta-2001244, Barcelona, Spain) for 3.3 hrs to generate PSE-like meat. The pH changes were monitored over time. After that, the carcasses were placed in separate plastic bags and stored at 4°C for up to 24 h for further analysis. The other group of samples (NORMAL; n=8) were held by the hocks on shackles in the commercial cold room for approximately 60 minutes. After cooling, the carcasses (4 °C) were placed in separate plastic bags and stored at 4°C for up to 24 h for further analysis. The pH and temperatures values of PSE and NORMAL breast samples were measured at 20, 80, 200 min and 24h *post-mortem*. Thereafter, breast muscles were dissected from carcasses and immediately subjected to color, texture and WHC measurements as described below. A slice (1 cm thickness) from the middle of each breast was placed in falcon test tubes and kept under refrigeration (4°C) for 7 days and analyzed for lipid and protein oxidation and WHC as described below. The remaining samples were placed in plastic bags and kept in a −80 °C freezer for enzymes determination.

Temperature and pH determination

The pH and temperature of carcasses were measured (in duplicate) using portable pH-meter (Crison PH25, Barcelona, Spain) and thermometer (Testo 735, Lenzkirch, Germany) by inserting electrodes into the *pectoralis major* as described in Carvalho et al. (2014).

Color determination

This evaluation was performed using a Minolta chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ) with illuminant D65 and 0º standard observer. Before each measurement the chromameter was calibrated on the CIE color space system using a white tile. Measurements were made at three different random reading points on the surface of the breast muscle and in the core after slicing as previously described.

Water Holding Capacity (WHC)

Press method: This measurement was carried out based on the technique of Hamm (1960), as described in Carvalho et al. (2014), with minor modifications. After 24 h *post-mortem*, samples were collected from the cranial side of the breast filets and cut into cubes 1.0 ± 0.01 g. A total of 16 samples were analyzed in duplicate. They were first carefully placed between 2 pieces of filter paper and then left under a 1-kg weight for 2 min. The samples were weighed and WHC was determined using the following equation: 100 - [(Wi - Wf/Wi) × 100], where Wi and Wf are the initial and final sample weight, respectively.

Centrifugation method: A total of 16 samples were analyzed in duplicate as described by Honikel, (1987), with minor modifications. The piece of breast meat (5g) was placed in plastic bag (Ziploc bags) and heated at 70 °C for 30 min in a water bath. After heating, samples were left at room temperature (21ºC for 45 min). The WHC was determined by a centrifugal method. Briefly, samples were centrifuged at 500 g for 15 min at 4 °C. WHC (%) was expressed as 100 - [(Wi - Wf/Wi) × 100], where Wi and Wf are the initial and final sample weight, respectively.

Centrifugation method with salt addition: A piece of breast meat (5g) was placed in falcon tubes and added 8 mL NaCl 0.6M (Barbut, 1993). Samples were then heated at 70 °C for 30 min in a water bath. After heating, samples were left at room temperature (21ºC for 45 min). The WHC was determined by the centrifugal method previously described.

Cooking loss: A piece of breast meat (5g) was placed in falcon tubes, weighed before and after 15 min of cooking, which was the time required for the internal temperature to reach 75 °C (Honikel, 1998).

Texture

Texture profile analysis (TPA) was performed at room temperature (21ºC) with a Texture Analyser TA-XT2i (Stable Micro Systems, Surrey, UK). Seven cube samples (1 × 1 × 1 cm) were taken from the middle of the breast and subjected to a two-cycle compression test. The samples (raw) were compressed to 40% of their original height with a cylindrical probe of 5 cm diameter and a cross-head speed of 5 mm/s. Texture profile parameters were determined following descriptions by Bourne (1978) and the SMS manual (Stable Micro Systems, Surrey, UK). All analyses were performed in heptaplicate in each breast sample.

Free thiols and disulphide bonds

Free thiols and disulphide bonds were analyzed following the methodology of Rysman et al. (2014). One g of sample was homogenized with 25 mL of 6 M guanidine hydrochloride (GuHCl) in 100 mM Tris buffer (pH 8.0) using an Ultra Turrax. The homogenates were centrifuged (for 20 min at 5000 g and 4 °C), and supernatants were filtered (qualitative filter paper, particle retention of 11 μm). Hence, an aliquot of 3 mL of filtrate was subjected to disulfide reduction by adding 50 μL of 1-octanol and 100 μL of freshly prepared 30% (w/v) sodium borohydride in 1 M NaOH. After incubation at 50 °C for 30 min, an aliquot of 1.35 mL of 6 M HCl was added, followed by stirring for 10 min. Free and total thiols were determined with 4,4'-dithiodipyridine (4-DPS) in the non-reduced and reduced filtrates, respectively. An aliquot of 500 µL of filtrate was mixed with 2 mL of 6 M GuHCl in 1 M citric acid buffer (pH 4.5) and 500 µL of 4-DPS solution (4 mM 4-DPS in 12 mM HCl). The absorbance was measured at 324 nm against 6 M GuHCl in 1 M citric acid buffer (pH 4.5) before the addition of 4-DPS (A_{pre}) and after 30 min of reaction with 4-DPS in the dark at room temperature (A_{post}). A mixture of 2.5 mL of 6 M GuHCl in 1 M citric acid buffer (pH 4.5) and 500 µL of 4-DPS solution was prepared as a blank sample (A_{blank}). The absorbance corresponding to the thiol concentration was calculated by subtracting A_{pre} and A_{blank} from A_{post}. The thiol concentration was calculated on the basis of a five-point standard curve ranging from 2.5 to 500 µM L-cysteine in 6 M GuHCl in 1 M citric acid buffer (pH 4.5). The thiol content was expressed as µmol of thiols per g of sample. The disulfide content was calculated as half of the difference between total and free thiols.

