UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS
DEPARTAMENTO DE TECNOLOGIA DE ALIMENTOS

INJEÇÃO POST-RIGOR DE CLORETO DE CÁLCIO NO MÚSCULO SEMIMEMBRANOSO DE NOVILHOS SUPLEMENTADOS COM ZILPATEROL: PROPRIEDADES FÍSICO-QUÍMICAS E SENSORIAIS DA CARNE

SÉRGIO BERTELLI PFLANZER JR.

Prof.Dr. Pedro Eduardo de Felício
Orientador

Tese apresentada à Faculdade de Engenharia de Alimentos para a obtenção do título de Doutor em Tecnologia de Alimentos.

CAMPINAS, 2012

Ficha catalográfica

FICHA CATALOGRÁFICA ELABORADA POR
LUCIANA P. MILLA – CRB8/8129- BIBLIOTECA DA FACULDADE DE ENGENHARIA DE ALIMENTOS – UNICAMP

P483i

Pflanzer, Sérgio Bertelli, 1983-
Injeção post-rigor de cloreto de cálcio no músculo semimenbranoso de novilhos suplementados com zilpaterol: propriedades físico-químicas e sensoriais da carne / Sérgio Bertelli Pflanzer. -- Campinas, SP: [s.n], 2012.

Orientador: Pedro eduardo de Felício.
Tese (doutorado) – Universidade Estadual de Campinas.Faculdade de Engenharia de Alimentos.

1. Músculo semimembranosus. 2. Cloreto de cálcio. 3. Hidrocloreto de zilpaterol. 4. Palatabilidade. 5. Cor. I. Felício, Pedro Eduardo de II. Universidade Estadual de Campinas.Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Título em inglês: Post-rigor injection of calcium chloride in semimembranosus muscle of steers suplemented with zilpaterol: physical-chemical and sensory properties
Palavras-chave em inglês (Keywords):
Beef inside round
Calcium chloride
Zilpaterol hydrochloride
Palatability
Color stability
Área de concentração: Tecnologia de Alimentos
Titulação: Doutor em Tecnologia de Alimentos
Banca examinadora:
Pedro Eduardo de Felício [Orientador]
Eduardo Francesquine Delgado
Manuel Pinto Neto
Guilherme Fernando Alleoni
Angélica Simone Cravo Pereira
Data da defesa: 09/02/2012
Programa de Pós Graduação: Tecnologia de Alimentos

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

SÉRGIO BERTELLI PFLANZER JÚNIOR

INJEÇÃO POST-RIGOR DE CLORETO DE CÁLCIO NO MÚSCULO SEMIMEMBRANOSO DE NOVILHOS SUPLEMENTADOS COM ZILPATEROL: PROPRIEDADES FÍSICO-QUÍMICAS E SENSORIAIS DA CARNE

TESE DE DOUTORADO APRESENTADA À FACULDADE DE ENGENHARIA DE ALIMENTOS UNICAMP PARA OBTENÇÃO DO TÍTULO DE DOUTOR EM TECNOLOGIA DE ALIMENTOS

Pedro Eduardo de Felício

ORIENTADOR

Este exemplar corresponde à versão final da tese defendida por Sérgio Bertelli Pflanzer Júnior, aprovada pela comissão julgadora em ___/___/___ e orientado pelo Prof. Dr. Pedro Eduardo de Felício.

_____
Assinatura do Orientador

CAMPINAS, 2012

BANCA EXAMINADORA

_____

Prof. Dr. Pedro Eduardo de Felício
Orientador

_____

Profa. Dra. Angélica Simone Cravo Pereira
Titular

_____

Prof. Dr. Eduardo Francisquine Delgado
Titular

_____

Dr. Guilherme Fernando Alleoni
Titular

_____

Dr. Manuel Pinto Neto
Titular

_____

Dr. Expedito Tadeu Facco Silveira
Suplente

_____

Prof. Dr. Marise Aparecida Rodrigues Pollonio
Suplente

_____

Prof. Dr. Renata Tieko Nassu
Suplente

AGRADECIMENTOS

À UNICAMP, em especial à Faculdade de Engenharia de Alimentos e ao Departamento de Tecnologia de Alimentos pela acolhida e pela estrutura oferecida para o desenvolvimento dos trabalhos.

Ao CNPq pela bolsa de doutorado no país e pela concessão da bolsa de doutorado sanduíche.

A todos os meus familiares e em especial a minha mãe e minha irmã que sempre estiveram por perto, trazendo carinho e apoiando minhas decisões.

A minha esposa Carolina pelo apoio e compreensão em todos os momentos bons ou difíceis dessa empreitada.

Ao Professor e orientador Pedro Eduardo de Felício pela orientação, pela amizade, pelos conselhos como professor, pesquisador e profissional, e pela experiência de vida passada ao longo dos anos, como homem e como pai, meus mais sinceros e profundos agradecimentos.

À todos os Professores do DTA pela amizade e ensinamentos para minha qualificação acadêmica.

À todos os colegas e funcionários do DTA/FEA/UNICAMP pela amizade e companheirismo.

Ao Prof. Dr. Mark Miller pela oportunidade proporcionada para que eu pudesse executar minha tese de doutorado na Texas Tech University e por todos seus ensinamentos acadêmicos e éticos.

Ao colega Argenis Rodas pela amizade e compreensão em quase todos os momentos que passei nos EUA, que além de ter colaborado muito na execução do projeto foi também um ótimo amigo.

Aos membros da banca examinadora, pelo tempo dedicado na avaliação deste trabalho e pelas valiosas sugestões, que enriqueceram o mesmo e que contribuíram imensamente para o aperfeiçoamento técnico do autor.

À todos aqueles que participaram, direta ou indiretamente, da realização deste trabalho.

MUITO OBRIGADO!

ÍNDICE

Página

LISTA DE ABREVIAÇÕES

AC	Adenilato ciclase
AMPc	Adenosina monofosfato cíclico
ARM	Atividade redutora da metamioglobina
ATP	Adenosina trifosfato
AβA	Agonista dos receptores beta adrenérgicos
βAA	Beta adrenergic receptor agonist
BG	Belt grill (tipo de forno)
d	dia
DMb	Deoxymyoglobin
DSM	Deep *Semimembranosus*
FDA	Food and Drug Administration
FG	Feeding group
GM	Músculo *Gluteus medius*
Gs	Proteína G
HCW	Hot carcass weight
HZ	Hidrocloreto de zilpaterol
IFM	Índice de fragmentação miofibrilar
IGF	Insulin-like growth factor
IT	Injection treatment
KDa	Kilodalton
LL	Músculo *Longissimus lumborum*
LM	Músculo *Longissimus*
M	Músculo ou Molar
MFI	Miofibrilar fragmentation index
MMb	Metmyoglobin
MRA	Metmyoglobin reducing activities
NAD	Nicotinamide adenine dinucleotide
PKA	Proteína quinase A
PVC	Polivinil chloride
QG	Quality grade
RNA	Ácido ribonucleico
RNAm	Ácido ribonucleico mensageiro
SL	Sarcomere length
SSM	Superficial *Semimembranosus*
T4	Tiroxina
TB	Músculo *Triceps brachii*
TBARS	Tiobarbituric acid reactive substances
USDA	United States Department of Agriculture
WBSF	Warner Bratzler Shear Force
YG	Yield grade
ZH	Zilpaterol hydrochloride

RESUMO

Dois estudos foram conduzidos para determinar o efeito da injeção de cloreto de cálcio nas características de palatabilidade e na estabilidade da cor de bifes de coxão mole (M. *Semimembranoso*) de bovinos suplementados com hidrocloreto de zilpaterol (HZ). No estudo 1 (palatabilidade), 466 bovinos foram divididos em 2 grupos, não-suplementados (n = 233) e suplementados (8,3 mg/kg de HZ nos últimos 20 dias do confinamento; n = 233). Após o abate e resfriamento, 39 carcaças *USDA Select* foram destinadas aleatoriamente para o teste (não-suplementados = 19; HZ = 20). No estudo 2 (estabilidade da cor), foram utilizados, 20 das 39 carcaças, em igual número de não-suplementados (n = 10) e suplementados HZ (n = 10). Os cortes de coxão mole das duas meias carcaças foram enviados para o laboratório, onde foram destinados alternadamente para os tratamentos não-injetado ou injetado com cloreto de cálcio (5% do peso com solução a 200 mM $CaCl_2$). No estudo 1 foram avaliados o comprimento de sarcômero, índice de fragmentação miofibrilar, maciez instrumental (força de cisalhamento por Warner Bratzler - WBSF) e atributos sensoriais dos bifes de coxão mole maturados por até 28 dias. No estudo 2 foram avaliadas a composição centesimal, atividade redutora da metamioglobina (MRA), cor visual e instrumental e oxidação lipídica (TBARS) dos bifes de coxão mole maturados por até 21 dias seguidos da exposição em “display” por 4 dias. No estudo 1 não houve diferença ($P > 0,05$) do comprimento de sarcômero entre tratamentos com ou sem HZ. Amostras de animais não suplementados com HZ e amostras injetadas com

cloreto de cálcio apresentaram maiores ($P < 0,05$) índices de fragmentação miofibrilar em relação a suplementados e a não-injetados, respectivamente. A suplementação com HZ não afetou ($P > 0,05$) as perdas de peso pela exsudação na embalagem, bem como a perda na cocção. A utilização de cloreto de cálcio aumentou ($P < 0,05$) as perdas de peso. O uso de HZ aumentou ($P < 0,05$) a WBSF somente dos bifes maturados por 7 dias. A injeção de cloreto de cálcio em amostras de animais suplementados com HZ anulou ($P > 0,05$) a diferença na WBSF após 7 dias de maturação e também reduziu ($P < 0,05$) a WBSF nas amostras dos não suplementados. O HZ não afetou ($P > 0,05$) as características sensoriais dos bifes maturados por 14 ou 21 dias. Amostras injetadas foram mais macias ($P < 0,05$) do que as não-injetadas, após a maturação por 14 dias, entretanto não houve diferença ($P > 0,05$) nas maturadas por 21 dias para os atributos sensoriais. Estudo 2: animais tratados com HZ resultaram em carne de cor vermelho-cereja mais claro, maior valor de L * e menores escores de descoloração ($P < 0,05$). No final da exposição em "display", os bifes de animais suplementados com HZ tiveram maiores valores de a * e b * que os controle ($P < 0,05$). A injeção de $CaCl_2$ resultou em carne de cor vermelha mais intensa e com maiores escores de descoloração ($P < 0,05$) em amostras maturadas por 14 e 21 dias, mas não influenciou ($P > 0,05$) a cor aos 7 dias. O valor de L * não foi afetado pela injeção de $CaCl_2$ ($P > 0,05$). Porém, ao final do período de exposição, as amostras injetadas apresentaram menores valores de a * b * ($P < 0,05$) que aquelas não-injetadas. A medida que se estendem os períodos de maturação a

carne apresentou-se mais escura e com maiores escores de descoloração ($P < 0,05$). Além disso, a maturação e a exposição continuadas diminuíram ($P < 0,05$) os valores de a* e b* em ambos os tratamentos de suplementação e injeção. A oxidação lipídica, medida por TBARS, não foi afetada ($P > 0,05$) pela suplementação com HZ. A injeção de $CaCl_2$ aumentou ($P < 0,05$) os valores de TBARS. A suplementação dos novilhos com zilpaterol reduziu a maciez das amostras em relação ao grupo não-suplementado. Entretanto a maturação por 14 dias, ou por 7, dias combinada com o tratamento pelo $CaCl_2$, pode equiparar as médias de maciez objetiva. Na primeira alternativa, o HZ contribuiu para uma maior estabilidade da cor relativamente aos não suplementados, e na segunda, não ocorreu alteração da cor em decorrência do $CaCl_2$ injetado.

Palavras-chave: coxão mole, cloreto de cálcio, hidrocloreto de zilpaterol, palatabilidade, estabilidade da cor.

SUMMARY

POST-RIGOR INJECTION OF CALCIUM CHLORIDE IN SEMIMEMBRANOSUS MUSCLE OF STEERS SUPPLEMENTED WITH ZILPATEROL: PHYSICAL-CHEMICAL AND SENSORY PROPERTIES OF THE MEAT

Two studies were conducted to determine the effect of calcium chloride injection on the palatability traits and color stability of steaks from inside round (M. *Semimembranosus*) of steers supplemented with zilpaterol hydrochloride (ZH). In study 1 (palatability), 466 steers were divided into two groups, non-supplemented and supplemented (8.3 mg/kg of ZH in the last 20 days of feedlot). After slaughter and cooling, 39 USDA Select grade carcasses were randomly destined for the test (non-supplemented = 19; ZH = 20). In study 2 (color stability), 20 from 39 carcasses, previously selected, were randomly destined for the test (non- supplemented = 10, ZH = 10). The inside round of both half-carcasses were sent to the laboratory where they were alternately selected to serve as non-injected or injected with calcium chloride (5 % with 200 mM $CaCl_2$ solution). In study 1 it were evaluated the sarcomere length, myofibrillar fragmentation index, instrumental tenderness (shear force by Warner Bratzler - WBSF) and sensory traits of inside round steaks aged up to 28 days. In study 2 it were evaluated the proximate composition, metmyoglobin reducing activity, visual and instrumental color and lipid oxidation (TBARS) of inside round steaks aged up to 21 days followed by exposure in the display for 4 days. In study 1 there is no difference ($P > 0.05$) in treatments with or without ZH for sarcomere length. Samples of not ZH

supplemented animals and injected samples had higher ($P < 0.05$) myofibrillar fragmentation index, than supplemented and non-injected, respectively. The ZH supplementation did not affect ($P > 0.05$) the losses during aging and cooking. The $CaCl_2$ injection increased ($P < 0.05$) both losses. The use of ZH increased ($P > 0.05$) WBSF of inside round steaks aged 7 d, however samples aged 14 d did not differ ($P > 0.05$). The $CaCl_2$ injection in samples from ZH supplemented steers cancelled ($P > 0.05$) the difference for WBSF after 7 d of aging and reduced ($P < 0.05$) WBSF in samples from non-supplemented. The ZH did not affect ($P > 0.05$) sensory traits of steaks aged 14 and 21 d. Injected samples were more tender ($P < 0.05$) than non-injected after 14 d aging, however there was no difference ($P > 0.05$) in steaks aged 21 d for sensory traits. In study 2 ZH steaks were lighter cherry red, had higher L* values, and had lower discoloration scores ($P < 0.05$). At the end of display, ZH steaks had greater ($P < 0.05$) a* and b* values than non-supplemented. $CaCl_2$ injection resulted in redder beef color, and increased discoloration scores ($P < 0.05$) in samples aged 14 and 21 d, however it did not affect color traits on d 7 ($P > 0.05$). L* values were not affected by $CaCl_2$ ($P > 0.05$), but at the end of display, injected samples had lower a* and b* values than non-injected samples ($P < 0.05$). Increasing aging and display time resulted in redder color and greater discoloration scores ($P < 0.05$). Moreover aging and display time decreased ($P < 0.05$) a* and b* values from both treatments of the two sources of variation. Lipid oxidation, evaluated by TBARS, was not affected by ZH ($P > 0.05$). The $CaCl_2$ injection increased ($P < 0.05$) TBARS

values. The ZH supplementation decreased tenderness of inside round steaks, however 14 d aging, or 7 d aging combined with $CaCl_2$ injection, can even objective tenderness means, and in the last one, there is no effect in color traits due to $CaCl_2$ injection, as it happens on d 14 and 21 of aging.

Keywords: beef inside round, calcium chloride, zilpaterol hydrochloride, palatability, color stability.

1. INTRODUÇÃO GERAL

A utilização do hidrocloreto de zilpaterol (**HZ**), composto sintético pertencente ao grupo dos agonistas dos receptores beta-adrenérgicos (**AβA**), tem sido amplamente estudada para determinar seus efeitos no desempenho e características de carcaça de bovinos. Assim, já é sabido que bovinos confinados e suplementados com HZ apresentam maior taxa de ganho de peso diário e melhor conversão alimentar, aumento no rendimento de carcaça, carcaças mais pesadas, incremento da área de olho de lombo, melhoria do rendimento de desossa (*yield grade* - **YG**) e diminuição na quantidade de gordura intramuscular (*quality grade* - **QG**) (BECKETT et al., 2009; DERINGTON et al., 2011; ELAM et al., 2009; GARMYN et al., 2010; MONTGOMERY et al., 2009a; MONTGOMERY et al., 2009b; VASCONCELOS et al., 2008). Em relação a seus efeitos na qualidade da carne, foi verificado que a utilização de HZ reduz os escores de maciez, quando avaliada sensorialmente, e aumenta os valores de força de cisalhamento, por medida instrumental, principalmente do contrafilé, M. *Longissimus* (BROOKS et al., 2009; HILTON et al., 2009; LEHESKA et al., 2009) e do coxão mole, M. *Semimembranoso* (GARMYN et al., 2010).

O efeito negativo dos AβA na maciez da carne tem sido atribuído principalmente ao aumento da atividade da calpastatina, enzima que inibe a ação das calpaínas (μ-calpaína e m-calpaína), prejudicando a degradação proteica, no processo de maturação (DUNSHEA et al., 2005; HOPE-JONES et al., 2010; KOOHMARAIE e SHACKELFORD, 1991; KRETCHMAR et al.,

1989; YANG e McELLIGOTT, 1989). Kretchmar et al. (1990) relataram 74% de aumento na atividade da calpastatina no músculo do contrafilé (M. *Longissimus dorsi*) de ovinos tratados com L-644,969 (AβA - Merck, Sharpe e Dohme Research Laboratories, Rahway NJ) nos dias 0 e 4 após o abate, além de uma redução de 10 e 14 % na atividade das calpaínas nestes dois períodos após o abate, respectivamente. A redução na atividade das calpaínas também foi relatada em estudo realizado por Wang e Beermann (1988), no qual a carne de animais tratados com cimaterol (AβA) tiveram entre 55 e 70 % de redução na atividade destas enzimas. Contudo, outros trabalhos indicaram inconsistência do efeito dos AβA na atividade das enzimas calpaínas e calpastatina (GEESINK et al., 1993; HOPE-JONES et al., 2010; SIMMONS et al., 1997; WHEELER e KOOHMARAIE, 1992). Outro complexo enzimático, o das proteases lisossomais, poderia igualmente apresentar sua atividade reduzida pelo uso dos AβA, como o L-644,969 (KRETCHMAR et al., 1990).

Em decorrência do efeito negativo a maciez causado pela utilização de AβA na alimentação de bovinos, algumas tecnologias pós-abate, como: maturação, *"enhancement"* (melhoramento da qualidade através de marinação ou injeção de soluções de diferentes sais), amaciamento mecânico e estimulação elétrica de carcaças, têm sido aplicadas na carcaça ou na carne para promover uma melhoria da qualidade, principalmente a maciez. Brooks et al. (2009) avaliaram o efeito da utilização HZ na força de cisalhamento dos músculos M. *Longissimus lumborum* (**LL**), M. *Triceps brachii* (**TB**) e M. *Gluteus medius* (**GM**) maturados por 7, 14 e 21 dias. Os autores concluíram

que a maturação melhorou a maciez da carne na mesma proporção em amostras de animais suplementados ou não com HZ. Entretanto, outros pesquisadores relataram que amostras de bovinos que receberam HZ, mesmo respondendo a maturação, foram mais duras (maiores valores de força de cisalhamento) em todos os períodos pós-abate estudados, do que as amostras de animais não tratados (HILTON et al., 2009; HOLMER et al., 2009; HOPE-JONES et al., 2010; KELLERMEIER et al., 2009; LEHESKA et al., 2009; MEHAFFEY et al., 2009; RATHMANN et al., 2009).

Estudos realizados com avaliação sensorial, com provadores treinados e com consumidores, confirmaram algumas das pesquisas realizadas por avaliações instrumentais de maciez, em amostras de bovinos que receberam HZ e que foram maturadas. Garmyn et al. (2010) concluíram que a maturação (14 e 21 dias) melhorou as características sensoriais da carne avaliada por provadores treinados, de animais suplementados com HZ na mesma intensidade que de animais não suplementados. Contudo, Leheska et al. (2009) descreveram menores escores de suculência, sabor e maciez para o contrafilé (maturado por 28 dias) de novilhas e novilhos que receberam HZ, quando comparados ao de animais controle. Da mesma maneira, Hilton et al. (2009) concluíram que contrafilé de animais suplementados com HZ e maturadas por 14 dias apresentaram menores escores de suculência, sabor e maciez em pesquisa com provadores treinados, mas, quando avaliados por consumidores. Ainda não houve diferença para proporção da aceitação global e aceitação da maciez entre amostras HZ ou controle. Entretanto, os consumidores apontaram

menores escores de maciez para amostras de contrafilé de animais que receberam HZ. Em outro estudo, avaliando a aceitação por teste com consumidores, Mehaffey et al. (2009) concluíram que o contrafilé de bovinos suplementados com HZ necessitam ser maturados por, no mínimo, 21 dias para apresentar os mesmos escores de aceitação que o de animais controle, pois aos 14 dias de maturação, as amostras controle foram mais aceitas.

A utilização de estimulação elétrica de alta voltagem (400 V após 30 min da sangria) em carcaças bovinas melhorou a maciez (diminuição da força de cisalhamento) do contrafilé com maior eficiência em amostras de animais tratados com HZ do que de animais não tratados. Essa melhoria foi atribuída à diminuição da atividade da calpastatina, mas isso não foi suficiente para igualar a maciez entre amostras de animais com ou sem HZ aos 3 ou 14 dias de maturação (HOPE-JONES et al., 2010).

Brooks et al. (2010) estudaram o efeito de três tecnologias para melhorar a maciez do contrafilé de bovinos alimentados com HZ, *"enhancement"* (melhoramento da qualidade através da injeção ou marinação com soluções de sais), amaciamento mecânico e maturação. Os resultados indicaram que o *"enhancement"* melhorou a maciez, mas não foi suficiente para superar os efeitos negativos da utilização do HZ sobre a força de cisalhamento, em amostras maturadas por até 21 dias; entretanto os consumidores atribuíram melhores escores sensoriais para as amostras que

foram tratadas com *"enhancement"* e amaciamento mecânico, maturadas por 14 e 21 dias.

Além das tecnologias citadas, outro método que pode ser proposto para reverter os prejuízos causados na qualidade da carne pela utilização de AβA é a utilização de cloreto de cálcio ($CaCl_2$), seja por injeção da carcaça pré-rigor ou injeção da carne pós-rigor. O uso de cloreto de cálcio é bem conhecido por melhorar a maciez da carne de animais que apresentam muita variação neste atributo, como os de genótipo *Bos indicus*, touros, castração tardia, e descartes (animais mais velhos) (DILES, MILLER e OWEN, 1994; LANSDELL et al., 1995; MORGAN et al., 1991; WHEELER, KOOHMARAIE e CROUSE, 1991). O efeito da utilização de cloreto de cálcio, juntamente com algum tipo de AβA é raramente descrito. Koohmaraie e Shackelford (1991) estudaram o efeito da injeçao de cloreto de cálcio em carcaças de ovinos suplementados com L644,969 (tipo de AβA) e apontaram que a utilização do AβA aumentou a força de cisalhamento, enquanto que o cloreto de cálcio promoveu uma melhoria na maciez. Os autores não verificaram interação significativa entre os tratamentos, e concluíram que o procedimento de injeção de cloreto de cálcio foi eficiente em reverter a dureza causada pela utilização do L644,969. Todavia, não existem pesquisas avaliando o efeito da utilização do cloreto de cálcio na qualidade da carne de bovinos que foram suplementados com HZ.

A maior parte das tecnologias pós-abate, aplicadas para melhorar a palatabilidade da carne, reduziram os valores de força de cisalhamento nas amostras de contrafilé de animais suplementados com diferentes tipos de AβA, até mesmo para o HZ. Entretanto, essas amostras foram maturadas por um longo período e muitas vezes os valores de força de cisalhamento ainda não atingiram os mesmos das amostras dos animais não tratados com HZ. Como foi verificado, poucas pesquisas foram feitas para avaliar o efeito do HZ na qualidade do coxão mole (M. *Semimembranoso*). Porém, não há pesquisa que tenha avaliado o efeito da injeção de cloreto de cálcio na qualidade do coxão mole de bovinos suplementados com HZ.

2. OBJETIVO

O objetivo desta pesquisa foi comprovar se a utilização de hidrocloreto de zilpaterol na suplementação de novilhos, nos últimos 20 dias de confinamento, diminui a maciez do coxão mole (M. *Semimembranoso*), e determinar a eficácia da maturação e da injeção pós-rigor de cloreto de cálcio na melhoria da maciez, sem prejuízo dos demais atributos de qualidade.

3. REVISÃO BIBLIOGRÁFICA

3.1. Agonistas dos receptores beta-adrenérgicos (AβA)

Os agonistas dos receptores beta-adrenérgicos (AβA), também conhecidos como agentes repartidores, têm sido utilizados e estudados em diferentes espécies de animais de produção por mais de duas décadas, principalmente por seus efeitos na produção de carcaças mais magras e musculosas. Esses compostos sintéticos são farmacologicamente similares às catecolaminas, como a dopamina, norepinefrina e epinefrina, que são compostos utilizados na medicina humana a mais de 30 anos como bronco dilatadores . Nos animais de produção, os AβA mais utilizados são: cimaterol, clenbuterol, L-644-969, ractopamina, salbutamol e zilpaterol, os quais são administrados na forma oral pela adição em ingredientes da ração (ANDERSON, MOODY e HANCOCK, 2005).

Nos Estados Unidos, onde esta pesquisa foi desenvolvida, apenas 2 AβA são aprovados pelo **FDA** (*US Food and Drug Administration*) para utilização na criação de bovinos (novilhas e novilhos). O hidrocloreto de ractopamina, comercialmente conhecido como Optaflexx 45 (Elanco Animal Health, Greenfield, IN), aprovado em 2003 (FDA, 2003), e o hidrocloreto de zilpaterol (HZ), comercializado com o nome de Zilmax (Intervet/Schering-Plough Animal Health, DeSoto, KS), aprovado em 2006 (FDA, 2006). Este último, HZ, tem sido alvo de diversas pesquisas com o objetivo de caracterizar seus efeitos, seja na etapa de produção animal, avaliando as

características de desempenho, nas características de carcaças, como os indicadores de qualidade e rendimento (*Quality grade* e *Yield grade*, respectivamente), ou, ainda, nos atributos de qualidade da carne. De maneira geral, os trabalhos indicaram que o HZ, quando suplementado durante a fase final de confinamento, promove uma maior taxa de ganho de peso diário, melhora a conversão alimentar, aumenta o peso das carcaças, aumenta os rendimentos de carcaça e de desossa, diminuindo a quantidade de gordura intramuscular (mármore) e subcutânea (acabamento), mas compromete a qualidade da carne, principalmente a maciez (BECKETT et al., 2009; DERINGTON et al., 2011; ELAM et al., 2009; GARMYN et al., 2010; MONTGOMERY et al., 2009a; MONTGOMERY et al., 2009b; VASCONCELOS et al., 2008).

Nesta revisão serão discutidos os aspectos relevantes sobre a utilização dos AβA, especialmente o hidrocloreto de zilpaterol, aplicados na produção de ruminantes, sobretudo em bovinos. Os pontos abordados serão os mecanismos de ação, e como as características de palatabilidade da carne, assim como a estabilidade da cor podem ser afetadas pela utilização destes compostos.

3.1.1. Mecanismo de ação dos AβA

Os efeitos dos AβA são mediados por modificações de alguns sinais metabólicos nas células musculares e de gordura, através da ligação destes compostos em receptores específicos na membrana celular.

Os receptores das substâncias βA (beta-adrenérgicos) são divididos em três subtipos, os receptores beta-1 (**β1**), beta-2 (**β2**) e beta-3 (**β3**), e estes estão presentes na maioria das células de mamíferos. Entretanto, a distribuição e proporção dos subtipos variam entre os tecidos e as espécies (MERSMANN, 1998). Nos bovinos, a predominância nas células musculares e adipócitos é dos receptores β2, podendo chegar à proporção de 75% de β2 e 25% de β1 nas células de gordura (SILLENCE e MATTHEWS, 1994; VAN LIEFDE et al., 1994). Essa relação pode explicar porque as respostas da suplementação com HZ, em bovinos, são mais pronunciadas do que a suplementação com ractopamina, visto que o HZ tem maior afinidade pelos receptores β2, enquanto a ractopamina age predominantemente em receptores β1 (MERSMANN, 1998; MOODY et al., 2000; WINTERHOLLER et al., 2007). Até o momento, não existem evidências sobre a presença de receptores β3 nos adipócitos de bovinos (MERSMANN, 1998; PIETRI-ROUXEL et al., 1995; VAN LIEFDE et al., 1994).

A ação dos AβA tem início com a ativação dos receptores βA, que se dá pela ligação dos AβA aos receptores específicos, que é mediada pelas proteínas **G**s, que por sua vez ativam a adenilato ciclase (**AC**), que tem habilidade em converter o **ATP** (adenosina trifosfato) em **AMPc** (adenosina monofosfato cíclico), que é um segundo mensageiro intracelular. O AMPc se liga a subunidade regulatória da proteína quinase A (**PKA**), causando fosforilação da mesma (liberando suas subunidades), deixando-a ativa para suas funções catalíticas (ANDERSON et al., 2005; MERSMANN, 1998;

MOODY et al., 2000). A ativação dos receptores βA pelos AβA, resulta em alteração do metabolismo nos adipócitos e fibras musculares como segue:

Tecido adiposo: a PKA, uma vez ativada, fosforila e ativa as lipases e inativa as enzimas lipogênicas que estão envolvidas nos processos de degradação e síntese dos ácidos graxos e triglicerídeos, respectivamente (MOODY et al., 2000; YANG e MCELLIGOTT, 1989). Essas atividades lipolíticas e anti-lipogênicas ativadas pelos AβA são facilmente comprovadas pela incubação *in vitro* de adipócitos e hepatócitos na presença destes compostos. As respostas lipolíticas dos AβA, *in vivo, são* normalmente determinadas pelo monitoramento dos níveis sanguíneos dos ácidos graxos não esterificados ou glicerol, os quais têm seus níveis aumentados quando algum tipo de AβA é utilizado (BLUM e FLUECKIGER, 1988; EISEMANN, HUNTINGTON e FERRELL, 1988).

A suplementação de ovinos com cimaterol, por longo período de tempo (10 ppm por 12 semanas), elevou o nível plasmático de ácidos graxos não esterificados e diminuiu a quantidade de gordura na carcaça, sugerindo a maior mobilização dos lipídios (BEERMANN et al., 1987). A diminuição na gordura da carcaça foi também relatada em bovinos suplementados com o AβA L644,969 por um período de 6 semanas (CHWALIBOG et al., 1996). Segundo Moloney et al. (1991), a resposta dos adipócitos frente aos AβA é diminuída quando a suplementação se torna crônica. De modo geral, a diminuição da gordura corporal pode ser consequência do aumento da mobilização da gordura pelo tecido adiposo ou pela diminuição da síntese de gordura pelos

tecidos adiposo e hepático, ou uma combinação dos dois (YANG e MCELLIGOTT, 1989).

