Universidade Estadual de Londrina

GUILHERME AGOSTINIS FERREIRA

ANÁLISE INTEGRADA DA CARNE DFD EM BOVINOS NELORE:
TERMOGRAFIA INFRAVERMELHA E AVALIAÇÃO SENSORIAL

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“Os tolos se multiplicam quando os sábios ficam em silêncio.”

Nelson Mandela

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RESUMO

Esta tese discute o fenômeno da carne de corte escuro, também conhecida como DFD na espécie bovina em condições tropicais e está dividida em três estudos acerca do tema. O primeiro estudo teve como objetivo investigar as diferenças entre carne bovina com valores intermediários e altos de pHu e de características físico-químicas, além de avaliar a correlação entre biomarcadores sanguíneos ligados ao estresse e o pH final (pHu) de carcaças. O estudo constatou que os bifes do grupo de pH intermediário e alto apresentaram valores de a* e b* mais baixos do que os bifes do grupo normal, com diferença apenas na luminosidade entre eles. Os grupos de pH intermediário e alto apresentaram maior conteúdo de Metamioglobina do que o normal. O grupo de pH alto foi o mais macio entre todos os grupos. Com relação aos biomarcadores sanguíneos, houve correlação moderada entre os biomarcadores sanguíneos e o pHu, indicando que a mensuração dos parâmetros sanguíneos no momento do abate pode ser uma ferramenta útil para identificar carcaças com diferentes pHu. O segundo estudo avaliou a percepção do consumidor brasileiro sobre carne bovina com diferentes valores de pHu por meio de uma análise sensorial. O estudo constatou que os consumidores gostaram mais dos bifes de pH mais elevado em termos de maciez, e houve uma tendência para o mesmo comportamento em termos de suculência. Não houve diferença nos demais parâmetros avaliados. Os modelos de regressão indicaram uma forte influência da cor e aparência de frescor, além de maciez e suculência nas pontuações gerais de preferência dos consumidores. O terceiro estudo explorou o uso da termografia infravermelha (IRT) para identificar o fenômeno DFD na carne bovina brasileira. Os resultados indicaram correlações significativas entre a termografia infravermelha (TRI) e vários preditores. O IRT max sozinho apresentou um valor notável de R-quadrado (R^2) de 0,75, indicando uma forte relação com a variável desfecho. A combinação da TRI máx com diferentes variáveis, como Glicose e Lactato, resultou em melhora dos valores de R^2, chegando até 0,80. Essas descobertas ressaltam o potencial da TRI como uma ferramenta valiosa para modelagem preditiva em nosso estudo. De forma geral, os estudos destacam a importância de entender o fenômeno DFD e desenvolver ferramentas eficazes para identificar carcaças com alto pHu para melhorar a qualidade da carne.

Palavras-chave: Anomalia da carne. Estresse. IRT. Qualidade da carne.

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ABSTRACT

This thesis discusses the phenomenon of dark cutting beef, also known as DFD in the bovine species under tropical conditions, and is divided into three studies on the subject. The first study aimed to investigate the differences between beef with intermediate and high pHu values and physical-chemical characteristics, as well as to evaluate the correlation between blood biomarkers related to stress and the final pH (pHu) of carcasses. The study found that steaks from the intermediate and high pH groups had lower a* and b* values than steaks from the normal group, with differences only in brightness between them. The intermediate and high pH groups had a higher content of Metmyoglobin than the normal group. The high pH group was the most tender among all the groups. Regarding blood biomarkers, there was a moderate correlation between blood biomarkers and pHu, indicating that measuring blood parameters at the time of slaughter can be a useful tool for identifying carcasses with different pHu.The second study evaluated the perception of Brazilian consumers regarding beef with different pHu values through sensory analysis. The study found that consumers liked steaks with higher pH values in terms of tenderness, and there was a trend for the same behavior in terms of juiciness. There were no differences in the other evaluated parameters. Regression models indicated a strong influence of color, freshness appearance, tenderness, and juiciness on consumers' overall preference scores. The third study explored the use of infrared thermography (IRT) to identify the DFD phenomenon in Brazilian beef. The results indicated significant correlations between infrared thermography (IRT) and various predictors. IRT max alone showed a notable R-squared (R^2) value of 0.75, indicating a strong relationship with the outcome variable. Combining IRT max with different variables, such as glucose and lactate, improved R^2 values, reaching up to 0.80. These findings highlight the potential of IRT as a valuable tool for predictive modeling in our study. Overall, the studies emphasize the importance of understanding the DFD phenomenon and developing effective tools to identify carcasses with high pHu to improve meat quality.

Keywords: Beef anomaly. IRT. Meat quality. Pre-slaughter stress.

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LISTA DE ABREVIATURAS E SIGLAS

a*	Intensidade De Vermelho-Verde
ABIEC	Associação Brasileira Das Indústrias Exportadoras De Carnes
AMP	Adenosina Monofosfato
ATP	Adenosina Trifosfato
b*	Intensidade De Amarelo-Azul
C*	Croma
CEP	Comitê De Ética Em Pesquisa Envolvendo Seres Humanos
CIELAB	Espaço De Cor
CK	Creatina Quinase
DBT	Temperatura De Bulbo Seco
DeoMb	Deoximioglobina
DFD	Carne escura, firme e seca
DFDa	Carne escura atípica
DFDt	Carne escura típica
H+	Íons Positivos
Hº	Ângulo Hue
IRT max	Temperatura Ocular Máxima
IRT min	Temperatura Ocular Mínima
IRT	Termografia De Infravermelho
L*	Luminosidade
LDH	Lactato Desidrogenase
LT	Músculo Longíssimus Thoracis
Mb	Mioglobina
MetMb	Metamioglobina
O2	Oxigênio

OxiMb	Oximioglobina
pH	Potencial Hidrogeniônico
pHu	Potencial Hidrogeniônico Final
RFN	Carne Normal Referência
RH	Umidade Relativa
SEM	Erro Padrão Médio
UEL	Universidade Estadual De Londrina
WBSF	Método Warner Bratzler Shear Force

SUMÁRIO

1 INTRODUÇÃO

O pH que a carne alcançará quando a glicólise estiver concluída é denominado pH final (pHu). Para ter certeza de que todas as carnes atingiram o pH mínimo, o pHu normalmente é mensurado de 24 a 48 horas após o abate na carne bovina e é usado como um indicador dos atributos de qualidade da carne (REIS; ROSENVOLD, 2014). Carnes que apresentam pH final menor que 5,8 são consideradas como pH final normal e apresentam atributos desejáveis de qualidade (VILJOEN; DE KOCK; WEBB, 2002b). Contudo, carnes com pH final maior que 5,8 apresentam cor mais escura (XIAO et al., 2015), uma vida útil mais curta (FAUCITANO et al., 2010), pode ser inferior em sabor (APPLE et al., 2011) e tendem a apresentar maciez variável (SILVA; PATARATA; MARTINS, 1999; WATANABE; DALY; DEVINE, 1996).

Casos de estresse muito prolongado podem levar ao esgotamento das reservas de glicogênio muscular e a baixa produção de ácido lático na glicólise anaeróbica (WATANABE; DALY; DEVINE, 1996). Assim, o pH que deveria cair em torno de 5,8 fica estabilizado acima de 6,0 caracterizando a carne *dark, firm and dry* (DFD). A carne DFD, pela aparência escura, é rejeitada pelo consumidor para o consumo “in natura”.

Fatores estressantes que ocorrem durante o manejo pré-abate resultam em perda da qualidade da carne, como o aparecimento de carnes DFD (carne de cor escura, textura dura e que retém muita água) (LOSADA-ESPINOSA et al., 2018). O estresse pré-abate pode ser provocado por vários fatores como mistura de animais não conhecidos, machos não castrados (sodomia e brigas), caminhões mal projetados com rampas de embarque muito inclinadas, densidade de transporte muito alta ou baixa, tempo e distância de transporte, tempo de jejum muito longo, uso de bastão elétrico, gritos, outros animais na condução dos animais, ou sendo simplesmente caracterizados como fisiológicos, morfométricos, comportamentais e relacionadas a qualidade da carne (LOSADA-ESPINOSA et al., 2018).

Pelos métodos tradicionais, a carne de bovinos é classificada por meio dos valores de pH, capacidade de retenção de água e cor (luminosidade). Entretanto, essas metodologias são demoradas e não permitem classificar a carne de bovinos na linha de abate. Estudos utilizando a Termografia por Infravermelho (IRT) vem se mostrando promissores, não só no controle da ambiência e conforto térmico dos animais mensurando radiação solar, temperatura do ar, umidade relativa, ventos e precipitação (BARRETO et al., 2020; GERSONY et al., 2018; KARVATTE et al., 2020; KIM et al., 2016), ou mensurando padrões de temperatura da superfície corporal objetivando identificar processos metabólicos (COOK et al., 2016;

MONTANHOLI et al., 2009, 2010), mas também na área da ciência da carne (HORCADA et al., 2019).

Apesar da escassez de estudos que abordem a temática da avaliação da qualidade da carcaça, alguns trabalhos mostraram a eficiência dessa tecnologia em predizer graus de acabamento de musculosidade da carcaça (CORRÊA, 2019), além de identificar carcaças que produzirão carne de maciez indesejada. Mensurações realizadas ainda na fazenda antes dos animais serem transportados por diferentes meios para identificar parâmetros de qualidade na carne também foram conduzidos na Austrália (CUTHBERTSON et al., 2020), apontaram a possibilidade da utilização da IRT para auxiliar no monitoramento tanto do bem-estar animal, quanto da qualidade da carne.

Em estudo conduzido no Canadá (SCHAEFER et al., 2018), objetivando testar a eficácia da termografia infravermelha na identificação de animais vivos com risco de anomalias na qualidade da carne bovina, como a DFD e animais febris, concluíram que a IRT associada a terapias nutricionais é capaz de identificar a anomalia em plantas comerciais.

Em resumo, o pH final da carne é um indicador importante dos atributos de qualidade da carne bovina, e o estresse pré-abate pode levar à carne DFD, que é de qualidade inferior. Métodos tradicionais de classificação de carne são demorados e não permitem classificação na linha de abate. No entanto, a IRT vem se mostrando uma tecnologia promissora na avaliação da qualidade da carne, permitindo a identificação de anomalias na qualidade da carne em animais vivos. Estudos têm mostrado a eficácia da IRT na identificação de anomalias como a DFD e na predição de graus de acabamento de musculosidade da carcaça, o que pode ajudar na melhoria da qualidade da carne bovina.

2 REFERENCIAL TEÓRICO

2.1 DEFINIÇÃO E DESCRIÇÃO DE CARNES DFD

A sigla DFD significa "Dark, Firm, and Dry" em inglês, que pode ser traduzido como "Escura, Firme e Seca" em português. A origem da nomenclatura se deve às características físicas e sensoriais da carne afetada pelo fenômeno, que apresenta uma cor mais escura do que o normal, é mais firme ao toque e menos exsudativa, o que a torna mais seca em sua superfície. Outro termo ligado a esse fenômeno é "Dark-cutting" em inglês, que poderia ser traduzido como carne escura, ou corte de carne escura, em português.

A carne DFD, ou carne escura, é caracterizada por uma coloração escura (Dark), firmeza excessiva (Firm) e pouca umidade superficial (Dry), resultantes da pequena queda do pH muscular após o abate do animal (PONNAMPALAM et al., 2017; TARRANT, 1989; TERLOUW et al., 2021). Esse fenômeno ocorre devido à rápida depleção dos estoques de glicogênio muscular e resulta na falta de acidificação normal da carne durante o desenvolvimento do rigor causando o pHu > 5,8. É geralmente definida como a carne com um pH final elevado (pHu).

A carne DFD pode ser encontrada em diferentes espécies animais, mas é mais comum em bovinos e ovinos, por conta da presença de fibras vermelhas – oxidativas – presentes nessas espécies animais (PONNAMPALAM et al., 2017), e é mais frequente em animais submetidos a situações estressantes antes do abate

Em muitos países, carcaças com um limite de pHu ≥ 6,0 no contra-filé são definidas como carne escura, incluindo a indústria e para experimentos laboratoriais (GAGAOUA et al., 2021). Existem exceções; por exemplo, a indústria de carne bovina australiana especifica um limite de pHu > 5,7 para carne escura e falha na classificação, não apenas devido à dureza da carne com pH intermediário (5,8 < pHu < 6,2) medida por instrumentos e painéis sensoriais (GRAYSON et al., 2016a), mas também porque compradores experientes de carne discriminam a carne com um pHu ≥ 5,8 (TRUSCOTT et al., 1988). Para consumidores chineses, o limite pode ser mais alto (> 6,2) (ZHANG et al., 2021).

Gagaoua et al. (2021) recentemente ressaltou que apesar da maioria dos estudos citarem o conteúdo de glicogênio muscular no abate como principal fator que influencia o pHu, o determinante é a inerente concentração de íons de hidrogênio, que são gerados predominantemente por meio da glicólise e da formação de lactato a partir do glicogênio, mas também da hidrólise da adenosina trifosfato (ATP), que é revisado em alguns estudos

(APAOBLAZA et al., 2020; SCHEFFLER; GERRARD, 2007). Os autores ainda citam três aspectos relacionados ao fenômeno DFD que devem ser levados em consideração, como tempo de classificação das carcaças, a taxa de queda de pH e temperatura, além do tipo de músculo, que influencia, entre outros, o conteúdo de glicogênio.

2.2 INCIDÊNCIA E IMPLICAÇÕES ECONÔMICAS DE CARNES DFD

A classificação de carcaças bovinas é influenciada pela cor e pH no momento da avaliação, o que pode resultar em carne de corte escuro. Isso é particularmente importante em esquemas de classificação, como o Meat Standards Australia, que podem ocorrer em até 12 horas após o abate (GAGAOUA et al., 2021). Carcaças que não são classificadas devido a um pHu elevado podem ter um impacto econômico significativo, uma vez que seu valor de mercado é reduzido.

Alguns estudos foram feitos objetivando avaliar o momento de avaliação da cor e pHu das carcaças. Um dos estudos australianos concluiu que a porcentagem de carcaças não conformes era alterada de acordo com o momento em que a avaliação era realizada, a medida que se aumentava o intervalo de mensuração em 16 horas, 5% a menos eram classificadas como não conforme (HUGHES; KEARNEY; WARNER, 2014). Outro estudo demonstrou que a redução do número de carcaças classificadas como não conforme foi aproximadamente 3% menor quando mensurado mais tardiamente, cerca de 22 horas após o abate (STEEL et al., 2021).

Embora possa parecer que o momento da classificação esteja diretamente relacionado ao pH final, alguns estudos indicam que ocorre algum desenvolvimento de cor e clareamento da superfície muscular em torno do rigor mortis, independentemente do pH (HUGHES et al., 2020). Portanto, embora seja recomendado classificar as carcaças em um momento posterior, isso nem sempre é viável. Sendo assim, o momento da classificação para cor da carne e pH deve ser considerado como um fator determinante do corte escuro (GAGAOUA et al., 2021).

Não há dados precisos sobre a incidência da carne DFD no Brasil e em outros países em números absolutos, pois as taxas de ocorrência variam amplamente dependendo de muitos fatores, como raça, manejo, dieta, abate e processamento. Além disso, a avaliação da carne DFD pode ser subjetiva e depende da metodologia de avaliação utilizada. No entanto, estudos realizados em diversos países sugerem que a carne DFD é um problema significativo na indústria da carne bovina em todo o mundo, variando de 1,3 a 13,9% (PONNAMPALAM et al., 2017), e em alguns casos, a taxa de incidência foi de até 25% (HUGHES et al., 2017a) ou superior (MILLER, 2007). Para exemplificar, em alguns países, como os Estados Unidos e Canada, a incidência de carne DFD pode variar de 2% a 4% da produção total de carne bovina (HOLDSTOCK et al., 2014; MOORE et al., 2012). No Brasil, a taxa de ocorrência pode variar de acordo com a região, mas também pode chegar a níveis significativos, podendo variar de 4 a 8% (BARÓN et al., 2021; ROSA et al., 2016).

2.3 CAUSAS BIOQUÍMICAS

A carne DFD (Dark, Firm, Dry) é causada por uma interação complexa entre vários processos bioquímicos que ocorrem no músculo do animal após o abate. Os processos bioquímicos envolvidos incluem a glicólise anaeróbica, o metabolismo do glicogênio, a atividade das enzimas proteolíticas e a atividade bacteriana (FINK; SCHOENFELD; NAKAZATO, 2017; LAWRENCE; FOWLER; NOVAKOFSKI, 2012; PONNAMPALAM et al., 2017).

Em condições normais, a glicólise aeróbica é a principal fonte de energia para os músculos durante o exercício, e a energia é gerada na forma de ATP. No entanto, após o abate, a glicólise anaeróbica continua ocorrendo nos músculos, mas sem o fornecimento contínuo de oxigênio, inviabilizando a utilização da cadeia respiratória. Então, a glicose gerada a partir do glicogênio muscular passa a ser metabolizada pela glicólise e gerar lactato, $H+$ e ATP, fazendo com que o pH do músculo caia, levando a uma redução no pH muscular (ENGLAND et al., 2016; SCHEFFLER et al., 2015).