Tryptophan concentration

The concentration of tryptophan was measured using fluorescence spectroscopy according to the methodology reported by Estévez, Kylli, Puolanne, Kivikari, & Heinonen (2008) with some modifications. Breast samples were homogenized (1:10 w/v) in sodium phosphate buffer pH 6.0 with 8 M urea using an Ultra Turrax. Two mL of 20 mM sodium phosphate buffer pH 6.5 with 6 M GuHCl was added to a 0.5 mL aliquot of the homogenate and stirred in vortex. After dilution (1:1000 v/v) samples were transferred to a 4 mL quartz cuvette with four flat walls (101-QS 10x10mm, Analytics Hellma). The emission spectrum for tryptophan fluorescence was recorded between 300 and 400 nm wavelength with excitation set at 283 nm (Perkin-Elmer LS 55 Luminescence spectrometer, Beaconsfield, UK). The excitation and emission slit widths were set at 10 nm and data were collected at 500 nm per minute. Tryptophan content was calculated on the basis of a five-point standard curve of N-acetyl-L-tryptophan amide (NATA) ranging from 0.1 to 0.5 μM. Results were expressed as mg NATA equivalents per 100 g of sample.

Protein carbonyls

Protein oxidation was measured by the total carbonyl content (DNPH) as described by Ganhão, Morcuende, & Estevez (2010) with minor modifications. Breast meat (1 g) was minced and then homogenized 1:10 (w/v) in 20 mM sodium phosphate buffer containing 0,6 M NaCl (pH 6.5) using an ultraturrax homogenizer for 45 s. Two equal aliquots of 0.15 mL were taken from the homogenates and dispensed in 2 mL eppendorf tubes. Proteins were precipitated by cold 10% TCA (1 mL) and subsequent centrifugation for 3 min at 4500g. One pellet was treated with 1 mL 2N HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) DNPH in 2N HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. The samples were precipitated by 10% TCA (1 mL) and washed two times with 1 mL ethanol:ethyl acetate (1:1, v/v) to remove excess DNPH. The pellets were then dissolved in 1.5 mL of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5) and centrifuged for 2 min at 2000g to remove insoluble fragments. Protein concentration was calculated from the absorption at 280 nm using BSA as standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein (nmol carbonyls/mg protein) using an absorption coefficient of 21.0 nM^{-1} cm^{-1} at 370 nm for protein hydrazones.

Schiff bases

The analysis of Schiff base structures was performed as described by Estévez, Kylli, Puolanne, Kivikari, & Heinonen (2008) with some modifications. Sample homogenates were prepared following a similar procedure to that previously described for tryptophan analysis. After dilution (1:20 v/v) samples were transferred to a 4 mL quartz cuvette with four flat walls (101-QS 10x10mm, Analytics Hellma). The emission spectrum for the Schiff base was recorded between 400 nm and 500 nm wavelength with excitation set at 350 nm (Perkin-Elmer LS 55 Luminescence spectrometer, Beaconsfield, UK). The excitation and emission slit widths were set at 10 nm and data were collected at 500 nm per minute. The results were expressed as units of fluorescence intensity emitted by Schiff base structures at 450 nm. These values were corrected according to the protein concentration of each sample by applying a correction factor ($C_f = P_t/P_p$) where P_t is the total average of the amount of protein from all samples and P_p is the content of protein in each type of sample.

TBA-RS

Lipid oxidation was determined in breast meat by the thiobarbituric acid-reactive substances (TBA-RS) assay using the method of Tarladgis et al. (1960). Briefly, 2.5 g of each breast meat was dispensed in falcon plastic tubes and homogenized with 7.5 mL of 3.86% perchloric acid and 0.25 mL BHT (4.2%), using an Omni-mixer homogenizer for 1 min. The homogenate blended was centrifuged (4500 g for 3 min) and filtered through double filter paper. Next, 2 mL aliquot of the distillate was mixed with 2 mL of 0.02 mol L^{-1} TBA in perchloric acid (3.86%) in test tubes (duplicate). The test tubes were homogenized by vortex and these together with the tubes from the standard curve were incubated at water bath (90-100°C) for 30 min, in order to develop the colour reaction. All tubes test were centrifuged (4500 g for 2 min) and the absorbance was measured at 532 nm using a spectrophotometer (Shimadzu UV-1800, Japan) against a blank containing 2 mL of 3.86% perchloric acid and 2 mL of TBA reagent. The results from the samples were plotted against a standard curve prepared with known concentrations of tetraethoxypropane (TEP). The results were expressed as TBA-RS numbers, mg malonodialdehyde (MDA) kg^{-1} breast meat.

Extraction for antioxidant enzyme's activity

Extraction was performed in breast meat following the method of Mahecha et al. (2011) with minor modifications. The extractions were performed twice with one replicate. The muscle (5 g) was mixed with 35 mL of ice cold phosphate buffer (extraction solvent, pH 7.0, 50 mM; disodium phosphate heptahydrate (Na_2HPO_4 x $7H_2O$) and KH_2PO4). Samples were homogenized by UltraTurrax (12,000 rpm, ca. 45 s). After centrifugation (4500g, 40 min, 4 °C), the supernatants were recovered and filtered over glass wool. These muscle extracts were used for the enzyme measurements of catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD).

Catalase (CAT)

CAT activity was measured according to the method described by Aebi (1974), with minor modifications. The aliquot of 100 µL extract of breast meat (kept in the ice-bath) were brought in a cuvette (1 cm path length), and 2.90 mL of H_2O_2 (hydrogen peroxide) were added. Immediately, the absorbance was monitored at 240 nm during 180 seconds using a spectrophotometer (Shimadzu UV-1800, Japan). CAT activity was expressed in µmol × min^{-1} × g^{-1} (U/g). One unit (U) of CAT activity was defined as the amount of extract needed to decompose l µol of H_2O_2 per min.

Glutathione peroxidase (GSH-Px)

GSH-Px was determined in breast meat using the method described by Mahecha et al. (2011), with minor modifications. The aliquot 600 µL of the muscle extracts was mixed with 2.35 mL of reaction solvent containing 1.13 mM reduced glutathione, 0.57 mM EDTA, 1.13 mM NaN_3 and 1.7 units glutathione reductase in 100 mL phosphate buffer (pH 7.0, 50 mM; disodium phosphate heptahydrate ($Na_2HPO_4 \times 7H_2O$) and KH_2PO_4 in MiliQ water). 26 µL NADPH solution (17.3 mM), and 20 µL H_2O_2 solution were dispensed in cuvettes (1 cm path length). The absorbance was monitored at 340 nm during 600 s using a spectrophotometer (Shimadzu UV-1800). The extinction coefficient of 6.220 µL μmol^{-1} cm^{-1} for NADPH at 340 nm and 25 °C was used for the calculation. GSH-Px activity was expressed as µmol of oxidized NADPH μL^{-1} min^{-1} g^{-1} (U/g).