Tecido muscular esquelético: as respostas dos AβA no músculo são hipertrofia celular e aumento da massa magra. A maior presença de PKA ativada, sinaliza para um aumento na quantidade total de **RNA** (ácido ribonucleico) e **RNAm** (ácido ribonucleico mensageiro) das proteínas miofibrilares, que é refletido em um aumento na taxa de síntese proteica *in vivo* (ANDERSON et al., 2005; MOODY et al., 2000). O aumento de RNA e RNAm foi reportado para a proteína miofibrilar alfa-actina (α-actina), quando ovinos foram tratados com L-644,969 (KOOHMARAIE et al., 1991) e para a proteína miofibrilar miosina em bovinos tratados com ractopamina (SMITH et al., 1989). Entretanto os trabalhos não foram conclusivos sobre o efeito do HZ sobre a síntese de RNA e RNAm para as proteínas miofibrilares (RATHMANN et al., 2009).

Em relação às taxas de degradação proteica, os estudos tem avaliado a atividade das enzimas proteolíticas frente ao uso dos AβA. As enzimas comumente estudadas são as calpaínas (μ e m), proteases cálcio dependentes intracelulares não lisossomais, responsáveis pela degradação das proteínas, e a calpastatina, que é o inibidor endógeno das calpaínas. A ação dos AβA tem sido explicada como aumento da atividade da calpastatina, juntamente com inibição da atividade das calpaínas, diminuindo a degradação proteica (DUNSHEA et al., 2005; HOPE-JONES et al., 2010; KOOHMARAIE e SHACKELFORD, 1991; KRETCHMAR et al., 1989; PARR et al., 1992;

YANG E MCELLIGOTT, 1989). Wang e Beermann (1988) citaram uma diminuição na atividade das calpaínas em torno de 55-70 % no contrafilé de animais tratados com cimaterol. Já, Kretchmar et al. (1990), observaram elevada atividade da calpastatina (+74 %) no contrafilé de ovinos tratados com L-644,969 por 6 semanas, com redução na atividade das calpaínas próximo a 15 %. Entretanto, alguns trabalhos indicaram haver inconsistência na redução da atividade das calpaínas pelo uso de AβA, a ação seria decorrente apenas do incremento na atividade da calpastatina (BERGEN et al., 1989; GEESINK et al., 1993; HOPE-JONES et al., 2010; SIMMONS et al., 1997; WHEELER e KOOHMARAIE, 1992). Ainda, de acordo com outros trabalhos, as proteases lisossomais, que podem estar envolvidas na degradação proteica, tiveram sua atividade reduzida pela utilização de L-644,969 em ovinos (KRETCHMAR et al., 1990).

Algumas pesquisas relataram que os AβA tem sua atividade principalmente sobre o anabolismo proteico, não afetando o catabolismo ou degradação das proteínas miofibrilares (ANDERSON et al., 2005; MOODY et al., 2000). Moloney et al. (1991), relataram que a síntese proteica foi aumentada e a degradação pode ou não ser inibida pela utilização de cimaterol, clenbuterol e L-644,969, enquanto Bergen et al. (1989) indicaram que apenas a síntese proteica foi afetada pela utilização de ractopamina. Uma possível explicação para estes resultados pode estar relacionada com os tipos de AβA citados (receptores específicos), os quais foram utilizados em

diferentes animais, que por sua vez não possuem as mesmas proporções dos receptores βA (MOLONEY et al., 1991; MOLONEY et al., 1990).

Outros tecidos: a administração oral dos AβA pode produzir respostas hormonais e fisiológicas capazes de originar eventos secundários que contribuem para os mecanismos dos próprios AβA. Devido à presença de vários tipos de receptores βA na membrana celular, é possível que múltiplos efeitos de um mesmo AβA ocorram no organismo animal (MERSMANN, 1998). Uma das principais repostas fisiológicas dos AβA é a vasodilatação periférica, que eleva o fluxo sanguíneo dos músculos esqueléticos e tecido adiposo. Um aumento no fluxo sanguíneo pode elevar o processo de hipertrofia muscular, pois aumenta a chegada de substrato e energia para a síntese proteica, além de facilitar a remoção dos ácidos graxos produzidos durante o processo de lipólise (MERSMANN, 1998; YANG e MCELLIGOTT, 1989). Um aumento agudo ou crônico do fluxo sanguíneo pela utilização de clenbuterol é citado em bovinos por 1 ou 9 dias de suplementação, respectivamente, com concomitante aumento de oxigênio, glicose, lactato e diminuição de nitrogênio na corrente sanguínea (EISEMANN e HUNTINGTON, 1993; EISEMANN, HUNTINGTON e FERRELL, 1988). Outras pesquisas apontaram um incremento no fluxo sanguíneo pelo uso de cimaterol em novilhos (BYREM, BEERMANN e ROBINSON, 1998), bem como em ovinos pelo uso de clenbuterol (AUROUSSEAU et al., 1993).

A utilização de AβA pode alterar também a concentração sanguínea de algumas substâncias endócrinas. Foi verificado que em bovinos tratados com

AβA houve estimulação da secreção de insulina pelo pâncreas (MOLONEY et al., 1991), mas outros pesquisadores relataram que a utilização de clenbuterol ou ractopamina não afetaram a concentração desta substância (EISEMANN, HUNTINGTON e FERRELL, 1988; EISEMANN e BRISTOL, 1998; WALKER et al., 2010), e em ovinos, a utilização de cimaterol e ractopamina, diminuiu a concentração sanguínea de insulina (BEERMANN et al., 1987; WALKER et al., 2007).

Hormônios do crescimento, fatores de crescimento semelhantes à insulina (*insulin-like growth factors* – **IGF**) e tiroxina (**T4**) podem ser afetados pela utilização de AβA. Beermann et al. (1987) suplementaram ovinos com cimaterol e verificaram aumento no hormônio de crescimento e T4, com diminuição de IGF após 6 semanas de administração. Diferente destes resultados, O'Connor et al. (1991) não detectaram alterações para IGF, entretanto a concentração de T4 foi diminuída quando ovinos foram suplementados com clenbuterol por 3 semanas. Young et al. (1995) também não detectaram alterações para IGF pela utilização de clenbuterol em ovinos. Dawson et al. (1993) não identificaram diferenças nas concentrações de hormônio de crescimento e IGF em bovinos tratados com cimaterol. Walker et al. (2010) determinaram que a utilização de ractopamina diminuiu a concentração de IGF em novilhas e aumentaram em novilhos. Já Winterholler et al. (2008) descreveram não haver alteração nas concentrações de IGF quando novilhos jovens foram suplementados com ractopamina. Portanto sugere-se que o aumento no crescimento muscular em animais suplementados

com ractopamina pode não estar associado às alterações de IGF. De acordo com Mersmann (1998), existe pouca ou nenhuma evidência que os AβA aumentam massa muscular e diminuem gordura na carcaça devido a alterações nos fatores de crescimento, isso porque não existe relação estrutural entre os seus receptores.

Para fêmeas, machos não castrados, machos castrados fisicamente ou imunocastrados, os AβA foram efetivos promotores de crescimento, indicando que os hormônios esteróides gonadais não estão envolvidos na hipertrofia muscular causada pelos AβA (YANG e MCELLIGOTT, 1989). Walker et al. (2010) indicaram não haver diferenças entre novilhos e novilhas nas respostas pela utilização de AβA.

3.1.2. Efeito dos AβA na palatabilidade da carne

Os efeitos na qualidade da carne devido à utilização de AβA variam muito entre pesquisas, mas todos concordam que mesmo afetando positivamente a produção de carne (desempenho), adversamente produzem uma redução nos escores de maciez e aumentam a força de cisalhamento (BROOKS et al., 2009).

Para suplementação com cimaterol, Fiems et al. (1990) avaliaram touros (Belgian Blue e Charolês) que receberam 60 µg/kg/d desta substância na dieta durante 246 dias e concluíram que a força de cisalhamento dos cortes cárneos aumentou em 31 % quando comparado com touros controle. Do mesmo modo, Chikhou et al. (1993) concluíram que a utilização de cimaterol por 4 semanas

elevou a força de cisalhamento na carne de animais abatidos com 275, 375 e 475 kg de peso vivo, em 55, 145 e 118 %, respectivamente, quando comparados com animais não suplementados. Boucqué et al. (1994) alimentaram touros Belgian White-Blue com 60 µg/kg/d de cimaterol por 136 dias, o que produziu um incremento de 30 % na força de cisalhamento, mas sem alterar as características sensoriais. Da mesma maneira, Vestergaard, Sejrsen e Klastrup (1994) relataram um aumento na força de cisalhamento em touros Friesian alimentados com cimaterol, mas neste caso a diferença foi detectada no teste sensorial.

Com a utilização de clenbuterol, Luño et al. (1999) concluíram que novilhas Charolês que receberam 1 ppm/d de clenbuterol por 5 semanas tiveram carne mais dura tanto por analises instrumental quanto sensorial, mesmo após 8 dias de maturação. Do mesmo modo, Schiavetta et al. (1990) observaram incremento de 19 % na força de cisalhamento na carne de animais que receberam clenbuterol (7 mg/cabeça/d) por 128 dias.

Outro AβA, o L644,969, tem sido também indicado por afetar negativamente a maciez. Kretchmar et al. (1990) alimentaram ovinos com este composto por 6 semanas e verificaram aumento da força de cisalhamento de 111 e 108 % em lombos maturados por 3 e 6 dias, respectivamente, quando comparados com lombos de animais não tratados. Resultados semelhantes foram encontrados por Koohmaraie et al. (1991), que avaliaram ovinos suplementados com L644,969 e concluíram que bifes de animais não tratados foram 52,6 % mais macios após 14 dias de maturação, enquanto que a

maturação dos cortes de animais que receberam L644,969 houve melhora em 18,3 %.

Resultados similares têm sido relatados pela utilização do hidrocloreto de zilpaterol (HZ). Brooks et al. (2009) avaliaram o efeito da suplementação com 6,8 g/t de HZ na dieta de bovinos por 0, 20, 30 e 40 d na qualidade da carne de 3 músculos (LL, TB, GM), maturados por 7, 14 e 21 d. Os autores concluíram que a força de cisalhamento aumentou de acordo com o aumento do período de suplementação, para os 3 músculos, e também que a porcentagem de bifes com valores de força de cisalhamento < 4,5 kg (macio) foi menor em animais suplementados. Em trabalho similar desenvolvido por Claus et al. (2010) onde foi avaliado a maciez da carne de novilhos e novilhas suplementados com HZ (7,56 g/ton) por até 40 dias. Os M. *longissimus lumborum*, M. G*luteus medius* e M. *triceps brachii* foram mais duros quando os animais receberam HZ por 30 e 40 dias do que dos animais que receberam HZ por 20 dias ou não receberam, mas apenas 40 % dos bifes do M. *Gluteus medius* de animais suplementados com HZ tiveram força de cisalhamento superior a 4,6 kg. O efeito da utilização de implantes hormonais de estrogênio (Revalor-S) em animais suplementados com HZ foi avaliado por Kellermeier et al. (2009). Os autores concluíram que não houve interação significativa entre os tratamentos, contudo o uso de HZ aumentou a força de cisalhamento em 60, 59 e 67 % em amostras maturadas por 7, 14 e 21 dias, respectivamente, quando comparadas com o controle nos mesmos tempos de maturação.

O prejuízo na qualidade da carne, quando avaliada por provadores treinados ou consumidores, também é atribuído a suplementação com HZ, acarretando em menores escores de maciez. Bifes maturados por 7 e 14 dias de bovinos que receberam ou não HZ (8,3 mg/kg) e monensina foram avaliados sensorialmente, e não foi verificado efeito do uso de monensina, entretanto a suplementação do HZ diminuiu os escores sensoriais de maciez (HILTON et al., 2009). Em outra pesquisa, realizada por Garmyn et al. (2010), constatou-se que a utilização de HZ (8,3 mg/kg) produziu bifes com menores escores de suculência e maciez, mesmo quando maturados por 14 e 21 dias. Entretanto, os autores concluíram que mesmo com a utilização de HZ as amostras foram consideradas macias quando avaliadas por provadores treinados.

Os diferentes AβA podem afetar a qualidade da carne de maneira diferente, pois tem distinta afinidade pelos receptores (β1 ou β2). Contudo, existem poucos trabalhos que comparam seus efeitos. Comparando-se HZ (60 mg/bovino/d) e ractopamina (300 mg/cabeça/d), Avendaño-Reyes et al. (2006) concluíram que ambos AβA aumentaram a força de cisalhamento do contrafilé quando comparados a amostras de animais que não foram suplementados, e que não houve diferença entre os tipos de AβA. Strydom et al. (2009) compararam o efeito de 3 AβA (HZ - 6 ppm, ractopamina - 30 ppm e clenbuterol - 2 ppm), suplementando bovinos por 30 dias e maturando o contrafilé por 2, 7 e 14 dias. Os autores concluíram que todos os AβA aumentaram a força de cisalhamento dos cortes, quando comparados com o

controle, sendo que amostras de animais que receberam clenbuterol foram mais duras, seguidas por HZ e ractopamina.

3.1.3. Efeito dos AβA na atividade enzimática, proteólise miofibrilar e diâmetro das miofibrilas

O processo responsável pela maturação da carne é adiado pela utilização de AβA, principalmente pela inibição do complexo enzimático, diminuindo a degradação proteica.

A administração de cimaterol em ovinos durante 3 ou 6 semanas inibiu a atividade de μ-calpaína em 55 e 70 %, respectivamente, quando comparados ao controle (WANG e BEERMANN, 1988). Novilhos suplementados com cimaterol por 16 semanas tiveram aumento na atividade da m-calpaína e da calpastatina, sem afetar a atividade da μ-calpaína (PARR et al., 1992). Outros AβA também são citados por afetar a atividade das calpaínas e calpastaina, como o clenbuterol (GARSSEN et al., 1995; GEESINK et al., 1993; LUÑO et al., 1999) e o L644,969 (KOOHMARAIE et al., 1991; KRETCHMAR et al., 1990; WHEELER e KOOHMARAIE, 1992). Strydom et al. (2009) compararam o efeito de 3 AβA na atividade enzimática, e concluíram que a atividade da calpastatina foi maior quando os animais foram suplementados com clenbuterol, seguidos de ractopamina e HZ. Os mesmos autores não encontraram diferença para atividade das calpaínas.

Alterações na atividade das proteases lisossomais causadas pela suplementação com AβA também é citada na literatura. Kretchmar et al.

(1990) indicaram que ovinos alimentados com L644,969 tiveram menor atividade de catepsina B e maior atividade de catepsina H, quando comparados com o controle. Koohmaraie et al. (1991) avaliando o mesmo L644,969, constataram elevadas atividades de catepsina B e catepsina B + L em ovinos não suplementados.

Outra forma importante de avaliar o efeito dos AβA na maturação *post-mortem* é através do índice de fragmentação miofibrilar (**IFM**). Fiems et al. (1990) encontraram menor IFM em amostras de touros jovens suplementados com cimaterol por 246 dias, quando comparados com o controle. Do mesmo modo, Kretchmar et al. (1990) observaram que o IFM de ovinos controle aumentou durante a maturação de 1 a 6 dias, enquanto que em amostras de animais tratados com L644,969, o aumento não foi significativo. Outros autores tem relatado essa diminuição do IFM em carne de ovinos e bovinos suplementados com L644,969 (KOOHMARAIE et al., 1991; WHEELER e KOOHMARAIE, 1992).

Para avaliar e comparar o efeito dos AβA sobre a degradação de proteínas específicas são utilizadas técnicas de biologia molecular, como a eletroforese que separa as proteína e o *western-blot,* que é utilizado para detectar e quantificar as proteínas alvo. Kretchmar et al. (1990) constataram que a banda de troponina-T, da carne de ovinos alimentados com L644,969, não desaparece com a maturação por até 6 dias, enquanto que em animais não tratados essa banda fica menos visível, ficando mais aparente os resíduos de 30 kDa. Para este mesmo AβA, Koohmaraie et al. (1991) relataram não haver

degradação de titina, desmina e troponina-T quando os ovinos foram suplementados com L644,969 por 6 semanas e a carne foi maturada por 7 dias. Entretanto, aos 14 dias alguma degradação pôde ser notada. O efeito do HZ sobre a degradação da desmina foi avaliado por Kellermeier et al. (2009). Os autores concluíram não haver diferença na degradação desta proteína quando os bovinos foram suplementados por 30 dias e a carne maturada por 7, 14 e 21 dias.

O diâmetro das fibras musculares também pode ser afetado pela utilização de AβA. Kellermeier et al. (2009) indicaram haver um importante incremento no diâmetro das fibras musculares do contrafilé de animais tratados com HZ. Outros pesquisadores também relataram este aumento do diâmetro das fibras musculares quando bovinos foram suplementados com cimaterol (VESTERGAARD, SEJRSEN e KLASTRUP, 1994) e ractopamina (GONZALEZ et al., 2007).

3.1.4. Efeito dos AβA na cor da carne

O efeito dos AβA na cor da carne normalmente é avaliada em condições de "display" (gôndolas), pois objetiva-se avaliar a cor nas mesmas condições encontradas pelo consumidor na aquisição da carne. Boucqué et al. (1994) e Fiems et al. (1990) não encontraram diferença nas coordenadas de L*, a* e b* quando touros Belgian Blue foram suplementados com cimaterol (60 µg/kg) por 136 ou 246 dias, respectivamente. Entretanto, Vestergaard, Sejrsen e Klastrup (1994) encontraram que a suplementação com cimaterol (0,056

mg/kg de peso vivo/d) por 90 dias produziu carne com maior valor de L*, sem interferir nos valores de a* e b*. Monson et al. (2007) não encontraram diferença nas coordenadas L*, a* e b* entre touros jovens que receberam ou não clenbuterol (5 ou 10 µg/kg de peso vivo/d) por 110 dias e tiveram sua carne maturada por 7 dias. No mesmo sentido, Quinn et al. (2008) não encontraram diferença na cor da carne (maturada por 14 dias e exposta em "display" por 7 dias) de bovinos que foram suplementados com ractopamina (200 mg/cabeça/d) por 28 d. Geesink et al. (1993) reportaram tendência para carne pálida em carne de animais que receberam AβA.

Avendano-Reyes et al. (2006) avaliaram a cor da carne (maturada por 5 e 14 dias) de novilhos suplementados com HZ (60 mg/cabeça/d) e ractopamina (300 mg/cabeça/d) por 33 dias, e concluíram que durante a exposição em "display", as amostras de todos os tratamentos ficaram mais escuras, sendo mais proeminente nas amostras de animais suplementados com HZ.

O efeito da utilização de HZ na cor da carne foi avaliado em diferentes condições, como tempo de suplementação, músculo, tipo de sistema de embalagem e presença de outros aditivos alimentares. Rogers et al. (2010) avaliaram o efeito do tempo de suplementação de HZ (6,8 g/ton) e a presença de atmosfera modificada (80 % de oxigênio e 20 % de dióxido de carbono) para carne de bovinos holandês, e concluíram que a presença de HZ não afetou a cor do contrafilé, seja por medidas instrumentais ou medida por um painel treinado, que avaliaram a cor e a descoloração durante a exposição das carnes por 5 dias no "display" nos dois sistemas de embalagem (tradicional e

modificada). Diferentes resultados foram obtidos por VanOverbeke et al. (2009) que encontraram maior valor de a* em alcatra (sistema tradicional de embalagem - **PVC**) de bovinos suplementados por 30 dias com HZ, mas não foi verificado diferença pela avaliação visual. Quando avaliados sob atmosfera modificada (80 % de oxigênio e 20 % de dióxido de carbono) as carnes de animais suplementados por 20 dias tiveram maior a* e b* do que os outros períodos de suplementação. Os autores recomendam um tempo de suplementação com HZ entre 20 e 30 dias para produzir carne com maior teor de vermelho.

Avaliando o efeito da suplementação com HZ (6,8 g/ton de ração) na cor de bifes de coxão mole, Gunderson et al. (2009) encontraram que os bifes de animais suplementados entre 20 e 30 dias tiveram as melhores características de cor, sendo considerada mais atraente pelos avaliadores, isso quando os bifes foram expostos em sistema tradicional de embalagem (PVC), sendo que quando embalados sob atmosfera modificada (69,6 % N_2, 30 % CO_2 e 0,4 % CO), todos os períodos de suplementação (20, 30 e 40 d) promoveram uma melhor estabilidade da cor da carne, quando comparados com amostras de animais não suplementados. Hilton et al. (2009) avaliaram a cor de bifes de contrafilé maturados por 14 dias, obtidos de bovinos suplementados ou não com HZ (8,3 mg/kg de ração) por 35 dias e utilizando ou não monensina. Os autores concluíram não haver interação entre os tratamentos para as características de cor avaliadas e que a utilização de HZ não afetou a uniformidade da cor, descoloração da superfície e escores de escurecimento.

3.1.5. Efeito dos AβA na capacidade de retenção de água

A utilização de AβA tem sido pontuada por afetar pouco ou não afetar a capacidade de retenção de água de cortes primários ou bifes, seja medida por gotejamento, descongelamento, cozimento ou a soma de todas as perdas. Fiems et al. (1990) e Boucqué et al. (1994) concluíram não haver efeito da suplementação de cimaterol nas perdas por gotejamento e cozimento, assim como na medida de pH, de touros jovens (Belgian Blue e Charolês). Maiores perdas por gotejamento foram verificadas em amostras de bovinos suplementados com cimaterol, quando comparados com animais não tratados, mesmo não havendo diferença no valor de pH final (CHIKHOU et al., 1993). Para a utilização de clenbuterol, Monson et al. (2007) verificaram um incremento no valor de pH quando comparado com animais não tratados. Entretanto, menores valores de retenção de água foram encontradas para amostras de animais suplementados.

Quinn et al. (2008) não detectaram diferenças para perdas por exsudação durante a exposição em "display" de bifes obtidos de bovinos tratados ou não com ractopamina. De outro lado, Avendano-Reyes et al. (2006) compararam a utilização de HZ e ractopamina por 33 dias de suplementação e concluíram que a capacidade de retenção de água diminuiu quando as amostras de animais controle e com ractopamina foram maturadas de 5 para 14 dias, e aumentou quando os animais foram suplementados com HZ.

Leheska et al. (2009) não encontraram diferenças nas perdas por cozimento de bifes de novilhas e novilhos que foram suplementados com HZ (8,3 mg/kg de ração) por 20 ou 40 dias. Da mesma maneira, Garmyn et al. (2010) não verificaram efeito da suplementação de HZ nas perdas por descongelamento, resfriamento e cozimento em bifes de contrafilé maturados por 14 e 21 dias. Hilton et al. (2009) também não encontraram diferenças para perdas por cocção entre contrafilé (maturados por 14 dias) de bovinos suplementados ou não com HZ e recebendo ou não monensina. Diferente destes resultados, Rogers et al. (2010) detectaram que cortes primários de animais não tratados ou tratados por 20 dias com HZ tiveram menores perdas por exsudação que cortes de animais tratados por 30 e 40 dias com HZ. Estes resultados também foram encontrados por Kellermeier et al. (2009), em que a suplementação com HZ por 30 dias aumentou as perdas por exsudação do contrafilé, quando comparados com animais não tratados. Na mesma linha, Rathmann et al. (2009) encontraram uma tendência para maiores perdas por exsudação em bovinos tratados com HZ.

3.1.6. Efeito dos AβA na composição centesimal e conteúdo de colágeno

A capacidade de repartição de nutrientes é bem documentada para os AβA. Um aumento na deposição de proteína e umidade, com diminuição na porcentagem de gordura tem sido demonstrada pela utilização de clenbuterol (BAKER et al., 1984; RICKS et al., 1984), cimaterol (BOUCQUÉ et al., 1994; FIEMS et al., 1990) e ractopamina (SCHROEDER et al., 2005).

Adicionalmente, Moloney et al. (1990), Vestergaard, Sejrsen e Klastrup (1994) e Schroeder et al. (2005) mostraram um aumento na carne magra com diminuição de gordura devido a suplementação com L644,969, cimaterol e ractopamina.

Resultados inconsistentes têm sido atribuídos à utilização de HZ na composição química da carne. Hilton et al. (2009) encontraram que a suplementação com HZ diminuiu a porcentagem de gordura, mas sem efeito sobre a proteína e umidade em bifes de contrafilé. Já, Leheska et al. (2009) não encontraram diferença para gordura, umidade e cinzas, mas relataram incremento na quantidade de proteína em amostra de animais suplementados com HZ. Maiores porcentagens de proteína e umidade e menores de gordura em amostras de bovinos que receberam HZ foram descritos por Kellermeier et al. (2009) e Rathmann et al. (2009).

Strydom et al. (2009) compararam o efeito de HZ, ractopamina e clenbuterol na composição química da carne. Os autores concluíram não haver efeito sobre proteína e cinzas, mas o teor de umidade foi maior em amostras de animais alimentados com HZ e clenbuterol, enquanto amostras controle tiveram maiores teores de gordura.

O efeito dos AβA sobre o teor de colágeno é controverso. Alguns autores relataram redução na quantidade de colágeno pelo uso de AβA (FIEMS et al., 1990; KELLERMEIER et al., 2009). Além da quantidade do colágeno, a proporção entre colágeno solúvel e insolúvel também deve ser avaliada. Vestergaard, Sejrsen e Klastrup (1994) citaram redução no colágeno total pelo

uso de cimaterol, mas o colágeno solúvel não foi afetado. Na mesma linha, Strydom et al. (2009) verificaram diminuição nos teores de colágeno total em amostras de animais suplementados com HZ e clembuterol. Entretanto, os autores não detectaram diferença na solubilidade das amostras de contrafilé. Mesmo com a diminuição no teor de colágeno total devido ao uso de AβA, o qual seria favorável à maciez da carne, os resultados de maciez e força de cisalhamento não refletem esta teoria.

3.2. Cloreto de cálcio ($CaCl_2$)

O cloreto de cálcio tem sido citado há muitos anos para promover a maciez de carnes que apresenta muita variação nesta característica, como a de animais com predominância genética *Bos indicus*, touros, castrados tardiamente, vacas de descarte, uso de AβA (L644,969) ou em cortes menos macios (coxão-mole) (DILES et al., 1994; KOOHMARAIE e SHACKELFORD, 1991; LANSDELL et al., 1995; MORGAN et al., 1991; WHEELER, KOOHMARAIE e CROUSE, 1991). A melhoria da maciez ocorre principalmente devido a ativação do sistema enzimático das calpaínas, que hidrolisa algumas proteínas musculares durante o período *post-mortem*, conhecido como maturação (KOOHMARAIE, CROUSE e MERSMANN, 1989; WHIPPLE e KOOHMARAIE, 1992). De maneira geral, a aplicação de $CaCl_2$ pode diminuir a atividade da calpastatina, enzima que inativa as calpaínas, e permitir maior ação dessas (WHIPPLE e KOOHMARAIE, 1992).

3.2.1. Efeito do $CaCl_2$ na força de cisalhamento e palatabilidade da carne

Após estabelecida a ação das proteases dependentes de cálcio no processo de maturação (KOOHMARAIE et al., 1988a), estudos envolvendo o cloreto de cálcio foram iniciados para tentar promover a maciez da carne por processos de injeção na carcaça ou músculo pré-rigor. Um dos primeiros estudos onde este processo foi utilizado foi citado por Koohmaraie et al. (1988b), em que os autores confirmaram que o processo de maturação pode

ser acelerado pela injeção de cloreto de cálcio em carcaças ovinas imediatamente após o abate (0,3 M – 10 % do peso vivo), diminuindo os valores de força de cisalhamento já no primeiro dia de maturação. Após um ano, os mesmos autores (KOOHMARAIE, CROUSE e MERSMANN, 1989) obtiveram resultados semelhantes quando avaliaram diferentes concentrações de cloreto de cálcio (0,075 M, 0,15 M ou 0,3 M) aplicadas em carcaças ovinas. Os autores concluíram que a aplicação de cloreto de cálcio na concentração de 0,3 M foi a mais eficiente para melhorar a maciez 24 horas após o abate.

Para avaliar o efeito do cloreto de cálcio em ovinos suplementados com L644,969, Koohmaraie e Shackelford (1991) infundiram carcaças pré-rigor com $CaCl_2$ (0,3 M - 10 % do peso vivo) e concluíram que nos dias 1, 7 e 14 *post-mortem*, o contrafilé de todas as carcaças, tratamento e controle, ficaram mais macias com a maturação, ou seja, o cloreto de cálcio foi eficiente para reverter a dureza causada pela utilização do L644,969.

Grande parte dos trabalhos realizados para avaliar os efeitos do cloreto de cálcio na maciez da carne foi realizada apenas com determinações químicas e físicas, como força de cisalhamento, cinéticas enzimáticas (atividade das calpaínas e calpastatina) e degradação proteica (titina, desmina, etc), mas sem avaliar as amostras sensorialmente. Morgan et al. (1991) observaram que a injeção da carcaça com cloreto de cálcio (0,3 M – 10% do peso vivo) reduziu a força de cisalhamento do contrafilé bovino, mesmo quando maturado por 14 dias, quando comparado a carcaças controle. Entretanto, na avaliação

sensorial com provadores treinados, as amostras com cálcio foram consideradas mais macias e com sabor metálico, amargo, e em alguns casos, sabor que lembra fígado.

Estudos posteriores foram realizados para verificar se a injeção do cloreto de cálcio em cortes cárneos (pós-rigor) teria o mesmo efeito benéfico na maciez da carne, quanto à injeção pré-rigor. Wheeler, Crouse e Koohmaraie (1992) injetaram o contrafilé 24 horas após o abate (0,3 M de $CaCl_2$ - 10% peso do corte) e concluíram que a maturação foi tão eficiente quanto se o cálcio fosse aplicado na fase pré-rigor.

Mesmo o cloreto de cálcio proporcionando excelente amaciamento na carne, seja por injeção pré-rigor ou injeção pós-rigor (KOOHMARAIE et al., 1988b; KOOHMARAIE et al., 1989; KOOHMARAIE e SHACKELFORD, 1991; MORGAN et al., 1991; WHEELER et al., 1992), há a desvantagem da presença de sabor estranho como amargo e metálico (MORGAN et al., 1991). Entretanto, Wheeler et al. (1993) determinaram que a injeção com cloreto de cálcio (200 mM – 5 %) em contrafilé, após 24 horas do abate, foi eficiente em melhorar a maciez, sem comprometer outras características de palatabilidade. Todavia, quando o cálcio foi injetado em concentrações superiores a 250 mM, os provadores detectaram a presença de sabor alterado. Resultados similares foram encontrados por Diles et al. (1994), que avaliaram a injeção com cloreto de cálcio em diferentes concentrações e tempos pós-abate. Os autores concluíram que 200 mM injetado 24 horas após o abate foi eficiente para promover a maciez e suculência, sem afetar o sabor. Outros pesquisadores têm

obtido resultados semelhantes com a aplicação do cloreto de cálcio, na concentração de 200 mM a 5 %, mesmo quando aplicado 24 ou 48 horas do abate, e os mesmos recomendam este protocolo para ser aplicado comercialmente pela indústria para promover a maciez sem afetar negativamente as características de sabor da carne bovina e ovina (KERTH, MILLER e RAMSEY, 1995; LANSDELL et al., 1995).

Para avaliar se o cloreto de cálcio tem efeito na melhoria da qualidade da carne de bovinos com predominância de genética indiana e sendo abatidos com mais de 5 anos, após serem utilizados para serviço pesado, Jaturasitha et al. (2004) realizaram a pesquisa injetando cloreto de cálcio, em diferentes concentrações (0, 0,2, 0,3 e 0,4 M a 10%) e tempos pós abate (45 min ou 24 h) no contrafilé. Os autores concluíram que a injeção pré-rigor foi mais eficiente para melhorar a maciez da carne e que a concentração mínima de 0,3 M foi necessária para uma maturação mais acelerada. Entretanto, para esta concentração ou superior, os provadores detectaram presença de sabor estranho, mas isso não alterou as notas de aceitação global. Mesmo a maciez sendo melhorada pelo uso de cálcio, a carne maturada por 7 dias ainda foi considerada dura, tanto por força de cisalhamento quanto pela avaliação sensorial.