A depleção do glicogênio muscular pode ser causada por atividades relacionadas ao estresse físico, principalmente, como transporte, restrição alimentar, ou mistura de lotes (WARRISS, 1990), levando ao aparecimento da carne escura, caso não haja tempo e condições de recuperação antes do abate (PONNAMPALAM et al., 2017). Os sistemas nervosos simpático e parassimpático regulam a resposta e a recuperação do estresse. Quando um animal percebe uma ameaça ou experimenta estresse, o sistema nervoso simpático é ativado, causando a liberação de catecolaminas (epinefrina e norepinefrina) da medula adrenal. Essas catecolaminas estimulam a resposta de fuga ou luta do animal, promovendo a glicogenólise e a lipólise, que geram a energia necessária para essa resposta. Eventualmente, essa estimulação leva à depleção dos estoques de glicogênio muscular e hepático e de ácidos graxos celulares (PONNAMPALAM et al., 2017).

Além dos mecanismos destacados acima, a ocorrência da carne escura em bovinos também está relacionada ao estresse pré-abate, que reduz o teor de glicogênio muscular e, assim, limita a queda do pH muscular (KIYIMBA et al., 2021; MAHMOOD et al., 2018; POLETI et al., 2018; PONNAMPALAM et al., 2017). Como indicado acima, um pH muscular mais elevado é um fator propício para um aumento do consumo de oxigênio mitocondrial, o que limita a disponibilidade de oxigênio para a mioglobina e permite que as fibras musculares retenham mais água (RAMANATHAN et al., 2012; TANG et al., 2005). Esses efeitos combinados explicam a cor mais escura e os níveis mais elevados de deoximioglobina (DeoMb) na carne escura em comparação com a carne normal (ENGLISH et al., 2016; HUGHES et al., 2014).

A quantidade de glicogênio muscular é afetada pelas características inerentes do músculo. Músculos que são predominantemente compostos por tipos de fibras vermelhas normalmente têm níveis mais baixos de glicogênio muscular (PICARD; GAGAOUA; GAGAOUA, 2020), resultando em glicólise *post-mortem* limitada, pHu mais alto e maior probabilidade de corte escuro.

Por outro lado, é importante observar que se uma carcaça bovina for classificada como de corte escuro com base na mensuração do pHu do músculo *Longissimus thoracis et lumborum*, isso não implica necessariamente que todos os músculos da carcaça terão a mesma propriedade (HOLMAN et al., 2019). Vários fatores, como diferenças no tipo de fibra e propriedades metabólicas (PICARD et al., 2014), variação na sensibilidade do músculo ao estresse (TERLOUW et al., 2021), a taxa de resfriamento *post mortem* da carcaça (HOPKINS et al., 2014), liberação de adrenalina e atividade contrátil (por exemplo, comportamento de monta) (TARRANT, 1989), podem afetar a manifestação dessa característica. Consequentemente, mesmo que o músculo *Longissimus* tenha pHu > 6,0, outros músculos como *Semimembranosus, Gluteus medius, Biceps femoris e o Psoas major* podem ter níveis de pHu mais baixos e não ser de corte escuro (KENNY; TARRANT, 1987).

2.4 FATORES QUE CONTRIBUEM PARA O APARECIMENTO DE DFD

2.4.1 Na Fazenda

A ocorrência da carne DFD em bovinos na fazenda pode ser afetada por diversos fatores que contribuem para o estresse dos animais, tais como variação climática, gênero, genética e temperamento (PONNAMPALAM et al., 2017).

A nutrição inadequada do gado pode afetar a ocorrência da carne DFD. Uma dieta desequilibrada e/ou pobre em nutrientes pode afetar a formação de glicogênio no músculo, o que pode levar à formação de carne DFD. Alguns estudos mostram que os maiores índices do aparecimento do fenômeno ocorre em fases de transição, e uma delas é a mudança para o pasto mais velho ou de baixa qualidade nutricional, ou mesmo jovem porem baixo (MCGILCHRIST et al., 2014). Da mesma forma, animais criados em pastejo têm acesso a uma dieta com maior teor proteico do que energético, o que pode influenciar diretamente o peso da carcaça, a espessura da gordura de acabamento, as reservas de glicogênio muscular, a taxa de depleção do glicogênio durante o transporte e o abate, bem como a taxa de resfriamento da carcaça durante o período pós-morte (PONNAMPALAM et al., 2017).

Não há um consenso em relação a haver maior incidência de carnes de corte escuro em sistemas de pastejo ou confinamento. Alguns trabalhos concluíram que animais criados em pastejo apresentam pHu mais elevado do que os animais criados em confinamento (HUGHES et al., 2014; MUIR; DEAKER; BOWN, 1998), mas o sistema de produção deve ser levado em consideração, porque a idade em que o animal será abatido irá interferir no aparecimento ou não do fenômeno DFD. Em alguns casos, os animais sob pastejo podem ser abatidos mais tardiamente e ter acesso a uma dieta de baixa densidade energética, e haver a necessidade de suplementação (MCPHAIL et al., 2014; VESTERGAARD; OKSBJERG; HENCKEL, 2000).

A raça do gado também pode afetar a ocorrência da carne DFD. Algumas raças podem ter uma maior predisposição a desenvolver carne DFD do que outras. Estudos mostraram que a incidência desse fenômeno pode estar relacionada ao fenótipo do animal, ao apresentar menor área de olho de lombo, peso de carcaça, gordura subcutânea (MCGILCHRIST et al., 2012). Outros estudos avaliando a incidência do fenômeno DFD em duas faixas diferentes de pHu, concluíram que carcaças de carne de corte escuro possuíam menores pesos de carcaça e musculatura em relação as carcaças normais (HOLDSTOCK et al., 2014; MAHMOOD et al., 2016a, 2016b; MCGILCHRIST et al., 2012). Os pesquisadores associaram estes resultados a uma possível ausência de situação de estresse adicional, ou até mesmo uma associação com uma menor resistência desses animais em se recuperar de situações de estresse.

Em relação a gênero, um estudo canadense conclui que novilhas são mais propensas a apresentarem carnes de corte escuro do que novilhos, por terem menores pesos de desmame e abate (MAHMOOD et al., 2016a, 2016b). Até o momento, não foi estabelecida uma relação genética clara para a ocorrência de corte escuro em bovinos. No entanto, foi relatada uma correlação genética de 0,69 entre o glicogênio muscular e os padrões de cor da carne bovina, enquanto a herdabilidade do glicogênio foi estimada em 0,34 para o gado preto japonês

(KOMATSU et al., 2014).

Em um estudo realizado por Muchenje et al. (2009), foi observado que algumas raças bovinas apresentaram uma resposta ao estresse mais elevada do que outras, mesmo quando criadas na mesma fazenda. No entanto, a raça com os níveis mais baixos de catecolaminas produziu carne bovina com o maior pH intramuscular médio e o menor valor médio de L∗, o que sugere que a redução da glicose plasmática e intramuscular pode diminuir a resposta ao estresse sistêmico.

As diferenças genéticas decorrentes da adaptação tropical, como as encontradas em bovinos das espécies *Bos taurus e Bos indicus*, também podem contribuir para a prevalência de carne com corte escuro (PONNAMPALAM et al., 2017). Em estudo conduzido nos Estados Unidos com mais de 700 mil cabeças de gado, relatou um aumento na prevalência de carne com corte escuro em frigoríficos que processaram animais provenientes dos cruzamentos *Bos taurus* e *Bos indicus* em comparação com aqueles que processaram apenas *Bos taurus* (KREIKEMEIER; UNRUH; ECK, 1998).

Por outro lado, Shackelford et al. (1994) e Page et al. (2001) não relataram diferenças significativas na cor do músculo da carcaça ou no pH devido à genética tropicalmente adaptada em bovinos. Em resumo, a prevenção da carne DFD começa com um manejo adequado do gado, incluindo o fornecimento de uma dieta equilibrada e uma gestão cuidadosa do estresse pré-abate.

2.4.2 Fora Da Fazenda:

Além dos fatores que afetam a carne DFD na fazenda, existem outros fatores que podem afetar a ocorrência dessa condição fora da fazenda, durante o transporte e abate dos animais. O transporte é uma etapa crítica na cadeia produtiva da carne e pode ser um fator importante na ocorrência de carne DFD (BRANDT; AASLYNG, 2015). O estresse gerado pelo transporte pode levar à liberação de hormônios como o cortisol, que pode afetar a reserva de glicogênio muscular e, consequentemente, a qualidade da carne (PONNAMPALAM et al., 2017).

O tempo de transporte é um fator importante na determinação do nível de estresse dos animais. Estudos mostram que animais transportados por longas distâncias têm maior probabilidade de apresentar sinais de estresse e desidratação do que animais transportados por períodos mais curtos (IMMONEN et al., 2000; MACH et al., 2008). Outros fatores relacionados ao transporte que podem afetar a qualidade da carne incluem as condições de transporte, como a temperatura, a umidade e a ventilação (BRAGA et al., 2020). Temperaturas elevadas durante o transporte podem levar à desidratação dos animais e, consequentemente, à redução da reserva de glicogênio muscular. Já as condições de ventilação inadequadas podem levar ao acúmulo de gases tóxicos no ambiente do transporte, o que pode afetar a saúde e o bem-estar dos animais.

No estudo conduzido por Bethancourt-Garcia et al., (2019), foram investigados os efeitos de diferentes densidades de carga na ocorrência de hematomas e contusões nas carcaças. Os resultados apontaram que densidades de carga superiores a 430 kg/m² são críticas para a manutenção do Bem-Estar Animal e da qualidade da carcaça. Em outro estudo, Tarumán et al., (2018) examinou a correlação entre as variáveis pH, densidade de carga e número de contusões. Os resultados indicaram que animais transportados em densidades de carga mais baixas apresentam valores de pH menores, o que sugere um adequado estabelecimento do rigor mortis.

O descanso pré-abate é um período durante o qual o gado fica em repouso antes de ser abatido. É uma etapa importante na cadeia produtiva da carne, pois permite que os animais se recuperem do estresse do transporte e se adaptem às condições do frigorífico antes do abate. Essa etapa é fundamental para garantir a qualidade da carne, especialmente em relação à ocorrência de carne DFD.

Em estudo para investigar os efeitos da duração do período de espera antes do abate sobre o comportamento de bovinos de corte, expressos durante o trajeto percorrido no corredor anterior ao box de insensibilização, os pesquisadores concluíram que o aumento do período de espera não contribuiu para a redução das intervenções do tratador e dos comportamentos indicativos de bem-estar reduzido dos animais (ÖZDEMIR; EKIZ; EKIZ, 2022).

Outro estudo que avaliou o efeito de três tempos de espera (24 h, 48 h e 72 h) sobre a qualidade da carne de bovinos Simental submetidos a transporte comercial longo de aproximadamente 1800 km, concluiu que à medida que a duração do tempo de espera aumentou, o pHu diminuiu (TEKE et al., 2014). De acordo com (NIELSEN; DYBKJR; HERSKIN, 2011), não é apenas a duração da viagem que afeta o bem-estar animal durante o transporte de animais de longa duração. Fatores como temperatura extrema, falta de comida, água e descanso também são relevantes e podem comprometer ainda mais o bem-estar dos animais. É importante reconhecer que esses fatores devem ser considerados ao avaliar os efeitos do transporte de longa duração sobre os animais.

Portanto, é importante que os produtores sigam as recomendações dos órgãos regulatórios em relação ao período de descanso pré-abate e monitorem cuidadosamente as condições dos animais durante esse período, garantindo que tenham acesso adequado à água e alimentos de qualidade, além de um ambiente confortável e livre de estresse.

2.5 ASPECTOS QUALITATIVOS DA CARNE DFD

2.5.1 Maciez Da Carne

A maciez é uma das características mais importantes da carne em relação à qualidade alimentar e é considerada um fator limitante nesse aspecto (BEKHIT et al., 2014). A carne com pH final alto (>6,2) é geralmente tão macia ou mais macia em comparação com a carne normal com pH final de cerca de 5,5 (BEKHIT et al., 2014; GRAYSON et al., 2016a; JEREMIAH; TONG; GIBSON, 1991; LAHUCKY et al., 1998; WATANABE; DALY; DEVINE, 1996). No entanto, na faixa de pH entre 5,8 e 6,2, carnes menos macias são encontradas em relação a carnes com pHu normal (JEREMIAH; TONG; GIBSON, 1991; PURCHAS, 1990; WATANABE; DALY; DEVINE, 1996; WULF et al., 1996), o que pode ser atribuído às baixas taxas de degradação de titina e nebulina (WATANABE; DALY; DEVINE, 1996).

Os resultados apresentados anteriormente, relacionados a maciez, foram confirmados em carne de bovinos jovens com pH intermediário, onde a carne com pH intermediário era mais dura do que a carne com pH > 6,1 ou carne normal (HOLDSTOCK et al., 2014) e novamente mais recentemente por Grayson et al., (2016). Em pH intermediário (5,7 < pHu < 6,3), em resposta à desnaturação de proteínas, as proteínas de choque térmico protegem as proteínas miofibrilares, impedindo sua clivagem enzimática por proteases (PULFORD et al., 2008). Por outro lado, em pH < 5,7, ocorre grande quantidade de desnaturação de proteínas, tornando as proteínas suscetíveis à ação enzimática de degradação.

O estudo de Lomiwes et al. (2013) relatou que a degradação de titina, filamina e desmina foi menor no músculo *Longissimus thoracis* com pH final intermediário (5,8 a 6,19) em comparação com amostras com pHu alto (6,2) e pH normal (5,79). Em resumo, a maciez da carne é influenciada por vários fatores, incluindo pH e degradação de proteínas, que são importantes para o desenvolvimento de estratégias de melhoria da qualidade da carne (PONNAMPALAM et al., 2017).

2.5.2 Cor Da Carne

Quando se aborda sobre a coloração da carne, refere-se à cor que ela apresenta quando cortada e exposta ao ar por alguns minutos. A carne fresca normalmente tem uma cor vermelho-cereja brilhante devido à presença de mioglobina, uma proteína que dá cor à carne. No entanto, se a carne for exposta ao oxigênio por muito tempo, pode sofrer com a oxidação do Fe da mioglobina, levando a formação da Metamioglobina, o que afeta a aparência e a qualidade da carne.

O sistema CieLab* é um espaço de cores comumente utilizado para descrever a cor da carne, incluindo carne bovina de corte escuro. O sistema Lab* define a cor em termos de três parâmetros: L* (luminosidade), a* (vermelho/verde) e b* (amarelo/azul).

No caso da carne de corte escuro, o valor de L* costuma ser menor que o normal, indicando que a carne está mais escura (IJAZ et al., 2020; MAHMOOD et al., 2017). O valor de a* também é menor, indicando que a carne está menos vermelha que o normal, podendo apresentar coloração acastanhada ou arroxeada. O valor de b* pode estar acima do normal, indicando que a carne está com um tom mais amarelado ou esverdeado do que o normal.

A cor da carne é determinada por dois mecanismos que contribuem para a condição de corte escuro: os efeitos do pH elevado pós-morte na estabilidade do estado redox da mioglobina e os efeitos do pH elevado pós-morte na desestruturação das proteínas sarcoplasmáticas e espaçamento da rede de miofilamentos, que afetam a difusão da luz na carne de corte escuro devido a maior quantidade de água interna (GAGAOUA et al., 2021).

Ao discutir a cor da carne de corte escuro, várias revisões se concentram nos efeitos da retenção de água no músculo em pH elevado na dispersão da luz (MANCINI; HUNT, 2005; PONNAMPALAM et al., 2017; RAMANATHAN et al., 2020; SUMAN; JOSEPH, 2013). As fontes acromáticas que causam variações na claridade ou escuridão da carne são principalmente devidas a variações na microestrutura, que afetam a quantidade de dispersão de luz (HUGHES et al., 2019).

Recentemente, Purslow et al. (2020) destacaram que as principais mudanças estruturais ocorrem no espaçamento entre os filamentos grossos e finos no sarcômero (espaçamento da rede de miofilamentos), afetando o diâmetro das miofibrilas e o espaçamento entre as miofibrilas dentro da fibra muscular. Outras fontes de variações incluem o comprimento do sarcômero (TORRES-BURGOS et al., 2019), que pode alterar o diâmetro das miofibrilas e das fibras musculares, e as variações no estado e na distribuição das proteínas sarcoplasmáticas.

É importante ressaltar também, como ocorre a oxigenação muscular e as diferentes tonalidades geradas a partir desta interação. O pH muscular *post-mortem* elevado é o principal fator que prolonga a funcionalidade mitocondrial na carne de corte escuro (RAMANATHAN et al., 2019). Além disso, a estrutura muscular afeta o fluxo de O_2, a interação com a mioglobina e consequentemente a cor da carne (BENDALL; TAYLOR, 1972; RENERRE, 1990). A diminuição da concentração de O_2 nas miofibrilas favorece a formação de deoximioglobina e altera a coloração da carne *in natura* (RAMANATHAN et al., 2019). Temperaturas mais altas aumentam a atividade mitocondrial e, portanto, reduzem o O_2 disponível para se ligar a mioglobina. Porém, a carne de corte escuro apresenta um efeito protetor na oxidação da mioglobina devido ao seu alto pHu (LU et al., 2020). Esse efeito protetor é endógeno e relacionado ao conteúdo de substratos, como NADH, sendo que a atividade da enzima redutase diminui com o tempo (RIBEIRO et al., 2021).