Superoxide Dismutase (SOD)

SOD was measured according to the procedures of Marklund & Marklund (1974) using inhibition of pyrogallol autoxidation in a basic medium. The supernatant fraction of the muscle homogenate was also used for the determination of SOD enzyme activity, 50 µl of pyrogallol (10 mM) were added to 2.9 ml of Tris– cacodylic buffer (pH =8.2, 50 mM with diethylenetriaminepentaacetic acid, DTPA). The rate of pyrogallol autoxidation in presence of 50 µl of muscle extract was compared to a blank (with 50 µl of buffer) by measuring the increase of absorbance at 420 nm during 360 s using a spectrophotometer (Shimadzu UV-1800). One unit was taken as the activity that inhibits the pyrogallol autoxidation by 50%.

Statistical analysis

Statistical analyses were performed using SPSS software and Student´s t test was used to determine level of significance among two treatments: PSE and Normal meat. Pearson correlations were used for testing correlations between variables.

RESULTS AND DISCUSSION

Biochemical and physicochemical parameters

Figure 1 shows the pH decline in NORMAL and PSE-induced broiler breast meat samples. Holding the warm broiler carcass at the near-body temperature (37 °C) for 200 min. right after slaughter led to a faster pH decline compared to that observed for the carcasses that followed the commercial process (immediate chilling). These results illustrate the effectiveness of the procedure reported by Lesiow & Xiong, (2013) for producing PSE meat by accelerating the glycolysis reactions after slaughter. In general, if the pH of breast meat falls to 5.8 or around ultimate pH while the carcass temperature is above 35 °C, the muscle is prone to PSE (Lesiow & Xiong, 2013). This condition was met because after 200 min at 37 °C, the pH of carcasses fell to 5.84 ± 0.14. The pH kept on falling to reach a minimum of 5.69 after 24 h. In contrast, the pHs of carcasses subjected to the common commercial procedure were 6.59 and 5.96 after 200 min. and 24 h, respectively. Significant differences were found between pHs of PSE-induced and NORMAL carcasses at all sampling points after 80 min of induction ($P<0.05$). The time course of the pH changes in both PSE-induced and NORMAL carcasses was consistent with that occurring in typical PSE and normal meat, respectively (Mckee & Sams, 1998; Lesiow and Xiong, 2013). The interrelationship between temperature and pH in the development of PSE meat characteristics is crucial and well established in chicken (Barbut et al. 2008; Wilhelm et al., 2010) and turkey (Mckee & Sams, 1998; Carvalho et al. 2014). Color measurements are also often used as a descriptor of PSE chicken (Owens et al. 2009; Barbut et al 2008), since it is directly affected by pH and protein denaturation in the muscle (Bendall & Wismer-Pedersen, 1962). The paleness of PSE meat can be attributed to the denaturation of sarcoplasmic proteins, which increases light scattering in the muscle (Swatland, 2008; Barbut et al. 2008). As presented in Table 1, the L* value of NORMAL (54.46) samples was significantly lower ($P<0.05$) than that of PSE-induced (57.39) breast meat. There was no significant differences in redness (a*) and yellowness (b*) between NORMAL and PSE-induced chicken breasts ($P>0.05$). Our results are in agreement with those reported by Lesiow and Xiong (2013) in pork and Mckee and Sams (1998) in turkey, who showed that normal meat had significantly lower color parameters than PSE. In line with the pH decline and lightness displayed by samples, significant differences were also found between NORMAL and PSE-induced samples for their ability to hold water (Table 2). The WHC of PSE-induced samples significantly decreased by 5.5%, 4.1% and 12.2% compared to that of NORMAL samples, as measured by the press, centrifugation and centrifugation + salt procedures, respectively. In line with these results, the cooking loss of PSE-induced samples was 1.9% higher than in the NORMAL counterparts. As a result of rapid pH fall while the carcass remains warm, denaturation of myofibrilar proteins (particularly myosin) occurs (Kauffman et al., 1998; Offer, 1991) along with decreased protein solubility, thus, contributing to poor WHC (Mackee & Sams, 1998; Lesiow & Xiong, 2013; Carvalho et al. 2014).

PSE-induced breast muscles displayed significantly lower hardness ($P<0.001$) chewiness ($P<0.01$), adhesiveness ($P<0.05$), cohesiveness ($P<0.05$) and gumminess ($P<0.05$) than the NORMAL samples (Table 2). The softness of meat is highly undesirable as this could have a great impact on consumer acceptability. Droval et al. (2012) reported that consumers are able to differentiate PSE breast meat from normal breast meat at the point of preferring and purchasing meat with a better consistency.

Enzymatic activity

Table 3 shows the effect of PSE-induction on the activity of antioxidant enzymes chicken breast muscle. CAT, GSH-Px and SOD were present in both samples and the activity of these enzymes was significantly lower in PSE-induced samples than in the NORMAL counterparts. To our knowledge, there is little information in the scientific literature regarding the activity of antioxidant enzymes in muscles from stressed animals. In opposition to our results, Sárraga, Carreras, & García Regueiro (2002) found higher GSH-Px activity in PSE pork meat than in normal meat. In line with the present results, Chen et al. (2010) and Delles, Xiong, True, Ao, & Dawson (2014) reported a reduction of the activities of antioxidant defense enzymes in stressed pigs and broilers, respectively, compared to non-stressed ones. To understand the apparent contradiction between studies, it is crucial to comprehend that the activity measured in *post-mortem* muscle may not necessarily reflect the concentration and activity of the enzymes in the muscle of living animals.