A aplicação de cloreto de cálcio nas primeiras horas ou dias do abate mostrou-se eficiente para melhorar a maciez da carne. Entretanto, para utilização na indústria, o ideal seria aplicar esta tecnologia após 2 ou 3 dias do abate, pois é quando as carcaças normalmente estão disponíveis para

desossa e processamento (WHEELER, KOOHMARAIE e SHACKELFORD, 1997). Por esta razão esses autores avaliaram a aplicação de cálcio no contrafilé aos 2 e 14 dias após o abate, e concluíram que mesmo aos 14 dias, o cloreto de cálcio ainda foi eficiente para reduzir a força de cisalhamento (0,7 kg) quando comparado a amostras não injetadas. Entretanto, amostras injetadas aos 2 dias de maturação foram 1,2 kg mais macias que àquelas controle, indicando que quanto antes o cálcio for aplicado melhor o benefício para maciez.

Um efeito negativo observado pela aplicação de cloreto de cálcio é a mais rápida descoloração da carne (WHEELER, KOOHMARAIE e SHACKELFORD, 1996), e isto pode estar associado a maior taxa de oxidação (St. ANGELO et al., 1991), que também poderia afetar negativamente a atividade das calpaínas, por modificação do sítio de ação destas cisteínas nas proteínas alvo (HARRIS et al., 2001). Por esta razão, um experimento foi conduzido para avaliar se o tratamento conjunto, através da suplementação alimentar de vitamina E (antioxidante) e aplicação de cloreto de cálcio na carne poderia controlar a oxidação e com isso aumentar a estabilidade da cor e atividade enzimática. Os autores concluíram que a vitamina E potencializou a ação do cloreto de cálcio no processo de maturação protegendo as calpaínas do processo oxidativo (HARRIS et al., 2001).

Como toda tecnologia aplicada para promover a maciez da carne tem por objetivo atender às expectativas do consumidor final, trabalhos com a aplicação de cloreto de cálcio foram desenvolvidos para avaliar se esta

tecnologia é eficiente frente aos consumidores. Miller et al. (1995) desenvolveu uma pesquisa onde avaliou-se a carne injetada com cloreto de cálcio (200 mM a 5%) e maturada por 7 dias por meio de testes sensoriais com consumidores. Os autores concluíram que a carne injetada foi mais macia e suculenta e que não foi detectada a presença de sabor estranho. Da mesma maneira, Hoover et al. (1995) verificaram que o contrafilé injetado com cloreto de cálcio (200 mM a 5%) teve maiores escores de maciez e sabor, sem a presença de sabor estranho. Avaliando a percepção dos consumidores de grandes cidades americanas (Los Angeles, CA; Baltimore, MD; Chicago, IL; e Dallas, TX), Carr et al. (2004) constataram que bifes de contrafilé injetados com cloreto de cálcio foram considerados mais macios, suculentos, com mais intensidade de sabor e foram melhor aceitos que os bifes não injetados. Neste mesmo experimento, os autores mostraram que os consumidores conseguiram diferenciar as classes de maciez e declararam estar dispostos a pagar até 0,95 U$/kg nas carnes injetadas e macias. Em um experimento onde os consumidores recebiam os bifes em sua residência, e eram solicitados a preparar conforme normalmente o fazem, os resultados indicaram não haver diferença quanto a utilização ou não de cloreto de cálcio, pois os consumidores classificaram todas as amostras como aceitáveis. Os autores atribuem estes resultados principalmente devido aos diferentes métodos e pontos (temperatura final) de cozimento utilizado pelos consumidores (BEHRENDS et al., 2005).

3.2.2. Efeito do $CaCl_2$ na atividade enzimática e proteólise miofibrilar

As proteases dependentes de cálcio são as principais responsáveis pelo amaciamento da carne após o abate. Seus efeitos têm inicio imediatamente após a sangria, e é a m-calpaína responsável pelo amaciamento nas primeiras 24 horas, pois sua atividade sofre grande alteração nesta etapa e é onde se verifica a maior taxa de fragmentação miofibrilar. Ao contrário, a μ -calpaína mantêm sua atividade constante durante a maturação e isto se deve à necessidade de pequenas concentrações de cálcio para ser ativada. Do outro lado, a calpastatina perde sua atividade rapidamente, sendo que após 24 h da sangria, sua atividade já é quase 80 % menor quando comparada ao momento da sangria, e aos 6 dias de maturação quase não se detecta atividade. Em relação à fragmentação miofibrilar, constata-se que as mudanças mais drásticas ocorrem até o 6º dia de maturação, com pouca alteração até o 14º dia (KOOHMARAIE et al., 1988a,b). Alguns estudos mostraram que as calpaínas são capazes de hidrolisar a calpastatina, mas mesmo assim a calpastatina mantem sua atividade contra as calpaínas (MELLGREN, MERICLE e LANE, 1986).

Koohmaraie et al. (1988b) confirmaram que o processo de maturação pode ser acelerado pela injeção da carcaça de cálcio imediatamente após o abate, devido a ativação das proteases dependente de cálcio. Os autores encontraram uma perda na atividade da μ-calpaína e calpastatina e diminuição significativa na atividade de m-calpaína nas primeiras 24 horas, quando as carcaças foram injetadas, o que só acontecem com as amostras não injetadas

no dia 7 de maturação. Neste mesmo trabalho foi verificada a presença de componentes com peso molecular de 30 kDa, nas primeiras 24 h após abate, em amostras injetadas, sendo que esses componentes só foram verificados na mesma intensidade em amostras não injetadas com 6 dias de maturação. Não foi verificada alteração da atividade das catepsinas B, H e L devido à aplicação de cloreto de cálcio.

Koohmaraie et al. (1989) relataram que diferentes concentrações do cloreto de cálcio (pré-rigor) atuam de maneira diferente na atividade das enzimas responsáveis pela maturação. Concentrações de 0,075 M de cloreto de cálcio não foram suficientes para alterar a atividade das enzimas (calpaínas e calpastaina). Entretanto, 0,15 M causou uma diminuição significativa da atividade da μ-calpaína, sem afetar a m-calpaína e calpastatina. Já, com 0,3 M, tanto a atividade da μ-calpaína quanto da calpastatina foram reduzidas, ou seja, a maturação ocorreu de maneira mais acelerada.

A atividade das enzimas proteolíticas quando sob efeito de AβA (L644,969) e cloreto de cálcio (pré-rigor) foi avaliada por Koohmaraie e Shackelford (1991). Os autores encontraram menor atividade para a μ-calpaína e m-calpaína em amostras com cloreto de cálcio, enquanto que nas amostras de animais que receberam o AβA, a atividade da calpastatina foi mais elevada. Nas amostras de animais com AβA, em que as carnes foram injetadas com cálcio, a atividade da calpastatina foi intermediária. Em relação à degradação de proteínas especificas, como a titina, troponina-T e desmina, a

utilização de cálcio acelerou o processo tanto nas amostras do tratamento, com AβA como no controle.

Em pesquisa com aplicação de cálcio pós-rigor, Wheeler et al. (1992) encontraram que a diminuição da força de cisalhamento nas amostras injetadas se deu mais pela ativação das calpaínas que pela inativação da calpastatina. Whipple e Koohmaraie (1992) mostraram que a utilização de cloreto de cálcio teve efeito positivo na maturação pela diminuição da atividade da calpastatina. Porém isso não pôde ser comprovado pela detecção das proteínas alvo (desmina e troponina-T), pois não foi verificada diferença na quantificação destas. Entretanto, a utilização de cálcio possibilitou uma maior formação de fragmentos de baixo peso molecular (28 a 32 kDa), o que indica uma melhoria no processo de proteólise.

Wheeler et al. (1997) sugeriram que a m-calpaína está disponível para ser ativada em qualquer período pós abate, pois sua atividade não se altera significativamente durante a maturação, mesmo após 14 dias do abate. Quanto a prevenção da oxidação pela utilização de vitamina E, Harris et al. (2001) descreveram que a utilização de cálcio associada à suplementação com vitamina E possibilitou a mais rápida maturação, demonstrada pela degradação da troponina-T e a formação mais intensa de componentes de baixo peso molecular (30 kDa).

3.2.3. Efeito do $CaCl_2$ na cor e outros fatores bioquímicos da carne

A ação do cloreto de cálcio em acelerar a maturação já foi descrita e comprovada em diversas pesquisas (KOOHMARAIE et al., 1988b; KOOHMARAIE et al., 1989; KOOHMARAIE e SHACKELFORD, 1991; MORGAN et al., 1991; WHEELER et al., 1992). Entretanto, alguns autores destacaram que o cloreto de cálcio pode afetar a cor da carne quando exposta em condições de "display" dependendo da concentração, quantidade injetada e momento *post-mortem* da injeção.

Em um experimento avaliando diferentes concentrações de cloreto de cálcio (175, 200 e 250 mM), quantidade injetada (5 e 10 %) e tempo *post-mortem* de injeção (30 min ou 24 h), Wheeler et al. (1993) não encontraram alteração na cor da carne após 3 dias de "display" quando a injeção foi realizada 24 h após o abate, enquanto os bifes que foram injetados aos 30 min ficaram mais escuros que os bifes não injetados. Outra conclusão foi que a concentração não afetou a cor superficial dos bifes quando a injeção foi realizada a 5 %, mas em injeções de 10 % e concentrações de 200 e 250 mM a cor e a taxa de descoloração foram afetadas. Diles et al. (1994) mostraram que bifes injetados 30 min após o abate foram considerados mais escuros, por avaliação visual, que os bifes injetados após 24 h, e apresentaram maior descoloração. Diferentes concentrações de cloreto de cálcio (150 ou 200 mM) não afetaram a cor da carne avaliada visualmente ou através de colorímetro, durante os 3 dias de "display". Já, Lansdell et al. (1995) concluíram que quando a carne foi injetada com cloreto de cálcio (200 mM a 5 %) aos 30 min

pós-abate, e os bifes foram avaliados após sete dias de maturação, os escores visuais de cor e as medidas de L*, a* e b* não sofreram alteração durante os 5 dias de "display", quando comparados a bifes não injetados.

Kerth et al. (1995) trabalharam com contrafilé injetado com cloreto de cálcio (200 ou 250 mM) 48 após o abate e maturado por 7 ou 14 dias antes da avaliação em "display". Os autores constataram que as amostras injetadas com 250 mM e maturadas por 14 dias, sofreram mais alteração durante os 5 dias de "display", diminuindo a uniformidade da cor e isso foi também verificado por uma alteração no valor de a*, que foi maior em amostras maturadas a 14 quando comparadas às maturadas por 7 dias, independente da concentração de cloreto de cálcio injetada. Os autores sugerem uma aplicação de 200 mM mesmo que as amostras sejam maturadas por 14 dias, mas mesmo assim pode ocorrer alteração da cor durante a exposição em "display". Amostras de contrafilé injetadas com cloreto de cálcio (200 mM – 5 %) aos 2 ou 14 dias após o abate foram avaliadas quanto à cor por Wheeler et al. (1997). Os autores concluíram que independente do momento de injeção, a coloração dos bifes não foi alterada nos 3 primeiros dias de "display", entretanto se a injeção foi aos 14 dias uma maior descoloração foi verificada após o quinto dia.

A aplicação de cloreto de cálcio pode acelerar a taxa de oxidação da mioglobina, alterando a coloração da carne (St. ANGELO et al., 1991). Para tentar prevenir esta oxidação, Wheeler et al. (1996) utilizaram vitamina C (1% de ácido ascórbico) incorporada a solução de cloreto de cálcio (200 mM a 5

%) quando injetada no coxão mole. Os autores concluíram que esta associação foi eficiente em prevenir a oxidação da mioglobina, deixando a carne com uma coloração mais vermelha e com menor taxa de descoloração, enquanto que nas amostras que receberam apenas cloreto de cálcio a manutenção da cor foi menos estável durante os 5 dias de "display". Também para prevenir a oxidação da carne causada pelo sal de cálcio, Harris et al. (2001) avaliaram o efeito da suplementação do gado com vitamina E. As amostras de animais não suplementados e que foram injetadas com cloreto de cálcio foram as menos estáveis durante o período de exposição em "display", apresentando menores valores de a* e L*, assim como visualmente foram consideradas com maior descoloração na superfície. Para amostras de animais suplementados e que foram injetadas, a cor foi mais estável, apresentando menor taxa de descoloração, demonstrando o efeito protetor da vitamina E em prevenir a oxidação. Esse efeito também foi verificado quando os valores de TBA (ácido tiobarbitúrico) foram determinados, onde amostras dos que receberam vitamina E, e que foram injetadas tiveram menores valores de **TBA** durante os 5 dias de "display" que as dos não suplementados.

Alguns dos fatores bioquímicos que podem afetar a cor e a estabilidade da cor quando o cloreto de cálcio é injetado na carne foram avaliados por Bekhit et al. (2005). Os autores estudaram o efeito da injeção de cloreto de cálcio (300 mM), cloreto de zinco (50 mM) ou água em carcaças ovinas na queda de pH, na atividade redutora de metamioglobina (**ARM**), avaliação instrumental da cor e oxidação lipídica. Observaram que em amostras que

receberam cloreto de cálcio a queda do pH foi mais acelerada que nos outros tratamentos. Porém, o pH final não diferiu entre os tratamentos. O processo de injeção de líquidos diminuiu a AMR, enquanto que a injeção com cloreto de zinco resultou em menores taxas de ARM. A utilização de cloreto de cálcio foi responsável pela menor estabilidade da cor da carne, com maiores taxas de ARM, menores valores de L*, a* e b* e maior taxa de oxidação lipídica.

3.2.4. Efeito do $CaCl_2$ na capacidade de retenção de água

A maior parte dos estudos indica que a utilização de cloreto de cálcio diminuiu a capacidade de retenção de água da carne, seja medida pela taxa de gotejamento, cozimento ou a perda total. Wheeler et al. (1992) demonstraram que a injeção de cloreto de cálcio (300 mM a 10%) 24 h após o abate proporcionou maiores perdas por cozimento que amostras injetadas aos 30 min pós-abate ou não injetadas. Avaliando a injeção de diferentes concentrações (175, 200 e 250 mM) e quantidades (5 e 10 %) de cloreto de cálcio, Wheeler et al. (1993) concluíram que o volume injetado não afetou a perda por gotejamento. Entretanto, amostras injetadas com 200 mM perderam mais peso por gotejamento. O volume injetado também não afetou as perdas por cocção, mas quando o cloreto de cálcio foi utilizado a 200 ou 250 mM as perdas foram maiores que em amostras não injetadas.

Boleman et al. (1995) relataram que a perda por gotejamento foi menor em amostras não injetadas quando comparada com amostras injetadas com cloreto de cálcio em diferentes tempos pós-abate (1, 12 e 24 h), e que as

amostras injetadas na 1ª hora *post-mortem* foram as que perderam menos peso entre as amostras injetadas. Morgan et al. (1991) não encontraram diferença nas perdas por cocção entre amostras injetadas (300 mM $CaCl_2$ a 10%) e não injetadas, mesmo maturadas por 1, 7 e 14 dias. Jaturasitha et al. (2004) concluíram que injeção com cloreto de cálcio em diferentes concentrações (200, 300 e 400 mM a 10%) proporcionou maiores perdas de peso por gotejamento e descongelamento, mas as amostras injetadas não diferiram do controle em relação as perdas por cozimento. Outra conclusão destes autores foi que amostras injetadas 45 min após o abate perderam duas vezes mais a quantidade de líquido (gotejamento de descongelamento) que as amostras injetadas 24 h pós-abate.

Diles et al. (1994) determinaram que amostras de contrafilé injetadas com cloreto de cálcio (150 ou 200 mM) perderam mais peso por exsudação que amostras não injetadas e que as amostras maturadas por 7 tiveram mais perdas que as maturadas por 14 dias, adicionalmente não foi verificado diferença entre as perdas por cocção e entre as duas concentrações de cloreto de cálcio utilizadas ou os tempos de maturação. Kerth et al. (1995) e Lansdell et al. (1995) demonstraram que a aplicação de cloreto de cálcio (200 mM a 5%) não afetaram as perdas por cocção, quando comparadas a amostras controle. Entretanto, as amostras injetadas 24 ou 48 h após o abate apresentaram maiores perdas de peso por exsudação durante os períodos de maturação de 7 e 14 dias.

O contrafilé, quando injetado com cloreto de cálcio (200 mM a 5 %) 2 ou 14 dias após o abate, teve maior porcentagem de perda por cocção que as amostras não injetadas, e entre os tempos de injeção, nas amostras injetadas 14 dias após o abate foi verificado maior perda por cocção que a injeção no segundo dia após o abate (WHEELER et al., 1997). No experimento de Harris et al. (2001) foi verificado que a aplicação de cloreto de cálcio, em carne de animais que foram suplementados com vitamina E, diminuiu a perda por cocção em todos os tempos de maturação avaliados (1, 3 ou 7 d), quando comparados com os outros tratamentos de suplementação e injeção.

3.3. Outras tecnologias pós-abate e AβA

A redução nos escores de maciez e aumento da força de cisalhamento resultante da administração de AβA, como o hidrocloreto de zilpaterol (HZ), tem sido amplamente documentada na literatura (BROOKS et al., 2009; GARMYN et al., 2010; HILTON et al., 2009; LEHESKA et al., 2009). Em consequência disso, algumas tecnologias (maturação, *"enhancement"*, amaciamento mecânico e estimulação elétrica) são avaliadas para tentar minimizar esses problemas que podem diminuir a aceitabilidade do produto. Brooks et al. (2009) avaliaram a eficiência da maturação por até 21 dias, para tentar reverter o aumento da força de cisalhamento causado pela suplementação debovinos com HZ (6,8 g/t de ração) durante períodos de até 40 dias. Os autores concluíram que a maturação diminuiu os valores de força de cisalhamento e melhorou os escores de maciez dos músculos M. *Longissimus lumborum*, M. *Triceps brachii* e M. *Gluteus medius*, tanto nas

amostras de animais suplementados como nos animais controle, e isto ocorreu na mesma intensidade nas amostras de carcaças *choice* e *select* (sistema de tipificação americana). Outros pesquisadores têm indicado que a maturação da carne por até 28 dias (contrafilé) de animais suplementados com HZ, diminuiu os valores de força de cisalhamento, entretanto não atingiu os mesmos valores de maciez das amostras de animais não suplementados, para um mesmo período de maturação (HILTON et al., 2009; HOLMER et al., 2009; HOPE-JONES et al., 2010; KELLERMEIER et al., 2009; LEHESKA et al., 2009; MEHAFFEY et al., 2009; RATHMANN et al., 2009; STRYDOM et al., 2009). Para outros AβA, como a ractopamina, Gonzalez et al. (2010) concluíram que a maturação da carne por 13 dias foi suficiente para equiparar a força de cisalhamento entre amostra de animais suplementados e não suplementados. Por outro lado, na suplementação com clenbuterol, a maturação por 21 dias não foi suficiente para reverter o endurecimento da carne causado por este AβA (LUÑO et al., 1999; MONSÓN et al., 2007).

Garmyn et al. (2010) não encontraram interação significativa entre a suplementação com HZ e o período de maturação da carne (14 e 21 dias) para as medidas de força de cisalhamento e características sensoriais, demonstrando que a taxa de maturação ocorre na mesma intensidade entre amostras de animais suplementados ou não. Leheska et al. (2009) relataram que o contrafilé de novilhas e novilhos suplementados com HZ tiveram menores escores de suculência, maciez e intensidade de sabor, até o dia 28 de maturação, quando comparados com o controle. Hilton et al. (2009)

conduziram análise sensorial com amostras maturadas (14 dias) de animais que foram submetidos a suplementação com HZ e monensina por 35 dias. Os autores concluíram que as amostras de animais suplementados com HZ foram menos suculentas e macias e com menor intensidade de sabor de carne quando avaliadas pelos provadores treinados, enquanto que para os consumidores, as amostras de animais que receberam HZ, foram menos aceitas quanto a maciez e aceitação global, quando comparadas com as amostras controle. Mehaffey et al. (2009) avaliaram a aceitação da carne de bovinos suplementados com HZ através de um teste sensorial com um grande número de consumidores (n = 3007), de 4 cidades americanas. Os autores chegaram à conclusão de que independente do "grade" (tipo) de carcaça (*choice* ou *select*), a carne deveria ser maturada por pelo menos 21 dias e a suplementação com HZ não deveria ultrapassar 20 dias, para que as amostras fossem aceitas pelos consumidores da mesma maneira que as amostras controle.

Outra tecnologia empregada para melhorar a maciez da carne é a estimulação elétrica de carcaças. Hope-Jones et al. (2010) avaliaram o efeito da estimulação elétrica de alta voltagem (400 V) em carcaças de animais suplementados com HZ (0,15 mg/kg de peso vivo). Os autores encontraram interação entre esses dois tratamentos para a força de cisalhamento, em que a estimulação elétrica foi mais eficiente em melhorar a maciez da carne de animais suplementados com HZ, mas mesmo assim a carne do controle foi mais macia que aquela proveniente de animais suplementados, mesmo aos 14 dias de maturação. A aplicação de estimulação de carcaças de baixa voltagem

(85 V, 14 Hz, 60 s) em carcaças de animais suplementados com clenbuterol (0,16 µg/kg peso vivo/d por 42 d) foi descrita por Geesink et al. (1993). O AβA elevou a força de cisalhamento da carne. Entretanto, a estimulação elétrica aplicada foi suficiente para reverter à dureza durante o período de maturação de 13 dias.

A utilização do processo de amaciamento mecânico, juntamente com a técnica de *"enhancement"* (melhoramento da qualidade através da injeção de soluções de sais) foi avaliada por Brooks et al. (2010) com o objetivo de promover a maciez da carne de animais que foram suplementados com HZ. Os autores encontraram que o *"enhancement"* e o amaciamento mecânico diminuíram a força de cisalhamento da carne, mas não foram suficientes para superar o endurecimento causado pelo HZ.

4. REFERÊNCIAS BIBLIOGRÁFICAS

Anderson, D. B., D. E. Moody, e D. L. Hancock. 2005. Beta Adrenergic Agonist. In: W. G. P. A. W. Bell (ed.) Encyclopedia of Animal Science. p 104-107. Taylor & Francis.

Aurousseau, B., A. Connell, D. K. Revell, H. J. Rocha, e G. E. Lobley. 1993. Hind-limb protein metabolism in growing sheep; effects of acute and chronic infusion of clenbuterol by close arterial and systemic routes. Comp Biochem Physiol C 106: 529-535.

Avendano-Reyes, L., V. Torres-Rodriguez, F. J. Meraz-Murillo, C. Perez-Linares, F. Figueroa-Saavedra, e P. H. Robinson. 2006. Effects of two beta-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J Anim Sci 84: 3259-3265.

Baker, P. K., R. H. Dalrymple, D. L. Ingle, e C. A. Ricks. 1984. Use of a {beta}-Adrenergic Agonist to Alter Muscle and Fat Deposition in Lambs. J. Anim Sci. 59: 1256-1261.

Beckett, J. L., R. J. Delmore, G. C. Duff, D. A. Yates, D. M. Allen, T. E. Lawrence, e N. Elam. 2009. Effects of zilpaterol hydrochloride on growth rates, feed conversion, and carcass traits in calf-fed Holstein steers. J Anim Sci 87: 4092-4100.

Beermann, D. H., W. R. Butler, D. E. Hogue, V. K. Fishell, R. H. Dalrymple, C. A. Ricks, e C. G. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. J Anim Sci 65: 1514-1524.

Behrends, J. M., K. J. Goodson, M. Koohmaraie, S. D. Shackelford, T. L. Wheeler, W. W. Morgan, J. O. Reagan, B. L. Gwartney, J. W. Wise, e J. W. Savell. 2005. Beef customer satisfaction: factors affecting consumer evaluations of calcium chloride-injected top sirloin steaks when given instructions for preparation. J Anim Sci 83: 2869-2875.

Bekhit, A. E., M. A. Ilian, J. D. Morton, L. Vanhanan, J. R. Sedcole, e R. Bickerstaffe. 2005. Effect of calcium chloride, zinc chloride, and water infusion on metmyoglobin reducing activity and fresh lamb color. J Anim Sci 83: 2189-2204.

Bergen, W. G., S. E. Johnson, D. M. Skjaerlund, A. S. Babiker, N. K. Ames, R. A. Merkel, e D. B. Anderson. 1989. Muscle protein metabolism in finishing pigs fed ractopamine. J Anim Sci 67: 2255-2262.

Blum, J. W., and N. Flueckiger. 1988. Early metabolic e endocrine effects of perorally administered beta-adrenoceptor agonists in calves. Eur J Pharmacol 151: 177-187.

Boleman, S. J., S. L. Boleman, T. D. Bidner, K. W. McMillin, e C. J. Monlezun. 1995. Effects of Postmortem time of calcium chloride injection on beef tenderness and drip, cooking, and total loss. Meat Sci 39: 35-41.

Boucqué, C. V., L. O. Fiems, R. J. Moermans, B. G. Cottyn, e M. Sommer. 1994. Effect of cimaterol on growth, carcass characteristics and meat quality in double-muscled Belgian White-blue bulls. Canad J Anim Sci 74: 707-709.

Brooks, J. C., H. C. Claus, M. E. Dikeman, J. Shook, G. G. Hilton, T. E. Lawrence, J. M. Mehaffey, B. J. Johnson, D. M. Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, D. A. Yates, e M. F. Miller. 2009. Effects of zilpaterol hydrochloride feeding duration and postmortem aging on Warner-Bratzler shear force of three muscles from beef steers and heifers. J Anim Sci 87: 3764-3769.

Brooks, J. C., J. M. Mehaffey, J. A. Collins, H. R. Rogers, J. Legako, B. J. Johnson, T. Lawrence, D. M. Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, D. A. Yates, e M. F. Miller. 2010. Moisture enhancement and blade tenderization effects on the shear force and palatability of strip loin

steaks from beef cattle fed zilpaterol hydrochloride. J Anim Sci 88: 1809-1816.

Byrem, T. M., D. H. Beermann, e T. F. Robinson. 1998. The beta-agonist cimaterol directly enhances chronic protein accretion in skeletal muscle. J Anim Sci 76: 988-998.

Carr, M. A., K. L. Crockett, C. B. Ramsey, e M. F. Miller. 2004. Consumer acceptance of calcium chloride-marinated top loin steaks. J Anim Sci 82: 1471-1474.

Chikhou, F. H., A. P. Moloney, P. Allen, R. L. Joseph, P. V. Tarrant, J. F. Quirke, F. H. Austin, e J. F. Roche. 1993. Long-term effects of cimaterol in Friesian steers: II. Carcass composition and meat quality. J Anim Sci 71: 914-922.

Chwalibog, A., K. Jensen, e G. Thorbek. 1996. Quantitative protein and fat metabolism in bull calves treated with beta-adrenergic agonist. Arch Tierernahr 49: 159-167.

Claus, H. L., M. E. Dikeman, L. Murray, J. C. Brooks, J. Shook, G. G. Hilton, T. E. Lawrence, J. M. Mehaffey, B. J. Johnson, D. M. Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, D. A. Yates, M. F. Miller, M. C. Hunt, e J. Killefer. 2010. Effects of supplementing feedlot steers and heifers with zilpaterol hydrochloride on Warner-Bratzler shear force interrelationships of steer and heifer longissimus lumborum and heifer triceps brachii and gluteus medius muscles aged for 7, 14 and 21 d. Meat Sci 85: 347-355.

Dawson, J. M., J. Craigon, P. J. Buttery, e D. E. Beever. 1993. Influence of diet and beta-agonist administration on plasma concentrations of growth hormone and insulin-like growth factor-1 in young steers. Br J Nutr 70: 93-102.

Derington, A. J., J. C. Brooks, A. J. Garmyn, L. D. Thompson, D. B. Wester, e M. F. Miller. 2011. Relationships of slice shear force and Warner-Bratzler shear force of beef strip loin steaks as related to the tenderness gradient of the strip loin. Meat Sci 88: 203-208.

Diles, J. J., M. F. Miller, e B. L. Owen. 1994. Calcium chloride concentration, injection time, and aging period effects on tenderness, sensory, and retail color attributes of loin steaks from mature cows. J Anim Sci 72: 2017-2021.

Dunshea, F. R., D. N. D'Souza, D. W. Pethick, G. S. Harper, e R. D. Warner. 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Sci 71: 8-38.

Eisemann, J. H., e D. G. Bristol. 1998. Change in insulin sensitivity or responsiveness is not a major component of the mechanism of action of ractopamine in beef steers. J Nutr 128: 505-511.

Eisemann, J. H., e G. B. Huntington. 1993. Effects of dietary clenbuterol on net flux across the portal-drained viscera, liver and hindquarters of steers (Bos taurus). Comp Biochem Physiol C 104: 401-406.

Eisemann, J. H., G. B. Huntington, e C. L. Ferrell. 1988. Effects of dietary clenbuterol on metabolism of the hindquarters in steers. J Anim Sci 66: 342-353.

Elam, N. A., J. T. Vasconcelos, G. Hilton, D. L. VanOverbeke, T. E. Lawrence, T. H. Montgomery, W. T. Nichols, M. N. Streeter, J. P. Hutcheson, D. A. Yates, e M. L. Galyean. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J Anim Sci 87: 2133-2141.

FDA, F. a. D. A. 2003. Rules and Regulations. In: F. Register (ed.) 21 CFR Part 668. 181. p 5468.

FDA, F. a. D. A. 2006. Fiscal Year 2006. In: U. S. D. o. H. a. H. Services (ed.) New Animal Drug Approvals.

Fiems, L. O., B. Buts, C. V. Boucqué, D. I. Demeyer, e B. G. Cottyn. 1990. Effect of a [beta]-agonist on meat quality and myofibrillar protein fragmentation in bulls. Meat Sci 27: 29-39.

Garmyn, A. J., J. N. Shook, D. L. VanOverbeke, J. L. Beckett, R. J. Delmore, D. A. Yates, D. M. Allen, e G. G. Hilton. 2010. The effects of zilpaterol hydrochloride on carcass cutability and tenderness of calf-fed Holstein steers. J Anim Sci 88: 2476-2485.

Garssen, G. J., G. H. Geesink, A. H. Hoving-Bolink, e J. C. Verplanke. 1995. Effects of dietary clenbuterol and salbutamol on meat quality in veal calves. Meat Sci 40: 337-350.

Geesink, G. H., F. J. Smulders, H. L. van Laack, J. H. van der Kolk, T. Wensing, e H. J. Breukink. 1993. Effects on meat quality of the use of clenbuterol in veal calves. J Anim Sci 71: 1161-1170.

Gonzalez, J. M., J. N. Carter, D. D. Johnson, S. E. Ouellette, e S. E. Johnson. 2007. Effect of ractopamine-hydrochloride and trenbolone acetate on longissimus muscle fiber area, diameter, and satellite cell numbers in cull beef cows. J Anim Sci 85: 1893-1901.

Gonzalez, J. M., S. E. Johnson, A. M. Stelzleni, T. A. Thrift, J. D. Savell, T. M. Warnock, e D. D. Johnson. 2010. Effect of ractopamine-HCl supplementation for 28 days on carcass characteristics, muscle fiber morphometrics, and whole muscle yields of six distinct muscles of the loin and round. Meat Sci 85: 379-384.