2.6 IDENTIFICAÇÃO DE CARNES DFD

A análise do pH é um dos métodos mais utilizados para a identificação da carne DFD (PONNAMPALAM et al., 2017). Normalmente, o pH da carne é medido utilizando um pHmetro portátil, após o abate e depois da maturação sanitária das carcaças, que pode variar de 24 a 48 horas em temperatura de 4ºC para resfriamento. Apesar de vários países adotarem a mensuração do pH final como parâmetro de referência para a determinação de carcaças DFD, não há um consenso com relação a um valor padrão.

A indústria australiana adotou em seu sistema um pHu > 5,7 para classificar as carcaças como DFD, sob a justificativa de não só haver variação na maciez de carnes de pH intermediário (5,8 < pHu < 6,2), avaliada por metodologias objetivas e equipes sensoriais (GRAYSON et al., 2016a), mas também porque consumidores experientes preterem carne com pHu ≥5,8 (Truscott, 1988).

No Brasil, Canadá, Chile e Estados Unidos, a classificação de carcaças DFD ocorre com pHu ≥ 5,8 (GALLO, 2004; HOLDSTOCK et al., 2014; MOORE et al., 2012; ROSA et al., 2016). Nos Estados Unidos o valor de pHu utilizado é de 5,9. Já para o mercado chinês, o valor é ainda maior (> 6,1) (DU et al., 2009), e em estudo recente com consumidores, foi identificado que este valor poderia saltar para 6,2 sem prejuízos nas características sensoriais (ZHANG et al., 2021).

A análise da cor também é uma técnica comum para a identificação da carne DFD. Existem vários métodos para a análise da cor da carne, sendo os mais comuns a avaliação visual e a mensuração instrumental. A avaliação visual é geralmente realizada por técnicos treinados que utilizam uma escala padrão de cores para comparar a cor da carne com a carne normal. É adotada em sistemas de classificação de países como Austrália, Canada e Japão (GOTOH et al., 2014; HOLDSTOCK et al., 2014; WARNER et al., 2014). Já a mensuração instrumental é realizada com um espectrocolorímetro, que mede a reflectância da luz na superfície da carne em diferentes comprimentos de onda, também conhecido como colorímetro e é utilizado majoritariamente por pesquisadores e pouco difundido no meio industrial.

A cor da carne que o consumidor percebe é determinada pela interação da luz com a superfície da carne, incluindo a luz refletida, absorvida e espalhada (RIBEIRO et al., 2021). A carne normal possui coloração vermelho-brilhante, cuja intensidade depende da presença, concentração e estado químico do pigmento central do tecido bovino, a mioglobina (MANCINI; HUNT, 2005). A presença mioglobina tende a ser associada aos tons de vermelho, enquanto a intensidade da luz refletida é influenciada pelas características estruturais do músculo (HUGHES et al., 2014)

A cor escura da carne de corte escuro pode estar relacionada a dois efeitos notáveis no estado redox da mioglobina (GAGAOUA et al., 2021). O primeiro é que, devido ao estado desoxigenado da mioglobina na carne de corte escuro, a cor escura não mudará para o estado vermelho brilhante da oximioglobina quando houver exposição ao ar (ou seja, não ocorrerá o chamado *blomming*, segundo (EGBERT; CORNFORTH, 1986). Além disso, a carne de corte escuro ao ser cozida à mesma temperatura interna que a carne de pH normal, tem uma cor mais avermelhada e pode parecer mal passada (GAŠPERLIN; ŽLENDER; ABRAM, 2000).

A carne de corte escuro apresenta um encolhimento na estrutura das miofibrilas muito reduzido, resultando em uma maior capacidade de hidratação pelas proteínas musculares, conferindo maior capacidade de retenção de água no músculo (HUGHES et al., 2019). O aumento do teor de água na estrutura muscular reduz a dispersão da luz pelas fibras, resultando em uma carne mais escura (HUGHES et al., 2019). Portanto, a relação entre o pHu e a cor da carne DFD é complexa e influenciada por uma série de fatores, incluindo a mioglobina, a estrutura muscular e a retenção de água.

2.7 ACEITABILIDADE DO CONSUMIDOR

Atender às expectativas do consumidor é uma parte crucial da sua satisfação e comportamento de compra, uma vez que eles representam a última etapa da cadeia produtiva. Por conseguinte, compreender os fatores que influenciam o comportamento do consumidor é essencial. De acordo com Font-i-Furnols & Guerrero (2014), que estudou o comportamento do consumidor, existem três tipos de fatores que explicam esse comportamento: psicológicos (individuais), sensoriais (específicos do produto) e de marketing (ambientais). Esses aspectos estão inter-relacionados e, por sua vez, são influenciados por outros fatores que afetam a tomada de decisão do consumidor. A importância de cada componente do modelo varia de acordo com o consumidor, contexto, cultura e informações disponíveis, e pode influenciar o comportamento individual de maneiras distintas.

A realização de estudos com consumidores é fundamental, pois há situações em que estes não conseguem perceber diferenças de sabor que são notáveis para equipe treinada. Por exemplo, em um estudo conduzido por Claborn et al. (2011), provadores treinados conseguiram detectar diferenças na maciez e sabor entre os diferentes graus de qualidade da carne bovina, enquanto os consumidores não treinados não conseguiram fazê-lo. Da mesma forma, em outro estudo realizado por Vierck et al. (2018), provadores treinados foram capazes de identificar diferenças de sabor e suculência entre bifes com diferentes níveis de marmorização, enquanto os consumidores não treinados não conseguiram detectar tais diferenças de palatabilidade.

Na indústria de carne bovina, é crucial aprimorar e prever o sabor da carne para se manter competitivo (BORGOGNO et al., 2015). Isso se deve ao fato de que a qualidade sensorial, composta por maciez, suculência e sabor, desempenha um papel determinante na preferência do consumidor (GRUNERT; BREDAHL; BRUNSØ, 2004; JEONG et al., 2010). Embora os principais componentes do músculo esquelético sejam responsáveis pela palatabilidade geral da carne cozida, eles afetam os atributos de qualidade sensorial de maneiras distintas (JEONG et al., 2010).

A cor da carne é frequentemente utilizada como critério de seleção pelos consumidores, embora a satisfação alimentar nem sempre esteja relacionada a ela (CARPENTER; CORNFORTH; WHITTIER, 2001; MEAT & LIVESTOCK AUSTRALIA, [s.d.]). As preferências de cor da carne são influenciadas por experiências anteriores e hábitos do consumidor (SANTOS et al., 2021). A cor é usada para prever o frescor, o sabor e a textura da carne, sendo que a cor vermelho-púrpura é associada ao maior frescor e a cor marrom ao menor frescor (HENCHION; MCCARTHY; RESCONI, 2017).

Entre as cores vermelhas, o vermelho brilhante é considerado melhor do que o pálido vermelho ou vermelho escuro em vários países, como Espanha, Itália, Austrália e Japão

(BORGOGNO et al., 2015; EGAN; FERGUSON; THOMPSON, 2001; REALINI et al., 2014). Porém, nos Estados Unidos e na Alemanha (GREBITUS et al., 2013; GREBITUS; JENSEN; ROOSEN, 2013; KILLINGER et al., 2004), a cor preferida pode ser o vermelho cereja. Já na Escócia, o vermelho brilhante pode ser considerado como uma coloração falsa (alterada) e indicar a presença de aditivos ou falta de maturação (CORCORAN et al., 2001).

Com relação a intenção de compra, alguns consumidores americanos pagariam mais por uma carne vermelha brilhante (KILLINGER et al., 2004), enquanto consumidores irlandeses do sexo masculino preferem carne vermelha intensa e bifes mais grossos, o que está relacionado à expressão da masculinidade na cultura (MCCARTHY et al., 2017; NEWCOMBE et al., 2012). No geral, a cor vermelha é preferida em relação à cor marrom, que é associada à carne não fresca ou estragada (CARPENTER; CORNFORTH; WHITTIER, 2001; CORCORAN et al., 2001), mas a aceitação do consumidor em relação à carne DFD pode variar de acordo com o mercado, cultura e conhecimento prévio dos consumidores sobre o fenômeno.

3 REFERÊNCIAS

APAOBLAZA, A. et al. Muscle from grass- and grain-fed cattle differs energetically. **Meat Science**, v. 161, p. 107996, 1 mar. 2020.

APPLE, J. K. et al. Lactic acid enhancement can improve the fresh and cooked color of dark-cutting beef. **Journal of Animal Science**, v. 89, n. 12, p. 4207–4220, 1 dez. 2011.

BAIRD, M. F. et al. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. **Journal of Nutrition and Metabolism**, v. 2012, 2012.

BARÓN, C. L. C. et al. Influence of ultimate pH on biochemistry and quality of Longissimus lumborum steaks from Nellore bulls during ageing. **International Journal of Food Science & Technology**, v. 56, n. 7, p. 3333–3343, 1 jul. 2021.

BARRETO, C. D. et al. Infrared thermography for evaluation of the environmental thermal comfort for livestock. **International Journal of Biometeorology**, v. 64, n. 5, p. 881–888, 1 maio 2020.

BEKHIT, A. A. et al. Exogenous Proteases for Meat Tenderization. **Critical Reviews in Food Science and Nutrition**, v. 54, n. 8, p. 1012–1031, 2014.

BEKHIT, A. E. D.; FAUSTMAN, C. Metmyoglobin reducing activity. **Meat Science**, v. 71, n. 3, p. 407–439, 1 nov. 2005.

BELLO ACEBRÓN, L.; CALVO DOPICO, D. The importance of intrinsic and extrinsic cues to expected and experienced quality: an empirical application for beef. **Food Quality and Preference**, v. 11, n. 3, p. 229–238, 1 maio 2000.

BENDALL, J. R.; TAYLOR, A. A. Consumption of oxygen by the muscles of beef animals and related species. II. Consumption of oxygen by post-rigor muscle. **Journal of the Science of Food and Agriculture**, v. 23, n. 6, p. 707–719, 1 jun. 1972.

BETHANCOURT-GARCIA, J. A. et al. Pre-slaughter factors affecting the incidence of severe bruising in cattle carcasses. **Livestock Science**, v. 222, p. 41–48, 1 abr. 2019.

BORGOGNO, M. et al. The role of product familiarity and consumer involvement on liking and perceptions of fresh meat. **Food Quality and Preference**, v. 44, p. 139–147, 1 set. 2015.

BRAGA, J. S. et al. **Ebook transporte legal bovinos — Ministério da Agricultura e Pecuária**. 1. ed. Jaboticabal: [s.n.].

BRANCACCIO, P.; MAFFULLI, N.; LIMONGELLI, F. M. Creatine kinase monitoring in sport medicine. **British Medical Bulletin**, v. 81–82, n. 1, p. 209–230, 1 jan. 2007.

BRANDT, P.; AASLYNG, M. D. Welfare measurements of finishing pigs on the day of slaughter: A review. **Meat Science**, v. 103, p. 13–23, 1 maio 2015.

CARPENTER, C. E.; CORNFORTH, D. P.; WHITTIER, D. Consumer preferences for beef color and packaging did not affect eating satisfaction. **Meat Science**, v. 57, n. 4, p. 359–363, 1 abr. 2001.

CHULAYO, A. Y.; BRADLEY, G.; MUCHENJE, V. Effects of transport distance, lairage time and stunning efficiency on cortisol, glucose, HSPA1A and how they relate with meat quality in cattle. **Meat Science**, v. 117, p. 89–96, 1 jul. 2016.

CHULAYO, A. Y.; MUCHENJE, V. Activities of some stress enzymes as indicators of slaughter cattle welfare and their relationship with physico-chemical characteristics of beef. **animal**, v. 11, n. 9, p. 1645–1652, 1 set. 2017.

CLABORN, S. W. et al. CONSUMER EVALUATION OF THE PALATABILITY OF USDA SELECT, USDA CHOICE AND CERTIFIED ANGUS BEEF STRIP LOIN STEAKS FROM RETAIL MARKETS IN LUBBOCK, TEXAS, U.S.A. **Journal of Food Quality**, v. 34, n. 6, p. 425–434, 1 dez. 2011.

COCKRAM, M. S.; CORLEY, K. T. T. Effect of pre-slaughter handling on the behaviour and blood composition of beef cattle. **British Veterinary Journal**, v. 147, n. 5, p. 444–454, 1 set. 1991.

COOK, N. et al. Assessing Consistency in Radiated Thermal Output of Beef Steers by Infrared Thermography. **Journal of Imaging 2016, Vol. 2, Page 21**, v. 2, n. 3, p. 21, 11 jul. 2016.

CORCORAN, K. et al. **Current consumer attitudes towards lamb and beef in Europe**. Zaragoza: CIHEAM, 2001.

CORRÊA, A. M. **Termografia infravermelha para a avaliação de carcaça e qualidade de**

carne bovina. [s.l.] Universidade Federal de Viçosa, 28 mar. 2019.

CUTHBERTSON, H. et al. Using infrared thermography on farm of origin to predict meat quality and physiological response in cattle (Bos Taurus) exposed to transport and marketing. **Meat Science**, v. 169, p. 108173, 1 nov. 2020.

DE FRESLON, I. et al. Caracterización de la reactividad conductual frente al manejo y su relación con el Cortisol sanguíneo, contusiones y pH de la carne en novillos. **Archivos de medicina veterinaria**, v. 46, n. 2, p. 229–237, 2014.

DE LA FUENTE, J. et al. Physiological response and carcass and meat quality of suckling lambs in relation to transport time and stocking density during transport by road. **Animal**, v. 4, n. 2, p. 250–258, 1 jan. 2010.

DE MORENO, L. A. et al. Attitudinal Determinants of Beef Consumption in Venezuela: A Retrospective Survey. **Foods 2020, Vol. 9, Page 202**, v. 9, n. 2, p. 202, 16 fev. 2020.

DEVINE, C. E. et al. Pre-slaughter stress arising from on-farm handling and its interactions with electrical stimulation on tenderness of lambs. **Meat Science**, v. 73, n. 2, p. 304–312, 1 jun. 2006.

DU, Y. et al. Effect of pre-slaughter conditions on beef quality. **Scientia Agricultura Sinica**, v. 42, n. 10, p. 3625–3632, 2009.

DUNNE, P. G.; MONAHAN, F. J.; MOLONEY, A. P. Current perspectives on the darker beef often reported from extensively-managed cattle: Does physical activity play a significant role? **Livestock Science**, v. 142, n. 1–3, p. 1–22, 1 dez. 2011.

EGAN, A. F.; FERGUSON, D. M.; THOMPSON, J. M. Consumer sensory requirements for beef and their implications for the Australian beef industry. **Australian Journal of Experimental Agriculture**, v. 41, n. 7, p. 855–859, 2001.

EGBERT, W. R.; CORNFORTH, D. P. Factors Influencing Color of Dark Cutting Beef Muscle. **Journal of Food Science**, v. 51, n. 1, p. 57–59, 1 jan. 1986.

EKIZ, B. et al. Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs. **Meat Science**, v. 90, n. 4, p. 967–976, 1 abr. 2012.

ENGLAND, E. M. et al. Excess glycogen does not resolve high ultimate pH of oxidative muscle. **Meat Science**, v. 114, p. 95–102, 1 abr. 2016.

ENGLISH, A. R. et al. Effects of aging on the fundamental color chemistry of dark-cutting beef. **Journal of Animal Science**, v. 94, n. 9, p. 4040–4048, 1 set. 2016.

FAUCITANO, L. et al. Shelf life of pork from five different quality classes. **Meat Science**, v. 84, n. 3, p. 466–469, 1 mar. 2010.

FERGUSON, D. M.; WARNER, R. D. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? **Meat Science**, v. 80, n. 1, p. 12–19, 1 set. 2008.

FINK, J.; SCHOENFELD, B. J.; NAKAZATO, K. The role of hormones in muscle hypertrophy. **The Physician and Sportsmedicine**, v. 46, n. 1, p. 129–134, 2 jan. 2017.

FONT-I-FURNOLS, M.; GUERRERO, L. Consumer preference, behavior and perception about meat and meat products: An overview. **Meat Science**, v. 98, n. 3, p. 361–371, 1 nov. 2014.

FOURY, A. et al. Alternative rearing systems in pigs: consequences on stress indicators at slaughter and meat quality. **animal**, v. 5, n. 10, p. 1620–1625, out. 2011.

GAGAOUA, M. et al. Clustering of sensory eating qualities of beef: Consistencies and differences within carcass, muscle, animal characteristics and rearing factors. 2018.

GAGAOUA, M. et al. Dark-cutting beef: A brief review and an integromics meta-analysis at the proteome level to decipher the underlying pathways. **Meat Science**, v. 181, p. 108611, 1 nov. 2021.

GALLO, C. Carnes de corte oscuro en bovinos (dark cutting beef). **Revista Americarne para América Latina y El Caribe (español-inglés)**, v. 3, p. 10, 2004.

GARCÍA-TORRES, S. et al. Assessment of Stress by Serum Biomarkers in Calves and Their Relationship to Ultimate pH as an Indicator of Meat Quality. **Animals 2021, Vol. 11, Page 2291**, v. 11, n. 8, p. 2291, 3 ago. 2021.

GAŠPERLIN, L.; ŽLENDER, B.; ABRAM, V. Colour of normal and high pH beef heated to different temperatures as related to oxygenation. **Meat Science**, v. 54, n. 4, p. 391–398, 1 abr.

2000.

GERSONY, J. T. et al. Scaling Thermal Properties from the Leaf to the Canopy in the Alaskan Arctic Tundra. **https://doi.org/10.1657/AAAR0016-013**, v. 48, n. 4, p. 739–754, 1 nov. 2018.