The appearance of PSE meat is associated with the animal susceptibility and its response to the stressful situation, and the specificity of biochemical processes in pork is caused by the development of a stress syndrome PSS (Porcine Stress Syndrome). Hence, it may be reasonable that muscles from stress-sensitive animals have higher concentration and activity of antioxidant enzymes as an adaptation defense mechanism (Poznyakovskij, Gorlov, Tikhonov & Shelepov, 2015). This is independent of the fate of these enzymes during the *post-mortem* transformation of muscle to meat.

The reduced activities of SOD, CAT, and GSH-Px in PSE-induced samples from the present study may be due to the denaturation of these enzymes as a result of the combination of high temperatures and fast pH decline in *post-mortem* muscle. Therefore, regardless of the concentration of antioxidant enzymes in the breast muscle prior to slaughter (presumably equivalent in both groups, NORMAL and PSE), the loss of activity took place during the events occurred during PSE-induction. It is well known, however, that CAT, SOD and GSH-Px contribute to endogenous antioxidant mechanisms for the prevention of cell damage induced by reactive oxygen species (Delles et al., 2014). Hence, the inactivation of antioxidant enzymes during fast pH decline may compromise the susceptibility of *post-mortem* PSE muscle to undergo oxidative reactions during storage/processing.

Interestingly, the significantly reduced activity of GSH-Px in PSE pork reported by Chen et al. (2010) occurred along with a significantly higher activity of PLA_2 in the same muscles. Taking into account that the latter enzyme promotes lipid peroxidation (Soares et al., 2003), these results support the higher oxidative instability of PSE-like meat compared to NORMAL meat.

Protein and lipid oxidation

In the present study, the susceptibility of PSE-induced meat to undergo protein oxidation during chilled storage assessed by means of assorted methods including the depletion of protein components (tryptophan and free thiols) and the formation of protein oxidation products (carbonyls, Schiff bases and disulphide bonds). The storage of chicken breasts at 4ºC for 7 days led to a significant depletion of tryptophan in both NORMAL and PSE-induced samples (Figure 2A). However, the loss of tryptophan was significantly higher in PSE-induced chicken breasts (~30%) than in the NORMAL counterparts (~15%).

The concentration of tryptophan was significantly higher in NORMAL chicken breasts than in PSE-induced ones at all sampling times. Similar results were obtained for the analysis of free thiols (Figure 2B). The depletion of free thiols was more intense in PSE-induced chicken breasts (~ 33%) than in the NORMAL counterparts (12%) with the latter having significantly higher free thiols than the former at days 4 and 7.

Tryptophan and thiol residues from sulfur-containing amino acids are known for being preferential targets of ROS and hence, are particularly susceptible to oxidative reactions (Davies, 2005). Both measurements are typically used as indicators of the oxidative damage to meat and poultry proteins (Estévez, 2015) and regarded as a relevant expression of the loss of meat quality owing to the negative consequences for the nutritional value of meat (Soladoye et al., 2015). Among the reasons that may explain a higher susceptibility of meat proteins from PSE-induced chicken breasts to undergo oxidative degradation, we propose i) the pro-oxidant effect of low pHs on the oxidizing cycle of iron (indispensable in metal-catalyzed protein oxidation mechanisms) (Soladoye et al., 2015), ii) the impact of fast pH decline on cell structure and compartmentalization than may facilitate the interaction of pro-oxidants with susceptible amino acid residues (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013) and iii) the influence that the protein denaturation that typically occurs in PSE meat may have on the susceptibility of proteins to suffer oxidative damage (Estévez, 2011).

This last point is particularly applicable to tryptophan residues in proteins as the loss if their native structure facilitates protein unfolding which leads to the exposure of non-polar residues usually buried in the hydrophobic core of the protein structure. And, it is worth to point out that for the first point, as observed previously, to enhance the Protox, there is a higher content of metamyoglobin in relation to oxymyoglobin in PSE- in comparison to NORMAL meat samples (Olivo et al., 2001).

The effect of PSE-induction on the formation of protein carbonyls (Figure 3A) is consistent with the results previously reported for the loss of susceptible amino acid residues. Protein carbonylation was considerably more intense in PSE samples (days 0 = 0.479; 4 = 1.316 and 7 = 3.967 nmol carbonyls/mg protein) than in NORMAL chicken breasts (days 0 = 0.215; 4 = 0.740 and 7 = 2.869 nmol carbonyls/mg protein). At all sampling days, PSE meat had significantly higher amounts of protein carbonyls than NORMAL meat.

Protein carbonyls are known to form in protein as a result of the oxidative deamination of alkaline amino acids (mainly lysine and arginine). Protein carbonylation in meat systems requires the catalytic effect of iron and hence, the mechanism previously proposed for the higher susceptibility if PSE-induced meat to suffer more intense protein oxidation is also applicable here. Moreover, the inactivation of the endogenous antioxidant enzymes during PSE-induction (aforementioned) may also be accounted as another plausible mechanism given the relationship between these enzymes and the susceptibility of muscle tissue to protein carbonylation.

Utrera, Parra, & Estévez (2014) found a protective effect of SOD and catalase enzymes against protein carbonylation in beef muscles and other authors reported congruent results in pork (Chen et al., 2010; Pastsart, De Boever, Claeys, & De Smet, 2013) and poultry (Delles et al., 2014; Mercier, Gatellier, Viau, Remignon, & Renerre, 1998). The accumulation of TBA-RS followed a similar trend to that reported for protein carbonyls (Figure 3B). TBA-RS numbers increased more intensively on the PSE-induced chicken (days 0 = 0.201; 4 = 0.275 and 7 = 0.414 mg MDA/kg muscle) than in normal meat (days 0 = 0.141; 4 = 0.219 and 7 = 0.309 mg MDA/kg muscle). In fact, higher TBA-RS numbers were found in PSE-induced breasts than in the NORMAL counterparts at all sampling days ($p<0.05$). According to these results, the susceptibility of PSE-induced meat to suffer more intense oxidative reaction during chilled storage affected similarly to both, proteins and lipids. Of notice, the differences between treatments for protein carbonylation were higher than for TBA-RS which is uncommon in meat samples.

According to several review articles (Estevez, 2015; Estévez, 2011; Lund, Heinonen, Baron, & Estévez, 2011; Soladoye et al., 2015), lipids are readily oxidized in a pro-oxidant environment and the oxidative damage is detected, compared to that in proteins, more easily and faster. The present results may indicate that under the conditions of the present study proteins were particularly affected by the oxidative reactions leading to a degree of protein carbonylation equivalent to that reported in processed meats (Soladoye et al., 2015).