Gunderson, J. A., M. C. Hunt, T. A. Houser, E. A. Boyle, M. E. Dikeman, D. E. Johnson, D. L. VanOverbeke, G. G. Hilton, C. Brooks, J. Killefer, D. M. Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, e D. A.

Yates. 2009. Feeding zilpaterol hydrochloride to calf-fed Holsteins has minimal effects on semimembranosus steak color. J Anim Sci 87: 3751-3763.

Harris, S. E., E. Huff-Lonergan, S. M. Lonergan, W. R. Jones, e D. Rankins. 2001. Antioxidant status affects color stability and tenderness of calcium chloride-injected beef. J Anim Sci 79: 666-677.

Hilton, G. G., J. L. Montgomery, C. R. Krehbiel, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, J. R. Blanton, Jr., e M. F. Miller. 2009. Effects of feeding zilpaterol hydrochloride with and without monensin and tylosin on carcass cutability and meat palatability of beef steers. J Anim Sci 87: 1394-1406.

Holmer, S. F., D. M. Fernandez-Duenas, S. M. Scramlin, C. M. Souza, D. D. Boler, F. K. McKeith, J. Killefer, R. J. Delmore, J. L. Beckett, T. E. Lawrence, D. L. VanOverbeke, G. G. Hilton, M. E. Dikeman, J. C. Brooks, R. A. Zinn, M. N. Streeter, J. P. Hutcheson, W. T. Nichols, D. M. Allen, e D. A. Yates. 2009. The effect of zilpaterol hydrochloride on meat quality of calf-fed Holstein steers. J Anim Sci 87: 3730-3738.

Hoover, L. C., K. D. Cook, M. F. Miller, K. L. Huffman, C. K. Wu, J. L. Lansdell, e C. B. Ramsey. 1995. Restaurant consumer acceptance of beef loin strip steaks tenderized with calcium chloride. J Anim Sci 73: 3633-3638.

Hope-Jones, M., P. E. Strydom, L. Frylinck, e E. C. Webb. 2010. The efficiency of electrical stimulation to counteract the negative effects of [beta]-agonists on meat tenderness of feedlot cattle. Meat Sci 86: 699-705.

Jaturasitha, S., P. Thirawong, V. Leangwunta, e M. Kreuze. 2004. Reducing toughness of beef from Bos indicus draught steers by injection of calcium chloride: Effect of concentration and time postmortem. Meat Sci 68: 61-69.

Kellermeier, J. D., A. W. Tittor, J. C. Brooks, M. L. Galyean, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, B. J. Johnson, e M. F. Miller. 2009. Effects of zilpaterol hydrochloride with or without an estrogen-trenbolone acetate terminal implant on carcass traits, retail cutout, tenderness, and muscle fiber diameter in finishing steers. J Anim Sci 87: 3702-3711.

Kerth, C. R., M. F. Miller, e C. B. Ramsey. 1995. Improvement of beef tenderness and quality traits with calcium chloride injection in beef loins 48 hours postmortem. J Anim Sci 73: 750-756.

Koohmaraie, M., A. S. Babiker, A. L. Schroeder, R. A. Merkel, e T. R. Dutson. 1988b. Acceleration of postmortem tenderization in ovine carcass through activation of Ca++-dependent proteases. J Food Sci 53: 1638-1641.

Koohmaraie, M., A. S. Babiker, R. A. Merkel, e T. R. Durson. 1988a. Role of Ca++-dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. J Food Sci 53: 1253-1257.

Koohmaraie, M., e S. D. Shackelford. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a beta-adrenergic agonist. J Anim Sci 69: 2463-2471.

Koohmaraie, M., J. D. Crouse, e H. J. Mersmann. 1989. Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: effect of concentration and ionic strength. J Anim Sci 67: 934-942.

Koohmaraie, M., S. D. Shackelford, N. E. Muggli-Cockett, e R. T. Stone. 1991. Effect of the beta-adrenergic agonist L644,969 on muscle growth, endogenous proteinase activities, and postmortem proteolysis in wether lambs. J Anim Sci 69: 4823-4835.

Kretchmar, D. H., M. R. Hathaway, R. J. Epley, e W. R. Dayton. 1989. In vivo effect of a beta-adrenergic agonist on activity of calcium-dependent

proteinases, their specific inhibitor, and cathepsins B and H in skeletal muscle. Arch Biochem Biophys 275: 228-235.

Kretchmar, D. H., M. R. Hathaway, R. J. Epley, e W. R. Dayton. 1990. Alterations in postmortem degradation of myofibrillar proteins in muscle of lambs fed a beta-adrenergic agonist. J Anim Sci 68: 1760-1772.

Lansdell, J. L., M. F. Miller, T. L. Wheeler, M. Koohmaraie, e C. B. Ramsey. 1995. Postmortem injection of calcium chloride effects on beef quality traits. J Anim Sci 73: 1735-1740.

Leheska, J. M., J. L. Montgomery, C. R. Krehbiel, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. Streeter, J. R. Blanton, Jr., e M. F. Miller. 2009. Dietary zilpaterol hydrochloride. II. Carcass composition and meat palatability of beef cattle. J Anim Sci 87: 1384-1393.

Luño, M., J. A. Beltrán, I. Jaime, and P. Roncalés. 1999. Textural assessment of clenbuterol treatment in beef. Meat Sci 51: 297-303.

Mehaffey, J. M., J. C. Brooks, R. J. Rathmann, E. M. Alsup, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, D. A. Yates, B. J. Johnson, e M. F. Miller. 2009. Effect of feeding zilpaterol hydrochloride to beef and calf-fed Holstein cattle on consumer palatability ratings. J Anim Sci 87: 3712-3721.

Mellgren, R. L., M. T. Mericle, e R. D. Lane. 1986. Proteolysis of the calcium-dependent protease inhibitor by myocardial calcium-dependent protease. Arch Biochem Biophys 246: 233-239.

Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. J Anim Sci 76: 160-172.

Miller, M. F., K. L. Huffman, S. Y. Gilbert, L. L. Hamman, e C. B. Ramsey. 1995. Retail consumer acceptance of beef tenderized with calcium chloride. J Anim Sci 73: 2308-2314.

Moloney, A. P., P. Allen, D. B. Ross, G. Olson, and E. M. Convey. 1990. Growth, feed efficiency and carcass composition of finishing Friesian steers fed the beta-adrenergic agonist L-644,969. J Anim Sci 68: 1269-1277.

Moloney, A., P. Allen, R. Joseph, e V. Tarrant. 1991. Influence of Beta Adrenergic Agonists and Similar Compounds on Growth. In: A. M. Pearson and T. R. Dutson (eds.) Growth Regulation in Farm Animals. p 455-513. Elsevier, New York.

Monsón, F., C. Sañudo, G. Bianchi, P. Albertí, A. Herrera, e A. Ariño. 2007. Carcass and meat quality of yearling bulls as affected by the use of clenbuterol and steroid hormones combined with dexamethasone J Muscle Foods 18: 173-185.

Montgomery, J. L., C. R. Krehbiel, J. J. Cranston, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, D. T. Bechtol, E. Johnson, T. TerHune, e T. H. Montgomery. 2009a. Dietary zilpaterol hydrochloride. I. Feedlot performance and carcass traits of steers and heifers. J Anim Sci 87: 1374-1383.

Montgomery, J. L., C. R. Krehbiel, J. J. Cranston, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, R. S. Swingle, e T. H. Montgomery. 2009b. Effects of dietary zilpaterol hydrochloride on feedlot performance and carcass characteristics of beef steers fed with and without monensin and tylosin. J Anim Sci 87: 1013-1023.

Moody, D. E., D. L. Hancock, e D. B. Anderson. 2000. Phenethanolamine repartitioning agents. In: J. P. F. D'Mello (ed.) Farm

animal metabolism and nutrition. p 65-96. CAB International, WallingfordOxon, UK,.

Morgan, J. B., R. K. Miller, F. M. Mendez, D. S. Hale, e J. W. Savell. 1991. Using calcium chloride injection to improve tenderness of beef from mature cows. J Anim Sci 69: 4469-4476.

O'Connor, R. M., W. R. Butler, K. D. Finnerty, D. E. Hogue, e D. H. Beermann. 1991. Acute and chronic hormone and metabolite changes in lambs fed the beta-agonist, cimaterol. Domestic Anim Endoc 8: 537-548.

Parr, T., R. G. Bardsley, R. S. Gilmour, e P. J. Buttery. 1992. Changes in calpain and calpastatin mRNA induced by beta-adrenergic stimulation of bovine skeletal muscle. Eur J Biochem 208: 333-339.

Pietri-Rouxel, F., G. Lenzen, A. Kapoor, M. F. Drumare, P. Archimbault, A. D. Strosberg, e B. S. Manning. 1995. Molecular cloning and pharmacological characterization of the bovine beta 3-adrenergic receptor. Eur J Biochem 230: 350-358.

Quinn, M. J., C. D. Reinhardt, E. R. Loe, B. E. Depenbusch, M. E. Corrigan, M. L. May, e J. S. Drouillard. 2008. The effects of ractopamine-hydrogen chloride (Optaflexx) on performance, carcass characteristics, and meat quality of finishing feedlot heifers. J Anim Sci 86: 902-908.

Rathmann, R. J., J. M. Mehaffey, T. J. Baxa, W. T. Nichols, D. A. Yates, J. P. Hutcheson, J. C. Brooks, B. J. Johnson, e M. F. Miller. 2009. Effects of duration of zilpaterol hydrochloride and days on the finishing diet on carcass cutability, composition, tenderness, and skeletal muscle gene expression in feedlot steers. J Anim Sci 87: 3686-3701.

Ricks, C. A., R. H. Dalrymple, P. K. Baker, e D. L. Ingle. 1984. Use of a {beta}-Agonist to Alter Fat and Muscle Deposition in Steers. J. Anim Sci. 59: 1247-1255.

Rogers, H. R., J. C. Brooks, M. C. Hunt, G. G. Hilton, D. L. VanOverbeke, J. Killefer, T. E. Lawrence, R. J. Delmore, B. J. Johnson, D. M. Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, D. A. Yates, J. N. Martin, e M. F. Miller. 2010. Effects of zilpaterol hydrochloride feeding duration on beef and calf-fed Holstein strip loin steak color. J Anim Sci 88: 1168-1183.

Schiavetta, A. M., M. F. Miller, D. K. Lunt, S. K. Davis, e S. B. Smith. 1990. Adipose tissue cellularity and muscle growth in young steers fed the beta-adrenergic agonist clenbuterol for 50 days and after 78 days of withdrawal. J Anim Sci 68: 3614-3623.

Schroeder, A., D. Hancock, D. Mowrey, S. Laudert, G. Vogel, e D. Polser. 2005a. Dose titration of Optaflexx® (ractopamine HCl) evaluating the effects on composition of carcass soft tissues in feedlot heifers. J. Anim. Sci. Vol. 83, Suppl. 1/J. Dairy Sci. Vol. 88, Suppl. 1: 114-172.

Sillence, M. N., e M. L. Matthews. 1994. Classical and atypical binding sites for beta-adrenoceptor ligands and activation of adenylyl cyclase in bovine skeletal muscle and adipose tissue membranes. Br J Pharmacol 111: 866-872.

Simmons, N. J., O. A. Young, P. M. Dobbie, K. Singh, B. C. Thompson, e P. A. Speck. 1997. Post-mortem calpain-system kinetics in lamb: Effects of clenbuterol and preslaughter exercise. Meat Science 47: 135-146.

Smith, S. B., D. K. Garcia, S. K. Davis, e D. B. Anderson. 1989. Elevation of a specific mRNA in longissimus muscle of steers fed ractopamine. J Anim Sci 67: 3495-3502.

St. Angelo, A. J., M. Koohmaraie, K. L. Crippen, and J. Crouse. 1991. Acceleration of tenderization inhibition of warmed-over flavor by calcium chloride-antioxidant infusion into lamb carcasses. J. Food Sci. 56: 359-362.

Strydom, P. E., L. Frylinck, J. L. Montgomery, e M. F. Smith. 2009. The comparison of three beta-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci 81: 557-564.

Van Liefde, I., A. Van Ermen, A. van Witzenburg, N. Fraeyman, e G. Vauquelin. 1994. Species and strain-related differences in the expression and functionality of beta-adrenoceptor subtypes in adipose tissue. Arch Int Pharmacodyn Ther 327: 69-86.

VanOverbeke, D. L., G. G. Hilton, J. Green, M. Hunt, C. Brooks, J. Killefer, M. N. Streeter, J. P. Hutcheson, W. T. Nichols, D. M. Allen, e D. A. Yates. 2009. Effect of zilpaterol hydrochloride supplementation of beef steers and calf-fed Holstein steers on the color stability of top sirloin butt steaks. J Anim Sci 87: 3669-3676.

Vasconcelos, J. T., R. J. Rathmann, R. R. Reuter, J. Leibovich, J. P. McMeniman, K. E. Hales, T. L. Covey, M. F. Miller, W. T. Nichols, e M. L. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J Anim Sci 86: 2005-2015.

Vestergaard, M., K. Sejrsen, e S. Klastrup. 1994. Growth, composition and eating quality of Longissimus dorsi from young bulls fed the [beta]-agonist cimaterol at consecutive developmental stages. Meat Sci 38: 55-66.

Walker, D. K., E. C. Titgemeyer, E. K. Sissom, K. R. Brown, J. J. Higgins, G. A. Andrews, e B. J. Johnson. 2007. Effects of steroidal implantation and ractopamine-HCl on nitrogen retention, blood metabolites and skeletal muscle gene expression in Holstein steers. J Anim Physiol Anim Nutr (Berl) 91: 439-447.

Walker, D. K., E. C. Titgemeyer, T. J. Baxa, K. Y. Chung, D. E. Johnson, S. B. Laudert, e B. J. Johnson. 2010. Effects of ractopamine and sex

on serum metabolites and skeletal muscle gene expression in finishing steers and heifers. J Anim Sci 88: 1349-1357.

Wang, S. Y., e D. H. Beermann. 1988. Reduced calcium-dependent proteinase activity in cimaterol-induced muscle hypertrophy in lambs. J Anim Sci 66: 2545-2550.

Wheeler, T. L., e M. Koohmaraie. 1992. Effects of the beta-adrenergic agonist L644,969 on muscle protein turnover, endogenous proteinase activities, and meat tenderness in steers. J Anim Sci 70: 3035-3043.

Wheeler, T. L., J. D. Crouse, e M. Koohmaraie. 1992. The effect of postmortem time of injection and freezing on the effectiveness of calcium chloride for improving beef tenderness. J Anim Sci 70: 3451-3457.

Wheeler, T. L., M. Koohmaraie, e J. D. Crouse. 1991. Effects of calcium chloride injection and hot boning on the tenderness of round muscles. J Anim Sci 69: 4871-4875.

Wheeler, T. L., M. Koohmaraie, e S. D. Shackelford. 1996. Effect of vitamin C concentration and co-injection with calcium chloride on beef retail display color. J Anim Sci 74: 1846-1853.

Wheeler, T. L., M. Koohmaraie, e S. D. Shackelford. 1997. Effect of postmortem injection time and postinjection aging time on the calcium-activated tenderization process in beef. J Anim Sci 75: 2652-2660.

Wheeler, T. L., M. Koohmaraie, J. L. Lansdell, G. R. Siragusa, and M. F. Miller. 1993. Effects of postmortem injection time, injection level, and concentration of calcium chloride on beef quality traits. J Anim Sci 71: 2965-2974.

Whipple, G., e M. Koohmaraie. 1992. Freezing and calcium chloride marination effects on beef tenderness and calpastatin activity. J Anim Sci 70: 3081-3085.

Winterholler, S. J., G. L. Parsons, C. D. Reinhardt, J. P. Hutcheson, W. T. Nichols, D. A. Yates, R. S. Swingle, e B. J. Johnson. 2007. Response to ractopamine-hydrogen chloride is similar in yearling steers across days on feed. J Anim Sci 85: 413-419.

Winterholler, S. J., G. L. Parsons, D. K. Walker, M. J. Quinn, J. S. Drouillard, e B. J. Johnson. 2008. Effect of feedlot management system on response to ractopamine-HCl in yearling steers. J Anim Sci 86: 2401-2414.

Yang, Y. T., e M. A. McElligott. 1989. Multiple actions of beta-adrenergic agonists on skeletal muscle and adipose tissue. Biochem J 261: 1-10.

Young, O. A., S. Watkins, J. M. Oldham, e J. J. Bass. 1995. The role of insulin-like growth factor I in clenbuterol-stimulated growth in growing lambs. J Anim Sci 73: 3069-3077.

5. ARTIGO 1 PARA PUBLICAÇÃO

Artigo formatado para publicação no *Journal of Animal Science*

Print ISSN: 0021-8812; Online ISSN: 1525-3163

Post rigor calcium chloride injection effects on palatability traits of inside round steaks from beef cattle fed with zilpaterol hydrochloride

S.B. Pflanzer[§], A. Rodas-González*, J.N. Martin*, J.C. Brooks*, B.J. Johnson*, J.D. Starkey*, P.E. de Felicio[§], M.N. Streeter[†], J.P. Hutcheson[†], D.A. Yates[†], R.J. Rathmann*, M.F. Miller*[1].

[§]State University of Campinas, Campinas, Brazil. *Department of Animal and Food Science, Texas Tech University, Lubbock, TX 79409, USA.

[†]Intervet/Schering-Plough Animal Health, DeSoto, KS 66018, USA

Corresponding author: mfmrraider@aol.com

ABSTRACT

To determine the effects of calcium chloride injection on palatability traits of cattle fed with zilpaterol hydrochloride (**ZH**), 39 USDA Select quality grade carcasses were selected from a pool of 466 steers that were ZH-fed 0 or 8.3 mg/kg DM basis (233 non-ZH-fed and 233 ZH-fed; respectively) for the last 20 d of the finishing period. At 72 h postmortem, inside rounds from both sides of carcass were selected alternatively in order to serve as control (non-injected) or injected (5% wt/wt) with a calcium chloride solution ($CaCl_2$-injected, at 200 mM). Before injecting the subprimals (at 72 h postmortem), two initial steak were cut for sarcomere length and myofibrillar fragmentation index (MFI) analysis. At 7 d postmortem aging (2°C), inside rounds were portioned into steaks to be aged until 28 d postmortem. Samples were used for MFI (at 7, 14, 21 and 28 d aging), sensory analysis by trained panelists (at 14 and 21 d aging) and Warner Bratzler shear force (WBSF; at 7, 14, 21 and 28 d aging). No differences on sarcomere length were found between non-ZH-fed and ZH-fed samples ($P > 0.05$). The non-ZH-fed and $CaCl_2$-injected steaks presented higher MFI values ($P < 0.05$). The ZH supplementation did not affect drip and cooking losses of inside round steaks. However, $CaCl_2$ caused great ($P < 0.05$) losses. Results indicated that ZH increased ($P < 0.05$) WBSF; however, 14 d postmortem aging could improve tenderness, and if treated with $CaCl_2$, 7 d of aging was sufficient to reach the same level of tenderness of non-ZH samples. $CaCl_2$ generates lower ($P < 0.05$) WBSF in samples at 7, 14 and 21 d postmortem. Sensory traits were not altered by ZH supplementation

at 14 or 21 postmortem. The $CaCl_2$ improved ($P < 0.05$) tenderness at 14 d postmortem of inside round steaks, but without any improvement on palatability traits at 21 d. In conclusion, inside round from either non-ZH or ZH-fed steaks get lower WBSF values at 7, 14 and 21 when injected with $CaCl_2$, in consequence, $CaCl_2$ can overcome the toughness induced by zilpaterol hydrochloride supplementation.

Key words: beef palatability, calcium chloride, inside round, shear force, zilpaterol hydrochloride.

5.1. Introduction

In 2006, it was approved zilpaterol hydrochloride (**ZH**; oral synthetic β-adrenergic receptor agonist) in the United States for being used in feedlot cattle (FDA, 2006), and since it has been commercially available as Zilmax (Intervet Schering Plough, DeSoto, KS). The ZH supplementation has been documented to cause a detrimental palatability effect on beef strip loin (Brooks et al., 2010; Brooks et al., 2009; Hilton et al., 2009; Leheska et al., 2009) and inside round (Garmyn et al., 2010), increasing Warner-Bratzler shear force (**WBSF**) and adversely affecting sensory evaluations. Some postmortem technologies such as postmortem aging have improved palatability attributes. Brooks et al. (2010) have indicated that strip loin WBSF were similar between ZH-treated and untreated steaks just after 21 d postmortem aging, however Garmyn et al. (2010) found greater inside round WBSF values in ZH-treated samples than control, but the authors did not find interaction between ZH treatments (0 or 8.3 mg/kg ZH) and aging (14 and 21 d) on sensory traits and WBSF. Thus, the magnitude of change over time was similar for control and treated steaks.

Some segments of the meat industry have utilized additional technologies for further improving of beef tenderness. Injection or infusion of calcium chloride ($CaCl_2$) solution used on beef carcass or muscle cuts is one of these. Application of $CaCl_2$ has been reported to improve or accelerate tenderness in the pre-rigor (Koohmaraie et al., 1988, 1989, 1990; Koohmaraie

and Shackelford, 1991; Morgan et al., 1991; Wheeler et al., 1991), and post rigor meat (Wheeler et al., 1992, 1993) reducing the variation in beef tenderness without affecting other beef quality or palatability traits. However, no research had been conducted to determine the effects of calcium chloride injection on beef from ZH-fed cattle.

Therefore, the objectives of this study were to examine 1) the effect of dietary administration of zilpaterol hydrochloride on the palatability of beef semimembranosus muscle and 2) the effectiveness of $CaCl_2$ injection in overcoming the potential toughness of meat resulting from zilpaterol hydrochloride feeding.

5.2. Materials and Methods

Animal Care and Use Committee approval was not obtained for this study because samples were taken from federally inspected slaughter facilities.

Cattle source and subprimal selection

A feedlot experiment was conducted using 466 crossbred steers. Cattle were weighed on arrival at the feedlot so that steers could be blocked into heavy animals (n = 234) fed 143 d; and light animals (n = 232) fed 163 d. Steers were implanted on arrival at the feedlot (d 0) and re-implanted on d 80 with a Revalor-XS (80 mg of trenbolone acetate and 16 mg of estradiol, Intervet/ Schering-Plough Animal Health). Diet containing ZH (ZH-fed, 8.3 mg/kg dry matter basis, Intervet/Schering-Plough Animal Health, DeSoto, KS) was fed for 20 of the last 23 d (control diet designated as 0 d - control-fed) of the feeding period. Harvesting occurred after a mandatory 3 d withdrawal of the supplementation.

Animals were slaughtered at two different commercial processing facilities and at two different dates (July and August, 2010 at the year). All carcasses were subject to electrical stimulation on the blood pit and hide pull (70 volts at 0.05 amps for 10 sec). Animals were identified at slaughter, and their identities were maintained throughout the grading and fabrication process. At 36 h postmortem, carcasses were graded and a subset of carcasses

(control-fed = 19; ZH-fed = 20) representing USDA Select quality grade were selected randomly. At 48 h postmortem, carcasses were fabricated and the boneless inside round (IMPS # 168; USDA, NAMP, 2010) were obtained from both side of each carcass, identified, boxed, and shipped refrigerated (1 to 3°C) to Texas Tech University Meat Laboratory.

Hot carcass weight (HCW), KPH (kidney, pelvic and heart fat percentage), fat thickness, longissimus muscle area, marbling and USDA yield grade were evaluated in the carcasses selected for the experiment.

Injection and Steaks Fabrication

At the meat lab, at 72 h postmortem, the subprimals were removed from boxes and trimmed of fat and connective tissue. The gracilis and adductor muscles were removed and the semimembranosus was used for the study (IMPS # 169A; USDA, NAMP, 2010).

Cuts from the alternating sides were randomly assigned to either a control (non-injected) or injected with calcium chloride (injected). Before injection, two steaks from each cut were assigned to sarcomere length (**SL**) and miofibrilar fragmentation index (**MFI**), and stored at -84°C in Whirl-pak bags (Nasco, Fort Atkinson, WI). Water was not applied to control cuts because previous work had shown it would have little, if any, influence on tenderness (Wheeler et al., 1993) and because water injection is not used commercially (Diles et al., 1994).

The inside rounds were injected at close than 5% (wt/wt) of 200 mM food grade $CaCl_2$ (Tetra Technologies, The Woodlands, TX) in 5°C distilled and de-ionized water. The injection was carried out once through a multiple-needle pickle injector (Wolf-Tec, Inc., Model Schroder//Imax 350, Kingston, NY, USA). Cuts were then allowed to equilibrate for 5 min post-pump drain period, each cut was reweighed to determine the actual percentage pump (6.15 ± 0.45) calculated as [(pumped and drained cut wt. – unpumped cut wt.) ÷ unpumped cut wt.] × 100 (Lansdell et al., 1995). Cuts were weighed, vacuum-packaged, and stored at 2°C until 7 d postmortem (4 d after injection).

At d 7 postmortem, each inside round was removed from the package, blotted with a paper towel to remove surface moisture, and weighed to acquire subprimal purge loss. Each inside round was then faced, portioned into halves (perpendicular) and 2.5 cm thick steaks were fabricated using a manual meat slicer (Berkel, Model X13E, 13", USA) to ensure uniformity and consistency.

Each steak was immediately vacuum packaged in a multilaminar, thermo-shrinkable bag (BHT 620 Sealed Air Inc. – Cryovac division, Duncan, SC) by use of a Koch Ultravac® vacuum packaging machine (Model UV-250, Kansas City, MO). Steaks assigned to the 7 d were frozen immediately (-20°C), and stored at that temperature for subsequent analysis. Steaks assigned to 14, 21 or 28 d aging treatments were placed in a cooler room at 3°C for the appropriate time, then frozen (-20°C) and stored at that temperature for subsequent analysis.

The assignment of steaks to analysis and postmortem aging treatment was rotated by one location for each subsequent cut to ensure all locations within the subprimal were represented in all aging periods and analysis. After fabrication, steaks were weighed, individually vacuum packaged, and aged for their respective time under refrigeration at 2°C. After the appropriate aging time, steaks were frozen (−20°C) until further analysis.

The steaks obtained at 7 d postmortem from each inside round were assigned to the following analysis and aging treatment: four steaks for MFI at 7, 14, 21 and 28 d postmortem, two steaks for sensory analysis by trained panelist at 14 and 21 d postmortem, and four steaks for WBSF at 7, 14, 21 and 28 d postmortem.

Sarcomere length (SL)

Sarcomere length was determined by the neon-laser diffraction method described by Cross et al. (1981) with modification.

A small piece of muscle (3.0 x 3.0 x 2.0 cm) was cut with the fibers running longitudinally, placed in scintillation vials, added 5% glutaraldehyde solution and fixed for 4 hours at 4°C. After that, the glutaraldehyde solution was poured and replaced with the 0.2 M sucrose solution. Samples were fixed overnight at 4°C. A very fine tipped dissecting forceps was used to “pinch” a small bundle of muscle; place that on the microscope slides with cover slips

and gently spread out the individual fibers. A drop of sucrose solution was used to keep moist.

The slides were exposed to the neon-laser beam (Spectra Physics Inc., Model 117A, CA, USA) of 632.8 nm. The lengths of at least 5 diffraction patterns from each sample were measured and sarcomere length was determined by averaging the measurement.

Myofibrillar fragmentation index (MFI)

The ease of fragmentation of myofibrils was assessed following the procedure of Culler et al. (1978). This procedure was conducted to provide an estimate of muscle fiber degradation. Duplicate 4 g samples were trimmed free of fat and connective tissue, knife-minced, and homogenized for 30 s with 40 ml of MFI buffer (100 mM KCl, 20 mM potassium phosphate [pH 7], 1 mM EGTA, 1 mM $MgCl_2$, 1 mM NaN_3). The homogenate was centrifuged at 1000 − g for 15 min, the supernatant discarded, the pellet suspended in 40 ml of MFI buffer, and centrifuged at 1000 − g for 15 min. The supernatant was again discarded and the pellet suspended in 10 ml of MFI buffer. Samples were poured through a strainer to remove connective tissue, and then assayed for protein content using the biuret method (Gornall et al., 1949). Samples were adjusted to a common protein content of 0.5 mg protein/ml of solution, and the absorbance of 8 ml was read at 540 nm on a spectrophotometer (DU-

640, Beckman Instruments, Inc., Fullerton, CA). To determine MFI the absorbance was multiplied by 200.

Cooking samples for WBSF and sensory analysis

Individually packaged frozen steaks were thawed at 4°C for 24 h before analysis and the steaks were weighted to calculate aging and thawing losses. Before cooking, the initial internal temperature of steaks were recorded (Cooper Instruments digital meat thermometer model SH66A, Middlefield, CT) and they were between 4 and 7°C.

Belt grill (**BG**) cooking was conducted with a Magi-grill (model TBG-60 Magigrill, Magi-Kitch'n Inc., Quakertown, PA). The BG settings (top heat = 163°C, bottom heat = 163°C, preheat = 149°C, height (gap between platens) = 2.16 cm, and cook time = 5.8 min) were designed to achieve a final internal temperature of 71°C. After the steaks exited the BG, a needle thermocouple probe was inserted into the geometric center of the chop and post cooking temperature rise was monitored with a handheld thermometer (Cooper Instruments digital meat thermometer model SH66A, Middlefield, CT). The final weight and the maximal internal temperature, which occurred about 2 min after the steak exited the BG, was recorded. The identification of each steak was maintained throughout the process (Shackelford et al., 1999).

Warner-Bratzler shear force analysis (WBSF)

After cooking, the steaks were placed on trays, covered with plastic film, and held overnight at 4°C for WBSF analysis.

In the other day, cores from each of the lateral, middle, and medial portions (for a total of 6 cores; 1.3 cm in diameter) from each steak were removed parallel to the longitudinal orientation of the muscle fibers. Cores were sheared using a Warner-Bratzler Shear Testing Machine (G-R Elec. Mfg. Co., Manhattan, KS), and the peak shear force was recorded and the average was determined. Peak load (kg) for all 6 cores was averaged, and mean peak load (kg) was analyzed for each sample.

Sensory analysis

After cooking, fat and connective tissue were removed and each steak was cut into cubes (1 cm x 1 cm x steak thickness) and placed on preheated pans kept warm with heated sand. Cubed samples were served warm (at 50ºC), unsalted and unspiced.

The sensory panel consisted of eight-member trained sensory students from Texas Tech University. Panelists were trained on tenderness, juiciness, and beef flavor attributes (Cross et al., 1978). Sensory sessions were conducted twice a day, and each session contained 10 samples.

Samples were evaluated using a ballot consisted of a numerical, 8-point scale (AMSA, 1995) for initial juiciness, sustained juiciness, initial

tenderness, sustained tenderness, flavor intensity, beef flavor, and overall mouth feel (1 = extremely dry, tough, bland, off-flavor, and uncharacteristic of young beef; 8 = extremely juicy, tender, intense, beef-like and characteristic of young beef).

During sessions, panelists were randomly seated in individual booths in a temperature controlled room with red lights. The 10 samples were served in a randomized order according to panelist. The panelists were provided water, apple juice, and unsalted crackers to cleanse their palate after tasting each sample.

Statistical Analysis

Data collected were analyzed using SAS (Cary, NC) version 9.2 (SAS, 2003). Because there were two industries in the experiment, they were included in the model as a random effect. Data were analyzed as a completely randomized design (CRD) without or with split-plot or split-split plot arrangements. The degrees of freedom in the denominator were adjusted using the Satterthwaite procedure. Least squares means were separated (F test, $P < 0.05$) by using least significant differences generated by the PDIFF option

Carcass data and sarcomere length. For analysis of variance, a CRD model was used to study the effects of feeding group (non-ZH-fed vs. ZH-fed) as main effect on these variables.