GOODSON, K. J. et al. Beef customer satisfaction: factors affecting consumer evaluations of clod steaks. **Journal of Animal Science**, v. 80, n. 2, p. 401–408, 1 fev. 2002.

GOTOH, T. et al. Meat produced by Japanese Black cattle and Wagyu. **Animal Frontiers**, v. 4, n. 4, p. 46–54, 1 out. 2014.

GRAYSON, A. L. et al. The effects of degree of dark cutting on tenderness and sensory attributes of beef,. **Journal of Animal Science**, v. 94, n. 6, p. 2583–2591, 1 jun. 2016a.

GRAYSON, A. L. et al. The effects of degree of dark cutting on tenderness and sensory attributes of beef. **Journal of Animal Science**, v. 94, n. 6, p. 2583–2591, 1 jun. 2016b.

GREBITUS, C. et al. Fresh Meat Packaging: Consumer Acceptance of Modified Atmosphere Packaging including Carbon Monoxide. **Journal of Food Protection**, v. 76, n. 1, p. 99–107, 1 jan. 2013.

GREBITUS, C.; JENSEN, H. H.; ROOSEN, J. US and German consumer preferences for ground beef packaged under a modified atmosphere – Different regulations, different behaviour? **Food Policy**, v. 40, p. 109–118, 1 jun. 2013.

GREGORY, N. G. Animal welfare at markets and during transport and slaughter. **Meat Science**, v. 80, n. 1, p. 2–11, 1 set. 2008.

GRUNERT, K. G.; BREDAHL, L.; BRUNSØ, K. Consumer perception of meat quality and implications for product development in the meat sector—a review. **Meat Science**, v. 66, n. 2, p. 259–272, 1 fev. 2004.

HENCHION, M. M.; MCCARTHY, M.; RESCONI, V. C. Beef quality attributes: A systematic review of consumer perspectives. **Meat Science**, v. 128, p. 1–7, 1 jun. 2017.

HOLDSTOCK, J. et al. The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade.

Meat Science, v. 98, n. 4, p. 842–849, 1 dez. 2014.

HOLMAN, B. W. B. et al. The identification of dark cutting beef carcasses in Australia, using Nix Pro Color Sensor^{TM} colour measures, and their relationship to bolar blade, striploin and topside quality traits. **Meat Science**, v. 148, p. 50–54, 1 fev. 2019.

HOPKINS, D. L. et al. The effect of pH decline rate on the meat and eating quality of beef carcasses. **Animal Production Science**, v. 54, n. 4, p. 407–413, 20 fev. 2014.

HORCADA, A. et al. Short communication: Using infrared ocular thermography as a tool to predict meat quality from lean cattle breeds prior to slaughter: Exploratory trial. **Spanish Journal of Agricultural Research**, v. 17, n. 4, p. e06SC01-e06SC01, 2019.

HUGHES, J. et al. **Improving beef colour at grading-Final report**. Disponível em: <https://www.ampc.com.au/getmedia/fb3a8f45-75c0-4791-acef-0da992fb4e3c/AMPC_ImprovingBeefColourAtGrading_FinalReport.pdf?ext=.pdf>. Acesso em: 14 mar. 2023a.

HUGHES, J. et al. High pH in beef longissimus thoracis reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. **Food Research International**, v. 101, p. 228–238, 1 nov. 2017b.

HUGHES, J. et al. Differences in light scattering between pale and dark beef longissimus thoracis muscles are primarily caused by differences in the myofilament lattice, myofibril and muscle fibre transverse spacings. **Meat Science**, v. 149, p. 96–106, 1 mar. 2019.

HUGHES, J. M. et al. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. **Meat Science**, v. 98, n. 3, p. 520–532, 1 nov. 2014.

HUGHES, J. M. et al. Meat color is determined not only by chromatic heme pigments but also by the physical structure and achromatic light scattering properties of the muscle. **Comprehensive Reviews in Food Science and Food Safety**, v. 19, n. 1, p. 44–63, 1 jan. 2020.

HUGHES, J. M.; KEARNEY, G.; WARNER, R. D. Improving beef meat colour scores at carcass grading. **Animal Production Science**, v. 54, n. 4, p. 422–429, 21 fev. 2014.

I. CAPPELLOZZA, B.; S. MARQUES, R. Effects of Pre-Slaughter Stress on Meat

Characteristics and Consumer Experience. **Meat and Nutrition**, 28 jul. 2021.

IJAZ, M. et al. Association between meat color of DFD beef and other quality attributes. **Meat Science**, v. 161, 1 mar. 2020.

IJAZ, M. et al. Changes in postmortem metabolites profile of atypical and typical DFD beef. **Meat Science**, v. 193, p. 108922, 1 nov. 2022.

IMMONEN, K. et al. Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. **Meat Science**, v. 55, n. 1, p. 25–31, 1 maio 2000.

JEONG, D. W. et al. Correlations of trained panel sensory values of cooked pork with fatty acid composition, muscle fiber type, and pork quality characteristics in Berkshire pigs. **Meat Science**, v. 86, n. 3, p. 607–615, 1 nov. 2010.

JEREMIAH, L. E.; TONG, A. K. W.; GIBSON, L. L. The usefulness of muscle color and pH for segregating beef carcasses into tenderness groups. **Meat Science**, v. 30, n. 2, p. 97–114, 1 jan. 1991.

JOHNSON, E. O. et al. Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. **Neuroscience & Biobehavioral Reviews**, v. 16, n. 2, p. 115–130, 1 jun. 1992.

KARVATTE, N. et al. Infrared thermography for microclimate assessment in agroforestry systems. **Science of The Total Environment**, v. 731, p. 139252, 20 ago. 2020.

KENNY, F. J.; TARRANT, P. V. The behaviour of young Friesian bulls during social re-grouping at an abattoir. Influence of an overhead electrified wire grid. **Applied Animal Behaviour Science**, v. 18, n. 3–4, p. 233–246, 1 out. 1987.

KILLINGER, K. M. et al. Consumer visual preference and value for beef steaks differing in marbling level and color. **Journal of Animal Science**, v. 82, n. 11, p. 3288–3293, 1 nov. 2004.

KIM, Y. et al. Canopy skin temperature variations in relation to climate, soil temperature, and carbon flux at a ponderosa pine forest in central Oregon. **Agricultural and Forest Meteorology**, v. 226–227, p. 161–173, 15 out. 2016.

KIYIMBA, F. et al. Changes in glycolytic and mitochondrial protein profiles regulates postmortem muscle acidification and oxygen consumption in dark-cutting beef. **Journal of Proteomics**, v. 232, p. 104016, 10 fev. 2021.

KOCH, R. M.; DIKEMAN, M. E.; CROUSE, J. D. Characterization of Biological Types of Cattle (Cycle III).III. Carcass Composition, Quality and Palatability. **Journal of Animal Science**, v. 54, n. 1, p. 35–45, 1 jan. 1982.

KOMATSU, T. et al. Effects of genetic and environmental factors on muscle glycogen content in Japanese Black cattle. **Animal Science Journal**, v. 85, n. 8, p. 793–798, 1 ago. 2014.

KOU, H. X. et al. Automated measurement of cattle surface temperature and its correlation with rectal temperature. **PLOS ONE**, v. 12, n. 4, p. e0175377, 1 abr. 2017.

KREIKEMEIER, K. K.; UNRUH, J. A.; ECK, T. P. Factors affecting the occurrence of dark-cutting beef and selected carcass traits in finished beef cattle. **Journal of Animal Science**, v. 76, n. 2, p. 388–395, 1 fev. 1998.

LAHUCKY, R. et al. Effect of preslaughter handling on muscle glycogen level and selected meat quality traits in beef. **Meat Science**, v. 50, n. 3, p. 389–393, 1 nov. 1998.

LAWRENCE, T. L. J.; FOWLER, V. R.; NOVAKOFSKI, J. E. **Growth of farm animals**. 3. ed. [s.l.] CABI, 2012.

LEIGHTON, P. L. A. et al. Towards improving classification of Canadian dark-cutting beef carcasses: Consumer sensory evaluation. **Meat Science**, v. 195, 1 jan. 2023.

LI, M. et al. Comparative profiling of sarcoplasmic phosphoproteins in ovine muscle with different color stability. **Food Chemistry**, v. 240, p. 104–111, 1 fev. 2018.

LIU, J. et al. Contributions of tenderness, juiciness and flavor liking to overall liking of beef in Europe. **Meat Science**, v. 168, p. 108190, 1 out. 2020.

LOMIWES, D. et al. Small heat shock proteins and toughness in intermediate pHu beef. **Meat Science**, v. 95, n. 3, p. 472–479, 1 nov. 2013.

LOMIWES, D. et al. The development of meat tenderness is likely to be compartmentalised by ultimate pH. **Meat Science**, v. 96, n. 1, p. 646–651, jan. 2014.

LÓPEZ-CAMPOS, Ó. et al. Packaging for retail appearance improvement of dark cutting beef. **Meat Science**, v. 1, n. 96, p. 121, jan. 2014.

LOSADA-ESPINOSA, N. et al. Pre-slaughter cattle welfare indicators for use in commercial abattoirs with voluntary monitoring systems: A systematic review. **Meat Science**, v. 138, p. 34–48, 1 abr. 2018.

LOWE, G. et al. Infrared Thermography—A Non-Invasive Method of Measuring Respiration Rate in Calves. **Animals 2019, Vol. 9, Page 535**, v. 9, n. 8, p. 535, 7 ago. 2019.

LU, X. et al. Association of ultimate pH and stress-related blood variables in cattle. **Meat Science**, v. 139, p. 228–230, 1 maio 2018.

LU, X. et al. Effect of oxygen concentration in modified atmosphere packaging on color changes of the M. longissimus thoraces et lumborum from dark cutting beef carcasses. **Meat Science**, v. 161, p. 107999, 1 mar. 2020.

MACH, N. et al. Association between animal, transportation, slaughterhouse practices, and meat pH in beef. **Meat Science**, v. 78, n. 3, p. 232–238, 1 mar. 2008.

MAHMOOD, S. et al. Can potential for dark cutting be predicted by phenotype? Relationship between sex, carcass characteristics, and the incidence of dark cutting beef. **Canadian Journal of Animal Science**, v. 96, n. 1, p. 19–31, 24 mar. 2016a.

MAHMOOD, S. et al. Relationship between phenotype, carcass characteristics and the incidence of dark cutting in heifers. **Meat Science**, v. 121, p. 261–271, 1 nov. 2016b.

MAHMOOD, S. et al. Understanding the quality of typical and atypical dark cutting beef from heifers and steers. **Meat Science**, v. 133, p. 75–85, 1 nov. 2017.

MAHMOOD, S. et al. Proteomics of dark cutting longissimus thoracis muscle from heifer and steer carcasses. **Meat Science**, v. 137, p. 47–57, 1 mar. 2018.

MANCINI, R. A.; HUNT, M. C. Current research in meat color. **Meat Science**, v. 71, n. 1, p. 100–121, 1 set. 2005.

MARTELLO, L. S. et al. Infrared thermography as a tool to evaluate body surface temperature and its relationship with feed efficiency in Bos indicus cattle in tropical conditions. **International Journal of Biometeorology**, v. 60, n. 1, p. 173–181, 1 jan. 2016.

MCCARTHY, S. N. et al. Evaluation of beef eating quality by Irish consumers. **Meat Science**, v. 132, p. 118–124, 1 out. 2017.

MCGILCHRIST, P. et al. Beef carcasses with larger eye muscle areas, lower ossification scores and improved nutrition have a lower incidence of dark cutting. **Meat Science**, v. 92, n. 4, p. 474–480, 1 dez. 2012.

MCGILCHRIST, P. et al. The incidence of dark cutting in southern Australian beef production systems fluctuates between months. **Animal Production Science**, v. 54, n. 10, p. 1765–1769, 2014.

MCKEITH, R. O. et al. Mitochondrial abundance and efficiency contribute to lean color of dark cutting beef. **Meat Science**, v. 116, p. 165–173, 1 jun. 2016.

MCPHAIL, N. G. et al. Factors influencing the occurrence of high ultimate pH in three muscles of lamb carcasses in Australia. **Animal Production Science**, v. 54, n. 10, p. 1853–1859, 19 ago. 2014.

MEAT & LIVESTOCK AUSTRALIA. **Annual Report 2016-17**. Disponível em: <https://www.mla.com.au/about-mla/how-we-are-governed/Planning-reporting/annual-reporting/annual-report-2016-17/>. Acesso em: 16 mar. 2023.

MILLER, M. **Dark, Firm and Dry Beef**. Disponível em: <https://fyi.extension.wisc.edu/wbic/files/2011/04/Dark-Firm-and-Dry-Beef.pdf>. Acesso em: 14 mar. 2023.

MINKA, N. S.; AYO, J. O. Physiological responses of food animals to road transportation stress. **African Journal of Biotechnology**, v. 9, n. 40, p. 6601–6613, 16 ago. 2010.

MOHAN RAJ, A. B. et al. Effect of mixing male sex types of cattle on their meat quality and stress-related parameters. **Meat Science**, v. 32, n. 4, p. 367–386, 1 jan. 1992.

MONTANHOLI, Y. R. et al. On the determination of residual feed intake and associations of infrared thermography with efficiency and ultrasound traits in beef bulls. **Livestock Science**, v. 125, n. 1, p. 22–30, 1 out. 2009.

MONTANHOLI, Y. R. et al. Assessing feed efficiency in beef steers through feeding behavior, infrared thermography and glucocorticoids. **Animal**, v. 4, n. 5, p. 692–701, 1 jan. 2010.

MOORE, M. C. et al. National Beef Quality Audit–2011: In-plant survey of targeted carcass characteristics related to quality, quantity, value, and marketing of fed steers and heifers,. **Journal of Animal Science**, v. 90, n. 13, p. 5143–5151, 1 dez. 2012.

MOTA-ROJAS, D. et al. Clinical Applications and Factors Involved in Validating Thermal Windows Used in Infrared Thermography in Cattle and River Buffalo to Assess Health and Productivity. **Animals 2021, Vol. 11, Page 2247**, v. 11, n. 8, p. 2247, 30 jul. 2021.

MUCHENJE, V. et al. Relationship between pre-slaughter stress responsiveness and beef quality in three cattle breeds. **Meat Science**, v. 81, n. 4, p. 653–657, 1 abr. 2009.

MUELLER, L. F. et al. Gender status effect on carcass and meat quality traits of feedlot Angus × Nellore cattle. **Animal Science Journal**, v. 90, n. 8, p. 1078–1089, 1 ago. 2019.

MUIR, P. D.; DEAKER, J. M.; BOWN, M. D. Effects of forage- and grain-based feeding systems on beef quality: A review. **New Zealand Journal of Agricultural Research**, v. 41, n. 4, p. 623–635, 1998.

NELIS, J. L. D. et al. Biomarkers and biosensors for the diagnosis of noncompliant pH, dark cutting beef predisposition, and welfare in cattle. **Comprehensive Reviews in Food Science and Food Safety**, v. 21, n. 3, p. 2391–2432, 1 maio 2022.

NEWCOMBE, M. A. et al. "Eat like a man". A social constructionist analysis of the role of food in men's lives. **Appetite**, v. 59, n. 2, p. 391–398, 1 out. 2012.

NIELSEN, B. L.; DYBKJR, L.; HERSKIN, M. S. Road transport of farm animals: effects of journey duration on animal welfare. **Animal**, v. 5, n. 3, p. 415–427, 1 jan. 2011.

O'LOUGHLIN, A. et al. Biomarker responses to weaning stress in beef calves. **Research in Veterinary Science**, v. 97, n. 2, p. 458–463, 1 out. 2014.

ÖZDEMIR, S.; EKIZ, E. E.; EKIZ, B. Effect of lairage duration on cattle behaviors and stockperson actions in the slaughter corridor in Simmental and Swiss Brown breeds. **Tropical Animal Health and Production**, v. 54, n. 2, p. 1–7, 1 abr. 2022.

PAGE, J. K.; WULF, D. M.; SCHWOTZER, T. R. A survey of beef muscle color and pH. **Journal of Animal Science**, v. 79, n. 3, p. 678–687, 1 mar. 2001.

PEDREIRA, C.; SILVA, L.; ALONSO, M. **Use of grazed pastures in the brazilian livestock industry: a brief overview**. (R. Carnevalli et al., Eds.) Forages in Warm Climates. **Anais**...2015. Disponível em: <https://www.researchgate.net/profile/Roberta-Carnevalli-2/publication/280612162_Silvopastoral_systems_with_Tifton-85_grass_and_tropical_fruit_trees/links/55bea9a908aec0e5f445e28f/Silvopastoral-systems-with-Tifton-85-grass-and-tropical-fruit-trees.pdf#page=7>. Acesso em: 1 mar. 2023

PICARD, B. et al. Inverse relationships between biomarkers and beef tenderness according to contractile and metabolic properties of the muscle. **Journal of Agricultural and Food Chemistry**, v. 62, n. 40, p. 9808–9818, 8 out. 2014.

PICARD, B.; GAGAOUA, M.; GAGAOUA, M. Muscle Fiber Properties in Cattle and Their Relationships with Meat Qualities: An Overview. **Journal of Agricultural and Food Chemistry**, v. 68, n. 22, p. 6021–6039, 3 jun. 2020.