Finally, proteins from chicken breasts were also analyzed for the formation of Schiff bases and disulphide bonds (Figures 4A and 4B, respectively). Both measurements are regarded as markers of the formation of protein cross-links as Schiff bases derive from the reaction of protein carbonyls with δ-amino group from alkaline amino acids and disulphide bonds are formed via the linkage of two oxidized cysteines (Lund et al., 2011).

In line with the previous results, PSE-induced samples had significantly higher amounts of Schiff bases and disulphide bonds than NORMAL chicken breasts at all days of storage (except for disulphide bonds at day 0). The consistency was expected given the connection between protein carbonylation and Schiff bases and the formation of disulphide bonds from free thiols. It is worth highlighting however, that the increase in disulphide bonds does not explain the total depletion of free thiols which means that the oxidation of the latter followed, partly, other mechanisms different to that of the formation of cross-links.

Connection between protein oxidation measurements and impaired quality in PSE poultry

Besides the loss of protein quality and digestibility, protein oxidation has also been linked to impaired protein functionality (Estévez, 2011). In particular, Utrera & Estévez (2012) reported plausible mechanisms by which protein carbonylation and the formation of protein cross-links would contribute to alter the ability of myofibrillar proteins to hold water, form emulsions and exert other functional properties. These findings were recurrent to previous proposals that pointed on the same direction (Bertram, Straadt, Jensen, & Dall Aaslyng, 2007; Estévez, Ventanas, Heinonen, & Puolanne, 2011; Z. Liu, Xiong, & Chen, 2010). While classical theories ascribe to denaturation processes the loss of protein functionality in PSE meat, the present results suggest that protein oxidation may also play a relevant role. In fact, this proposal is perfectly compatible with the classical theories given the proven connection between protein oxidation and protein denaturation (Estévez, 2011). It is of great scientific interest to comprehend the nature and chemical pathways of all mechanisms implicated in the impaired quality of PSE meat and hence, oxidation phenomena may not be disregarded. The implication of protein oxidation on the altered texture of PSE meat remains unclear. The formation of protein crosslinks in muscle systems (both Schiff bases and disulphide bonds) has been proposed as a relevant mechanism of meat toughening (Lund et al., 2011). The present results indicate that the soft texture of PSE meat does not respond to oxidative modification in proteins as PSE-induced chicken breasts had, in fact, higher concentration of disulphide bonds than the NORMAL counterparts. In this case, the disruption of the muscle tissue and sarcomere structure as a result of the fast pH decline and a likely increased proteolysis (Wilhelm et al., 2010), may be more influential on the rheological properties of meat than the formation of protein crosslinks. Further experiments may be required to confirm this extent.

CONCLUSIONS

The present results situate protein oxidation in the center of the biochemical reactions having an influence on the altered quality of PSE meat. PSE chicken breasts are more susceptible to oxidative stress due to lower concentration of antioxidant enzymes (CAT, GSH-Px and SOD) and as a consequence of the biochemical changes occurred in *post-mortem* meat. In turn, the oxidative damage to meat proteins may contribute to the loss of protein functionality and the impaired WHC of PSE meat.

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FINANCIAL SUPPORT

The authors are thankful to the CNPq for the support through the projects 474300/2011-0. RHC is under CNPq graduate scholarships 203165/2015-2.

FIGURE CAPTIONS

Figure 1. Evolution of pH in normal and PSE-induced breast broiler muscle during 24 hours following slaughter.

Significant differences between means for a sampling day in Student t test are denoted on top of bars; ns: no significant differences.

Figure 2. Tryptophan (mg NATA/g protein) (A) and free thiols (µmol thiols/mg sample) (B) depletion during chilled storage of normal and PSE-induced breast broiler muscle.

Significant differences between means for a sampling day in Student t test are denoted on top of bars; ns: no significant differences.

Figure 3. Formation of protein carbonyls (nmol carbonyls/mg protein) (A) and TBARS (mg MDA/kg muscle) (B) during chilled storage at 4ºC of normal and PSE-induced breast broiler muscle.

Significant differences between means for a sampling day in Student t test are denoted on top of bars; ns: no significant differences.

Figure 4. Formation of Schiff bases (fluorescence intensity) (A) and disulphide bonds (µmol thiols/mg sample) (B) during chilled storage at 4ºC of normal and PSE-induced breast broiler muscle.

Significant differences between means for a sampling day in Student t test are denoted on top of bars; ns: no significant differences.

Table 1. pH measurements and color (mean ± standard deviation) displayed by normal and PSE-induced breast broiler muscle.

	NORMAL	PSE	p-value[A]
pH 200 min	6.58±0.08	5.81±0.15	**
pH 24h	5.94±0.12	5.75±0.09	*
L*_Surface	54.46±2.68	57.39±2.15	*
a*_Surface	1.97±0.47	2.24±0.47	ns
b*_Surface	1.07±0.95	0.67±1.37	ns
L*_Inner	56.86±1.93	58.90±1.11	*
a*_Inner	3.89±0.58	3.80±0.83	ns
b*_Inner	1.09±1.18	0.63±0.76	ns

[A]Statistical significance in Student T test: *p<0.05; **p<0.01;; ns: no significant differences.

Table 2. Water holding capacity (WHC) and texture properties (mean ± standard deviation) of normal and PSE-induced breast poultry muscle.

	NORMAL	PSE	p-value[A]
WHC_press (%)	85.43±2.08	79.84±1.86	***
WHC_g (%)	82.94±1.55	78.82±1.50	***
WHC_salt (%)	134.34±5.69	122.11±2.97	***
Drip loss (%)	95.64±0.94	94.29±0.84	ns
Cooking loss (%)	75.27±1.40	73.30±1.42	*
Hardness	17.58±1.85	11.96±2.76	***
Adhesiveness	0.21±0.05	0.11±0.08	*
Springiness	0.70±0.09	0.69±0.07	ns
Cohesiveness	0.40±0.03	0.32±0.04	*
Gumminess	6.11±0.84	4.35±0.93	*
Chewiness	4.37±0.86	3.01±1.00	**
Resilience	0.31±0.03	0.27±0.01	ns

[A]Statistical significance in Student t test: *p<0.05; **p<0.01; ***p<0.001; ns: no significant differences. WHC_press = press method. WHC_g = centrifugation method. WHC_salt = centrifugation method with salt addition.