Injection traits. Were analyzed as a CRD with split-plot arrangement; where the whole-plot portion were 2 feeding groups, and in the sub-plot, it was assigned to injection treatments. Thus, feeding groups, injection treatments, and their interaction were considered to be fixed.

Myofibrillar fragmentation index. Because some confounding effects of injection treatment and postmortem aging were expected, data were divided into two sub-sets for conducting separate analyses. The first data sub-set corresponded only to the non-injected samples from both feeding groups present at 3 d postmortem (CRD). The second model represented injected and non-injected steaks from both feeding group aged at 7, 14, 21 and 28 d postmortem (CRD with split split-plot arrangement)

WBSF, drip and cooking losses: These data were analyzed as CRD with split split-plot arrangement; where the whole-plot portion were both feeding groups (non-ZH-fed vs. ZH-fed), and in the sub-plot, it was assigned to injection treatments (non-injected vs. $CaCl_2$-injected). In the sub-subplot, postmortem aging days (7, 14, 21 vs. 28 d); thus, feeding groups, injection treatments, postmortem aging and their interaction were considered to be fixed. The internal cooked temperature did not affect treatments involved ($P > 0.05$) so; it was not included in the model as a covariate.

Trained sensory evaluation: Data across postmortem aging period (14 or 21 d) were analyzed separately because trained panel were served within aging treatment. The model used for each postmortem day was a CRD with split-plot arrangement; where the whole-plot portion were 2 feeding groups,

and in the sub-plot, it was assigned to injection treatments. Thus, feeding groups, injection treatments, and their interaction were considered to be fixed.

5.3. Results and Discussion

Carcass data

The feeding group had effect ($P < 0.05$) on HCW (hot carcass weight), longissimus area and USDA calculated yield grade (Table 1). The ZH-fed group had heavier carcasses, larger LM area and lower numerical USDA yield grade than carcasses from control-fed. Fat thickness, KPH (kidney, pelvic and heart fat percentage) and marbling score were not affected ($P > 0.05$) by ZH supplementation. The lack of difference on marbling is due to carcasses being selected within the same quality grade.

Generally, ZH-fed cattle had greater HCW and LM area due to increased protein deposition (Avendano-Reyes et al., 2006; Beckett et al., 2009; Elam et al., 2009; Vasconcelos et al., 2008), although fat deposition may not be affected as it happened in this research (Beckett et al., 2009; Montgomery et al., 2009; Casey et al., 1997; Plascencia et al., 1999). However, ZH-supplementation had been reported to decrease the marbling score (Elam et al., 2009; Hilton et al., 2009; Vasconcelos et al., 2008)

Sarcomere length (SL)

The feeding group did not have effect ($P > 0.05$) on SL, nevertheless in both cases the SL means could be considered as shortened (Table 1). Results from Hopes-Jones et al., (2010) agree with the current study in that ZH did not cause any effect on SL. Strydom et al. (2009) did not found any difference

on longissimus and semitendinosus muscles SL while comparing the effects of three βAA. Williams et al. (1987) suggested that the leanness caused by β-agonists may allow rapid postmortem cooling inducing cold shortening which could result in tougher meat. Although Geesink et al. (1993) agreed that the effect of βAA on meat tenderness is only partly explained by its effect on calcium-dependent proteases, they found no significant effect of βAA treatment on SL.

Injection traits

The effects for initial portion weights, injection percentages, and 7 d postmortem purge loss are presented in Table 2. The feeding group had effect ($P < 0.05$) on whole weight of inside round. Inside rounds from ZH-fed were heavier than those from control-fed cattle. Many articles have shown that ZH increases percentage yield in several cuts (Hilton et al., 2009; Kellermeier et al., 2009). Rathmann et al. (2009) found that although ZH manifested its effect within every whole primal region in the carcass, the most consistent ZH effect was seen in the round where ZH increased the percentage yield of every subprimal recorded. The percentage of solution that was injected was higher but not statistically different ($P = 0.27$) in inside round from ZH-fed than control-fed cattle.

In the current study, the feeding group did not have effect ($P = 0.46$) on inside round purge loss. Other studies have indicated none or little variations

on drip loss using a different βAA (Boucqué et al., 1994; Fiems et al., 1990). Other results, higher purge loss in ZH-treated beef cattle was reported by Kellermeier et al., (2009) and Rathmann (2009). Equally, Strydom (2009) found higher drip loss in ZH-fed steaks than control steaks.

The effect of injection treatments was significant on subprimal purge loss at 7 d postmortem ($P < 0.01$). The $CaCl_2$-injected inside round showed higher purge loss. Many reports describe an increased drip loss in $CaCl_2$ injected, particularly when is measured as purge (Dikeman et al., 2003; Koohmaraie et al., 1990; Pringle et al., 1999). Previous studies have indicated that $CaCl_2$ injection reduced the water holding capacity of meat. Wheeler et al. (1993) found that the amount of drip loss was not affected by injection amount (5 or 10 %), but it was affected by $CaCl_2$ concentration being greatest in muscle injected with 200 mM. Jaturasitha et al. (2004) reported the injection of $CaCl_2$ at any concentration (0.2, 0.3 and 0.4 M at 10%) increased drip loss.

Myiofibrillar fragmentation index (MFI)

Analysis of variance (Table 3) indicated effect of feeding group ($P < 0.01$), injection treatment ($P = 0.02$) and postmortem aging ($P < 0.01$) on MFI, but there was no effect of triple interaction ($P = 0.93$), that was not shown in tabular form.

Samples from ZH-fed had 23% lower MFI than those from control-fed cattle, indicating a low postmortem proteolysis activity. Previous researches had confirmed the finding of this study. Fiems et al. (1990) reported that diets with cimaterol reduced myofibrillar protein degradation via a low activity of the proteolytic enzymes in young bulls. Wheeler and Koohmaraie (1992) suggested that the inhibition of calpain activity by a high calpastatin activity probably is responsible for the lower than normal postmortem tenderization in steers fed βAA. The MFI is a direct index of the extent of postmortem proteolysis, and normally βAA feeding decreases MFI (Beermann, 1985; Fiems et al., 1990; Kretchmar et al., 1990; Koohmaraie and Shackelford, 1991; Koohmaraie et al., 1991; Wheeler and Koohmaraie, 1992; Berge et al., 1993; Lee and Kim, 1994; Vestergaard et al., 1994).

$CaCl_2$ increased the total MFI in 15% when compared with non-injected. Consequently, the higher levels of myofibril fragmentation found in samples treated with $CaCl_2$ could be considered as a result of the action of endogenous proteolytic enzymes that are activated at an accelerated rate reducing the time necessary for the post mortem aging (Cottin et al., 1991; Pérez et al., 1998). Our data are supported by those of Gonzalez et al. (2001) that showed MFI values of treated cutaneous trunci muscle aged 3 d similar to those obtained for the injected inside round. However, Lawrence et al. (2003) found no differences in MFI of semitendinosus or longissimus muscles injected with calcium lactate, where $CaCl_2$ increased the MFI at 28 d postmortem when compared with non-injected.

For aging period, MFI increased up to 50% from 7 to 28 d postmortem. According Olson et al. (1976), the index values tend to increase with aging time, with the most significant increase occurring in the first week, and a slow increase after that.

During all aging periods, injected samples from non-ZH-fed cattle had higher ($P < 0.05$) MFI than those non-injected from ZH-fed cattle (Figure 1). After injection with $CaCl_2$, samples from ZH-fed and non-ZH-fed cattle increase the MFI when compared with non-injected samples, however the great increasing was seen in ZH-fed cattle, that reached the same MFI level of non-injected samples from control-fed cattle at day 7.

It demonstrated that regards lower MFI at the beginning of aging due to βAA supplementation, the extended aging can improve tenderness by increasing MFI.

Drip and cooking losses

No aging period effects were found for the drip and cooking losses data ($P > 0.05$). Therefore, data were pooled and are presented on Table 4. Similarly, no aging effects on drip loss percentages have been reported by Savell et al. (1978) and Boleman et al. (1995). Parrish et al. (1969) and Morgan et al. (1991) found no differences in cooking loss due to aging when semimembranosus muscles were aged for 4, 7, and 11 d or 1, 7, and 14 d postmortem, respectively. Hamm (1986) stated that it is possible that an

increase of pH and/or proteolytic disintegration during aging may result in increased water retention.

There was no effect of ZH-supplementation or ZH x $CaCl_2$ interaction for drip and cooking losses ($P > 0.05$). On the other hand, $CaCl_2$ injection increased drip and cooking losses ($P < 0.05$). Previous studies have indicated none or little variations on cooking loss of steaks from βAA treated animals. Fiems et al. (1990) worked with cimaterol did not find any change on cooking loss in the meat of young bulls and Leheska et al. (2009), Garmyn et al. (2010) and Hilton et al. (2009) did not find any difference on cooking loss percentage of ZH treated samples vs. control.

Koohmaraie et al. (1990), Wheeler et al. (1991), Diles et al. (1994), Kerth et al. (1995), Wheeler et al. (1997), Miligan et al. (1997), and Kong et al. (2006) showed that cooking losses were higher in cuts injected with $CaCl_2$. Miligan et al. (1997) found that drip losses were greater for the $CaCl_2$ injected inside round roasts than for the non-injected roasts.

The greater drip and cooking losses found in $CaCl_2$ samples are related to the increasing of free water in the muscle, and the excess water is not hold and is released during aging or cooking.

WBSF

Analysis of variance (Table 5) indicated effect of feeding group ($P = 0.04$), injection treatment ($P < 0.01$) and postmortem aging time ($P < 0.01$) on

WBSF. Significant effect of interaction was found between feeding group x postmortem aging period ($P < 0.001$) and injection treatment x postmortem aging period ($P = 0.01$) There were no interaction between feeding x injection treatment ($P = 0.58$), and neither triple interaction ($P = 0.72$; Figure 2).

Non-injected inside round from ZH-supplemented cattle had higher WBSF than all other treatments at 7 d postmortem. However, at 14 and 21 d postmortem the WBSF was similar to samples non-injected from control-fed cattle, but still higher than both $CaCl_2$ injected ZH and control samples. Just at 28 d of aging the WBSF reached the same level for all treatments (Figure 2). The results evidenced that zilpaterol had higher WBSF with respect to control group in each aging time. These results indicate that ZH has a similar effect to other βAA, such as clenbuterol in increasing WBSF (Miller et al., 1988; Schiavetta et al., 1990). Higher increases in WBSF due to the use of ZH have been reported in the literature. Buys and Strydom (2000) found a 20%, while Strydom et al. (2002) found 28% increases when ZH was fed. More recently, Hilton et al. (2009) and Leheska et al. (2009) observed increases in WBSF from 3.29 to 4.01 kg and 3.18 to 3.45 kg, respectively, when steers and heifers were supplemented with ZH for 0 and 30 d. Studying the effects of ZH on inside round, Garmyn et al. (2010) found 15% increase on WBSF in aged steaks (7 and 14 d postmortem) from ZH-fed cattle.

The greater WBSF of ZH-fed steaks are being associated with the lower MFI. When $CaCl_2$ is injected, it accelerates protein degradation and MFI, and in consequence reduces WBSF.

According to these results, steaks from both control-fed cattle injected or not did not reduced WBSF with aging (7 through 28 d postmortem), in the other hand ZH-feed steaks diminished WBSF values among 7 and 14 d when injected or not and 21 and 28 d postmortem just when not injected. In agreement with the present study, Hilton et al. (2009) reported a decrease in shear force attributed to postmortem aging of ZH-treated beef from 7 to 21 d postmortem that exceeded the control group (1.3 kg reduction vs. 0.4 kg reduction for control steaks), agreeing with the interaction observed in our study.

At 7 d postmortem non-ZH-feed steaks were much tender and were not improved by aging, however ZH-feed steaks had high WBSF at 7 d postmortem, and the WBSF was very well enhanced by aging.

When inside round from ZH-fed cattle were injected with $CaCl_2$, the WBSF at 7, 14 and 21 d postmortem was diminished up to 0.6 kg, 0.5 kg and 0.4 kg, respectively, and already at 7 d postmortem the WBSF was similar to control-fed cattle non-injected, been higher just when compared with injected control-fed. The WBSF advantage of steaks injected with $CaCl_2$ is in agreement with other results from loin steaks (Wheeler et al., 1993; Lansdell et al., 1995) as well as Wheeler et al. (1991) and Milligan et al. (1997) in which $CaCl_2$ injection decreased WBSF values of semimembranosus muscle. Kerth et al. (1995) reported 16 and 22% improvement on WBSF comparing when steaks were injected 200 and 250 mM $CaCl_2$, respectively, and a 13% improvement in tenderness from 7 to 14 d postmortem aging.

Sensory analysis

The effect of feeding group and injection treatments on the trained sensory panel scores of inside round steaks aged 14 or 21 d postmortem are presented in Table 6 and 7, respectively. Either at 14 or 21 d postmortem aging, data analysis revealed no significant interactions between feeding group and injection treatments ($P > 0.05$), also no interactions were found between feeding group or injection treatments and days of aging for any sensory trait ($P > 0.05$). As well, feeding group did not affect any sensory trait of steaks aged 14 or 21 d postmortem ($P > 0.05$). Only, the main effect of injection treatment tend to affect initial ($P = 0.06$) and affect sustained ($P = 0.01$) tenderness of steaks aged 14 d, however at 21 d postmortem significant effect was found just on off-flavor ($P < 0.01$). A 200 mM $CaCl_2$ injection at 72 h postmortem improved initial and sustained tenderness at 14 d postmortem inside round steaks, but at 21 d postmortem no more improvement was verified to tenderness score. $CaCl_2$ induced development of off-flavors when compared with control samples in both 14 and 21 d aging period.

The lacking of difference on tenderness scores of inside round steaks from ZH-fed or control-fed steers at 14 and 21 d postmortem agree with results obtained in WBSF, in which similar values were found for both groups. In the same way, Garmyn et al. (2010) found similar initial and sustained juiciness and initial tenderness regardless of ZH supplementation or aging period, however panelists tended to assign greater overall tenderness scores to control steaks, yet they found no difference in connective tissue

amount between control and ZH steaks. Leheska et al. (2009) and Hilton et al. (2009) reported a decrease in scores for trained sensory traits of tenderness, juiciness, and flavor of ZH-fed cattle, attributing these differences to modifications in protein structure caused by ZH.

With regard to $CaCl_2$, Lawrence et al. (2003) showed that calcium salts increased sensory tenderness scores. Other reports have shown increased sensory panel tenderness due to $CaCl_2$ marination (Kerth et al., 1995; Morgan et al., 1991; Wheeler et al., 1993, 1997). For juiciness and flavor Carr et al. (2004) found that $CaCl_2$ improved sustained juiciness, overall mouthfeel, and beef flavor scores. Diles et al. (1994) and Lansdell et al. (1995) also reported that $CaCl_2$ injection improved juiciness. Milligan et al. (1997) found overall beef mouthfeel scores were increased by $CaCl_2$ injection. However, Wheeler et al. (1993) found no difference in beef flavor from injected steaks.

The development of off-flavors induced by $CaCl_2$ was described in previous reports. Morgan et al. (1991) reported increased metallic, bitter, and livery flavors (0.3 M $CaCl_2$ solution - 10% of the sub primal weight), while Wheeler et al. (1993) reported increased sour and bitter flavors (0.2 and 0.25 M $CaCl_2$ solution – 5 and 10% of the sub primal weight). Morris et al. (1997) reported increased livery, soured, and oniony flavors; Lawrence et al (2003) indicated that $CaCl_2$ increase bitter, metallic, sour, soapy, and astringent flavors (0.1, 0.2 and 0.3 M $CaCl_2$ solution - 11% of the sub primal weight). In contrast with the present study, Milligan et al. (1997), Kerth et al. (1995), Lansdell et al. (1995), and Carr et al. (2004), using 0.2 mM $CaCl_2$ solution -

5% of the sub primal weight, found no effects of $CaCl_2$ on off-flavor perception.

In conclusion, ZH supplementation increased WBSF of inside round steaks, while aging time and $CaCl_2$ decreased WBSF. $CaCl_2$ improvements on tenderness sensory scores were detected just at 14 d, but not at 21 d postmortem by the trained panelists, in the other hand HZ did not affect sensory traits. Inside round from either control or ZH-fed steaks could get lower WBSF values at 7, 14 and 21 when injected with $CaCl_2$, in consequence, $CaCl_2$ could overcome the toughness induced by zilpaterol hydrochloride supplementation.

5.4. Literature Cited

AMSA. 1995. Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness of Fresh Meat. Natl. Live Stock and Meat Board, Chicago, IL.

Avendano-Reyes, L., V. Torres-Rodriguez, F. J. Meraz-Murillo, C. Perez-Linares, F. Figueroa-Saavedra, and P. H. Robinson. 2006. Effects of two beta-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 84: 3259-3265.

Beckett, J. L., R. J. Delmore, G. C. Duff, D. A. Yates, D. M. Allen, T. E. Lawrence, and N. Elam. 2009. Effects of zilpaterol hydrochloride on growth rates, feed conversion, and carcass traits in calf-fed Holstein steers. J. Anim. Sci. 87: 4092-4100.

Beermann, D. H. 1985. Mechanisms responsible for repartitioning tissue growth in meat animals. Proc. Recip. Meat Conf. 38:105.

Berge, P., J. Culioli, and A. Ouali. 1993. Performance, muscle composition and meat texture in veal calves administered a b-agonist (Clenbuterol). Meat Sci. 33:191.

Boleman, S. J., S. L. Boleman, T. D. Bidner, K. W. McMillin, and C. J. Monlezun. 1995. Effects of Postmortem Time of Calcium Chloride Injection on Beef Tenderness and Drip, Cooking, and Total Loss. Meat Sci. 39:35-41.

Boucqué, C. V., L. O. Fiems, R. J. Moermans, B. G. Cottyn, and M. Sommer. 1994. Effect of cimaterol on growth, carcass characteristics and meat quality in double-muscled Belgian White-blue bulls. Can. J. Animal Sci. 74: 707-709.

Brooks, J. C., H. C. Claus, M. E. Dikeman, J. Shook, G. G. Hilton, T. E. Lawrence, J. M. Mehaffey, B. J. Johnson, D. M. Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, D. A. Yates, and M. F. Miller. 2009. Effects of zilpaterol hydrochloride feeding duration and postmortem aging on Warner-Bratzler shear force of three muscles from beef steers and heifers. J. Anim. Sci. 87:3764–3769.

Brooks, J. C., J. M. Mehaffey, J. A. Collins, H. R. Rogers, J. Legako, B. J. Johnson, T. Lawrence, D. M. Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, D. A. Yates, and M. F. Miller. 2010. Moisture Enhancement and Blade Tenderization Effects on the Shear Force and Palatability of Strip Loin Steaks from Beef Cattle fed Zilpaterol Hydrochloride. J. Anim. Sci. 88:1809-1816.

Buys, E. M., and P. E. Strydom. 2000. The effect of Zilpaterol on growth performance, meat tenderness and meat colour of beef. Meat Industry Centre-ARC-Animal Nutrition and Animal Products Institute, Irene, South Africa Zilpaterol Report.

Carr, M. A., K. L. Crockett, C. B. Ramsey, and M. F. Miller. 2004. Consumer acceptance of calcium chloride-marinated top loin steaks. J. Anim. Sci. 82:1471-1474.

Casey, N. H., T. H. Montgomery, and M. L. Scheltons. 1997. The effect of zilpaterol on feedlot performance, carcass quality, USDA carcass grades and meat quality. Pages 262-263 in Proc. 43rd Int. Congr. Meat Sci. Technol., Auckland, New Zealand.

Cottin, P., J. P. Poussard, D. Desmazes, H. Georgescauld, and A. Ducastaing. 1991. Free calcium and calpain I activity. Biochimica et Biophysica Acta. 1079:139-140.

Cross, H. R., R. L. West, and T. R. Dutson. 1981. Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. Meat Sci. 5:261-266.

Cross, H. R., R. Moen, and M. S. Stanfield. 1978. Training and testing of judges for sensory analysis of meat quality. Food Technol. 32:48-54.

Culler, R. D., F. C. Parrish, Jr., G. C. Smith, and H. R. Cross. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177–1180.

Dikeman, M. E., M. C. Hunt, P. B. Addis, H. J. Schoenbeck, M. Pullen, E. Katsanidis, and E. J Yancey. 2003. Effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on quality and sensory traits of steaks and ground beef. J. Anim. Sci. 81:156–166.

Diles, J. J. B., M. F. Miller, and B. L. Owen. 1994. Calcium chloride concentration, injection time, and aging period effect on tenderness, sensory, and retail color attributes of loin steaks from mature cows. J. Anim. Sci. 72:2017–2021.

Elam, N. A., J. T. Vasconcelos, G. Hilton, D. L. VanOverbeke, T. E. Lawrence, T. H. Montgomery, W. T. Nichols, M. N. Streeter, J. P. Hutcheson, D. A. Yates, and M. L. Galyean. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87: 2133-2141.

FDA. 2006. Freedom of Information Summary. Original New Animal Drug Application NADA 141–258. ZILMAX (Zilpaterol Hydrochloride). Type A Medicated Article for Cattle Fed in Confinement for Slaughter. http://www.fda.gov/cvm/FOI/141-258o08102006.pdf Accessed Aug. 14, 2008.

Fiems, L. O., B. Buts, and B. G. Cottyn. 1990. Effect of a b-agonist on meat quality and myofibrillar protein fragmentation in bulls. Meat Sci. 27:29-39.

Garmyn, A. J., J. N. Shook, D. L. VanOverbeke, J. L. Beckett, R. J. Delmore, D. A. Yates, D. M. Allen, and G. G. Hilton. 2010. The effects of zilpaterol hydrochloride on carcass cutability and tenderness of calf-fed Holstein steers. J. Anim. Sci. 88:2476-2485.

Geesink, G. H., F. J. M. Smulders, H. L. J. M. Van Laack, J. H. Van der Kolk, Th. Wensing, and H. J. Breukink. 1993. Effects on meat quality of the use of clenbuterol in veal calves. J. Anim. Sc. 71:1161–1170.

Gonzalez, C. B., V. A. Salitto, F. J. Carduza, A. A. Pazos, and J. A. Lasta. 2001. Effect of calcium chloride marination on bovine Cutaneus trunci muscle. Meat Sci. 57:251-256.

Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. J. Biological Chem. 177:751–766.

Hamm, R. 1986. In Muscle as Food, ed. P. J. Bechtel. Academic Press, Orlando, p. 135.

Hilton, G. G., J. L. Montgomery, C. R. Krehbiel, J. Cranston, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. Streeter, J. R. Blanton Jr., and M. F. Miller. 2009. Dietary zilpaterol hydrochloride. IV. Carcass cutability and meat palatability of beef cattle with and without monensin and tylosin. J. Anim. Sci. 87:1394–1406.

Hope-Jones, M., P. E. Strydom, L. Frylinck, and E. C. Webb. 2010. The efficiency of electrical stimulation to counteract the negative effects of beta-agonists on meat tenderness of feedlot cattle. Meat Sci. 86: 699-705.

Jaturasitha, S., P. Thirawong, V. Leangwunta, and M. Kreuze. 2004. Reducing toughness of beef from Bos indicus draught steers by injection of calcium chloride: Effect of concentration and time postmortem. Meat Sci. 68: 61-69.

Kellermeier, J. D., A. W. Tittor, J. C. Brooks, M. L. Galyean, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, B. J. Johnson, and M. F. Miller. 2009. Effects of zilpaterol hydrochloride with or without an estrogen-trenbolone acetate terminal implant on carcass traits, retail cutout, tenderness, and muscle fiber diameter in finishing steers. J. Anim. Sci. 87:3702–3711.

Kerth, C. R., M. F. Miller, and C. B. Ramsey. 1995. Improvement of beef tenderness and quality traits with calcium chloride injection in beef loins 48 hours postmortem. J. Anim. Sci. 73:750–756.

Kong, B., X. Diao, and Y. L. Xiong. 2006. Postmortem calcium chloride injection alters ultrastructure and improves tenderness of mature chinese yellow cattle longissimus muscle. J. Food Sci. 71:124-129.

Koohmaraie, M., A. S. Babiker, A. L. Schroeder, R. A. Merkel, and T. R. Dutson. 1988. Acceleration of postmortem tenderization in ovine carcasses through activation of Ca2+-dependent proteases. J. Food Sci. 53:1638-1641.

Koohmaraie, M., and S. D. Shackelford. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a B-adrenergic agonist. J. Anim. Sci. 69:2463-2471.

Koohmaraie, M., G. Whipple, and J. D. Crouse. 1990. Acceleration of postmortem tenderization in lamb and Brahman-cross beef carcasses through infusion of calcium chloride. J. Anim. Sci. 68:1278-1283.

Koohmaraie, M., J. D. Crouse, and H. J. Mersmann. 1989. Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and ionic strength. J. Anim. Sci. 67:934-942.

Koohmaraie, M., S. D. Shackelford, N. E. Muggli-Cockett, and R. T. Stone. 1991. Effect of the b-adrenergic agonist L-644,969 on muscle growth, endogenous proteinase activities, and postmortem proteolysis in wether lambs. J. Anim. Sci. 69:4823-4835.

Kretchmar, D. H., M. R. Hathaway, R. J. Epley, and W. R. Dayton. 1990. Alterations in postmortem degradation of myofibrillar proteins in muscle of lambs fed a P-adrenergic agonist. J. Anim. Sci. 68:1760-1772.

Lansdell, J. L., M. F. Miller, T. L. Wheeler, M. Koohmaraie, and C. B. Ramsey. 1995. Postmortem injection of calcium chloride effects on beef quality traits. J. Anim. Sci. 73:1735–1740.

Lawrence, T. E., M. E. Dikeman, M. C. Hunt, C. L. Kastner, and D. E. Johnson. 2003. Staged injection marination with calcium lactate, phosphate and salt may improve beef water-binding ability and palatability traits. Meat Sci. 65:967-972.

Lee, Y. B., and Y. S. Kim. 1994. Muscle characteristics and meat tenderness of cimaterol-fed lambs. J. Food Sci. 59:33-37.

Leheska, J. M., J. L. Montgomery, C. R. Krehbiel, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. Streeter, J. R. Blanton Jr., and M. F. Miller. 2009. Dietary zilpaterol hydrochloride. II. Carcass composition and meat palatability of beef cattle. J. Anim. Sci. 87:1384–1393.

Miller, M. F., D. K. Garcia, M. E. Coleman, P. A. Ekeren, D. K. Lunt, K. A. Wagner, M. Procknor, T. H. Welsh Jr., and S. B. Smith. 1988. Adipose tissue, longissimus muscle and anterior pituitary growth and function in clenbuterol-fed heifers. J. Anim. Sci. 66:12–20.

Milligan, S. D., M. F. Miller, C. N. Oats, and C. B. Ramsey. 1997. Calcium chloride injection and degree of doneness effects on the sensory characteristics of beef inside round roasts. J. Anim. Sci. 75:668–672.

Montgomery, J. L., C. R. Krehbiel, J. J. Cranston, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, D. T. Bechtol, E. Johnson, T. TerHune, and T. H. Montgomery. 2009. Dietary zilpaterol hydrochloride. I. Feedlot performance and carcass traits of steers and heifers. J. Anim. Sci. 87: 1374-1383.

Morgan, J. B., R. K. Miller, F. M. Mendez, D. S. Hale, and J. W. Savell. 1991. Using calcium chloride injection to improve tenderness of beef from mature cows. J. Anim. Sci. 69:4469-4476.

Morris, C. A., R. L. Theis, R. K. Miller, G. R. Acuff, and J. W. Savell. 1997. Improving the flavor of calcium chloride and lactic acid injected mature beef top round steaks. Meat Sci. 45:531–537.

Olson, D. G., J. R. Parrish, and M. H. Stromer. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. J. Food Sc. 41:1036–1041.

Parrish Jr., F. C., R. E. Rust, G. R. Popenhagen, and B. E. Miner. 1969. Effect of postmortem aging time and temperature on beef muscle attributes. J. Anim. Sci. 29:398-403.

Pérez, M. L., H. Escalona, and I. Guerrero. 1998. Effect of calcium chloride marination on calpain and quality characteristics of meat from chicken, horse, cattle and rabbit. Meat Sci. 48:125-134.

Plascencia, A., N. Torrentera, and R. A. Zinn. 1999. Influence of the β-agonist, zilpaterol, on growth performance and carcass characteristics of feedlot steers. Proc. Western Sect. Am. Soc. Anim. Sci. 50: 331-334.

Pringle, T. D., J. M. Harrelson, R. L. West, S. E. Williams, and D. D. Johnson. 1999. Calcium-activated tenderization of strip loin, top sirloin, and top round steaks in diverse genotypes of cattle. J. Anim. Sci. 77:3230–3237.

Rathmann, R. J., J. M. Mehaffey, T. J. Baxa, W. T. Nichols, D. A. Yates, J. P. Hutcheson, J. C. Brooks, B. J. Johnson, and M. F. Miller. 2009. Effects of duration of zilpaterol hydrochloride and days on the finishing diet on carcass cutability, composition, tenderness, and skeletal muscle gene expression in feedlot steers. J. Anim Sci. 87:3686-3701.

SAS Institute. 2003. SAS proprietary software release 9.1. SAS Institute Inc., Cary, NC.

Savell, J. W., G. C. Smith, and Z. L. Carpenter. 1978. Beef quality and palatability as affected by electrical stimulation and cooler aging. J. Food Sci. 43:1666-1668.

Schiavetta, A. M., M. F. Miller, D. K. Lunt, S. K. Davis, and S. B. Smith. 1990. Adipose tissue cellularity and muscle growth in young steers fed the β-adrenergic agonist clenbuterol for 50 days and after 78 days of withdrawal. J. Anim. Sci. 68:3614–3623.

Shackelford, S. D., T. L.Wheeler, and M. Koohmaraie. 1999. Tenderness classification of beef. II: Design and analysis of a system to measure beef longissimus shear force under commercial processing conditions. J. Anim. Sci. 77:1474–1481.

Strydom, P. E., L. Frylinck, J. L. Montgomery, and M. F. Smith. 2009. The comparison of three [beta]-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci. 81:557-564.

Strydom, P. E., L. Loots, K. J. Leeuw, K. van Rooyen, J. Anderson, L. Frylinck, J. D. Snyman, J. Kruger, R. Greebe, S. M. van Heerden, E. Visser, and J. van Niekerk. 2002. The impact of three beta agonists on growth

performance, carcass characteristics and meat quality of feedlot cattle. Animal Nutrition and Animal Products Institute, Irene, the Republic of South Africa. Report # Intervet SA 2001/3.

USDA. 2010. Institutional Meat Purchase Specifications. 100 - Fresh Beef. Agric. Marketing Serv., USDA, Washington, DC.

Vasconcelos, J. T., R. J. Rathmann, R. R. Reuter, J. Leibovich, J. P. McMeniman, K. E. Hales, T. L. Covey, M. F. Miller, W. T. Nichols, and M. L. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J. Anim. Sci. 86: 2005-2015.

Vestergaard, M., K. Sejrsen, and S. Klastrup. 1994. Growth, composition and eating quality of Longissimus dorsi from young bulls fed the b-agonist cimaterol at consecutive developmental stages. Meat Sci. 38:55-66.