PIGHIN, D. G. et al. Relationship between changes in core body temperature in lambs and post-slaughter muscle glycogen content and dark-cutting. **Animal Production Science**, v. 54, n. 4, p. 459–463, 15 maio 2013.

POLETI, M. D. et al. Label-free quantitative proteomic analysis reveals muscle contraction and metabolism proteins linked to ultimate pH in bovine skeletal muscle. **Meat Science**, v. 145, p. 209–219, 1 nov. 2018.

POLKINGHORNE, R. et al. Development of a commercial system to apply the Meat Standards Australia grading model to optimise the return on eating quality in a beef supply chain. **Australian Journal of Experimental Agriculture**, v. 48, n. 11, p. 1451–1458, 16 out. 2008.

PONNAMPALAM, E. N. et al. Causes and Contributing Factors to “Dark Cutting” Meat: Current Trends and Future Directions: A Review. **Comprehensive Reviews in Food Science and Food Safety**, v. 16, n. 3, p. 400–430, 1 maio 2017.

PULFORD, D. J. et al. The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. **Meat Science**, v. 79, n. 4, p. 623–630, 1 ago.

2008.

PURCHAS, R. W. An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. **Meat Science**, v. 27, n. 2, p. 129–140, 1 jan. 1990.

PURCHAS, R. W.; AUNGSUPAKORN, R. Further investigations into the relationship between ultimate pH and tenderness for beef samples from bulls and steers. **Meat Science**, v. 34, n. 2, p. 163–178, 1 jan. 1993.

PURSLOW, P. P. et al. Variations in meat colour due to factors other than myoglobin chemistry; a synthesis of recent findings (invited review). **Meat Science**, v. 159, p. 107941, 1 jan. 2020.

RAMANATHAN, R. et al. Effects of 4-hydroxy-2-nonenal on beef heart mitochondrial ultrastructure, oxygen consumption, and metmyoglobin reduction. **Meat Science**, v. 90, n. 3, p. 564–571, 1 mar. 2012.

RAMANATHAN, R. et al. Mitochondrial functionality and beef colour: A review of recent research. **South African Journal of Animal Science**, v. 49, n. 1, p. 9–19, 1 abr. 2019.

RAMANATHAN, R. et al. Recent Updates in Meat Color Research: Integrating Traditional and High-Throughput Approaches. **Meat and Muscle Biology**, v. 4, n. 2, p. 1–24, 28 jul. 2020.

REALINI, C. E. et al. Relative importance of cues underlying Spanish consumers' beef choice and segmentation, and consumer liking of beef enriched with n-3 and CLA fatty acids. **Food Quality and Preference**, v. 33, p. 74–85, 1 abr. 2014.

REIS, M. M.; ROSENVOLD, K. Early on-line classification of beef carcasses based on ultimate pH by near infrared spectroscopy. **Meat Science**, v. 96, n. 2, p. 862–869, 1 fev. 2014.

RENERRE, M. Factors involved in the discoloration of beef meat. **International Journal of Food Science & Technology**, v. 25, n. 6, p. 613–630, 1 dez. 1990.

RIBEIRO, C. C. DE S. et al. New alternatives for improving and assessing the color of dark–cutting beef – a review. **Scientia Agricola**, v. 79, n. 1, p. 1–16, 18 jan. 2021.

ROSA, A. et al. Incidence of DFD meat on Brazilian beef cuts. **Meat Science**, v. 112, p. 132–

133, fev. 2016.

RUSSELL, E. et al. Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. **Psychoneuroendocrinology**, v. 37, n. 5, p. 589–601, 1 maio 2012.

SANTANA, M. L. et al. Structure and genetic diversity of Brazilian Zebu cattle breeds assessed by pedigree analysis. **Livestock Science**, v. 187, p. 6–15, 1 maio 2016.

SANTOS, D. et al. The most important attributes of beef sensory quality and production variables that can affect it: A review. **Livestock Science**, v. 250, p. 104573, 1 ago. 2021.

SCHAEFER, A. et al. 497 The automated and real time use of infrared thermography in the detection and correction of DFD and fevers in cattle. **Journal of Animal Science**, v. 96, n. suppl_3, p. 275–275, 7 dez. 2018.

SCHEFFLER, T. L. et al. Mitochondria influence postmortem metabolism and pH in an in vitro model. **Meat Science**, v. 110, p. 118–125, 1 dez. 2015.

SCHEFFLER, T. L.; GERRARD, D. E. Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. **Meat Science**, v. 77, n. 1, p. 7–16, 1 set. 2007.

SEPULVEDA, C. A. et al. Cooking Method and USDA Quality Grade Affect Consumer Palatability and Flavor of Beef Strip Loin Steaks. **Meat and Muscle Biology**, v. 3, n. 1, 1 out. 2019.

SHACKELFORD, S. D. et al. Effect of biological type of cattle on the incidence of the dark, firm, and dry condition in the longissimus muscle. **Journal of Animal Science**, v. 72, n. 2, p. 337–343, 1 fev. 1994.

SHAW, F. D.; TUME, R. K. The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents—A review of recent work. **Meat Science**, v. 32, n. 3, p. 311–329, 1 jan. 1992.

SILVA, J. A.; PATARATA, L.; MARTINS, C. Influence of ultimate pH on bovine meat tenderness during ageing. **Meat Science**, v. 52, n. 4, p. 453–459, 1 ago. 1999.

STEEL, C. C. et al. Abattoir Factors Influencing the Incidence of Dark Cutting in Australian Grain-Fed Beef. **Animals 2021, Vol. 11, Page 474**, v. 11, n. 2, p. 474, 10 fev. 2021.

SUMAN, S. P.; JOSEPH, P. Myoglobin Chemistry and Meat Color. **Annual Review of Food Science and Technology** , v. 4, p. 79–99, 2013.

SUTHERLAND, M. A. et al. Evaluation of infrared thermography as a non-invasive method of measuring the autonomic nervous response in sheep. **PLOS ONE**, v. 15, n. 5, p. e0233558, 1 maio 2020.

TADICH, N. et al. Effects of transport and lairage time on some blood constituents of Friesian-cross steers in Chile. **Livestock Production Science**, v. 93, n. 3, p. 223–233, 1 maio 2005.

TANG, F. et al. Non-Invasive Glucose Measurement by Use of Metabolic Heat Conformation Method. **Sensors 2008, Vol. 8, Pages 3335-3344**, v. 8, n. 5, p. 3335–3344, 21 maio 2008.

TANG, J. et al. Postmortem Oxygen Consumption by Mitochondria and Its Effects on Myoglobin Form and Stability. **Journal of Agricultural and Food Chemistry**, v. 53, n. 4, p. 1223–1230, 23 fev. 2005.

TANG, J.; FAUSTMAN, C.; HOAGLAND, T. A. Krzywicki revisited: Equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. **Journal of Food Science**, v. 69, n. 9, 2004.

TARRANT, P. V. Animal Behaviour and Environment in the Dark-Cutting Condition in Beef - A Review. **Irish Journal of Food Science and Technology**, v. 13, n. 1, p. 1–21, 1989.

TARRANT, P. V.; SHERINGTON, J. An investigation of ultimate pH in the muscles of commercial beef carcasses. **Meat Science**, v. 4, n. 4, p. 287–297, 1980.

TARUMÁN, J. A. et al. Risk Factors for Bruises and High Muscle pH in Lamb Carcasses of Tierra Del Fuego, Chilean Patagonia. **Open Access Library Journal**, v. 5, n. 1, p. 1–11, 4 jan. 2018.

TEKE, B. et al. Effects of different lairage times after long distance transportation on carcass and meat quality characteristics of Hungarian Simmental bulls. **Meat Science**, v. 96, n. 1, p. 224–229, 1 jan. 2014.

TERLOUW, C. E. M. et al. Understanding the Determination of Meat Quality Using Biochemical Characteristics of the Muscle: Stress at Slaughter and Other Missing Keys. **Foods 2021, Vol. 10, Page 84**, v. 10, n. 1, p. 84, 4 jan. 2021.

TESTA, M. L. et al. Color and Marbling as Predictors of Meat Quality Perception of Argentinian Consumers. **Foods 2021, Vol. 10, Page 1465**, v. 10, n. 7, p. 1465, 24 jun. 2021.

TORRES-BURGOS, Y. I. et al. pH Variability and its Relationship with Sarcomere Length and Free Calcium in Beef from Commercial Cattle in Puerto Rico. **Meat and Muscle Biology**, v. 3, n. 2, 30 nov. 2019.

VESTERGAARD, M.; OKSBJERG, N.; HENCKEL, P. Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. **Meat Science**, v. 54, n. 2, p. 177–185, 1 fev. 2000.

VIERCK, K. R. et al. Marbling Texture's Effects on Beef Palatability. **Meat and Muscle Biology**, v. 2, n. 1, 30 abr. 2018.

VILJOEN, H. F.; DE KOCK, H. L.; WEBB, E. C. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. **Meat Science**, v. 61, p. 181–185, 2002a.

VILJOEN, H. F.; DE KOCK, H. L.; WEBB, E. C. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. **Meat Science**, v. 61, n. 2, p. 181–185, 1 jun. 2002b.

WARNER, R. D. et al. Acute stress induced by the preslaughter use of electric prodders causes tougher beef meat. **Australian Journal of Experimental Agriculture**, v. 47, n. 7, p. 782–788, 2 jul. 2007.

WARNER, R. D. et al. Factors influencing the incidence of high rigor temperature in beef carcasses in Australia. **Animal Production Science**, v. 54, n. 4, p. 363–374, 28 fev. 2014.

WARNER, R. D. et al. Meat tenderness: advances in biology, biochemistry, molecular mechanisms and new technologies. **Meat Science**, v. 185, p. 108657, 1 mar. 2022.

WARRIS, P. D. The extraction of haem pigments from fresh meat. **International Journal of Food Science & Technology**, v. 14, n. 1, p. 75–80, 1 fev. 1979.

WARRISS, P. D. The handling of cattle pre-slaughter and its effects on carcass and meat quality. **Applied Animal Behaviour Science**, v. 28, n. 1–2, p. 171–186, 1 nov. 1990.

WATANABE, A.; DALY, C. C.; DEVINE, C. E. The effects of the ultimate pH of meat on tenderness changes during ageing. **Meat Science**, v. 42, n. 1, p. 67–78, 1 jan. 1996.

WEAVER, A. D.; BOWKER, B. C.; GERRARD, D. E. Sarcomere length influences μ-calpain-mediated proteolysis of bovine myofibrils. **Journal of Animal Science**, v. 87, n. 6, p. 2096–2103, 1 jun. 2009.

WERNER, M. et al. Effects of a long distance transport and subsequent recovery in recently weaned crossbred beef calves in Southern Chile. **Livestock Science**, v. 152, n. 1, p. 42–46, 1 mar. 2013.

WHEELER, T. L. et al. Characterization of biological types of cattle (Cycle V): carcass traits and longissimus palatability. **Journal of Animal Science**, v. 79, n. 5, p. 1209–1222, 1 maio 2001.

WHEELER, T. L. et al. Characterization of biological types of cattle (Cycle VI): Carcass, yield, and longissimus palatability traits. **Journal of Animal Science**, v. 82, n. 4, p. 1177–1189, 1 abr. 2004.

WHEELER, T. L. et al. Characterization of biological types of cattle (Cycle VIII): Carcass, yield, and longissimus palatability traits. **Journal of Animal Science**, v. 88, n. 9, p. 3070–3083, 1 set. 2010.

WHEELER, T. L.; SHACKELFORD, S. D.; KOOHMARAIE, M. Cooking and palatability traits of beef longissimus steaks cooked with a belt grill or an open hearth electric broiler. **Journal of Animal Science**, v. 76, n. 11, p. 2805–2810, 1 nov. 1998.

WULF, D. M. et al. Genetic influences on beef longissimus palatability in charolais- and limousin-sired steers and heifers. **Journal of Animal Science**, v. 74, n. 10, p. 2394–2405, 1 out. 1996.

XIAO, L. et al. A Review of Previous Studies on DFD Beef. **Food Science**, v. 36, n. 19, p. 271, 15 out. 2015.

YANG, X. et al. Investigation of the physicochemical, bacteriological, and sensory quality of beef steaks held under modified atmosphere packaging and representative of different ultimate pH values. **Meat Science**, v. 174, p. 108416, 1 abr. 2021.

ZHANG, Y. et al. Determination of a pH threshold for dark cutting beef based on visual evaluation by Asian consumers. **Meat Science**, v. 172, p. 108347, 1 fev. 2021.

4 OBJETIVOS

4.1 OBJETIVOS GERAIS

Investigar a caracterização química, avaliação sensorial e uso da termografia infravermelha para identificar a anomalia DFD na carne, visando compreender suas características e impactos na qualidade do produto e na percepção do consumidor.

4.2 OBJETIVOS ESPECÍFICOS

a) Avaliar marcadores de estresse por meio de análise sanguínea e correlacionar com carcaças de diferentes valores de pH final.
b) Avaliar as diferenças físico-químicas entre carnes com pHu ≥ 5,8 e pHu ≥ 6.
c) Avaliar a utilização da termografia de infravermelho para a identificação da anomalia DFD por meio da correlação da temperatura máxima ocular dos animais *ante-mortem* com parâmetros sanguíneos post-mortem e de qualidade da carne.
d) Analisar a preferência do consumidor em relação à presença do fenômeno DFD por meio de análise sensorial da carne crua e grelhada.

5 ARTIGO A – EXPLORING MEAT QUALITY IN NELLORE BULLS UNDER TROPICAL CONDITIONS: PHU VARIATIONS AND THEIR RELATIONSHIP WITH PRE-SLAUGHTER BLOOD BIOMARKERS OF STRESS

Esta seção apresentará a versão do artigo que será submetida para publicação

Exploring meat quality in Nellore bulls under tropical conditions: pHu variations and their relationship with pre-slaughter blood biomarkers of stress

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Abstract

The present study investigated the quality characteristics of meat derived from Nellore cattle slaughtered under tropical conditions at various final pH (pHu) ranges. Additionally, we aimed to establish a correlation between blood biomarkers associated with stress and the final pH of meat. A total of 389 male Nellore cattle carcasses were studied, with 113 selected based on pHu values (Normal pHu < 5.8; n = 37, atypical dark, firm, and dry (DFD) 5.8 > pHu < 6.0; n = 48, typical DFD pHu ≥ 6.0; n = 28). Blood cortisol, creatine kinase (CK), and lactate dehydrogenase (LDH) levels were higher in the typical DFD pH group than in the normal and atypical DFD groups. Blood glucose concentration was higher in the normal and typical DFD group, whereas blood lactate levels were higher (*P = 0.01*) in the typical DFD group than in the normal group. The incidence of typical DFD was 7.2%, which increased to 20.8% in carcasses with atypical DFD. Atypical DFD steaks had lower redness and yellowness (*P < 0.001*) but no significant differences in lightness. The atypical and typical DFD groups had higher metmyoglobin (MetMb) levels (*P < 0.01*) compared to normal levels. The typical DFD group exhibited the highest tenderness. The R^2 values exceeding 0.8, indicating a strong predictive capacity, suggesting that measuring blood parameters at slaughter can help identify carcasses with different pHu.

Keywords: Beef tenderness; Cortisol; DFD; Myoglobin; Pre-slaughter stress

1. Introduction

The process of slaughtering animals is complex and requires a series of steps and procedures that are potentially stressful for the animals (NELIS et al., 2022). These steps range from handling to transport, where the animals are exposed to unavoidable situations during the pre-slaughter period (FERGUSON; WARNER, 2008; NELIS et al., 2022; WARNER et al., 2007). These stressors can be related to pain, injuries, exacerbated exercise (physical stressors), extreme temperatures, and changes in the environment, or linked to fear and anxiety (physiological stressors). The stressors vary in duration and can be chronic, intermittent, or acute (Cappellozza & Marques, 2021; Johnson et al., 1992).

An physical effort and vigilance of the animal can be enhanced by these reactions; however, they can also result in physical exhaustion and dark cutting (PONNAMPALAM et al., 2017). Consequently, psychological stress may be a factor in the differences observed among individual animals in a cohort regarding dark cutting outcomes, whereas the overall potential of a cohort to develop dark cutting is determined by its nutritional plane and physical stressors (NELIS et al., 2022).

Brazilian meat production is one of the largest in the international market and meat is exported to several countries worldwide (ABIEC, 2022). Ensuring the competitiveness of Brazilian meat in international markets requires meeting demands not only in terms of price, but also in upholding quality standards. One crucial factor in this regard is the final pH (pHu) of meat, which varies depending on the market and serves as an indicator of the expected quality parameters of normal meat (PONNAMPALAM et al., 2017), particularly with respect to color. For example, in countries such as Canada and Chile, for a carcass to qualify as normal, the pHu must be <5.8 (GALLO, 2004; HOLDSTOCK et al., 2014). In the United States, this value is 5.9 (Natl. Beef Quality Audit, 2000), whereas in Europe and Australia, this value decreases to 5.7 (Warner et al., 2014). In Asian countries such as China, this value is slightly higher, approximately 6.2 (ZHANG et al., 2021).

The Nellore breed of the *Bos taurus indicus* subfamily is the most commonly used among Brazilian beef cattle producers (MUELLER et al., 2019). However, it possesses characteristics distinct from those utilized in European, North American, and Australian production systems, which predominantly involve European breeds (British or Continental) or crossbreeds with only 25% *Bos indicus* (POLKINGHORNE et al., 2008). It is essential to recognize these peculiarities, as the Nellore breed is known for its lower tenderness and higher susceptibility to color instability, leading to dark meat (Koch et al., 1982; Mahmood et al., 2017; Warner et al., 2022; Wheeler et al., 2001, 2004, 2010).