Table 3. Enzyme activities (mean ± standard deviation) in normal and PSE-induced breast poultry muscle.

	NORMAL	PSE	p-value[A]
CAT[B]	43.84±3.31	38.15±2.71	*
GSH[C]	0.66±0.04	0.54±0.04	***
SOD[D]	84.38±4.67	70.08±3.42	***

[A]Statistical significance in Student T test: *p<0.05; **p<0.01; ***p<0.001; ns: no significant differences.

[B]Catalase activity expressed as μmol x min^{-1} x g^{-1} (U/g); [C] Glutathione peroxidase activity expressed as μmol of oxidised NADPH μL^{-1} min g^{-1} (U/g); [D]Superoxide dismutase activity expressed as U/g of sample, SOD activity that inhibits the reaction by 50%.

Figure 1.

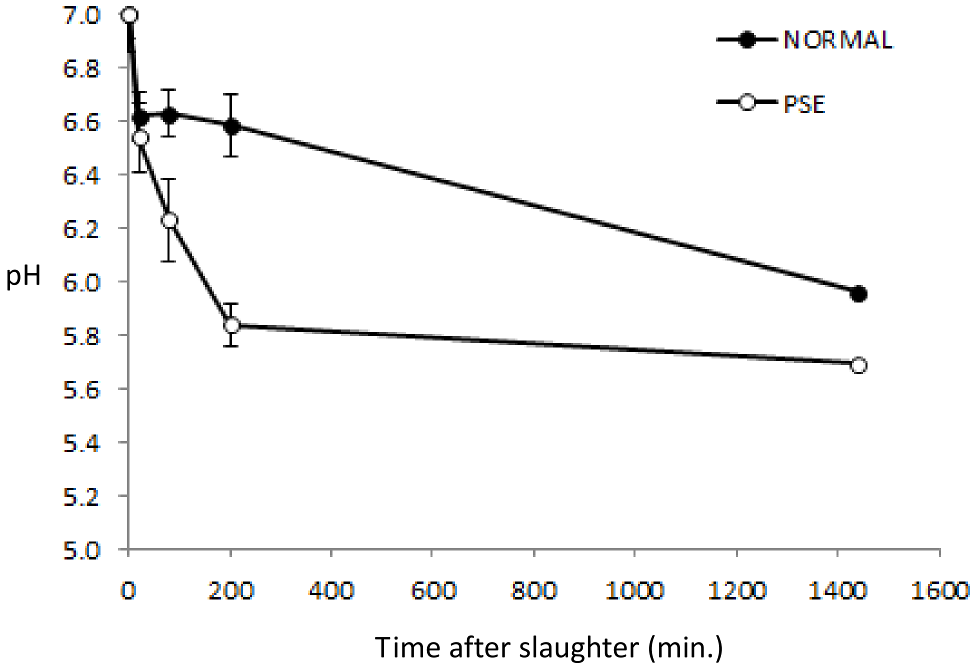
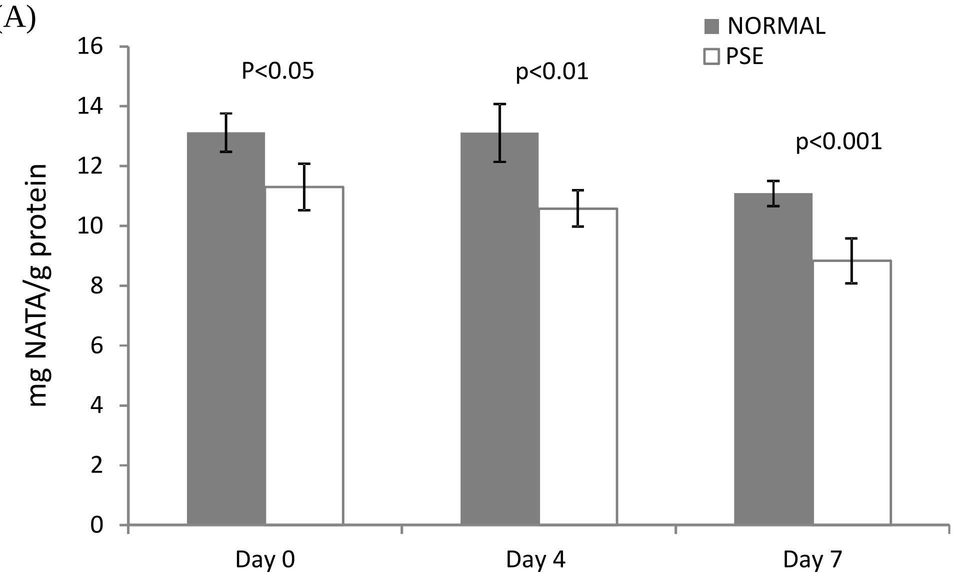

7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
pH
0
200
400
600
800
1000
1200
1400
1600
Time after slaughter (min.)
NORMAL
PSE

Figure 2.