Wheeler, T. L., and M. Koohmaraie. 1992. Effects of the b-adrenergic agonist L-644,969 on muscle protein turnover, endogenous proteinase activities, and meat tenderness in steers. J. Anim. Sci. 70:3035-3043.

Wheeler, T. L., J. D. Crouse, and M. Koohmaraie. 1992. The effect of postmortem time of injection and freezing on the effectiveness of calcium chloride for improving beef tenderness. J. Anim. Sci. 70:3451-3457.

Wheeler, T. L., M. Koohmaraie, and J. D. Crouse. 1991. Effects of calcium chloride injection and hot boning on the tenderness of round muscles. J. Anim. Sci. 69:4871-4875.

Wheeler, T. L., M. Koohmaraie, and S. D. Shackelford. 1997. Effect of postmortem injection time and postinjection aging time on the calcium-activated tenderization process in beef. J. Anim. Sci. 75:2652–2660.

Wheeler, T. L., M. Koohmaraie, J. L. Lansdell, G. R. Siragusa, and M. F. Miller. 1993. Effect of postmortem injection time, injection level, and concentration of calcium chloride on beef quality traits. J. Anim. Sci. 71:2965–2974.

Williams, P. E. V., L. Pagliani, G. M. Innes, K. Pennie, C. I. Harris, and P. Garthwaite. 1987. Effects of a b-agonist (clenbuterol) on growth, carcass composition, protein and energy metabolism of veal calves. Br. J. Nutr. 57:417–428.

5.5. Figure and tables

Table 1. Effect of feeding zilpaterol hydrochloride[1] (ZH) on USDA Select carcass characteristics and sarcomere length (n = 39[a]).

Item	Feeding group		*P*-value	Pooled SEM[2]
	Non-ZH-fed (n = 19)	ZH-fed (n = 20)		
Hot carcass weight, kg	375.77	402.72	< 0.01	4.38
Marbling score[3]	356.32	361.50	0.58	4.64
Fat thickness, cm	1.20	1.21	0.93	0.06
Longissimus area, cm^2	88.97	101.05	< 0.01	1.59
KPH[5] fat, %	3.31	3.40	0.71	0.11
USDA yield grade[4]	2.36	1.80	0.02	0.12
Sarcomere length, µm	1.74	1.80	0.50	0.03

[a] Data were calculated after the selection of carcasses.
[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] As determined by Texas Tech personnel: 300 = Slight[00]; 400 = Small[00]; 500 = Modest[00].
[4] Yield grade as calculated by the regression equation (USDA, 1997).
[5] Kidney, pelvic and heart fat percentage.

Table 2. Effect of $CaCl_2$ injection on weight measures of inside round portions from steers fed or not zilpaterol hydrochloride[1] (n = 78).

	Feeding group (FG)		Injection treatment (IT)		Pooled SEM[2]	*P*-value		
	Non-ZH-fed	ZH-fed	Non-injected	Injected[3]		FG	IT	Interaction
Pre-injection weight, kg	6.14	7.22	6.74	6.66	0.11	< 0.01	0.65	0.46
Weight added by injection[a], %	5.58	6.70	-	6.15	0.45	0.27	-	-
Purge loss on d 7[b], %	4.30	4.95	2.02	5.97	0.34	0.46	< 0.01	0.16

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[a] Calculated by [(pre-injection weight - weight after injection) / pre-injection weight] x 100.
[b]Calculated by [(pre-injection weight - weight on d 7) / pre-injection weight] x 100.

Table 3. Effect of zilpaterol hydrochloride[1] (ZH) and $CaCl_2$ injection on myofibrillar fragmentation index (MFI) on inside round aged 7, 14, 21 and 28 d (n = 312).

	MFI
Feeding group	
Non-ZH-fed	77.78
ZH-fed[1]	59.50
SEM[2]	5.49
P-value	< 0.01
Injection treatment	
Non-injected	63.43
$CaCl_2$-injected[3]	73.84
SEM	5.48
P-value	0.02
Postmortem age, d	
7	48.29[a]
14	57.75[ab]
21	63.36[b]
28	99.14[c]
SEM	6.42
P-value	< 0.01

[a,b,c] Least squares means within a column lacking a common superscript letter differ ($P < 0.05$).
[1]Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.

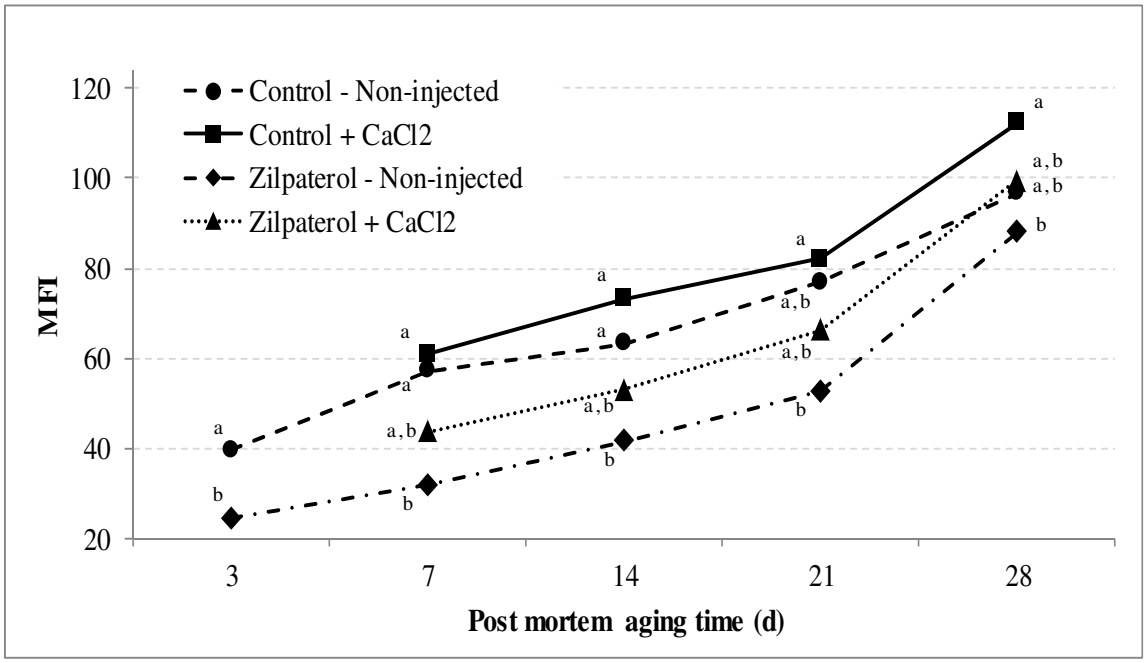

Figure 1. Effect of zilpaterol hydrochloride and $CaCl_2$ injection on myofibrillar fragmentation index (MFI) of inside round steaks aged 3, 7, 14, 21 and 28 days postmortem (n = 351; P = 0.93; SEM = 6.79). ^{a,b} Means in the same aging time with unlike superscripts are different ($P < 0.05$).

Table 4. Effect of feeding group (FG) and injection treatments (IT) on drip and cooking losses of inside round steaks with pooled aging period[c] (n = 312).

%	Non-ZH-fed		ZH-fed[1]		Pooled	P-value[2]		
	Non-injected	Injected[3]	Non-injected	Injected[3]	SEM[2]	FG	IT	Interaction
Drip[a]	8.20	9.92	8.57	9.53	0.11	0.95	< 0.01	0.09
Cooking[b]	24.85	26.24	25.35	26.33	0.16	0.37	< 0.01	0.54

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Observed significance levels for the main effects of FG, IT and FG x IT interaction.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[a] Calculated by [(weight before ageing - weight after ageing and thaw) / weight before ageing] x 100.
[b] Calculated by [(weight before cooking - weight after cooking) / weight before cooking] x 100.
[c] Pooled aging period (7, 14, 21 and 28 days).

Table 5. Effect of feeding group (FG), injection treatment (IT) and x ageing time (AT) on inside round WBSF[4], kg (n = 312).

	WBSF
Feeding group	
Non-ZH-fed	4.52
ZH-fed[1]	4.72
SEM[2]	0.09
P-value	0.04
Injection treatment	
Non-injected	4.79
$CaCl_2$-injected[3]	4.37
SEM	0.08
P-value	< 0.01
Postmortem age, d	
7	5.04[a]
14	4.60[b]
21	4.53[b,c]
28	4.32[c]
SEM	0.10
P-value	< 0.01

[a,b,c] Least squares means within a column lacking a common superscript letter differ ($P < 0.05$).
[1]Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[4] Warner Bratzler Shear Force

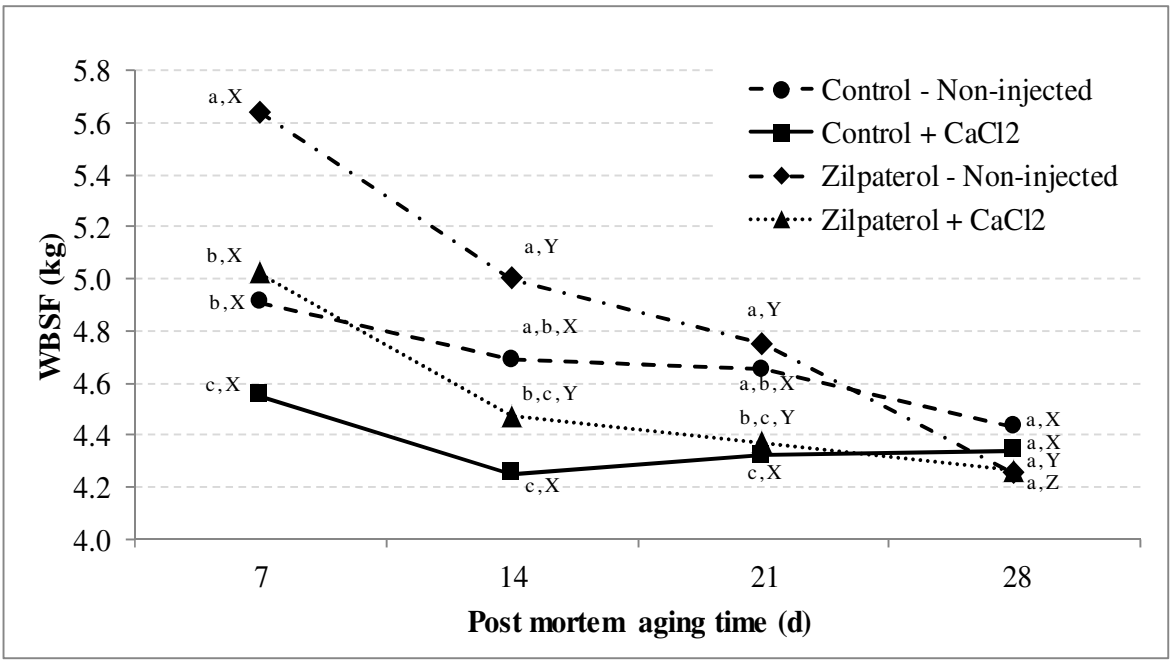

Figure 2. Effect of zilpaterol hydrochloride and $CaCl_2$ injection on WBSF (kg) of inside round steaks aged 7, 14, 21 and 28 days postmortem (n = 312; P = 0.72; SEM = 0.18). ^{a,b,c,d} Means in the same aging time with unlike superscripts are different ($P < 0.05$). ^{X,Y,Z} Means in the same treatment with unlike superscripts are different ($P < 0.05$).

Table 6. Effect of feeding group (FG) by injection treatments (IT) on sensory attributes[2] of inside round at 14 d postmortem (n = 78).

FG	Non-ZH-fed		ZH-fed[1]		Pooled	*P*-value[4]		
IT	Non-injected	Injected[3]	Non-injected	Injected[3]	SEM[5]	FG	IT	Interaction
Initial juiciness	5.51	5.57	5.78	5.67	0.07	0.22	0.87	0.55
Sustained juiciness	5.59	5.65	5.85	5.67	0.07	0.33	0.66	0.41
Initial tenderness	5.24	5.66	5.25	5.58	0.07	0.83	0.06	0.75
Sustained tenderness	5.15	5.62	5.17	5.57	0.07	0.90	0.01	0.79
Flavor intensity	6.12	6.31	6.22	6.25	0.05	0.86	0.34	0.50
Beef flavor	6.22	6.37	6.31	6.26	0.06	0.95	0.69	0.41
Overall beef mouth feel	4.66	4.92	4.81	4.90	0.07	0.66	0.26	0.57
Off flavor	1.00	1.07	1.04	1.10	0.01	0.17	0.50	0.93

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] 1 = extremely dry, extremely tough, bland beef flavor, and uncharacteristic beef mouth feel; 8 = extremely juicy, extremely tender, intense beef flavor, and characteristic beef mouth feel.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[4] Observed significance levels for the main effects of FG, IT and FG x IT interaction.
[5] Pooled (largest) SE of LS means.

Table 7. Effect of feeding group (FG) by injection treatments (IT) on sensory attributes[2] of inside round at 21 d postmortem (n = 78).

FG	Non-ZH-fed		ZH-fed[1]		Pooled	*P*-value[4]		
IT	Non-injected	Injected[3]	Non-injected	Injected[3]	SEM[5]	FG	IT	Interaction
Initial juiciness	5.32	5.22	5.47	5.38	0.05	0.19	0.42	0.96
Sustained juiciness	5.27	5.19	5.45	5.36	0.05	0.12	0.46	0.97
Initial tenderness	5.43	5.32	5.21	5.40	0.07	0.67	0.79	0.33
Sustained tenderness	5.39	5.28	5.11	5.34	0.08	0.51	0.70	0.32
Flavor intensity	6.07	6.14	6.16	6.16	0.05	0.58	0.76	0.75
Beef flavor	6.09	6.11	6.23	6.03	0.05	0.79	0.41	0.29
Overall beef mouth feel	4.79	4.78	4.82	4.52	0.06	0.41	0.27	0.30
Off flavor	1.09[a]	1.11[a]	1.01[b]	1.13[a]	0.01	0.26	< 0.01	0.06

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS
[2] 1 = extremely dry, extremely tough, bland beef flavor, and uncharacteristic beef mouth feel; 8 = extremely juicy, extremely tender, intense beef flavor, and characteristic beef mouth feel.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[4] Observed significance levels for the main effects of FG, IT and FG x IT interaction.
[5] Pooled (largest) SE of LS means.

6. ARTIGO 2 PARA PUBLICAÇÃO

Artigo formatado para publicação no *Journal of Animal Science*

Print ISSN: 0021-8812; Online ISSN: 1525-3163

Post rigor calcium chloride injection effects on the color stability, proximate analysis and lipid oxidation of inside round steaks from beef cattle fed zilpaterol hydrochloride

S.B. Pflanzer[§], A. Rodas-González*, J.N. Martin*, J.C. Brooks*, B.J. Johnson*, J.D. Starkey*, P.E. de Felicio[§], M.N. Streeter[†], J.P. Hutcheson[†], D.A. Yates[†], R.J. Rathmann*, M.F. Miller*[1].

[§]State University of Campinas, Campinas, Brazil. *Department of Animal and Food Science, Texas Tech University, Lubbock, TX 79409, USA.

[†]Intervet/Schering-Plough Animal Health, DeSoto, KS 66018, USA

Corresponding author: mfmrraider@aol.com

ABSTRACT

To determine the effects of calcium chloride injection on color stability of cattle fed zilpaterol hydrochloride (**ZH**), carcasses (n = 20) were selected from a pool of 232 steers that were ZH-fed 0 or 8.3 mg/kg (DM basis) for the last 20 d of the finishing period. Inside rounds from the alternating sides were injected (5 %) with a calcium chloride solution (200 mM). Before injection, one steak was cut for proximate analysis. At 7 d, two steaks, one for proximate analysis and other for color evaluation were taken, and the remainder portion was vacuum packed. Treatments were evaluated at 7, 14, and 21 d postmortem using color analysis, pH, purge loss during display, metmyoglobin reducing activity (**MRA**) and lipid oxidation (**TBARS**) on steaks which were PVC-overwrapped and placed in retail cabinets for three days at 3 °C. The deep (**DSM**) and superficial (**SSM**) portions of steaks were evaluated for initial color, display color, discoloration, and L*, a* and b* values. MRA was not affected by feeding group (**FG**) or injection treatment (**IT**) ($P > 0.05$). ZH steaks were brighter red, had lower discoloration scores, and had higher L* values than Non-ZH steaks, even aged by 14 or 21 d ($P < 0.05$). On d 3 of simulated display, ZH steaks had greater a* and b* values than Non-ZH steaks ($P < 0.05$), without difference on d 0 of display ($P > 0.05$). $CaCl_2$ injection produced darker steaks and increased discoloration than non-injected steaks ($P < 0.05$). However, on d 7 postmortem no differences in sensory color were detected ($P > 0.05$). L* values were not affected by IT ($P > 0.05$), however on d 3 of display, injected samples had lower a* and b*

values than non-injected samples ($P < 0.05$), been less stable throughout display. SSM area had higher color scores (darker), was more stable to discoloration, and had lower L* value when compared with DSM area ($P < 0.05$), however SSM had higher a* values in steaks aged for 7 and 14 d, than DSM aged for the same time. The increasing of display time decreased both a* and b* values from both FG and both IT ($P < 0.05$), additionally the increasing of aging decreased a* and b* values ($P < 0.05$), without any effect on L* values ($P > 0.05$). TBARS was not affected by FG ($P > 0.05$), however it was increased by $CaCl_2$ injection ($P < 0.05$). In conclusion, inside round from ZH-fed steers produced great color attributes, however $CaCl_2$ injection cause detrimental effects on color stability if samples were aged by 14 d or more.

Key words: beef color, calcium chloride, inside round, display stability, zilpaterol hydrochloride.

6.1. Introduction

Meat purchasing decisions are influenced by color more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness, and as well-known beef color can be affected by several pre and post-harvest factors (Mancini and Hunt, 2005).

Recognized experimental technique used to improve beef tenderness is the calcium chloride injection, however there are some studies that indicated $CaCl_2$ can affect retail color display depending on $CaCl_2$ concentration, injection amount, time of injection, and time post-injection (Wheeler et al., 1993; Diles et al., 1994; Lansdell et al., 1995; Kerth et al., 1995; Wheeler et al., 1997; Bekhit et al., 2005).

The effects of different β-adrenergic receptor agonists (**βAA**) on beef color traits and stability have been documented by several researchers, however different conclusion have been described (Fiems et al., 1990; Geesink et al., 1993; Boucqué et al., 1994; Vestergaard et al., 1994). The latter βAA approved for use in the United States (FDA, 2006), zilpaterol hydrochloride (**ZH**), commercially available as Zilmax (Intervet Schering Plough, DeSoto, KS), has been reported to affect meat color and shelf life, but these research are usually conduced on strip loin steaks (Strydom et al., 2000; VanOverbeke et al., 2009; Hilton et al., 2009; Rogers et al., 2010). Few studies have analyzed the effects of feeding ZH on color development and stability of semimembranosus muscle (**SM**) (Gunderson et al., 2009a,b), and

no research has evaluated the combined effects of $CaCl_2$ injection and ZH on color stability of beef semimembranosus steaks.

Therefore, this study was conducted to determine the effect of $CaCl_2$ injection on the simulated retail display color stability, proximate analysis and lipid oxidation of beef semimembranosus muscle from cattle feed or not zilpaterol hydrochloride.

6.2. Materials and methods

Animal Care and Use Committee approval was not obtained for this study because samples were taken from federally inspected slaughter facilities.

Cattle Source and Subprimal Selection

A feedlot experiment was conducted using 232 black English-type steers that were grain fed for 163 d. Steers were implanted on arrival at the feedlot (d 0) and again on d 80 with a Revalor-XS (80 mg of trenbolone acetate and 16 mg of estradiol, Intervet/ Schering-Plough Animal Health). Diet containing ZH (ZH-fed**,** 8.3 mg/kg - 90% dry matter basis, Intervet/Schering-Plough Animal Health, DeSoto, KS) was fed for the last 20 d (control diet designated as 0 d - control-fed) of the feeding period, and animals were slaughtered after a mandatory 3 d withdrawal of the supplement.

Animal were slaughtered at a commercial processing facility at two different dates (July and August). All carcasses were subject to electrical stimulation on the blood pit and hide pull (70 volts at 0.05 amps for 10 sec).

A subset of carcasses representing USDA maturity score A, and Select quality grade were selected randomly at 36 h postmortem, and at 48 h postmortem the boneless inside round (IMPS # 168; USDA, NAMP, 2010) were obtained from both side of each carcass, identified, boxed, and shipped refrigerated (1 to 3 °C) to Texas Tech University Meat Laboratory. Carcass

data for each treatment were calculated after the selection of carcasses (Table 1).

Hot carcass weight (HCW), KPH (kidney, pelvic and heart fat percentage), fat thickness, longissimus muscle area, marbling and USDA yield grade were evaluated in the carcasses selected for the experiment.

Injection and Steaks Fabrication

At the meat lab, on d 3 postmortem, the subprimals were removed from their boxes and trimmed of fat and connective tissue. The gracillis muscle was removed and the semimembranosus and adductor muscles were used for the study (IMPS # 169A; USDA, NAMP, 2010).

Cuts from the alternating sides were randomly assigned to either a control (non-injected) or injected with calcium chloride (injected). Before injection, one steak from each cut was assigned to proximate analysis, and stored at -20 °C in Whirl-pak bags (Nasco, Fort Atkinson, WI).

The inside rounds were injected at 5% (wt/wt) of 200 mM food grade $CaCl_2$ (Tetra Technologies, The Woodlands, TX) in 5°C distilled and de-ionized water. The injection was carried out once through a multiple-needle pickle injector (Wolf-Tec, Inc., Model Schroder//Imax 350, Kingston, NY, USA). After injection cuts were allowed to equilibrate for 5 min post-pump drain period, each cut was reweighed to determine the actual percentage pump calculated as [(pumped and drained cut wt. – unpumped cut wt.) ÷ unpumped

cut wt.] × 100 (Lansdell et al., 1995). Cuts were weighed, vacuum-packaged, and stored at 2 °C until 7 d postmortem (4 d after injection).

On d 7 postmortem, two steaks (1.5 cm-thick) from each inside round were taken, one for proximate analysis that was stored at -20 °C and the other for color evaluation and metmyoglobin reducing activity (**MRA**) that was used immediately after faced. Then a portion (5 cm-thick) for color evaluation and MRA at 14 and 21 d postmortem was obtained and immediately vacuum-packaged in a multilaminar, thermo-shrinkable, B620 CryO-Vac® bag by use of a Koch Ultravac® vacuum packaging machine, identified, and stored at 2 °C until next sampling. For MRA, a 3 cm x 3 cm square sample was cutting from steaks previously assigned to color evaluation.

In each other sampling occasion (14 and 21 d postmortem), the primal portion was not exposed in its totality, rather only the packaging was peeled off, 1.5 cm-thick, in order to not contaminate the rest of the primal surface. Once samples were taken (sliced), the remnant portion of the sub-primal was immediately repackaged (Barrier bag BH620T, Sealed Air Inc. – Cryovac division, Duncan, SC) under vacuum condition for storage until next evaluation, avoiding oxygenating the surface. To obtain a fresh cut surface for color evaluation, it was established a repeated exposure and repackaging of the remnant portion, trying to maintain in the package a very low oxygen tension.

Retail display

Steaks assigned to color evaluation were overwrapped with polyvinyl chloride film (MAPAC L, oxygen transmission rate [OTR] = 21,700 cc of oxygen per m^2 per 24 h; Borden Packaging and Industrial Products, North Andover, MA.) in black foam trays (expanded polystyrene), taking care that the fresh cut surface (face up on the tray) was not touched by hands. The trays were placed in coffin-style (Model M1, Hussmann) retail cabinets at 3 °C filled with one layer, under fluorescent light (2140 lux) for three days using high-output bulbs with a color temperature rating of 3500 Kelvin and a color rendering index of 70.

Once the trays were prepared for retail display, and the steaks bloomed (1 hour after faced), color evaluation was carried out by trained panelist and Hunter colorimeter. The color evaluation was repeated every 24 hours for 3 days. Display cases were rotated daily to minimize effects of package location in the case. At the end of the display period, the steaks were removed of retail cabinets, weighed, measured the pH, flushed with nitrogen, packaged and frozen at -20 °C for subsequent lipid oxidation analysis.

Proximate analysis

Steaks were thawed, trimmed of external fat, and homogenized in a food processor (Kitchen Aid with grinder adapter, Model KP26M1XER Professional 600, USA). Compositional analysis was conducted using AOAC-

approved (Official Method 2007.04) near-infrared spectrophotometer (FOSS Food ScanTM 78800; Dedicated Analytical Solutions, DK-3400 Hilleroed, Denmark). Moisture, fat, protein and collagen percentages were determined for each sample at 72 h (prior to inject) and 7 d postmortem (after injection) to evaluate changes in the composition by injection effects.

pH Analysis

Muscle pH was measured on d 0 and 3 of display, by inserting the tip of a pH meter probe previously calibrated (IQ Scientific Instruments, Inc, Model IQ 150, Carlsbad, CA.) twice into the steak.

Display purge loss

Steaks for color evaluation were weighed prior being overwrapped at the end of display period, and the purge loss was calculated ([initial weight – weight after retail display/ initial weight] x 100).

Metmyoglobin reducing activity

Metmyoglobin-reducing activity (MRA) was determined at 7, 14 and 21 d of aging according to a procedure described by Sammel (2002). Samples were submerged for 20 min in a 0.3 % solution of sodium nitrite to facilitate nitric oxide metmyoglobin (**MMb**) formation, then removed, blotted dry, and

vacuum packaged (Barrier bag BH620T, Sealed Air Inc. – Cryovac division, Duncan, SC). On the light-exposed display surface, samples were scanned twice with a Hunter Lab Miniscan XE Plus (Hunter laboratories Model MSXP'4500C, Reston, VA) (D/8-S, 2.54-cm diameter aperture) to obtain 400-700 nm reflectance data.

Samples were incubated at 30 °C for 2 h to induce nitric oxide MMb reduction to deoxymyoglobin (**DMb**). Upon removal from the incubator, samples were rescanned twice immediately to determine the percentage of remaining surface MMb, using K/S ratios and equations from AMSA (1991). The following equation was used to calculate MRA: (Chg % surface MMb/preincubation % surface MMb) X 100.

Sensory and instrumental color

Visual and instrumental color evaluations were carried out on the superficial SM (**SSM** - the outer 1/3 of the SM, which typically chills faster and is darker) and the deep SM (**DSM** - the inner 1/3 of the SM, which chills slower and is often more pale).

The sensory panel was composed of 6 to 8 trained panelists from Animal and Food Science department at Texas Tech University who passed the Farnsworth-Munsell 100-hue test (Macbeth, Newsburgh, NY). On d 0 of display, initial color evaluations were made, whereas display color and

discoloration scores were recorded daily on 1 through 3 d of simulated display.

The initial color scale used was 1 = purplish pink or red or reddish tan of vacuum packages; 2 = bleached, pale red; 3 = slightly cherry red; 4 = moderately light cherry red; 5 = cherry red; 6 = slightly dark red; 7 = moderately dark red; 8 = dark red; and 9 = very dark red. Panelists scored to half-point increments.

The display color scale, for evaluating color stability, also rated to the nearest half-point, was 1 = very bright red; 2 = bright red; 3 = dull red; 4= slightly dark red; 5 = moderately dark red; 6 = dark red; 7 = tannish red; 8 =tan to brown.

For surface discoloration, a scale indicating the percentage of surface discoloration due to metmyoglobin formation, to the nearest whole point, was used (1 = no discoloration, 0%; 2 = slight discoloration, 1-19%; 3 = small discoloration, 20-39%; 4 = modest discoloration, 40-59%; 5 = moderate discoloration, 60-79%; 6 = extensive discoloration, 80-99%; 7 = total discoloration, 100%). Daily scores from each panelist for initial color, display color, and discoloration were averaged before statistical analysis.

Instrumental color was determined using a Hunter Lab Miniscan XE Plus (Hunter laboratories Model MSXP'4500C, Reston, VA) using illuminant D65 and the 10 standard observer angles and 2.54 cm aperture (CIE, 1978). Meat color was measured at twice locations across the DSM and 3 times

across the SSM portions of the steaks at 1 h post-bloom (d 0 - zero) and at the end of display (d 3). CIE L* (lightness), a* (redness) and b* (yellowness) values were measured.

Lipid oxidation analysis

Oxidative rancidity was evaluated in the samples by measuring Thiobarbituric Acid Reactive Substances (**TBARS**) at the end of retail display at 7, 14 and 21 d of aging using the modified extraction method described by Buege and Aust (1978). Samples were prepared by blending 10 g of meat sample with 30 ml of distilled water for approximately 30 sec. Samples were centrifuged at 3000 rpm for 10 min. After the reagents were added, samples were heated in a hot water bath (97 °C) for 15 min, followed by an ice water bath for 10 min. Optical density was read using a spectrophotometer (DU-640, Beckman Instruments, Inc., Fullerton, CA) at a wavelength of 531 nm. Samples were analyzed in duplicate and results were averaged prior to statistical analysis. The TBARS value was expressed as milligram of malonaldehyde produced per kilogram of sample.

Statistical Analysis

Data collected were analyzed using SAS (Cary, NC) version 9.2 (SAS, 2003). In CRD with split-plot or split-split plot arrangements, the degrees of freedom in the denominator were adjusted using the Satterthwaite procedure.

Carcass data: for analysis of variance, a complete randomized design model was used to study the effects of feeding group (non-ZH-fed vs. ZH-fed) as main effect. Individual carcass identification was included in the model as random effect. Least squares means were separated (F test, $P < 0.05$) by using least significant differences generated by the PDIFF option.

Proximate analysis: Two models were used because samples were processed separately for each postmortem day (3 d or 7 d). At 3 d postmortem, samples were taken prior to injection of the strip loins. A CRD model was used to study the effects of feeding group as main effect and as random variable was included individual steak identification. Least squares means were separated (F test, $P < 0.05$) by using least significant differences generated by the PDIFF option.

The second model represented steaks from the injected strip loin at 7 d postmortem. This model was analyzed as a CRD with split-plot arrangement; where the whole-plot portion were two feeding groups, and in the sub-plot, it was assigned to injection treatments. Thus, feeding groups, injection treatments, and their interaction were considered to be fixed. Random variable was included individual steak identification and its interaction with feeding groups, injection treatments. Least squares means were separated (F test, $P < 0.05$) by using least significant differences generated by the PDIFF option.

pH, steak purge loss, metmyoglobin reducing activity and lipid oxidation: data were analyzed as CRD with split split-plot arrangement; where the whole-plot portion were both feeding groups, and in the sub-plot, it

was assigned to injection treatments. In the sub-subplot, postmortem aging days (7, 14 and 21 d); thus, feeding groups, injection treatments, postmortem aging and their interaction were considered to be fixed. Random variable was included individual steak identification and its interaction with feeding groups, injection treatments and postmortem aging. Least squares means were separated (F test, $P < 0.05$) by using least significant differences generated by the PDIFF option.

Color evaluation data: Two models were used for each **SSM** and **DSM** portions. First model represents steaks that were obtained at 0 day in each ageing period. Color evaluation by trained panelist (initial color) and instrumental color (CIE L*, a*, b*, Hue angle and Chroma) were analyzed as a completely randomized design (CRD) with split-split plot arrangement. In the whole-plot portion were two feeding groups. In the sub-plot, it was assigned to each injection treatment. In the sub-subplot, steaks obtained at 7, 14 and 21 d postmortem. Thus, feeding treatment, injection treatments, ageing time and their interactions were al considered to be fixed. Random variable was included individual steak identification and its interaction with feeding groups, injection treatments and postmortem aging.