Dark meat, characterized by its dark, firm, and dry (DFD) attributes, is commonly associated with the final color of meat cuts, which exhibit high pH values and low lightness, redness, and yellowness levels (COCKRAM; CORLEY, 1991; MCKEITH et al., 2016; VILJOEN; DE KOCK; WEBB, 2002a). In addition to color-related factors, another important parameter for consumers is tenderness, which is also affected by high pHu, mainly because of the depletion of glycogen reserves in the pre-slaughter period, preventing muscle acidification and the consequent decrease in pH (HOLDSTOCK et al., 2014; MCKEITH et al., 2016). In addition, issues related to the fiber type have also been reported as important factors in this process.

To achieve a comprehensive understanding of meat production, it is crucial to investigate the behavior of animals, their production systems, and their impact on meat quality. Numerous studies (APAOBLAZA et al., 2020; GAGAOUA et al., 2018; IJAZ et al., 2022; MCKEITH et al., 2016) have explored various factors influencing the complex parameter of meat pHu, including energy metabolism, oxidative processes, genomics, and proteomics. However, most of these studies did not specifically focus on *Bos taurus indicus*, which are predominant in Brazil. Therefore, this study aimed to investigate the differences in blood parameters and meat quality traits between meats with different pHu values in the largest breed found in Brazil.

2. Material and Methods

2.1 Animals, slaughter, and experimental design

This study was conducted in March 2022 at a commercial slaughterhouse under federal inspection, situated in Nova Andradina, Mato Grosso do Sul, Brazil. The specific coordinates of the area are latitude 22° 14' 6''' South and longitude 53° 19' 54''' West, with an altitude of 401 meters. The average temperature in the city is 23.9 °C, and the average annual rainfall is 1455 mm (CLIMATE DATA, 2022). Carcasses were collected over a period of three consecutive days. A rigorous selection process was applied, limiting the choice to batches of Nelore bulls, resulting in a total of 389 animals from seven batches that met these specific criteria. Among these seven batches, only two of them had animals that fell within all three pH ranges evaluated in this study. The remaining batches that did not meet all the pH criteria were excluded from the primary analysis. However, they were considered in measuring the incidence of the DFD anomaly in this study. For slaughter, the animals were stunned using a pneumatic penetration gun with air injection. Subsequently, one paw was immediately lifted to facilitate slaughter by cutting the large vessels and adhering to an established humane slaughter protocol.

After 48 h of sanitary maturation at chilling temperature (4 ºC), 113 carcasses were selected for evaluation based on their pHu values. The pHu was measured at the exit of the sanitary maturation chamber in the *Longissimus thoracis* muscle at 12th rib using the Hanna HI98163® professional pH meter from Hanna Instruments Inc, Barueri, Brazil. The pH meter was calibrated at chiller temperature beforehand using standard commercial solutions at pH 4 and 7.

The selected carcasses were categorized into three groups based on their pH values. The first group, denoted as the "normal" group, consisted of carcasses with pH values of <5.8. The second group, named the "atypical DFD" group, consisted of carcasses exhibiting pH values ranging 5.8–5.99. Finally, the third group, named the “typical DFD” group, encompassed carcasses with pH values of ≥6.0 (Table 1). Whenever possible, the selection process involved utilizing all carcasses with atypical DFD and typical DFD final pH values, along with an equal number of carcasses with normal pH from the same slaughtered batch. The left halves of the carcasses were sectioned at the 12th rib to remove approximately 15 cm of the sample (*m. Longissimus thoracis*). Each portion was deboned and divided into steaks of varying sizes for subsequent analyses. The steaks were placed in polyamide/polyethylene bags, vacuum packed, and frozen at -20 °C for transportation to the meat laboratory at the State University of Londrina and preserved until further analysis.

Table 1. Minimum and maximum pHu values from Nellore bulls carcasses.

	pHu values				
	n	Mean	SEM	Minimum	Maximum
Normal	37	5.68	0.010	5.48	5.79
Atypical DFD	48	5.86	0.008	5.80	5.98
Typical DFD	28	6.20	0.035	6.00	6.94

Normal pHu (< 5.8). Atypical DFD (pHu < 6.0). Typical DFD (pHu ≥ 6.0).

2.2 Blood collection and measurements

Blood samples were collected during the bleeding of the animals after cutting the large vessels and stored in 10 mL tubes (BD Vacutainers®, Curitiba, Paraná, Brazil) containing sodium fluoride for the evaluation of lactate and cortisol, and anticoagulant to assess levels of creatine kinase (CK) and lactate dehydrogenase (LDH). To obtain plasma, the samples were centrifuged at 3000 rpm for 15 min within 30 min of collection, so that there was no metabolic activity that would interfere with the results. The serum was also collected after centrifugation, and both were divided into aliquots and stored at -20 °C until analysis, which was performed at the Clinical Pathology Laboratory of the Veterinary Hospital of the State University of Londrina.

Serum and plasma levels were determined in duplicate using commercial kits. The detection of glucose, CK, LDH, and lactate were analyzed using the Dimension® Xpand Plus device (Siemens Healthcare Diagnostics Inc., USA) with the commercial kits Gluc Ver Flex®, CK-NAC IFCC®, LDI Flex®, Dimension LA®, respectively. Cortisol concentrations in plasma samples were measured using a commercial cortisol ELISA kit (Jiaxing Korain Biotech Co., Zhejiang, China). The absorbance was determined using an ELISA reader (iMark™ Microplate Absorbance Spectrophotometer, Bio-Rad Laboratories Inc.) at 450 nm.

2.3 Collection of infrared thermography (IRT) images

The eye temperature of the animals was non-invasively measured inside a stunning box at the slaughterhouse, adhering to standard industrial procedures. IRT images were obtained using a Flir T440 portable infrared camera (FLIR Systems Inc., Wilsonville, OR, USA) equipped with a 640 × 480-pixel detector and a 15° × 11° lens (40 mm) in the right eye and captured at a 90° angle from a distance of 60 cm. The camera had a thermal sensitivity of < 40 mK (<0.04 °C at 30 °C ambient temperature) and a temperature range of -20–350 °C. The camera was set to automatic focus adjustment mode by a trained technician, and an emissivity value of 0.98, recommended for mammalian studies, was used (Hoffmann et al., 2013; Bernard, Staffa, Mornstein, & Bourek, 2013; Kastberger & Stachl, 2003). The FLIR TOOLS® software (Teledyne FLIR LLC, Wilsonville, Oregon, USA) was used to analyze the IRT images and obtain the maximum temperature (°C) within specific eye areas, including the posterior medial palpebral border, lower lid, and lacrimal caruncle (Fig. 1).

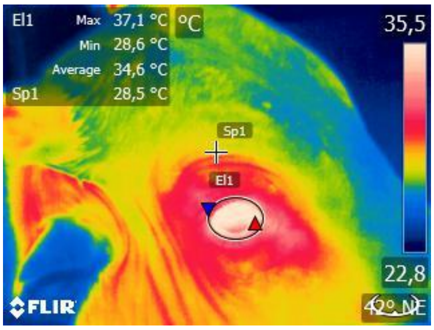

Figure 1. Ilustrative infrared image of the right side of the animal with the ocular area marked.

2.4 Meat color

Color analysis was performed after portioning the steaks and before freezing the samples. Beef samples were exposed to oxygen for 30 min at 4 °C (blooming time). The color was measured using the CIELab system of the CR-10® colorimeter (Konica Minolta, Inc., São Paulo, Brazil) illuminant D65, observation angle of 10° and opening size of 8.0 mm, performing three readings on the surface of the LT muscle sample. An average of three measurements was generated for each variable (L^*, a^*, and b^*), where L^* = lightness, a^* = redness, and b^* = yellowness. The colorimetric chromatic index (C*), which determines the color saturation, was calculated by the formula $[(a^*)^2 + (b^*)^2]$ 0.5, and the hue angle (Hº) $[\text{tang}^{-1}$ (b */a*) × [360°/ (2 × 3.14)] (Minolta, 1993).

2.5 Thawing and cooking loss, and shear force

The samples were weighed and thawed at a cooling temperature of 2–4 °C for 24 h, then weighed again to determine the fluid loss during thawing through the difference in weight. Next, a thermocouple was inserted in the geometric center of the samples, coupled to a digital thermometer ThermPro 4® Probes Tp17h to monitor the internal temperature of the samples. The steaks were roasted in a preheated oven (Paniz Indústria de Equipamentos para Comida Ltd., Caxias do Sul, RS, Brazil) equipped with a thermostat to avoid temperature fluctuations. When the internal temperature of the steak reached 40 °C, the sample was turned over and remained in the oven until it reached an internal temperature of 71 °C, based on the methodology described by Wheeler et al. (1996). Then, the samples were refrigerated at 4 °C for 24 h and weighed. Cooking loss was defined as the difference in the weight before and after cooking.

After cooling, eight cylinders measuring 1.27 cm in diameter were removed in the direction of the muscle fiber using a punch attached to an industrial drill FSB 16 Pratika® (Schultz Compressores S.A., Joinville, Brazil). The cylinders were sheared using a Brookfield CT-3

Texture Analyzer (Brookfield, São Paulo, Brazil). Eight repeated measurements were performed per steak to increase precision, and the results were obtained by averaging the values.

2.6 Sarcomere length

For each sample, a 2 × 2 × 1 cm piece was cut along the fiber direction. The piece was fixed for 4 h in a glutaraldehyde solution in 5% buffer (0.1 M Na_2HP0_4, pH 7.2). After 4 h, the samples were washed with 0.2 M sucrose buffer (0.1 M Na_2HP0_4, pH 7.2) until measurements (KOOLMEES et al., 1986). Tweezers were used to obtain a small muscle bundle following the fiber direction and placed it on a microscope slide with a drop of sucrose solution. The fabric was spread on a slide to identify at least ten individual fibers. The slides were covered with a coverslip and placed horizontally under a vertically oriented laser beam (Spectra Physics Inc., Model 117A, CA, USA) to generate a series of diffraction bands. Sarcomere lengths was calculated using the following formula:

Sarcomere length μm = $(0.0006328 \times D \times \sqrt{(T^2/D^2)} + 1)/T \times 1000$

Where "D" is the distance (mm) between the blade and the measurement site, and "T" is the measured distance between two diffraction bands (mm).

2.7 Myoglobin estimation

Myoglobin levels in the samples were estimated according to the methods described in the previous studies by Tang et al. (2004) and Warriss et al. (1979). To perform the estimation, 10 ± 0.5 g of tissue, which was free from fat depots and connective tissues, was taken in duplicate from each sample steak that was thawed at 4 °C for 2 h. The tissue was then homogenized for 40 s in 50 mL of ice-cold phosphate buffer (pH 6.8), and the mixture was first stored at 4 °C for 1 h, followed by centrifugation for 10 min at 6500×g and 4 °C. The homogenates were then filtered with Whatman® filter paper No. 4, and the supernatants were collected for measurement of absorbance at 503, 525, 557, 582, and 730 nm against a 0.04 M phosphate buffer blank using a spectrophotometer (Evolution 60S, UV–Visible Spectrophotometer, Thermo Scientific, USA). The dilution factor was computed by dividing the volume of buffer (mL) by the mass (g) of the sample. The ratios of oxy-myoglobin (OMb), deoxy-myoglobin (DMb), and met-myoglobin (MMb) were calculated as described by Tang et al. (2004), and the total myoglobin concentration was estimated using the following equation:

$$Mb\ (mg\ g^{-1}) = [(\text{Absorption at } 525 - \text{Absorption at } 730) / 2.2303] \times [\text{dilution factor}]$$

2.8 Statistical analysis

Statistical analysis was performed using the Jamovi® program (The Jamovi Project, version 2.2). Initially, the data were examined to identify outliers. In this study, each carcass served as an experimental unit, and the pHu values were treated as fixed effects and collection day as random effect. For the analysis of biochemical parameters, one-factor analysis of variance (ANOVA) was performed, with statistical significance set at $P \leq 0.05$. Post hoc analysis was conducted using Tukey's test to determine the specific pairwise differences between the groups. To understand the predictive relationship between serum and plasma biomarkers and beef pHu, both principal component analysis (PCA) and linear regression analyses were conducted. For the construction of prediction models, ultimate pH (pHu) was used as the fixed effect, and the collection day and batch were considered as random terms for all covariates (lactate, glucose, CK, LDH, and cortisol), which were analyzed individually and in different combinations.

3. Results and discussion

3.1 Incidence and group pHu

For sample collection, 389 Nellore cattle carcasses were evaluated, and the incidence of carcasses presenting with a DFD anomaly was calculated based on this total. Considering only carcasses with a pHu > 6, the incidence was 7.2% (Table 2). When atypical DFD carcasses were considered, the percentage increased to 20.8%.

Table 2. Minimum and maximum pHu values of bovine carcasses and percentage of incidence of each pHu class.

	pHu values					
	N	Mean	SEM	Minimum	Maximum	DFD incidence (%)
Normal	308	5.71	0.003	5.48	5.79	79.2
Atypical DFD	53	5.86	0.008	5.80	5.98	13.6
Typical DFD	28	6.20	0.035	6.00	6.94	7.2

Normal pHu (< 5.8). Atypical DFD (pHu < 6.0). Typical DFD (pHu ≥ 6.0).

This incidence is considered high (MAHMOOD et al., 2017) compared to that reported in other studies. Moore (2012) reported a value of 3.2% in the United States. Mach et al. (2008) reported 13.9% in a study carried out in Spain, reaching 17.1% when considering carcasses with a pHu above 6. Hughes et al. (2017), in a study conducted on Australian plants, reported 12% DFD beef, identifying that the variation in this incidence ranged from 0.6 to 40.1% among plants. evaluated with a history of high incidence. Similar values, ranging 17–40 %, were reported by Gallo (2004) in a study conducted in Chile. In Brazil, Rosa et al. (2016) evaluated crossbred animals and observed a lower value than that found in the present study (4.53%).

3.2 Blood variables

There was a significant difference between the groups with different pHu values and all blood parameters evaluated (Table 3). Blood cortisol levels were higher in the typical DFD group than in the normal and atypical DFD groups, but did not differ from each other.

The cortisol concentration in blood plasma is used to identify stress conditions in animals because it is released by the hypothalamic-adrenocortical axis, which is the main neuroendocrine system that responds to stressful situations, such as pre-slaughter stress (FOURY et al., 2011; RUSSELL et al., 2012). Activation of this system leads to the release of cortisol and a consequent increase in blood concentration. Pre-slaughter stress has been indicated as the primary cause of high pHu in cattle by causing muscle glycogen depletion, also resulting in a reduced amount of blood lactate (LU et al., 2018), also observed in this study. Studies have shown that transport, lairage, and weaning interfere with cortisol concentration (CHULAYO; BRADLEY; MUCHENJE, 2016; GARCÍA-TORRES et al., 2021; LU et al., 2018; O'LOUGHLIN et al., 2014), leading us to believe that they may explain the results obtained in this study as well, since breed and sexual class factors can be discarded from this population because they are all male Zebu cattle of the Nellore breed.

Table 3. Means and standard error of serum and plasma biomarkers evaluated in male Nellore cattle with different final pH (pHu) ranges.

Parameter	**Normal pHu < 5.8**	**Atypical DFD pHu < 6.0**	**Typical DFD pHu ≥ 6.0**	**P - value**
IRTmin (ºC)	28.12 ± 0.18b	28.25 ± 0.16b	29.17 ± 0.25a	0.003
IRTmax (ºC)	35.30 ± 0.12b	36.01 ± 0.16a	35.87 ± 0.16 ab	0.002
Cortisol (nmol/L)	20.48 ± 1.04b	21.13 ± 1.25b	29.33 ± 2.63a	0.019
Glucose (mmol/L)	117.12 ± 3.71a	103.90 ± 2.21b	115.52 ± 4.73 ab	0.005
Lactate (mmol/L)	5.88 ± 0.30 b	6.91 ± 0.26 ab	7.79 ± 0.72 a	0.01
CK (U/L)	612.20 ± 31.50b	875.83 ± 66.52a	1021.00 ± 160.99a	0.001
LDH (U/L)	1366.09 ± 15.25b	1464.21 ± 23.48a	1479.94 ± 34.76a	< 0.001

Different letters on the same line represent a significant difference (*P* < 0.05). CK = creatine kinase; LDH = lactate dehydrogenase. CK: creatine kinase. LDH: lactate dehydrogenase. IRTmin: Minimum Temperature. IRTmax: Maximum temperature.

Blood glucose concentration was higher in the normal group than in the atypical and typical DFD groups, which did not differ from each other. A similar result was observed for blood lactate levels, with a higher level in the normal group than in the Typical DFD group, with no difference between the atypical DFD group and the other two groups. The reduction in ATP levels during the pre-slaughter period results in glycogen depletion and leads to increased blood glucose production (MINKA; AYO, 2010). Furthermore, glucose levels are directly related to the nutritional status of the animals (SHAW; TUME, 1992). Studies have also indicated that prolonged transport causes a reduction in glucose levels and an increase in blood cortisol (CHULAYO; BRADLEY; MUCHENJE, 2016). With increasing lairage time, the glucose concentration (TADICH et al., 2005). This leads us to believe that the results of this study may be related to the good nutritional status of the normal group and the greater susceptibility of the typical DFD group to pre-slaughter stress.