(A)


NORMAL
PSE
P<0.05
p<0.01
p<0.001
mg NATA/g protein
16
14
12
10
8
6
4
2
0
Day 0
Day 4
Day 7

(B)

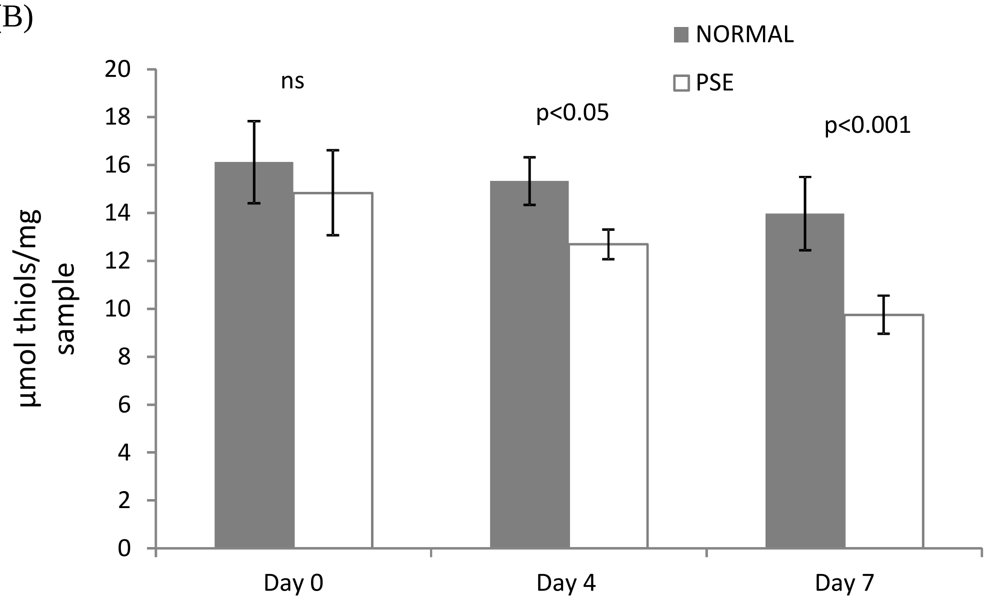
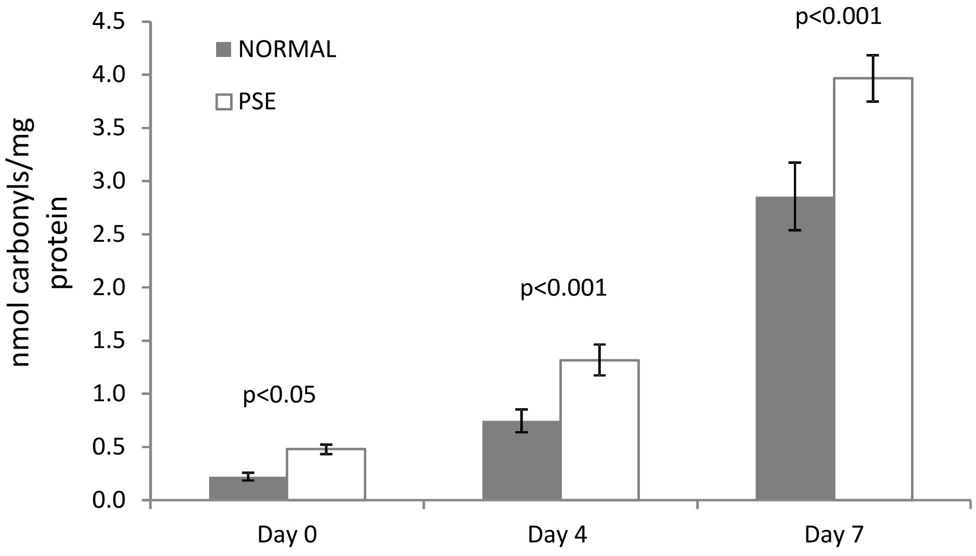

NORMAL
PSE
ns
p<0.05
p<0.001
µmol thiols/mg sample
20
18
16
14
12
10
8
6
4
2
0
Day 0
Day 4
Day 7

Figure 3.

(A)


4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
nmol carbonyls/mg protein
NORMAL
PSE
p<0.05
p<0.001
p<0.001
Day 0
Day 4
Day 7

(B)