The second model represents steaks that were displayed for 3 days. This model also was analyzed as a CRD with split-split-plot arrangement (display color and discoloration scores by trained panelist and instrumental color). In the whole-plot portion were two feeding groups. In the sub-plot, it was assigned to each injection treatment. In the sub-subplot, steaks obtained at 7,

14 and 21 d postmortem. In the sub-sub-subplot, retail display-time (1, 2, 3 days; repeated measure). Thus, feeding treatment, injection treatments, ageing time, retail display-time and their interactions were all considered to be fixed. Random variable was included individual steak identification and its interaction with feeding groups, injection treatments and postmortem aging. Least squares means were separated (F test, $P < 0.05$) by using least significant differences generated by the PDIFF option.

6.3. Results and discussion

Carcass data

The FG had a significant effect ($P < 0.05$) in longissimus area (Table 1). The ZH-fed group had larger LM area than carcasses from non-ZH-fed. A trend ($P = 0.06$) was found to heavier carcasses on ZH-fed group. Fat thickness, KPH, marbling score and USDA calculated yield grade were not affected ($P > 0.05$) by ZH supplementation. The lacking of difference on marbling is due to all carcasses being selected within the same quality grade.

ZH-fed cattle normally have greater HCW and LM area (Avendano-Reyes et al., 2006; Vasconcelos et al., 2008; Beckett et al., 2009; Elam et al., 2009), although fat deposition may not be affected (Casey et al., 1997; Plascencia et al., 1999; Beckett et al., 2009; Montgomery et al., 2009). In the other hand, decrease in fat content due to ZH-supplementation had been reported by other authors (Vasconcelos et al., 2008; Elam et al., 2009; Hilton et al., 2009).

Injection and Purge

The effects for initial muscle weights, injection percentages, and purge loss on d 7 postmortem are presented in Table 2. The FG had a significant effect ($P < 0.01$) on whole weight of inside round. Inside rounds from ZH-fed were heavier (+ 1.05 kg) than those from control-fed cattle. It occurs by reason of ZH increased protein deposition and decreased fat accumulation.

Many articles have shown ZH increase percentage yield in several cuts (Hilton et al., 2009; Kellermeier et al., 2009). Rathmann et al. (2009) found that although ZH manifested its effect within every whole primal region in the carcass, the most consistent ZH effect was seen in the round region, where ZH increased the percentage yield of every subprimal recorded.

The effect of IT was significant on subprimal purge loss on d 7 postmortem ($P < 0.01$). $CaCl_2$-injected inside round showed higher purge loss. Many reports described an increased drip loss in $CaCl_2$ injected meat particularly that measured as purge (Koohmaraie et al., 1990; Pringle et al., 1999; Dikeman et al., 2003). Previous studies have indicated that $CaCl_2$ injection reduce the water holding capacity (higher drip loss). Wheeler et al. (1993) found the amount of drip loss was not affected by injection amount (5 or 10 %), but it was affected by $CaCl_2$ concentration, being greatest in muscle injected with 200 mM. Jaturasitha et al. (2004) reported the injection of $CaCl_2$ at any concentration (0.2, 0.3 and 0.4 M at 10%) increased drip loss.

There was no interaction effect between IT and FG ($P = 0.93$) for purge loss. Higher purge loss in ZH-treated beef cattle were reported by Kellermeier et al. (2009) and Rathmann et al. (2009). Conversely, Strydom et al. (2009) found higher drip loss (> 1.79 %) in ZH-fed steaks than control steaks. In contrast, other studies have indicated none or little variations on drip loss using a different βAA (Fiems et al., 1990; Boucqué et al., 1994).

Proximate analysis

ZH-supplementation had increased ($P < 0.001$) protein content of inside round steaks when measured on d 3 postmortem, while no difference were found in fat, moisture, and collagen contents ($P > 0.05$; Table 3). A few articles have reported the effects of ZH on chemical composition. Hilton et al. (2009), Kellermeier et al. (2009) and Rathmann et al. (2009) evaluated the predicted carcass chemical composition, as described by Hankins and Howe (1946), and found higher protein and moisture contents in ZH-fed carcass, while fat content was decreased. Leheska et al. (2009) described, by carcass dissection, an increase on protein not affecting fat and moisture content, when cattle were fed ZH, while Hilton et al. (2009) reported, by proximate analysis on LD muscle, that feeding ZH decreased fat percentage, whereas protein and moisture percentage were not affected. The lack of difference on fat content, in the present research, was probably due to all carcasses were selected within the same quality grade (Select), nevertheless a trend ($P = 0.09$) was found to lower fat content in inside round from ZH-fed cattle.

Compositional analyses on d 7 postmortem were evaluated again to determine the effects of calcium chloride on protein, fat, moisture and collagen contents. The same effects were found for FG, in which ZH-supplementation increased protein content ($P < 0.001$) but did not change fat, moisture and collagen contents ($P > 0.05$). $CaCl_2$-injected samples had a slight increment of moisture content (0.5 %; $P = 0.02$), without any effect on

the other chemical components ($P > 0.05$). The analysis of variance did not detect interaction effect on proximal composition ($P > 0.05$).

When ZH is supplemented, the protein and fat metabolisms are altered, and the adipocytes and muscular cells work to produce more protein and less fat, by activating lipases and stimulating protein RNA synthesis.

Metmyoglobin Reducing activity

Feeding and injection treatments did not affect MRA ($P > 0.05$), however an effect was observed for postmortem aging time ($P < 0.05$) (Table 4). The analysis of variance did not detect interaction effect ($P > 0.05$). There were not found studies about βAA supplementation effect on MRA for comparison with our results. Different from the present, previous studies about $CaCl_2$ have demonstrated that infusion of $CaCl_2$ lowers MRA in beef (Bekhit et al., 2005). Stewart et al. (1965) found that sodium chloride inhibited enzymatic reducing activity in beef muscle.

MRA was increased after vacuum-aging storage, between 7 and 21 d. That increase in MMb reducing activity after storage was reported earlier in beef muscles (Echevarne et al., 1990) and confirmed in vacuum-packed lamb (Bekhit et al., 2001). One possible explanation for that increase was the presence of myofibrillar MMb reducing activity which may have been translocated to the sarcoplasmic fraction during aging (Bekhit et al., 2002, 2003).

pH and display purge loss

There was no significant interaction among the main factors FG, IT and aging time for initial pH ($P > 0.05$; Table 4). FG did not affect initial pH (non-ZH-fed: 5.70 and ZH-fed: 5.68; $P > 0.05$), however IT ($P < 0.01$) and ageing time ($P < 0.001$) did affect this trait. The pH values (5.61 to 5.81) were typical of beef muscle and were not likely a factor in any color differences. Regards to FG, previous studies have also reported no difference in pH values of SM steaks (Gunderson et al., 2009b) and LD steaks (Vestergaard et al., 1994; Avendaño-Reyes et al., 2006) when cattle were fed or not with different βAA, including ZH, cimaterol and ractopamine. In this study, pH was measured on the center of the steak, assuming there would be no difference between SSM and DSM, because according Gunderson et al. (2009a), who did not found differences in pH for the DSM and SSM portions.

Steaks from calcium chloride injected samples (5.65) had lower ($P < 0.05$) initial pH than those from non-injected steaks (5.72). Hunt et al. (2003) found no difference for pH at 48 h postmortem on LL, SSM, DSM, and psoas major when beef carcasses were infused (pre-rigor) with $CaCl_2$. Similar results were found by Jaturasitha et al. (2004) and Bekhit et al. (2005), in which LD from carcass infused with $CaCl_2$ had the same pH (24 h postmortem) than those from non-infused carcass.

Aging increased ($P < 0.05$) initial pH from 7 d (5.61) to 14 d (5.81), however decreased from 14 d to 21 d (5.66). Similar changing in pH due to ageing (increasing following by dropping) was reported by Pierson and Fox

(1976) and Kong et al. (2006). This varying has been attributed to an altered ion-protein interaction due to an increased influx of K^+ (Lawrie, 1998) and to the conversion of nitrogenous compounds into ammonia that neutralize lactic acid produced from glycolysis (Pierson and Fox, 1976).

At the end of display there were interactions between FG and aging time, and between IT and aging time ($P < 0.05$). However, all values were within the accepted normal range of pH; therefore, these changes are not large enough to suggest an alteration of meat pH. Samples from non-ZH-fed cattle had greater (5.64) final pH values on d 7 postmortem than samples from ZH-fed cattle (5.60), though at 14 and 21 d postmortem no difference was detected anymore. For IT and aging time, in samples aged 21 d postmortem, $CaCl_2$ decreased the final pH when compared with non-injected samples, though at 7 and 14 d postmortem no difference was found. The final pH decreased with aging time on samples injected (5.63 through 5.53; $P < 0.05$), on the other hand non-injected samples had similar final pH for all times of postmortem aging (5.62; $P > 0.05$).

All through the display, the pH values decreased ($P < 0.001$, not shown in tabular form) from 5.69 on d 0 (initial pH) to 5.60 on d 3 (final pH).

There was no significant interaction among the main factors FG, IT and aging time for steak purge loss during display ($P > 0.05$). Purge loss was not affected by either FG or IT ($P > 0.05$), in which the average loss was 2.26 %, but an effect of aging was detected on purge loss during display ($P < 0.001$), where steaks aged 14 d (1.85 %) and 21 d (1.60 %) had lower values for purge

than steaks aged 7 d (3.35 %); however no difference was detected between 14 and 21 d of aging. The lower values for purge loss found on samples aged 14 and 21 d probably occurred because with these times of age, the loss of water was higher in the vacuum packaged and steaks could not lose more water during display.

Studying the effect of βAA on purge loss during display of LD steaks, Rogers et al. (2010) found no difference on samples from cattle fed or not ZH, and Quinn et al. (2008) for ractopamine. Relative to $CaCl_2$, the lack of difference in purge loss during display can be explained by the purge loss before cutting the steaks that balance the purge during display. Many reports describe a reduced WHC in $CaCl_2$-injected meat particularly that measured either as purge (weight loss during storage) or drip loss (Koohmaraie et al., 1990; Wheeler et al., 1993; Pringle et al., 1999; Dikeman et al., 2003).

Sensory Color

Main effects FG and IT did affect initial color, display color and discoloration scores evaluated by trained panelist ($P < 0.01$; Table 5), however no interaction between them was detected ($P > 0.05$). A muscle area x postmortem aging interaction existed for the initial color, display color and discoloration ($P < 0.01$). Other interaction was muscle area x display day for display color ($P < 0.01$) and discoloration ($P < 0.05$; Table 6). A FG x aging time x display day interaction existed for the display color and discoloration

($P < 0.01$; Table 7), and IT x aging time x display day for display color and discoloration ($P < 0.01$; Table 8).

The data indicated ZH steaks (4.91) had lower scores for initial color, evaluated on d 0 of display, been lighter ($P < 0.05$), than non-ZH steaks (5.11). For display color, evaluated during display (days 1, 2 and 3 together) ZH steaks (4.84) had lower ($P < 0.05$) scores than non-ZH steaks (5.60). These results differed from those reported by Gunderson et al. (2009a), which indicated no differences in initial color scores of SM steaks attributable to ZH feeding. In the same way Van Overbeke et al. (2009) observed no difference for top sirloin butt steaks from cattle treated or not with ZH. For LD steaks, Rogers et al. (2010) found ZH supplementation for 0, 20, and 40 d produced similar initial color scores, which were significantly darker than LD steaks from cattle fed ZH for 30 d. The initial color differences among muscles are likely the result of differing muscle fiber types composition and the metmyoglobin-reducing ability.

The effects of ZH supplementation on discoloration show that ZH steaks (2.26) were more ($P < 0.05$) stable than non-ZH steaks (2.81). Disagreeing with these results, Gunderson et al. (2009) and Van Overbeke et al. (2009) found no difference for discoloration between steaks from cattle fed or not with ZH, when measuring inside round and top sirloin butt steaks, respectively.

The $CaCl_2$ injection increased ($P < 0.05$) initial color (non-injected = 4.89; $CaCl_2$-injected = 5.13), display color (non-injected = 5.01; $CaCl_2$-

injected = 5.43) and discoloration (non-injected = 2.20; $CaCl_2$-injected = 2.87) scores, been darker and less stable than non-injected steaks during retail display. In the other hand, Lansdell et al. (1995) found lower initial color score, in SM $CaCl_2$-injected (5.70) when compared with non-injected (6.0). Diles et al. (1994) and Kerth et al. (1995) found no difference on initial color of strip loin steaks when injecting $CaCl_2$.

Superficial semimembranosus area (SSM) had higher (darker) scores for initial color and display color for all aging periods evaluated (7, 14 and 21 d), than deep semimembranosus area (DSM) ($P < 0.05$). As aging increased, the SSM initial color and SSM and DSM display color scores increased, however on DSM initial color, the increasing aging decreased color scores. On d 7 of aging, muscle area had similar discoloration scores (SSM = 1.49; DSM = 1.39; $P > 0.05$). However, on d 14 and 21, DSM had greater discoloration scores than SSM ($P < 0.05$). SSM had greater display color scores than DSM for all display days (days 1, 2 and 3; $P < 0.05$), and the increasing of display time increased the color scores in both SSM and DSM ($P < 0.05$). Discoloration scores for DSM were higher than SSM for all days during display, and the display time increased discoloration ($P < 0.05$). Some researchers, Sammel et al. (2002b) and Seyfert et al. (2006), reported a lighter red initial color for the DSM than the SSM. The more pale color typical of the DSM (compared with the SSM) can be attributed to high temperatures early postmortem found within the DSM. Seyfert et al. (2006) noted that the DSM had a decreased oxygen consumption rate allowing for a bright red initial

color to develop upon exposure to oxygen. In general, our initial color scores were similar to those of Gunderson et al. (2009a,b), who reported reduced initial color scores for the DSM (4.3) than the SSM (5.0).

The steaks from ZH-fed cattle had lower display color for all aging periods in all display days ($P < 0.05$) than steaks from non-ZH-fed cattle. Among display days, non-ZH steaks aged 7 and 14 d, and ZH steaks aged 14 d, increased ($P < 0.05$) display color during all days of display (day 1 to 2 and, day 2 to 3). However, ZH steaks aged 7 d had similar display color on d 1 and 2 ($P > 0.05$), and higher on d 3 ($P < 0.05$). Display color on non-ZH steaks aged 21 d and ZH steaks aged 21, increased scores from d 1 and 2 ($P < 0.05$), however no difference was detected between d 2 and 3 ($P > 0.05$). Regarding aging time, non-ZH steaks, for all display days, and ZH steaks, on d 2 and 3, increased ($P < 0.05$) color scores when increasing aging (d 7 to 14, and d 14 to 21), however ZH steaks on d 1 of display had an increasing from d 7 to 14 ($P < 0.05$), but no difference was found between d 14 and 21 of aging ($P > 0.05$).

Steaks from ZH-fed cattle had lower discoloration scores than steaks from non-ZH-fed cattle just on d 14 and 21 of aging, for all display days ($P < 0.05$); however on d 7 of aging there was no differences between non-ZH and ZH discoloration ($P > 0.05$). Among display days, non-ZH and ZH steaks aged 7 d, had the same discoloration scores on d 1 and 2 ($P > 0.05$), and higher on d 3 ($P < 0.05$). Non-ZH and ZH steaks aged 14 d and ZH steaks aged 21 d, increased discoloration scores during all days of display (d 1 to 2, and d 2 to

3; $P < 0.05$), however non-ZH steaks aged 21 d increased discoloration scores from d 1 to 2 ($P < 0.05$), but not from d 2 to 3 ($P > 0.05$). Regarding aging time, non-ZH steaks, for all display days, and ZH steaks, on d 2 and 3, increased discoloration when increasing aging (d 7 to 14, and d 14 to 21; $P < 0.05$), however ZH steaks on d 1 of display and aged 7 and 14 d had the same discoloration score ($P > 0.05$), while steaks aged 21 days had higher score ($P < 0.05$).

Different from our results, Gunderson et al. (2009a) found that in the latter days of display (d 4 to 5), semimembranosus steaks from beef cattle supplemented with ZH for 0 and 40 d had increased color scores (darker red) when compared with steaks from cattle supplemented with ZH for either 20 or 30 d. Rogers et al. (2010) suggested that supplementation of ZH for 20 and 30 d resulted in steaks with a more red lean color than 0 d of supplementation on d 2 and 3 of display. On d 4 of display, steaks from beef cattle fed ZH for 20 d were redder than those from beef cattle fed ZH for 0, 30, and 40 d. Hilton et al. (2009), reported that ZH supplementation increased the LM color scores of trained panelists throughout a 5 d display period.

Steaks injected with $CaCl_2$, on d 7 of aging, during all days of display, and on d 14 of aging, on d 1 of display, had the same color score than those non-injected steaks ($P > 0.05$); however, when aged 14 d, on d 2 and 3 of display, and aged 21 d, in all days of display, injected steaks had higher color scores than non-injected steaks ($P < 0.05$). Among display days, non-injected steaks aged 7 d, and $CaCl_2$-injected steaks aged 14 and 21 d, increased color

scores during all day of display (d 1 to 2 and d 2 to 3; $P < 0.05$), however non-injected steaks aged 14 d and $CaCl_2$-injected steaks aged 7 d had the same color score on d 1 and 2 of display ($P > 0.05$), but higher on d 3 ($P < 0.05$). Color scores for non-injected steaks aged 21 d increased from d 1 to 2 of display ($P < 0.05$), however no difference was detected between d 2 and 3 ($P > 0.05$). Regarding aging time, non-injected steaks, on d 2 and 3 of display, and $CaCl_2$-injected steaks, for all display days, increased color scores when increasing aging (d 7 to 14 and d 14 to 21; $P < 0.05$), however non-injected steaks on d 1 of retail display had an increase from d 7 to 14 ($P < 0.05$), but no difference was found between days 14 and 21 ($P > 0.05$).

$CaCl_2$-injected steaks had higher discoloration scores than steaks from non-injected just on d 14 and 21 of aging, for all display days ($P < 0.05$); however on d 7 of aging there were no difference between non-injected and $CaCl_2$-injected steaks for discoloration in all display days ($P > 0.05$). Among display days, non-injected and $CaCl_2$-injected steaks aged 7 d, had the same discoloration scores on d 1 and 2 of display ($P > 0.05$), and higher on d 3 ($P < 0.05$), however non-injected and $CaCl_2$-injected steaks aged 14 and 21 d increased discoloration scores during all days of display (d 1 to 2 and d 2 to 3; $P < 0.05$). Regarding aging time, non-injected steaks, on d 2 and 3 of displays, and $CaCl_2$-injected steaks, for all display days, increased discoloration scores when increasing aging (d 7 to 14 and d 14 to 21; $P < 0.05$); however non-injected steaks on d 1 of retail display had similar

discoloration score on d 7 and 14 of aging ($P > 0.05$), however a higher score on d 21 ($P < 0.05$).

Different from our results, Hunt et al. (2003) found inside round steaks from beef carcasses that were cardiovascular infused with $CaCl_2$ (300 mM) did not differ from samples non-infused for color score during display, however, the authors agreed that the increasing time of display increased the color scores for both infused or not with $CaCl_2$. Harris et al. (2001) reported $CaCl_2$-injected LD samples (250 mM) had no differences in the percentage surface discoloration at d 1 of display; however, on d 4 of display, steaks injected with $CaCl_2$ had lower discoloration scores than non-injected steaks.

Instrumental Color

Means of instrumental color (L*, a* and b*) for main effects muscle area, FG, IT, aging time and days of display are presented on table 9. Muscle area had affected L* ($P < 0.001$), a* ($P < 0.001$) and b* ($P = 0.01$) values, while FG just affected L* value ($P < 0.001$). In the other hand, IT affected a* value ($P = 0.05$), while aging time and display day affected a* ($P < 0.001$) and b* ($P = 0.01$) values. The interaction muscle area x aging time x display day existed for a* ($P = 0.01$) and b* ($P = 0.04$) values, however there is no effect on L* value ($P = 0.98$; Table 10). The interaction display day x FG, and display day x IT did affect a* ($P < 0.001$) and b* ($P < 0.001$) values, in the other hand, the interaction display day x aging time did affect L*, a* and b*

values ($P < 0.01$; Table 11). There was no interaction FG x IT for neither L*, a* or b* values ($P > 0.05$).

The deep semimembranosus area (44.29) had higher L* value than the superficial area (36.67), and both areas were slight affected by aging time and display day ($P < 0.05$). Similarly, Sammel et al. (2002a,b) cited higher L* value for DSM when comparing with SSM, and it was seem in steaks aged 5, 9 or 14 d and in all days of display (0 to 6 d). Similar results were found by Seyfert et al. (2004), who reported higher L* value for DSM from steaks aged for 21 d. Mac-Dougall (1982) suggested that muscles that chill slowly (DSM) have a more open structure and greater scattering coefficients, which creates a paler appearance.

On d 0 of display, SSM and DSM presented higher a* and b* values when compared with d 3 of display, for all aging periods (7, 14 and 21 d; $P < 0.05$), however it was seen (numerically) that DSM was more affected by display time than SSM for a* value ($P > 0.05$). Sammel et al. (2002a) described that at the end of display, DSM had lower a* and b* values, and the more desirable appearance was lost quickly.

Progressive aging caused a decreasing in a* and b* values, for both SSM and DSM ($P < 0.05$), yet on d 0 of display the decreasing was similar between SSM and DSM ($P > 0.05$), however, on d 3, a* value decreased faster in SSM than DSM ($P < 0.05$), showing that SSM was more affected by aging than DSM. Sammel et al. (2002b) found both SSM and DSM areas were more color stable after storage of 5 d rather than 14 d.

On d 0 of display, a* value was similar between SSM and DSM of steaks aged 7 (25.73), 14 (26.21) and 21 (22.19) d ($P > 0.05$). For b* value, SSM had similar scores than DSM of steaks aged 7 (24.19) and 14 (25.14) d ($P > 0.05$), however, when aged 21 d, SSM (19.06) b* value was lower ($P < 0.05$) than DSM (20.68). At the end of display (d 3), SSM presented higher a* value than DSM in steaks aged 7 and 14 d ($P < 0.05$), however, steaks aged 21 d presented similar ($P > 0.05$) a* value (10.95). There were no difference for b* value between SSM and DSM for all aging days (7, 14 and 21; $P > 0.05$). Disagreeing with the present results, Sammel et al. (2002a,b) reported that DSM had higher a* and b* values than SSM, and Gunderson et al. (2009a) and Seyfert et al. (2006) found the SSM was redder (greater a* values) than the DSM.

Steaks from Non-ZH-fed cattle (38.89) had lower ($P < 0.05$) L* values than steaks from ZH-fed cattle (42.07). The greater L* values in ZH-fed steaks could be associated with the higher deposition of miofibrilar protein that could improve the blooming, and the muscle will became brighter. Gunderson et al. (2009a) found greater L* value on DSM at d 0 of display from cattle fed with ZH for 20 days when compared with 40 days, however no effect was verified on DSM or SSM on d 3 of display. Avendaño-Reyes et al. (2006) displayed LD steaks from cattle fed ZH, ractopamine, or no β-agonist for 14 d and reported no treatment difference in L* values on d 1 or 14; however, control steaks were darker (reduced L* value) on d 5 than steaks from either β-agonist treatment. Different to our results, VanOverbeke et al.

(2009), Hilton et al. (2009) and Rogers et al. (2010) did not detect difference by feeding ZH on color, of different beef muscle evaluated.

At the beginning of display (d 0), Non-ZH samples had similar a* (24.62) and b* (23.07) values than ZH samples ($P > 0.05$), however, on d 3 of display, higher a* and b* values were verified for ZH samples when compared with Non-ZH samples ($P < 0.05$), been more stable during display. The increasing of display decreased both a* and b* values from both feeding treatments ($P < 0.05$). VanOverbeke et al. (2009) found similar results, in that ZH supplementation had increased a* and b* values of top sirloin but steaks. In the same way, Gunderson et al. (2009a) found the DSM portion of steaks from beef steers fed ZH for 40 d had higher a* and b* values than the DSM portion of steaks from non-supplemented steers, however it was verified just on d 0 of display. In contrast, Avendaño-Reyes et al. (2006) reported a significant difference due to ZH treatment, in that ZH group had reduced a* values compared with control cattle, however no difference was detected for b* value. Reduced a* and b* values, for LM muscle, due to ZH-supplementation, were described by Hilton et al. (2009) and Rogers et al. (2010).

The effect feeding group x display day was also verified by Avendaño-Reyes et al. (2006). The authors reported that meat darkened with time, but the effect was more evident in the ZH group than Non-ZH. Seyfert et al. (2006) and Sawyer et al. (2007) also reported a loss in yellowness (lower b* value) from d 0 to 3 of display.

$CaCl_2$ injected samples had similar ($P > 0.05$) L* value than non-injected samples (40.37 vs 40.60). There are no difference ($P > 0.05$) among non-injected and injected samples for a* and b* values at the beginning of display (d 0), although on d 3 of display, injected samples had lower a* and b* values than non-injected samples ($P < 0.05$). The increasing of display time decreased both a* and b* values from both injection treatments ($P < 0.05$). Hunt et al. (2003) agreed in part with the present study. The authors did not find difference for L*, a* and b* values, on d 0 of display, between non-infused and $CaCl_2$-infused beef inside round, however at the end of display, the authors still did not find any difference between treatments. Lansdell et al. (1995) suggested that SM $CaCl_2$-injected steaks had higher L*, a* and b* values during all display time than non-injected samples. At the same way Harris et al. (2001) and Kerth et al. (1995), found higher L*, a* and b* values for injected LD steaks. Bekhit et al. (2005), infusing $CaCl_2$ in lamb carcasses, had lower L*, a* and b* values when comparing with chops from water-infused on d 0 through 7 of display. According Bekhit et al. (2005), the negative effects of calcium chloride on color, could be explained by the lower amounts of unbound water, shorter sarcomere length, lower NAD concentrations, and higher lipid peroxidation.

The increasing of aging, from 7 to 21 d, did not affect L* value (40.75) of steaks on d 0 of display ($P > 0.05$), however lower L* was found on d 3 of display in steaks aged 21 d, when compared with steaks aged 7 and 14 d ($P < 0.05$). Regarding a* and b* values on d 0 of display, steaks aged 7 and 14 d

had similar (25.97 and 24.66 for a* and b*, respectively; $P > 0.05$), and had higher values than steaks aged 21 d (21.93 and 19.87 for a* and b*, respectively; $P < 0.05$). On d 3 of display, the increasing aging decreased both a* (18.17 to 10.95) and b* (18.95 to 15.92) values ($P < 0.05$). In all aging periods (7, 14 and 21 d), a* and b* values were lower at the end of display (d 3), when compared with d 0 ($P < 0.05$). Kerth et al. (1995) found no difference for L* value, when studying LD muscle, due to aging time or display day, however steaks at 7 d aging had higher a* value (more red) than steaks at 14 d aging, and b* value was decreased, with increasing display, just in steaks aged 14 d, without affect steaks aged 7 d.

Lipid oxidation

The amount of thiobarbituric acid reactive substances (TBARS; mg/kg) at the end of display was not affected by FG ($P > 0.05$), however was affected by IT ($P < 0.05$) and postmortem aging time ($P < 0.05$) as main effects (Table 12). No interactions between them were detected ($P > 0.05$). The data indicated $CaCl_2$-injected steaks resulted in greater ($P < 0.05$) TBARS values (0.17 mg/kg) than non-injected steaks (0.14 mg/kg). For postmortem aging days, the TBARS were similar ($P > 0.05$) between days 7 (0.13) and 14 (0.14), however were higher ($P < 0.05$) at 21 d postmortem (0.19 mg/kg).

There is rare information about βAA supplementation on TBARS. In one study, Luque et al. (2011) reported that ground beef from ZH-treated animals had smaller TBARS values than non-ZH-fed at 7 d of dark storage; however,

no differences in TBARS values were observed between them at d 14 and 21. On relation to calcium chloride, Harris et al. (2001) observed that $CaCl_2$–injected steaks exhibited greater TBARS values than non-injected steaks. Other investigators have also reported increased lipid oxidation in meat products that have been injected with $CaCl_2$ (St. Angelo et al., 1991; Bekhit et al., 2005). Finally, other studies have shown increases in the TBARS values during the storage period (Gokalp et al., 1983; Igene and Pearson, 1979).

In conclusion, zilpaterol hydrochloride supplementation increased the amount of protein on inside round steaks, and had brighter red lean beef without affect metmyoglobin reducing activity (MRA) and lipid oxidation. The injection of $CaCl_2$ (5 % - 200 mM) on inside rounds did not affect beef composition and MRA. However it stimulated lipid oxidation, and produced dark lean beef and stimulate discoloration if steaks were aged more than 14 d, without affect steaks aged 7 d.

6.4. Literature cited

AMSA. 1991. Guidelines for Meat Color Evaluation. In Proc. 44th Reciprocal Meat Conf., Manhattan, KS. Am. Meat Sci. Assoc., Natl. Livest. Meat Board, Chicago, IL.

Avendano-Reyes, L., V. Torres-Rodriguez, F. J. Meraz-Murillo, C. Perez-Linares, F. Figueroa-Saavedra, and P. H. Robinson. 2006. Effects of two beta-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 84: 3259-3265.

Beckett, J. L., R. J. Delmore, G. C. Duff, D. A. Yates, D. M. Allen, T. E. Lawrence, and N. Elam. 2009. Effects of zilpaterol hydrochloride on growth rates, feed conversion, and carcass traits in calf-fed Holstein steers. J. Anim. Sci. 87: 4092-4100.

Bekhit, A. E. D., Bickerstaffe, R., Illian, M., Morton, J. D., Stevenson, B. J., (2002). Effect of calcium chloride, zinc chloride and water infusion on fresh meat colour. In Proceedings 48th international conference of meat science and technology (pp. 462–463), 25–30 August 2002, Rome, Italy.

Bekhit, A. E. D., Geesink, G. H., Morton, J. D., & Bickerstaffe, R. (2001). Metmyoglobin reducing activity and colour stability of ovine Longissimus muscle. Meat Science, 57, 427–435.

Bekhit, A. E. D., Illian, M. A., Morton, J. D., Sedcole, J. R., & Bickerstaffe, R. (2003). Particulate metmyoglobin reducing activity and its relationship with meat colour. Journal of Agricultural and Food Chemistry, 51, 6026–6035.

Bekhit, A. E., M. A. Ilian, J. D. Morton, L. Vanhanan, J. R. Sedcole, and R. Bickerstaffe. 2005. Effect of calcium chloride, zinc chloride, and water

infusion on metmyoglobin reducing activity and fresh lamb color. J Anim Sci 83: 2189-2204.

Boucqué, C. V., L. O. Fiems, R. J. Moermans, B. G. Cottyn, and M. Sommer. 1994. Effect of cimaterol on growth, carcass characteristics and meat quality in double-muscled Belgian White-blue bulls. Can. J. Animal Sci. 74: 707-709.

Buege, J. A., and S. D. Aust. 1978. Microsomal lipid peroxidation. Methods in Enzymology 52: 302-304.

Casey, N. H., T. H. Montgomery, and M. L. Scheltons. 1997. The effect of zilpaterol on feedlot performance, carcass quality, USDA carcass grades and meat quality. Pages 262-263 in Proc. 43rd Int. Congr. Meat Sci. Technol., Auckland, New Zealand.