The amount of CK was higher in the typical and atypical DFD groups than in the normal group. The same was true for the amount of LDH. Blood CK and LDH levels indicate tissue injury, increasing cellular permeability that can occur during various operations in pre-slaughter handling, such as physical stress caused by transport, injuries, loading, and unloading problems, or poor handling of animals in the slaughterhouse (BAIRD et al., 2012; CHULAYO; MUCHENJE, 2017; DE LA FUENTE et al., 2010; EKIZ et al., 2012). CK is an enzyme that occurs exclusively in muscle cells that catalyzes the phosphorylation of creatine to phosphocreatine and ADP to ATP (BRANCACCIO; MAFFULLI; LIMONGELLI, 2007; LU et al., 2018).

Injuries during the pre-slaughter period are quickly released into the blood and are therefore used as indicators of stress related to fatigue and muscle metabolism (LU et al., 2018). In a previous study, its activity increased after animal transport, suggesting that preslaughter stress factors may increase its activity in cattle (WERNER et al., 2013). Just as increased CK activity can be related to pre-slaughter stress, extracellular LDH can also be detected, as it is an enzyme detected in the blood after vigorous exercise or muscle trauma (LU et al., 2018). The results of this study are in line with those of previous studies that indicate an increase in the activities of these enzymes in stressful situations and are positively correlated with pHu

(GARCÍA-TORRES et al., 2021; LU et al., 2018; MOHAN RAJ et al., 1992).

Temperature variables varied among the groups. Animals in the typical DFD group exhibited higher minimum temperatures than those in the other two groups. When analyzing the maximum ocular IRT, the atypical DFD group presented higher temperatures than the normal group and did not differ from the typical DFD group. No significant differences were observed between the typical DFD and Normal groups.

Cuthbertson et al. (2020) studied the use of IRT on a farm video to predict meat quality and observed similar results to animals that presented higher temperatures with meat with high pHu and darker color. The authors hypothesized that this was because stressed cattle use glycogen stores in their muscles to maintain homeostasis, which can cause an increase in brain temperature. This temperature rise can be identified through eye IRT, because the eyes are close to the brain (Tang et al., 2008). After slaughter, glycogen reserves are converted into pyruvate, resulting in the production of lactic acid. Lactic acid produces hydrogen ions, which decrease meat pH. Consequently, inadequate production of lactic acid due to depleted glycogen stores at slaughter can lead to high meat pH, resulting in DFD (DEVINE et al., 2006; GREGORY, 2008).

Limited research has been conducted on the potential application of IRT to predict carcass quality and meat characteristics, particularly in live animals. A study conducted in Spain (HORCADA et al., 2019), observed a correlation between IRT measurements and pHu of light carcasses. Another Canadian study (SCHAEFER et al., 2018), utilized real-time IRT technology and found that normal animals had an average ocular temperature of 33.6 °C, whereas animals with DFD carcasses displayed lower and higher temperatures (30.4 ± 1.85 and 35.8 ± 1.28 °C, respectively). However, in our study, animals with atypical or typical DFD carcasses had higher minimum and maximum ocular IRT. Notably, the exact timing of image acquisition in the study by Schaefer et al. (2018) remains unclear. Our findings indicate that normal animals had a higher mean temperature; however, it should be acknowledged that the elevated temperatures in normal animals may be attributed to stress and movement during transportation to the stunning box (PIGHIN et al., 2013).

3.3 Meat quality

The results obtained for the color parameters support the hypothesis that pHu affects not only lightness but also other variables (Table 4). The variables L*, a*, b*, C*, and H* differed according to Hu. The means of color parameters and water loss supported the existence of differences between steaks classified as atypical and typical DFD, except for cooking loss, which did not show differences between the groups (P = 0.3). The atypical DFD steaks had similar color characteristics as the typical DFD steaks, showing lower values of redness and yellowness than the normal steaks. They differed only in lightness (L*), indicating that the atypical DFD group was intermediate between the other two groups.

Table 4. Means and standard error of Meat Characteristics in Nellore Cattle with Varying pHu levels.

Parameters	Normal	Atypical DFD	Typical DFD	P - value
*L**	$36.3^{a} \pm 0.25$	$35.4^{b} \pm 0.22$	$34.2^{c} \pm 0.32$	<0.001
*a**	$11.9^{a} \pm 0.32$	$9.7^{b} \pm 0.31$	$8.6^{b} \pm 0.29$	<0.001
*b**	$12.9^{a} \pm 0.15$	$12.1^{b} \pm 0.17$	$11.6^{b} \pm 0.16$	<0.001
Hue angle	$47.8^{a} \pm 0.54$	$51.8^{b} \pm 0.53$	$53.7^{b} \pm 0.62$	<0.001
Chroma	$17.8^{a} \pm 0.32$	$15.5^{b} \pm 0.32$	$14.6^{b} \pm 0.32$	<0.001
Mb (mg g^{-1})	$4.67^{a} \pm 0.103$	$4.08^{b} \pm 0.096$	$3.70^{c} \pm 0.128$	<0.001
OxyMb (%)	$60.5^{a} \pm 0.012$	$56.3^{b} \pm 0.010$	$54.6^{b} \pm 0.014$	0.006
DeoMb (%)	$9.8^{b} \pm 0.003$	$10.6^{ab} \pm 0.002$	$11.1^{a} \pm 0.004$	0.028
MetMb (%)	$26.7^{b} \pm 0.008$	$29.2^{a} \pm 0.006$	$30.7^{a} \pm 0.007$	0.003
WBSF (N)	$92.87^{b} \pm 040$	$100.22^{b} \pm 0.37$	$71.49^{a} \pm 0.43$	<0.001
Sarcomere length (µm)	$1.62^{b} \pm 0.013$	$1.63^{b} \pm 0.014$	$1.70^{a} \pm 0.014$	<0.001
Thawing loss (%)	$14.20^{b} \pm 0.61$	$13.94^{b} \pm 0.46$	$9.83^{a} \pm 0.85$	<0.001
Cooking loss (%)	20.99 ± 0.47	19.95 ± 0.44	20.46 ± 0.66	0.289

Different letters on the same line represent a significant difference ($P < 0.05$). Normal pHu (< 5.8). Atypical

DFD (pHu < 6.0). Typical DFD (pHu ≥ 6.0). DeoMb = deoxmyoglobin. OxyMb = deoxymyoglobin. MetMb = metmyoglobin. Mb = myoglobin.

The color of meat is not just an aesthetic feature but can also provide information on its quality and freshness. The normal group showed a fresh red appearance, which can be attributed to metabolic processes occurring during the *postmortem* period of the muscle (RAMANATHAN et al., 2019). The results obtained from our study suggest that the color parameters of meat may be influenced by the phosphorylation process of proteins that regulate glycolysis and the redox stability of myoglobin, which is impacted by myoglobin phosphorylation (LI et al., 2018).

Our findings are consistent with those of Li et al. (2018), who revealed that the majority of proteins related to color stability were glycolytic enzymes and that myoglobin phosphorylation was inversely related to meat color stability. As the level of myoglobin phosphorylation increased, the color stability of meat, based on the a* value, decreased, and the content of metmyoglobin (MetMb) increased. Furthermore, we found an inverse relationship between pHu and the parameters related to meat color, including a*, b*, chroma, and hue angle. As pHu increased, there was a decrease in the values of these parameters and a greater MetMb content. These results provide new insights into the mechanisms underlying color changes in meat, and highlight the importance of considering pHu in the assessment of meat color stability.

High values of chroma are beneficial to color stability; however, chroma values were negatively associated with pH due to the higher myoglobin oxidative potential in beef with a high ultimate pH, which led to the formation of MetMb and favored meat discoloration (HUGHES et al., 2017b).

There were significant differences in the total myoglobin content among the three groups ($P < 0.001$). The Normal group exhibited higher average myoglobin levels than the atypical and typical DFD groups, which also showed distinct values. Additionally, the oxygenated Mb

(OxyMb) content was higher in the normal group, whereas no difference was observed between the atypical and typical DFD groups. Conversely, the MetMb content was higher in the atypical and typical DFD groups than in the normal group, with no significant difference between the two groups. The terms of deoxymyoglobin Mb (DeoMb) content, the typical DFD group displayed a higher average than in the control group, whereas the atypical DFD group did not differ significantly from either group.

The concentration of myoglobin, as well as the pHu, can be affected by a series of factors, including age, testosterone concentration, muscle and fiber type, and the genetic composition of the animals (RIBEIRO et al., 2021). In addition, the concentration of Mb has been correlated with the high expression of cytochrome C oxidase in the mitochondria (APAOBLAZA et al., 2020). In addition, it can be influenced by the finishing system of the animals, which, when finished on pasture, have a higher concentration of Mb (DUNNE; MONAHAN; MOLONEY, 2011; PICARD; GAGAOUA; GAGAOUA, 2020).

In a study involving Angus × Hereford crossbred cattle that focused on the *Longissimus* muscle, animals fed on pasture had a higher concentration of myoglobin than those fed on grain (APAOBLAZA et al., 2020). Similarly, a separate study examining mitochondrial metabolism in steaks with varying degrees of dark cutting observed similar Mb concentrations in control steaks and steaks with severe DFD characteristics (MCKEITH et al., 2016).

In the current study, steaks from the atypical DFD group exhibited a lower content of OxyMb, which imparts a bright cherry-red color to the meat; a higher content of MetMb, which contributes to the brown color; and atypical DFD levels of DeoMb. These findings can be attributed to the shorter sarcomere length (Table 4), which can result in compression of the myofibrillar structure and a decrease in myoglobin oxygenation (Hughes et al., 2019). The decrease in OxyMb and the higher proportion of MetMb in the typical DFD group may suggest a higher rate of oxygen consumption, a characteristic previously observed in dark-cut meats (Tang et al., 2005).

Contrary to the common misconception that DFD meat is tough due to the term “firm,” our evaluation results indicate that the meat from the typical DFD group was the most tender compared to all other groups examined. Interestingly, our findings suggest that the tenderness of steaks from the atypical DFD group was similar to that of steaks from the normal group. Furthermore, the greater sarcomere length observed in DFD steaks supports an inverse relationship between sarcomere length and tenderness (WEAVER; BOWKER; GERRARD, 2009). This finding aligns with that of a similar study conducted on Puerto Rican cattle, which also reported greater sarcomere length in the typical DFD group, without further discussion of possible reasons (TORRES-BURGOS et al., 2019). During the thawing process, a significant difference was observed, with steaks from the typical DFD group losing less water compared to the other two groups, which did not exhibit differences between them.

3.4 The relationship between blood biomarkers and pHu

In the present study, principal component analysis (PCA) revealed three main components. The first component was characterized by the strong contribution of LDH variables, with a weight of 0.92, CK with a weight of 0.57, and pHu with a weight of 0.75. The high positive loadings of these variables suggest an association between a higher final pH and elevated concentrations of LDH and CK.

The second component was characterized by the variables CK, glucose, and lactate with respective weights of 0.35, 0.94, and 0.37. This component revealed that elevated levels of glucose are associated with CK and lactate, indicating a potential relationship between glucose metabolism and meat characteristics. This association may be related to the use of glucose as an energy source in muscular metabolic processes.

The third component showed a strong contribution of pHu with a weight of 0.40, lactate with a weight of 0.79, and cortisol with a weight of 0.58. This association suggests a relationship between the final meat pH, lactate concentration, and cortisol, which may be related to metabolic and stress processes during slaughter.

It is important to highlight that the absence of significant cross-loadings between these components strengthens the interpretability and validity of the observed relationships. The variance values of Component 1 (29.1), Component 2 (22.8), and Component 3 (20.0) indicate that these components capture a substantial part of the total variation in the data.

The results of the linear regression analyses (Table 5) demonstrate that blood markers such as Lactate, Glucose, CK, LDH, and Cortisol have significant associations with the final pH of beef, as indicated by the significant R^2 values ($P < 0.001$). This indicates that these blood markers play significant roles in influencing pHu. The adjusted R^2 was also considered to assess the ability of the predictors to explain the variation in the beef pHu while accounting for the number of predictors in the model. The high adjusted R^2 values indicate that the regression models are robust, and the selected predictors contribute significantly to the variation in the final pH.

Table 5. Regression analysis results for blood biomarkers and pHu in Nellore bulls.

Predictors	R^2	R^2 adjusted	RMSE	P
Lactate	0.78	0.77	0.11	<0.001
Glucose	0.76	0.75	0.12	<0.001
CK	0.72	0.70	0.13	<0.001
LDH	0.71	0.70	0.12	<0.001
Cortisol	0.87	0.85	0.08	<0.001
Lactate + Cortisol	0.87	0.85	0.07	<0.001
Cortisol + Glucose	0.88	0.86	0.08	<0.001
Cortisol + Glucose + Lactate	0.86	0.80	0.08	<0.001
Lac + Glu + Ck + LDH + Cor	0.83	0.68	0.06	0.015

CK: creatina kinase. LDH: lactate dehydrogenase. Glu: glucose. Cor: cortisol

Furthermore, the Root Mean Square Error (RMSE) provides a measure of the accuracy of the regression models. Low RMSE values suggest that the models have a good fit to the data. It is interesting to note that the combination of blood markers, such as Cortisol + Glucose and Cortisol + Glucose + Lactate, yielded an even higher R^2, indicating that including multiple predictors may enhance the ability to predict the final pH. However, it is important to note that the model "Lac + Glu + Ck + LDH + Cor" exhibited a lower R^2 compared to the previous models and a significant p-value (0.015). This suggests that including all blood markers together may not be the most effective approach in predicting the final pH of beef.

The association of blood biomarker parameters in cattle presents different behaviors according to the breed and study design (GARCÍA-TORRES et al., 2021; LU et al., 2018), but in general, it can vary from weak to moderate, and in some cases, it can be strong. However, it must be analyzed carefully when making comparisons between studies.

There are indications of an association between the pHu and blood cortisol levels. De Freslon et al. (2014) evaluated the association of behavioral tests for the incidence of dark cutting (DE FRESLON et al., 2014). The results showed a moderate correlation ($r = 0.34$; $P < .05$) between cortisol levels on the farm and crash test scores, which indicate the behavior of an animal. The study also revealed a high correlation ($r = 0.81$- 0.87; $P < 0.01$) between the behavior test and pHu, indicating that combining physiological measurements with behavior observations could be beneficial.

Lu et al. (2018) reported a direct correlation between plasma lactate levels during exsanguination and dark cutting, with a correlation coefficient (r) of 0.80. Additionally, a strong correlation was identified between cortisol ($r = 0.96$), lactate dehydrogenase ($r = 0.84$), and creatine kinase ($r = 0.97$) plasma levels and intermediate correlations with glucose ($r = 0.62$) plasma levels and final pH.

The correlation studies mentioned above demonstrate associations between variables but do not establish a direct cause-and-effect relationship. Associations between blood biomarkers, animal behavior, and pHu can vary in intensity, ranging from weak to moderate, and in some cases, they can be strong. However, it is crucial to remember that correlation does not imply causality. Our prediction models were developed based on these observed associations and provided significant results in explaining the variation in meat pHu. However, they represent a predictive tool and do not establish definitive cause-and-effect relationships. Other factors not considered in our analysis may influence the results.

4. Conclusion

Based on the findings of this study, it can be concluded that meat with atypical and typical DFD values differ in terms of meat quality traits. The analysis revealed variations in blood biomarkers such as cortisol, CK, LDH, glucose, and lactate among the various pHu groups. The atypical and typical DFD groups exhibited higher MetMb content, potentially influencing meat color and oxidative stability. The typical DFD group exhibited the highest tenderness. The relationship between blood biomarkers and the beef pHu emphasizes the potential to be used as a predictive tool to assess carcasses with different pHu levels at slaughter.

References

APAOBLAZA, A. et al. Muscle from grass- and grain-fed cattle differs energetically. **Meat Science**, v. 161, p. 107996, 1 mar. 2020.

APPLE, J. K. et al. Lactic acid enhancement can improve the fresh and cooked color of dark-cutting beef. **Journal of Animal Science**, v. 89, n. 12, p. 4207–4220, 1 dez. 2011.

BAIRD, M. F. et al. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. **Journal of Nutrition and Metabolism**, v. 2012, 2012.

BARÓN, C. L. C. et al. Influence of ultimate pH on biochemistry and quality of Longissimus lumborum steaks from Nellore bulls during ageing. **International Journal of Food Science & Technology**, v. 56, n. 7, p. 3333–3343, 1 jul. 2021.

BARRETO, C. D. et al. Infrared thermography for evaluation of the environmental thermal comfort for livestock. **International Journal of Biometeorology**, v. 64, n. 5, p. 881–888, 1 maio 2020.

BEKHIT, A. A. et al. Exogenous Proteases for Meat Tenderization. **Critical Reviews in**

Food Science and Nutrition, v. 54, n. 8, p. 1012–1031, 2014.

BEKHIT, A. E. D.; FAUSTMAN, C. Metmyoglobin reducing activity. **Meat Science**, v. 71, n. 3, p. 407–439, 1 nov. 2005.

BELLO ACEBRÓN, L.; CALVO DOPICO, D. The importance of intrinsic and extrinsic cues to expected and experienced quality: an empirical application for beef. **Food Quality and Preference**, v. 11, n. 3, p. 229–238, 1 maio 2000.