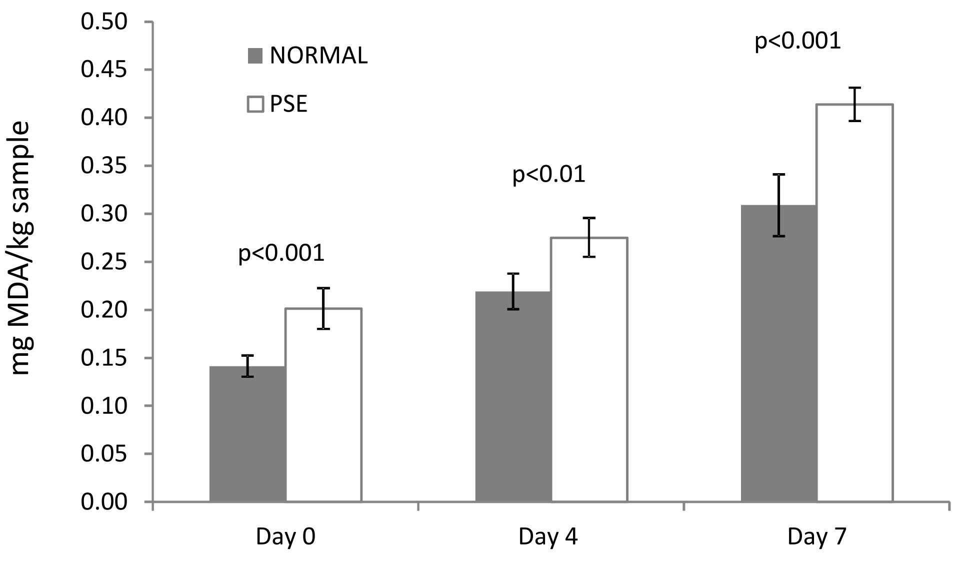
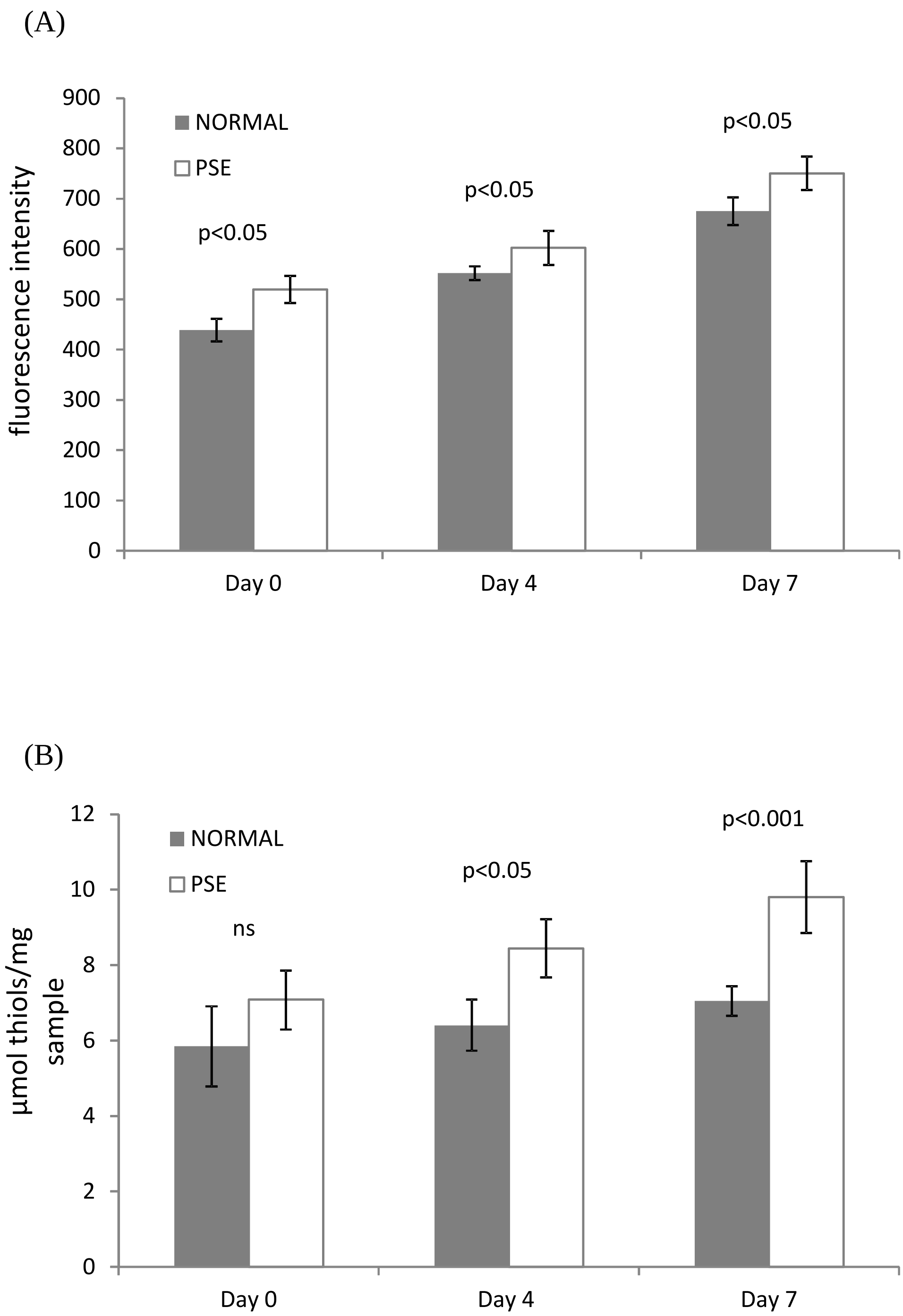

0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
mg MDA/kg sample
NORMAL
PSE
p<0.001
p<0.01
p<0.001
Day 0
Day 4
Day 7

Figure 4.


(A)
900
800
700
600
500
400
300
200
100
0
fluorescence intensity
NORMAL
PSE
p<0.05
p<0.05
p<0.05
Day 0
Day 4
Day 7
(B)
12
10
8
6
4
2
0
µmol thiols/mg sample
NORMAL
PSE
ns
p<0.05
p<0.001
Day 0
Day 4
Day 7

11 CONCLUSÕES

Experimento A – Caracterizamos e mapeamos a incidência de carne PSE em perus na região sul do Brasil, durante o verão e verificamos uma incidência de 41% da anomalia. Os resultados demostraram que são perdidos aproximadamente 5% de peso nas carnes PSE, aumentando a perspectiva de perdas econômicas para a indústria de carne de peru.

Experimento B – Sob condições industriais a taxa de declínio do pH muscular é diretamente afetada pela temperatura, o completo *rigor mortis* foi alcançado depois de 24 horas post-mortem sob refrigeração por ar, indicando que após este período de tempo os filés de peito de perus podem ser processados, a fim de manter a funcionalidade das proteínas miofibrilares.

Experimento C - Colheita mecânica e manual possui eficácia semelhante na colheita de perus. O uso da colheita mecânica não influencia nas taxas de mortalidade (DOA) e hematomas das aves, entretanto a colheita manual apresentou maior incidência de carne PSE.

Experimento D – *Wind Chill* e umidade relativa do ar são fatores importantes para o bem-estar de perus durante a estação de inverno. Os resultados demonstram que nestas condições de transporte a qualidade da carne do peito é afetada, ocasionado anormalidades como carnes PSE e DFD.

Experimento E - Para o transporte de peru durante o verão no Brasil, vários fatores influenciam o aparecimento de DOA e incidência de carnes PSE, o banho de água pré-transporte aplicado nas aves ainda na fazenda reduz a ocorrência de DOA e carnes PSE. Durante o transporte foi observada a formação de um núcleo térmico na parte inferior do caminhão nas posições meio e traseira.

Experimento F – O transporte de perus durante o verão e inverno no Brasil é influenciado por vários fatores e ocasionam o desenvolvimento de carnes PSE e DFD. O uso do banho de água enquanto na fazenda reduz a ocorrência de carne PSE no verão, entretanto aumenta no inverno em aves transportadas em curta distância. Já o uso do banho aumenta a ocorrência de carne DFD em longa distância. Longas distância/tempo no verão foram prejudiciais ao bem-estar das aves, produzindo mais carne PSE e DOA.

Experimento G - A incidência de carnes DFD é maior no inverno e da carne PSE no verão, assim como o índice de DOA é alto no verão.

Experimento H - A oxidação de proteínas situa-se no centro das reações bioquímicas e exerce influência sobre a qualidade da carne PSE. Peitos de frango PSE foram mais suscetíveis ao estresse oxidativo devido à menor concentração de enzimas antioxidantes (catalase, glutationa e sódio dismutase). O dano oxidativo proteico contribui para a perda da funcionalidade das proteínas da carne PSE.