CIE. 1978. Recommendations on uniform colorspaces—color equations, psychometric color terms. Supplement No. 2 to CIE Publ. No. 15 (E-1.3.L) 1971 (9TC-1-3) CIE, Paris.

Dikeman, M. E., M. C. Hunt, P. B. Addis, H. J. Schoenbeck, M. Pullen, E. Katsanidis, and E. J Yancey. 2003. Effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on quality and sensory traits of steaks and ground beef. J. Anim. Sci. 81:156–166.

Diles, J. J. B., M. F. Miller, and B. L. Owen. 1994. Calcium chloride concentration, injection time, and aging period effect on tenderness, sensory, and retail color attributes of loin steaks from mature cows. J. Anim. Sci. 72:2017–2021.

Echevarne, C., Renerre, M., & Labas, R. (1990). Metmyoglobin reductase activity in bovine muscles. Meat Science, 27, 161–172.

Elam, N. A., J. T. Vasconcelos, G. Hilton, D. L. VanOverbeke, T. E. Lawrence, T. H. Montgomery, W. T. Nichols, M. N. Streeter, J. P. Hutcheson, D. A. Yates, and M. L. Galyean. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87: 2133-2141.

FDA. 2006. Freedom of Information Summary. Original New Animal Drug Application NADA 141–258. ZILMAX (Zilpaterol Hydrochloride). Type A Medicated Article for Cattle Fed in Confinement for Slaughter. http://www.fda.gov/cvm/FOI/141-258o08102006.pdf Accessed Aug. 14, 2008.

Fiems, L. O., B. Buts, and B. G. Cottyn. 1990. Effect of a b-agonist on meat quality and myofibrillar protein fragmentation in bulls. Meat Sci. 27:29-39.

Geesink, G. H., F. J. M. Smulders, H. L. J. M. Van Laack, J. H. Van der Kolk, Th. Wensing, and H. J. Breukink. 1993. Effects on meat quality of the use of clenbuterol in veal calves. J. Anim. Sc. 71:1161–1170.

Gokalp, H. T., H. W. Ockerman, P. F. Plimpton, and W. J. Harper. 1983. Fatty acids of neutral and phospholipids, rancidity scores and TBA values as influenced by packaging and storage. Journal Food Science 48: 829-834.

Gunderson, J. A., M. C. Hunt, T. A. Houser, E. A. Boyle, M. E. Dikeman, D. E. Johnson, D. L. VanOverbeke, G. G. Hilton, C. Brooks, J. Killefer, D. M. Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, and D. A. Yates. 2009a. Effects of zilpaterol hydrochloride feeding duration on crossbred beef semimembranosus steak color in aerobic or modified atmosphere packaging. J Anim Sci 87: 3739-3750.

Gunderson, J. A., M. C. Hunt, T. A. Houser, E. A. Boyle, M. E. Dikeman, D. E. Johnson, D. L. VanOverbeke, G. G. Hilton, C. Brooks, J.

Killefer, D. M. Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, and D. A. Yates. 2009b. Feeding zilpaterol hydrochloride to calf-fed Holsteins has minimal effects on semimembranosus steak color. J Anim Sci 87: 3751-3763.

Hankins, O. G., and P. E. Howe. 1946. Estimations of the composition of beef carcasses and cuts. USDA Tech. Bull. 926. Washington, DC.

Harris, S. E., E. Huff-Lonergan, S. M. Lonergan, W. R. Jones, and D. Rankins. 2001. Antioxidant status affects color stability and tenderness of calcium chloride-injected beef. J Anim Sci 79: 666-677.

Hilton, G. G., J. L. Montgomery, C. R. Krehbiel, J. Cranston, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. Streeter, J. R. Blanton Jr., and M. F. Miller. 2009. Dietary zilpaterol hydrochloride. IV. Carcass cutability and meat palatability of beef cattle with and without monensin and tylosin. J. Anim. Sci. 87:1394–1406.

Hunt, M. C., J. J. Schoenbeck, E. J. Yancey, M. E. Dikeman, T. M. Loughin, and P. B. Addis. 2003. Effects of postexsanguination vascular infusion of carcasses with calcium chloride or a solution of saccharides, sodium chloride, and phosphates on beef display-color stability. J Anim Sci 81: 669-675.

Igene, J. O., and A. M. Pearson. 1979. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. Journal Food Science 44: 1285–1290.

Jaturasitha, S., P. Thirawong, V. Leangwunta, and M. Kreuze. 2004. Reducing toughness of beef from Bos indicus draught steers by injection of calcium chloride: Effect of concentration and time postmortem. Meat Sci. 68: 61-69.

Kellermeier, J. D., A. W. Tittor, J. C. Brooks, M. L. Galyean, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, B. J. Johnson, and M.

F. Miller. 2009. Effects of zilpaterol hydrochloride with or without an estrogen-trenbolone acetate terminal implant on carcass traits, retail cutout, tenderness, and muscle fiber diameter in finishing steers. J. Anim. Sci. 87:3702–3711.

Kerth, C. R., M. F. Miller, and C. B. Ramsey. 1995. Improvement of beef tenderness and quality traits with calcium chloride injection in beef loins 48 hours postmortem. J. Anim. Sci. 73:750–756.

Kong, B., X. Diao, and Y. L. Xiong. 2006. Postmortem calcium chloride injection alters ultrastructure and improves tenderness of mature chinese yellow cattle longissimus muscle. J. Food Sci. 71:124-129.

Koohmaraie, M., G. Whipple, and J. D. Crouse. 1990. Acceleration of postmortem tenderization in lamb and Brahman-cross beef carcasses through infusion of calcium chloride. J. Anim. Sci. 68:1278-1283.

Lansdell, J. L., M. F. Miller, T. L. Wheeler, M. Koohmaraie, and C. B. Ramsey. 1995. Postmortem injection of calcium chloride effects on beef quality traits. J. Anim. Sci. 73:1735–1740.

Leheska, J. M., J. L. Montgomery, C. R. Krehbiel, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. Streeter, J. R. Blanton Jr., and M. F. Miller. 2009. Dietary zilpaterol hydrochloride. II. Carcass composition and meat palatability of beef cattle. J. Anim. Sci. 87:1384–1393.

Luque, L. D., B. J. Johnson, J. N. Martin, M. F. Miller, J. M. Hodgen, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, D. A. Yates, D. M. Allen, and J. C. Brooks. 2011. Zilpaterol hydrochloride supplementation has no effect on the shelf life of ground beef. J Anim Sci 89: 817-825.

Mancini, R. A., and M. C. Hunt. 2005. Current research in meat color. Meat Science 71: 100-121.

MacDougal DB. 1982. Changes in the colour and opacity of meat. Food. Chem. 9(1):72-88.

Montgomery, J. L., C. R. Krehbiel, J. J. Cranston, D. A. Yates, J. P. Hutcheson, W. T. Nichols, M. N. Streeter, D. T. Bechtol, E. Johnson, T. TerHune, and T. H. Montgomery. 2009. Dietary zilpaterol hydrochloride. I. Feedlot performance and carcass traits of steers and heifers. J. Anim. Sci. 87: 1374-1383.

Pierson CJ, Fox JD. 1976. Effect of postmortem ageing time and temperature on pH, tenderness and soluble collagen fractions in bovine longissimus muscle. J Anim Sci 43:1206–10.

Plascencia, A., N. Torrentera, and R. A. Zinn. 1999. Influence of the β-agonist, zilpaterol, on growth performance and carcass characteristics of feedlot steers. Proc. Western Sect. Am. Soc. Anim. Sci. 50: 331-334.

Pringle, T. D., J. M. Harrelson, R. L. West, S. E. Williams, and D. D. Johnson. 1999. Calcium-activated tenderization of strip loin, top sirloin, and top round steaks in diverse genotypes of cattle. J. Anim. Sci. 77:3230–3237.

Quinn, M. J., C. D. Reinhardt, E. R. Loe, B. E. Depenbusch, M. E. Corrigan, M. L. May, and J. S. Drouillard. 2008. The effects of ractopamine-hydrogen chloride (Optaflexx) on performance, carcass characteristics, and meat quality of finishing feedlot heifers. J Anim Sci 86: 902-908.

Rathmann, R. J., J. M. Mehaffey, T. J. Baxa, W. T. Nichols, D. A. Yates, J. P. Hutcheson, J. C. Brooks, B. J. Johnson, and M. F. Miller. 2009. Effects of duration of zilpaterol hydrochloride and days on the finishing diet on carcass cutability, composition, tenderness, and skeletal muscle gene expression in feedlot steers. J. Anim Sci. 87:3686-3701.

Rogers, H. R., J. C. Brooks, M. C. Hunt, G. G. Hilton, D. L. VanOverbeke, J. Killefer, T. E. Lawrence, R. J. Delmore, B. J. Johnson, D. M.

Allen, M. N. Streeter, W. T. Nichols, J. P. Hutcheson, D. A. Yates, J. N. Martin, and M. F. Miller. 2010. Effects of zilpaterol hydrochloride feeding duration on beef and calf-fed Holstein strip loin steak color. J Anim Sci 88: 1168-1183.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister, and D. E. Johnson. 2002a. Comparison of assays for metmyoglobin reducing ability in beef inside and outside semimembranosus muscle. J. of F. Sci. 67: 978-984.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister, C. L. Kastner, and D. E. Johnson. 2002b. Influence of chemical characteristics of beef inside and outside semimembranosus on color traits. J. Food Sci. 67:1323–1330.

SAS Institute. 2003. SAS proprietary software release 9.1. SAS Institute Inc., Cary, NC.

Sawyer, J. T., Baublits, R. T., Apple, J. K., Meullenet, J. F., Johnson, Z. B., & Alpers, T. K. 2007. Lateral and longitudinal characterization of color stability, instrumental tenderness, and sensory characteristics in the beef semimembranosus. Meat Sci. 75(4): 575–584.

Seyfert, M., R. A. Mancini, M. C. Hunt, J. L. Tang, C. Faustman, and M. Garcia. 2006. Color stability, reducing activity, and cytochrome c oxidase activity of five bovine muscles. J. Agric. Food Chem. 54:8919–8925.

Stewart, M. R., Hutchins, B. K., Zipser, M. W., & Watts, B. M. (1965). Enzymatic reduction of metmyoglobin by ground beef. Journal of Food Science, 30, 487–491.

St. Angelo, A. J., M. Koohmaraie, K. L. Crippen, and J. Crouse. 1991. Acceleration of tenderization inhibition of warmed-over flavor by calcium chloride-antioxidant infusion into lamb carcasses. J. Food Sci. 56: 359-362.

Strydom, P. E. 2000. The effect of different withdrawal period of Zilpaterol on growth and performance, meat quality and muscle glycogen. Meat Industry Centre—ARC—Animal Nutrition and Animal Products Institute, Irene, South Africa.

Strydom, P. E., L. Frylinck, J. L. Montgomery, and M. F. Smith. 2009. The comparison of three [beta]-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci. 81:557-564.

USDA. 2010. Institutional Meat Purchase Specifications. 100 - Fresh Beef. Agric. Marketing Serv., USDA, Washington, DC.

VanOverbeke, D. L., G. G. Hilton, J. Green, M. Hunt, C. Brooks, J. Killefer, M. N. Streeter, J. P. Hutcheson, W. T. Nichols, D. M. Allen, and D. A. Yates. 2009. Effect of zilpaterol hydrochloride supplementation of beef steers and calf-fed Holstein steers on the color stability of top sirloin butt steaks. J Anim Sci 87: 3669-3676.

Vasconcelos, J. T., R. J. Rathmann, R. R. Reuter, J. Leibovich, J. P. McMeniman, K. E. Hales, T. L. Covey, M. F. Miller, W. T. Nichols, and M. L. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J. Anim. Sci. 86: 2005-2015.

Vestergaard, M., K. Sejrsen, and S. Klastrup. 1994. Growth, composition and eating quality of Longissimus dorsi from young bulls fed the b-agonist cimaterol at consecutive developmental stages. Meat Sci. 38:55-66.

Wheeler, T. L., M. Koohmaraie, and S. D. Shackelford. 1997. Effect of postmortem injection time and postinjection aging time on the calcium-activated tenderization process in beef. J. Anim. Sci. 75:2652–2660.

Wheeler, T. L., M. Koohmaraie, J. L. Lansdell, G. R. Siragusa, and M. F. Miller. 1993. Effect of postmortem injection time, injection level, and

concentration of calcium chloride on beef quality traits. J. Anim. Sci. 71:2965–2974.

6.5. Figure and tables

Table 1. Effect of feeding zilpaterol hydrochloride on USDA Select carcass characteristics (n = 20[a]).

Item	Feeding group		*P*-value	Pooled SEM[2]
	Non-ZH-fed	ZH-fed[1]		
Hot carcass weight, kg	383.64	406.23	0.06	6.13
Marbling score[3]	372.00	350.00	0.11	6.93
Fat thickness, cm	1.37	1.37	1.00	0.08
Longissimus area, cm^2	89.68	101.33	< 0.01	2.17
KPH fat[5], %	3.40	3.20	0.51	0.14
USDA yield grade[4]	2.97	2.60	0.28	0.16

[a] Data were calculated after the selection of carcasses.
[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] As determined by Texas Tech personnel: 300 = $Slight^{00}$; 400 = $Small^{00}$; 500 = $Modest^{00}$.
[4] Yield grade as calculated by the regression equation (USDA, 1997).
[5] Kidney, pelvic and heart fat percentage.

Table 2. Effect of $CaCl_2$ injection on weight measures of inside round portions from steers fed or not zilpaterol hydrochloride[1] (n = 40).

		Pre-injection weight, kg	Weight added by injection[a], %	Purge loss[b] on d 7, %
			Feeding group (FG)	
	Non-ZH-fed	6.17	6.08	4.08
	ZH-fed	7.22	5.83	3.76
			Injection treatment (IT)	
	Non-injected	6.74	-	2.02
	$CaCl_2$-injected[3]	6.65	5.95	5.82
	Pooled SEM[2]	0.14	0.50	0.36
P - value	*FG*	< 0.01	0.70	0.44
	IT	0.72	-	< 0.01
	FG x IT	0.65	-	0.93

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[a] Calculated by [(pre-injection weight - weight after injection) / pre-injection weight] x 100.
[b] Calculated by [(pre-injection weight - weight on d 7) / pre-injection weight] x 100.

Table 3. Feeding group and injection treatment means for proximate analysis of inside round steaks before injection (d 3; n = 20) and after injection (d 7; n = 40).

	Protein, %	Fat, %	Moisture, %	Collagen, %
		Day 3 (before injection)		
		Feeding group (FG)		
Non-ZH-fed	22.29	2.36	72.93	1.66
ZH-fed[1]	23.68	1.65	72.37	1.71
P - value	< 0.001	0.09	0.24	0.51
Pooled SEM[2]	0.23	0.21	0.23	0.03
		Day 7 (after injection)		
		Feeding group (FG)		
Non-ZH-fed	23.20	1.43	73.29	1.69
ZH-fed	24.11	1.25	73.16	1.71
P - value	< 0.001	0.35	0.71	0.83
		Injection treatment (IT)		
Non-injected	23.90	1.28	72.97	1.72
$CaCl_2$-injected[3]	23.46	1.38	73.47	1.68
P - value	0.13	0.69	0.02	0.41
Pooled SEM[2]	0.12	0.09	0.11	0.01

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.

Table 4. Feeding group, injection treatment, and postmortem aging time means for purge loss, initial (d 0) and final (d 3) pH and metmyoglobin reducing activity (MRA) of inside round steaks (n = 120).

	Purge loss (%)	Initial pH	Final pH	MRA
		Feeding group (FG)		
Non-ZH-fed	2.17	5.70	5.60	59.04
ZH-fed[1]	2.36	5.68	5.61	60.01
P - value	0.13	0.36	0.44	0.22
		Injection treatment (IT)		
Non-injected	2.27	5.72	5.62	59.07
$CaCl_2$-injected[3]	2.26	5.65	5.59	59.89
P - value	0.95	0.003	0.03	0.40
		Postmortem aging time (d)		
7	3.35[a]	5.61[c]	5.62	58.00[b]
14	1.85[b]	5.81[a]	5.61	59.34[ab]
21	1.60[b]	5.66[b]	5.58	61.15[a]
P - value	< 0.001	< 0.001	0.09	0.04
Pooled SEM[2]	0.009	0.01	0.007	0.52

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[a,b,c] Within a column, means with a different superscript letter differ (P < 0.05).

Table 5. Feeding group and injection treatment means for initial color (n = 240), display color (n = 720) and discoloration (n = 720) of inside round steaks packaged in polyvinyl chloride.

	Initial color[a]	Display color[b]	Discoloration[c]
		Feeding group	
Non-ZH-fed	5.11	5.60	2.81
ZH-fed[1]	4.91	4.84	2.26
P - value	< 0.001	< 0.001	< 0.001
		Injection treatment	
Non-injected	4.89	5.01	2.20
$CaCl_2$-injected[3]	5.13	5.43	2.87
P - value	< 0.001	< 0.001	< 0.001
Pooled SEM[2]	0.06	0.09	0.07

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[a] Initial color score: 4 = moderately light cherry red; 5 = cherry red; 6 = slightly dark red.
[b] Display color score: 4 = slightly dark red; 5 = moderately dark red; 6 = dark red.
[c] Discoloration score: 1 = 0%; 2 = 1-19%; 3 = 20-39%.

Table 6. Muscle area x postmortem aging time and muscle area x display day means for initial color (n = 240), display color (n = 720) and discoloration (n = 720) of inside round steaks packaged in polyvinyl chloride.

	Initial color[1]		Display color[2]		Discoloration[3]	
Muscle area[5]	SSM	DSM	SSM	DSM	SSM	DSM
	5.62	4.40	5.48	4.95	2.39	2.68
Aging time (d)						
7	5.29 ^{a,z}	4.76 ^{b,x}	4.83 ^{a,z}	4.10 ^{b,z}	1.49 ^{a,z}	1.39 ^{a,z}
14	5.56 ^{a,y}	4.14 ^{b,y}	5.40 ^{a,y}	4.84 ^{b,y}	2.27 ^{b,y}	2.55 ^{a,y}
21	6.02 ^{a,x}	4.30 ^{b,y}	6.21 ^{a,x}	5.93 ^{b,x}	3.42 ^{b,x}	4.10 ^{a,x}
P - value	< 0.001		< 0.001		< 0.001	
Display day (d)						
1	-		5.10 ^{a,z}	4.12 ^{b,z}	1.57 ^{b,z}	1.76 ^{a,z}
2	-		5.40 ^{a,y}	5.02 ^{b,y}	2.47 ^{b,y}	2.70 ^{a,y}
3	-		6.01 ^{a,x}	5.66 ^{b,x}	3.13 ^{b,x}	3.59 ^{a,x}
P - value	-		< 0.001		0.02	
Pooled SEM[4]	0.08		0.10		0.08	

[1] Initial color score: 4 = moderately light cherry red; 5 = cherry red; 6 = slightly dark red.
[2] Display color score: 4 = slightly dark red; 5 = moderately dark red; 6 = dark red.
[3] Discoloration score: 1 = 0%; 2 = 1-19%; 3 = 20-39%; 4 = 40-59%; 5 = 60-79%.
[4] Pooled (largest) SE of LS means.
[a,b] Within a row and color trait, means with a different superscript letter differ (P < 0.05).
[x,y,z] Within a column and main effect, means with a different superscript letter differ (P < 0.05).
[5] SSM – Superficial semimembranosus; DSM – Deep semimembranosus.

Table 7. Feeding group x post-mortem aging time x display day means for display color ($P < 0.001$) and discoloration ($P < 0.001$) of inside round steaks packaged in polyvinyl chloride (n = 720).

	Feeding group	Aging time (d)	Display day (d)		
			1	2	3
Display color[3] SE[2] = 0.12	Non-ZH-fed	7	4.23 ^{c,z,A}	4.61 ^{b,z,A}	5.30 ^{a,z,A}
		14	4.97 ^{c,y,A}	5.38 ^{b,y,A}	6.31 ^{a,y,A}
		21	5.83 ^{b,x,A}	6.67 ^{a,x,A}	6.99 ^{a,x,A}
	ZH-fed[1]	7	3.79 ^{c,y,B}	3.88 ^{bc,z,B}	4.98 ^{a,y,B}
		14	4.34 ^{c,x,B}	4.58 ^{b,y,B}	5.15 ^{a,y,B}
		21	4.51 ^{b,x,B}	6.05 ^{a,x,B}	6.28 ^{a,x,B}
Discoloration[4] SE[2] = 0.11	Non-ZH-fed	7	1.15 ^{b,z,A}	1.35 ^{b,z,A}	1.97 ^{a,z,A}
		14	1.59 ^{c,y,A}	2.49 ^{b,y,A}	4.04 ^{a,y,A}
		21	2.96 ^{b,x,A}	4.78 ^{a,x,A}	4.97 ^{a,x,A}
	ZH-fed[1]	7	1.07 ^{b,y,A}	1.24 ^{b,z,A}	1.87 ^{a,z,A}
		14	1.29 ^{c,y,B}	2.06 ^{b,y,B}	3.00 ^{a,y,B}
		21	1.94 ^{c,x,B}	3.60 ^{b,x,B}	4.31 ^{a,x,B}

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] Display color score: 3 = dull red; 4 = slightly dark red; 5 = moderately dark red; 6 = dark red.
[4] Discoloration score: 1 = 0%; 2 = 1-19%; 3 = 20-39%; 4 = 40-59%; 5 = 60-79%.
^{a,b,c} Within a row, means with a different superscript letter differ ($P < 0.05$).
^{x,y,z} Within a column, among aging time for color attribute, means with a different superscript letter differ ($P < 0.05$).
^{A,B} Within a column, among feeding group for color attribute, means with a different superscript letter differ ($P < 0.05$).

Table 8. Injection treatment x post-mortem aging time x display day means for display color (P = 0.01) and discoloration (P = 0.02) of inside round steaks packaged in polyvinyl chloride (n = 720).

	Injection treatment	Aging time (d)	Display day (d)		
			1	2	3
Display color[3] SE[2] = 0.12	Non-injected	7	3.93 ^{c,y,A}	4.33 ^{b,z,A}	5.16 ^{a,z,A}
		14	4.61 ^{b,x,A}	4.75 ^{b,y,B}	5.42 ^{a,y,B}
		21	4.72 ^{b,x,B}	6.05 ^{a,x,B}	6.12 ^{a,x,B}
	$CaCl_2$-injected[1]	7	4.09 ^{b,z,A}	4.16 ^{b,z,A}	5.12 ^{a,z,A}
		14	4.70 ^{c,y,A}	5.20 ^{b,y,A}	6.04 ^{a,y,A}
		21	5.63 ^{c,x,A}	6.77 ^{b,x,A}	7.15 ^{a,x,A}
Discoloration[4] SE[2] = 0.11	Non-injected	7	1.14 ^{b,y,A}	1.32 ^{b,z,A}	1.89 ^{a,z,A}
		14	1.27 ^{c,y,B}	2.00 ^{b,y,B}	3.00 ^{a,y,B}
		21	1.74 ^{c,x,B}	3.45 ^{b,x,B}	3.99 ^{a,x,B}
	$CaCl_2$-injected[1]	7	1.07 ^{b,z,A}	1.28 ^{b,z,A}	1.95 ^{a,z,A}
		14	1.61 ^{c,y,A}	2.55 ^{b,y,A}	4.04 ^{a,y,A}
		21	3.16 ^{c,x,A}	4.93 ^{b,x,A}	5.29 ^{a,x,A}

[1] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[2] Pooled (largest) SE of LS means.
[3] Display color score: 3 = dull red; 4 = slightly dark red; 5 = moderately dark red; 6 = dark red.
[4] Discoloration score: 1 = 0%; 2 = 1-19%; 3 = 20-39%; 4 = 40-59%; 5 = 60-79%.
^{a,b,c} Within a row, means with a different superscript letter differ (P < 0.05).
^{x,y,z} Within a column, among aging time for color attribute, means with a different superscript letter differ (P < 0.05).
^{A,B} Within a column, among injection treatment for color attribute, means with a different superscript letter differ (P < 0.05).

Table 9. Muscle area, feeding group, injection treatment, aging time and display day means for L*, a* and b* of inside round steaks packaged in polyvinyl chloride (n = 480).

	L*	a*	b*
		Muscle area	
Superficial SM[4]	36.67	21.09	19.61
Deep SM	44.29	17.99	20.53
P - value	< 0.001	< 0.001	0.01
		Feeding group	
Non-ZH-fed	38.89	19.22	19.98
ZH-fed[1]	42.07	19.85	20.15
P - value	< 0.001	0.31	0.11
		Injection treatment	
Non-injected	40.37	20.13	20.28
$CaCl_2$-injected[3]	40.60	18.94	19.86
P - value	0.45	0.05	0.23
		Aging time (d)	
7	40.60	21.95 [a]	21.57 [a]
14	40.58	20.22 [a]	20.74 [a]
21	40.26	16.44 [b]	17.89 [b]
P - value	0.77	< 0.001	0.01
		Display day (d)	
0	40.75	24.62	23.07
3	40.21	14.45	17.07
P - value	0.21	< 0.001	0.01
Pooled SEM[2]	0.21	0.31	0.21

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[4] SM –semimembranosus muscle.

Table 10. Muscle area[2] x postmortem aging time x display day means for L* (P = 0.98), a* (P = 0.01) and b* (P = 0.04) of inside round steaks packaged in polyvinyl chloride (n = 480).

	Feeding group	Display day (d)	Aging time (d)		
			7	14	21
L*	SSM (SE[1] = 0.16)	0	35.99 ^{b,x,B}	36.35 ^{b,x,AB}	38.39 ^{b,x,A}
		3	37.43 ^{b,x,A}	36.73 ^{b,x,AB}	35.16 ^{b,y,B}
	DSM (SE = 0.21)	0	43.83 ^{a,x,A}	44.45 ^{a,x,A}	45.51 ^{a,x,A}
		3	45.17 ^{a,x,A}	44.78 ^{a,x,A}	42.00 ^{a,y,B}
a*	SSM (SE = 0.43)	0	26.63 ^{a,x,A}	27.44 ^{a,x,A}	22.69 ^{a,x,B}
		3	21.32 ^{a,y,A}	16.55 ^{a,y,B}	11.90 ^{a,y,C}
	DSM (SE = 0.40)	0	24.83 ^{a,x,A}	24.99 ^{a,x,A}	21.70 ^{a,x,B}
		3	15.02 ^{b,y,A}	11.90 ^{b,y,A}	10.00 ^{a,y,B}
b*	SSM (SE = 0.25)	0	23.64 ^{a,x,A}	24.92 ^{a,x,A}	19.06 ^{b,x,B}
		3	18.90 ^{a,y,A}	15.74 ^{a,y,B}	15.39 ^{a,y,B}
	DSM (SE = 0.24)	0	24.74 ^{a,x,A}	25.36 ^{a,x,A}	20.68 ^{a,x,B}
		3	19.01 ^{a,y,A}	16.93 ^{a,y,B}	16.44 ^{a,y,B}

[1] Pooled (largest) SE of LS means.
^{a,b} Within a column, among muscle area for color attribute, means with a different superscript letter differ ($P < 0.05$).
^{x,y} Within a column, among display day for color attribute, means with a different superscript letter differ ($P < 0.05$).
^{A,B,C} Within a row, means with a different superscript letter differ ($P < 0.05$).
[2] SSM – Superficial semimembranosus; DSM – Deep semimembranosus.

Table 11. Display day x feeding group, display day x injection treatment and display day x post-mortem aging time means for L*, a* and b* of inside round steaks packaged in polyvinyl chloride (n = 480).

	L*		a*		b*	
Display day	0	3	0	3	0	3
			Feeding group			
Non-ZH-fed	39.04	38.74	24.94 ^{a,x}	13.51 ^{b,y}	23.40 ^{a,x}	16.56 ^{b,y}
ZH-fed[1]	42.46	41.68	24.31 ^{a,x}	15.39 ^{b,x}	22.73 ^{a,x}	17.58 ^{b,x}
P - value	0.34		< 0.001		< 0.001	
			Injection treatment			
Non-injected	40.83	39.90	24.86 ^{a,x}	15.40 ^{b,x}	23.02 ^{a,x}	17.54 ^{b,x}
$CaCl_2$-injected[3]	40.67	40.52	24.39 ^{a,x}	13.50 ^{b,y}	23.11 ^{a,x}	16.60 ^{b,y}
P - value	0.23		0.026		< 0.001	
			Aging time (d)			
7	39.91 ^{a,x}	41.30 ^{a,x}	25.73 ^{a,x}	18.17 ^{b,x}	24.19 ^{a,x}	18.95 ^{b,x}
14	40.40 ^{a,x}	40.76 ^{a,x}	26.21 ^{a,x}	14.23 ^{b,y}	25.14 ^{a,x}	16.34 ^{b,y}
21	41.95 ^{a,x}	38.58 ^{b,y}	21.93 ^{a,y}	10.95 ^{b,z}	19.87 ^{a,y}	15.92 ^{b,y}
P - value	< 0.001		< 0.001		< 0.001	
Pooled SEM[2]	0.31	0.30	0.17	0.35	0.17	0.14

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
^{a,b} Within a row, for color attribute, means with a different superscript letter differ (P < 0.05).
^{x,y,z} Within a column, for main effect, means with a different superscript letter differ (P < 0.05).

Table 12. Amount of thiobarbituric acid reactive substances (TBARS; mg/kg) in inside round steaks according to feeding group, injection treatment and aging time (n = 120).

	Feeding group
Non-ZH-fed	0.17
ZH-fed[1]	0.14
P - value	0.14
	Injection treatment
Non-injected	0.14
$CaCl_2$-injected[3]	0.17
P - value	0.01
	Aging time (d)
7	0.13 [b]
14	0.14 [b]
21	0.19 [a]
P - value	<0.0001
Pooled SEM[2]	0.01

[1] Zilpaterol hydrochloride 8.3 mg/kg DM basis for 20 d; Zilmax®, Intervet Schering Plough, DeSoto, KS.
[2] Pooled (largest) SE of LS means.
[3] Calcium chloride food grade at 200 mM at 5% (wt/wt); Tetra Technologies, The Woodlands, TX.
[a,b] Within a column, means with a different superscript letter differ (P < 0.05)

7. CONCLUSÃO GERAL

A suplementação com hidrocloreto de zilpaterol nos últimos 20 dias de confinamento resulta em prejuízo da maciez objetiva do coxão mole, porém torna a cor da carne mais estável durante a exposição em "display".

A maturação, por períodos de 14, 21 e 28 dias, progressivamente reduz a WBSF das amostras de coxão mole em relação às determinações obtidas aos 7 dias. Contudo há prejuízo a estabilidade da cor das amostras expostas em "display" por até 3 dias.

A maturação por períodos prolongados (14 dias) dos bifes de coxão mole de novilhos suplementados com HZ equipara as médias de WBSF e dos atributos avaliados sensorialmente em relação às de animais não-suplementados.

A aplicação de $CaCl_2$, por injeção pós-rigor, reduz a WBSF das amostras de coxão mole, contudo prejudica a estabilidade da cor durante a exposição em "display". As amostras de coxão mole de novilhos não-suplementados e que foram injetadas com $CaCl_2$ tiveram as menores médias de WBS em relação a todas as outras aos 7 dias de maturação.

A injeção de $CaCl_2$ em amostras de bovinos suplementados com HZ seguida de maturação por 7 dias consegue equiparar as médias de WBSF em relação aos não-suplementados e não-injetados, sem alterar a estabilidade da cor.