BENDALL, J. R.; TAYLOR, A. A. Consumption of oxygen by the muscles of beef animals and related species. II. Consumption of oxygen by post-rigor muscle. **Journal of the Science of Food and Agriculture**, v. 23, n. 6, p. 707–719, 1 jun. 1972.

BETHANCOURT-GARCIA, J. A. et al. Pre-slaughter factors affecting the incidence of severe bruising in cattle carcasses. **Livestock Science**, v. 222, p. 41–48, 1 abr. 2019.

BORGOGNO, M. et al. The role of product familiarity and consumer involvement on liking and perceptions of fresh meat. **Food Quality and Preference**, v. 44, p. 139–147, 1 set. 2015.

BRAGA, J. S. et al. **Ebook transporte legal bovinos — Ministério da Agricultura e Pecuária**. 1. ed. Jaboticabal: [s.n.].

BRANCACCIO, P.; MAFFULLI, N.; LIMONGELLI, F. M. Creatine kinase monitoring in sport medicine. **British Medical Bulletin**, v. 81–82, n. 1, p. 209–230, 1 jan. 2007.

BRANDT, P.; AASLYNG, M. D. Welfare measurements of finishing pigs on the day of slaughter: A review. **Meat Science**, v. 103, p. 13–23, 1 maio 2015.

CARPENTER, C. E.; CORNFORTH, D. P.; WHITTIER, D. Consumer preferences for beef color and packaging did not affect eating satisfaction. **Meat Science**, v. 57, n. 4, p. 359–363, 1 abr. 2001.

CHULAYO, A. Y.; BRADLEY, G.; MUCHENJE, V. Effects of transport distance, lairage time and stunning efficiency on cortisol, glucose, HSPA1A and how they relate with meat quality in cattle. **Meat Science**, v. 117, p. 89–96, 1 jul. 2016.

CHULAYO, A. Y.; MUCHENJE, V. Activities of some stress enzymes as indicators of slaughter cattle welfare and their relationship with physico-chemical characteristics of beef. **animal**, v. 11, n. 9, p. 1645–1652, 1 set. 2017.

CLABORN, S. W. et al. CONSUMER EVALUATION OF THE PALATABILITY OF USDA SELECT, USDA CHOICE AND CERTIFIED ANGUS BEEF STRIP LOIN STEAKS FROM RETAIL MARKETS IN LUBBOCK, TEXAS, U.S.A. **Journal of Food Quality**, v. 34, n. 6, p. 425–434, 1 dez. 2011.

COCKRAM, M. S.; CORLEY, K. T. T. Effect of pre-slaughter handling on the behaviour and blood composition of beef cattle. **British Veterinary Journal**, v. 147, n. 5, p. 444–454, 1 set. 1991.

COOK, N. et al. Assessing Consistency in Radiated Thermal Output of Beef Steers by Infrared Thermography. **Journal of Imaging 2016, Vol. 2, Page 21**, v. 2, n. 3, p. 21, 11 jul. 2016.

CORCORAN, K. et al. **Current consumer attitudes towards lamb and beef in Europe**. Zaragoza: CIHEAM, 2001.

CORRÊA, A. M. **Termografia infravermelha para a avaliação de carcaça e qualidade de carne bovina**. [s.l.] Universidade Federal de Viçosa, 28 mar. 2019.

CUTHBERTSON, H. et al. Using infrared thermography on farm of origin to predict meat quality and physiological response in cattle (Bos Taurus) exposed to transport and marketing. **Meat Science**, v. 169, p. 108173, 1 nov. 2020.

DE FRESLON, I. et al. Caracterización de la reactividad conductual frente al manejo y su relación con el Cortisol sanguíneo, contusiones y pH de la carne en novillos. **Archivos de medicina veterinaria**, v. 46, n. 2, p. 229–237, 2014.

DE LA FUENTE, J. et al. Physiological response and carcass and meat quality of suckling lambs in relation to transport time and stocking density during transport by road. **Animal**, v.

4, n. 2, p. 250–258, 1 jan. 2010.

DE MORENO, L. A. et al. Attitudinal Determinants of Beef Consumption in Venezuela: A Retrospective Survey. **Foods 2020, Vol. 9, Page 202**, v. 9, n. 2, p. 202, 16 fev. 2020.

DEVINE, C. E. et al. Pre-slaughter stress arising from on-farm handling and its interactions with electrical stimulation on tenderness of lambs. **Meat Science**, v. 73, n. 2, p. 304–312, 1 jun. 2006.

DU, Y. et al. Effect of pre-slaughter conditions on beef quality. **Scientia Agricultura Sinica**, v. 42, n. 10, p. 3625–3632, 2009.

DUNNE, P. G.; MONAHAN, F. J.; MOLONEY, A. P. Current perspectives on the darker beef often reported from extensively-managed cattle: Does physical activity play a significant role? **Livestock Science**, v. 142, n. 1–3, p. 1–22, 1 dez. 2011.

EGAN, A. F.; FERGUSON, D. M.; THOMPSON, J. M. Consumer sensory requirements for beef and their implications for the Australian beef industry. **Australian Journal of Experimental Agriculture**, v. 41, n. 7, p. 855–859, 2001.

EGBERT, W. R.; CORNFORTH, D. P. Factors Influencing Color of Dark Cutting Beef Muscle. **Journal of Food Science**, v. 51, n. 1, p. 57–59, 1 jan. 1986.

EKIZ, B. et al. Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs. **Meat Science**, v. 90, n. 4, p. 967–976, 1 abr. 2012.

ENGLAND, E. M. et al. Excess glycogen does not resolve high ultimate pH of oxidative muscle. **Meat Science**, v. 114, p. 95–102, 1 abr. 2016.

ENGLISH, A. R. et al. Effects of aging on the fundamental color chemistry of dark-cutting beef. **Journal of Animal Science**, v. 94, n. 9, p. 4040–4048, 1 set. 2016.

FAUCITANO, L. et al. Shelf life of pork from five different quality classes. **Meat Science**, v. 84, n. 3, p. 466–469, 1 mar. 2010.

FERGUSON, D. M.; WARNER, R. D. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? **Meat Science**, v. 80, n. 1, p. 12–19, 1 set. 2008.

FINK, J.; SCHOENFELD, B. J.; NAKAZATO, K. The role of hormones in muscle hypertrophy. **The Physician and Sportsmedicine**, v. 46, n. 1, p. 129–134, 2 jan. 2017.

FONT-I-FURNOLS, M.; GUERRERO, L. Consumer preference, behavior and perception about meat and meat products: An overview. **Meat Science**, v. 98, n. 3, p. 361–371, 1 nov. 2014.

FOURY, A. et al. Alternative rearing systems in pigs: consequences on stress indicators at slaughter and meat quality. **animal**, v. 5, n. 10, p. 1620–1625, out. 2011.

GAGAOUA, M. et al. Clustering of sensory eating qualities of beef: Consistencies and differences within carcass, muscle, animal characteristics and rearing factors. 2018.

GAGAOUA, M. et al. Dark-cutting beef: A brief review and an integromics meta-analysis at the proteome level to decipher the underlying pathways. **Meat Science**, v. 181, p. 108611, 1 nov. 2021.

GALLO, C. Carnes de corte oscuro en bovinos (dark cutting beef). **Revista Americarne para América Latina y El Caribe (español-inglés)**, v. 3, p. 10, 2004.

GARCÍA-TORRES, S. et al. Assessment of Stress by Serum Biomarkers in Calves and Their Relationship to Ultimate pH as an Indicator of Meat Quality. **Animals 2021, Vol. 11, Page 2291**, v. 11, n. 8, p. 2291, 3 ago. 2021.

GAŠPERLIN, L.; ŽLENDER, B.; ABRAM, V. Colour of normal and high pH beef heated to different temperatures as related to oxygenation. **Meat Science**, v. 54, n. 4, p. 391–398, 1 abr. 2000.

GERSONY, J. T. et al. Scaling Thermal Properties from the Leaf to the Canopy in the Alaskan Arctic Tundra. **https://doi.org/10.1657/AAAR0016-013**, v. 48, n. 4, p. 739–754, 1 nov. 2018.

GOODSON, K. J. et al. Beef customer satisfaction: factors affecting consumer evaluations of clod steaks. **Journal of Animal Science**, v. 80, n. 2, p. 401–408, 1 fev. 2002.

GOTOH, T. et al. Meat produced by Japanese Black cattle and Wagyu. **Animal Frontiers**, v. 4, n. 4, p. 46–54, 1 out. 2014.

GRAYSON, A. L. et al. The effects of degree of dark cutting on tenderness and sensory attributes of beef,. **Journal of Animal Science**, v. 94, n. 6, p. 2583–2591, 1 jun. 2016a.

GRAYSON, A. L. et al. The effects of degree of dark cutting on tenderness and sensory attributes of beef. **Journal of Animal Science**, v. 94, n. 6, p. 2583–2591, 1 jun. 2016b.

GREBITUS, C. et al. Fresh Meat Packaging: Consumer Acceptance of Modified Atmosphere Packaging including Carbon Monoxide. **Journal of Food Protection**, v. 76, n. 1, p. 99–107, 1 jan. 2013.

GREBITUS, C.; JENSEN, H. H.; ROOSEN, J. US and German consumer preferences for ground beef packaged under a modified atmosphere – Different regulations, different behaviour? **Food Policy**, v. 40, p. 109–118, 1 jun. 2013.

GREGORY, N. G. Animal welfare at markets and during transport and slaughter. **Meat Science**, v. 80, n. 1, p. 2–11, 1 set. 2008.

GRUNERT, K. G.; BREDAHL, L.; BRUNSØ, K. Consumer perception of meat quality and implications for product development in the meat sector—a review. **Meat Science**, v. 66, n. 2, p. 259–272, 1 fev. 2004.

HENCHION, M. M.; MCCARTHY, M.; RESCONI, V. C. Beef quality attributes: A systematic review of consumer perspectives. **Meat Science**, v. 128, p. 1–7, 1 jun. 2017.

HOLDSTOCK, J. et al. The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade. **Meat Science**, v. 98, n. 4, p. 842–849, 1 dez. 2014.

HOLMAN, B. W. B. et al. The identification of dark cutting beef carcasses in Australia, using

Nix Pro Color Sensor^{TM} colour measures, and their relationship to bolar blade, striploin and topside quality traits. **Meat Science**, v. 148, p. 50–54, 1 fev. 2019.

HOPKINS, D. L. et al. The effect of pH decline rate on the meat and eating quality of beef carcasses. **Animal Production Science**, v. 54, n. 4, p. 407–413, 20 fev. 2014.

HORCADA, A. et al. Short communication: Using infrared ocular thermography as a tool to predict meat quality from lean cattle breeds prior to slaughter: Exploratory trial. **Spanish Journal of Agricultural Research**, v. 17, n. 4, p. e06SC01-e06SC01, 2019.

HUGHES, J. et al. **Improving beef colour at grading-Final report**. Disponível em: <https://www.ampc.com.au/getmedia/fb3a8f45-75c0-4791-acef-0da992fb4e3c/AMPC_ImprovingBeefColourAtGrading_FinalReport.pdf?ext=.pdf>. Acesso em: 14 mar. 2023a.

HUGHES, J. et al. High pH in beef longissimus thoracis reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. **Food Research International**, v. 101, p. 228–238, 1 nov. 2017b.

HUGHES, J. et al. Differences in light scattering between pale and dark beef longissimus thoracis muscles are primarily caused by differences in the myofilament lattice, myofibril and muscle fibre transverse spacings. **Meat Science**, v. 149, p. 96–106, 1 mar. 2019.

HUGHES, J. M. et al. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. **Meat Science**, v. 98, n. 3, p. 520–532, 1 nov. 2014.

HUGHES, J. M. et al. Meat color is determined not only by chromatic heme pigments but also by the physical structure and achromatic light scattering properties of the muscle. **Comprehensive Reviews in Food Science and Food Safety**, v. 19, n. 1, p. 44–63, 1 jan. 2020.

HUGHES, J. M.; KEARNEY, G.; WARNER, R. D. Improving beef meat colour scores at carcass grading. **Animal Production Science**, v. 54, n. 4, p. 422–429, 21 fev. 2014.

I. CAPPELLOZZA, B.; S. MARQUES, R. Effects of Pre-Slaughter Stress on Meat Characteristics and Consumer Experience. **Meat and Nutrition**, 28 jul. 2021.

IJAZ, M. et al. Association between meat color of DFD beef and other quality attributes. **Meat Science**, v. 161, 1 mar. 2020.

IJAZ, M. et al. Changes in postmortem metabolites profile of atypical and typical DFD beef. **Meat Science**, v. 193, p. 108922, 1 nov. 2022.

IMMONEN, K. et al. Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. **Meat Science**, v. 55, n. 1, p. 25–31, 1 maio 2000.

JEONG, D. W. et al. Correlations of trained panel sensory values of cooked pork with fatty acid composition, muscle fiber type, and pork quality characteristics in Berkshire pigs. **Meat Science**, v. 86, n. 3, p. 607–615, 1 nov. 2010.

JEREMIAH, L. E.; TONG, A. K. W.; GIBSON, L. L. The usefulness of muscle color and pH for segregating beef carcasses into tenderness groups. **Meat Science**, v. 30, n. 2, p. 97–114, 1 jan. 1991.

JOHNSON, E. O. et al. Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. **Neuroscience & Biobehavioral Reviews**, v. 16, n. 2, p. 115–130, 1 jun. 1992.

KARVATTE, N. et al. Infrared thermography for microclimate assessment in agroforestry systems. **Science of The Total Environment**, v. 731, p. 139252, 20 ago. 2020.

KENNY, F. J.; TARRANT, P. V. The behaviour of young Friesian bulls during social re-grouping at an abattoir. Influence of an overhead electrified wire grid. **Applied Animal Behaviour Science**, v. 18, n. 3–4, p. 233–246, 1 out. 1987.

KILLINGER, K. M. et al. Consumer visual preference and value for beef steaks differing in marbling level and color. **Journal of Animal Science**, v. 82, n. 11, p. 3288–3293, 1 nov. 2004.

KIM, Y. et al. Canopy skin temperature variations in relation to climate, soil temperature, and carbon flux at a ponderosa pine forest in central Oregon. **Agricultural and Forest Meteorology**, v. 226–227, p. 161–173, 15 out. 2016.

KIYIMBA, F. et al. Changes in glycolytic and mitochondrial protein profiles regulates postmortem muscle acidification and oxygen consumption in dark-cutting beef. **Journal of Proteomics**, v. 232, p. 104016, 10 fev. 2021.

KOCH, R. M.; DIKEMAN, M. E.; CROUSE, J. D. Characterization of Biological Types of Cattle (Cycle III).III. Carcass Composition, Quality and Palatability. **Journal of Animal Science**, v. 54, n. 1, p. 35–45, 1 jan. 1982.

KOMATSU, T. et al. Effects of genetic and environmental factors on muscle glycogen content in Japanese Black cattle. **Animal Science Journal**, v. 85, n. 8, p. 793–798, 1 ago. 2014.

KOU, H. X. et al. Automated measurement of cattle surface temperature and its correlation with rectal temperature. **PLOS ONE**, v. 12, n. 4, p. e0175377, 1 abr. 2017.

KREIKEMEIER, K. K.; UNRUH, J. A.; ECK, T. P. Factors affecting the occurrence of dark-cutting beef and selected carcass traits in finished beef cattle. **Journal of Animal Science**, v. 76, n. 2, p. 388–395, 1 fev. 1998.

LAHUCKY, R. et al. Effect of preslaughter handling on muscle glycogen level and selected meat quality traits in beef. **Meat Science**, v. 50, n. 3, p. 389–393, 1 nov. 1998.

LAWRENCE, T. L. J.; FOWLER, V. R.; NOVAKOFSKI, J. E. **Growth of farm animals**. 3. ed. [s.l.] CABI, 2012.

LEIGHTON, P. L. A. et al. Towards improving classification of Canadian dark-cutting beef carcasses: Consumer sensory evaluation. **Meat Science**, v. 195, 1 jan. 2023.

LI, M. et al. Comparative profiling of sarcoplasmic phosphoproteins in ovine muscle with different color stability. **Food Chemistry**, v. 240, p. 104–111, 1 fev. 2018.

LIU, J. et al. Contributions of tenderness, juiciness and flavor liking to overall liking of beef in Europe. **Meat Science**, v. 168, p. 108190, 1 out. 2020.

LOMIWES, D. et al. Small heat shock proteins and toughness in intermediate pHu beef. **Meat Science**, v. 95, n. 3, p. 472–479, 1 nov. 2013.

LOMIWES, D. et al. The development of meat tenderness is likely to be compartmentalised by ultimate pH. **Meat Science**, v. 96, n. 1, p. 646–651, jan. 2014.

LÓPEZ-CAMPOS, Ó. et al. Packaging for retail appearance improvement of dark cutting beef. **Meat Science**, v. 1, n. 96, p. 121, jan. 2014.

LOSADA-ESPINOSA, N. et al. Pre-slaughter cattle welfare indicators for use in commercial abattoirs with voluntary monitoring systems: A systematic review. **Meat Science**, v. 138, p. 34–48, 1 abr. 2018.

LOWE, G. et al. Infrared Thermography—A Non-Invasive Method of Measuring Respiration Rate in Calves. **Animals 2019, Vol. 9, Page 535**, v. 9, n. 8, p. 535, 7 ago. 2019.

LU, X. et al. Association of ultimate pH and stress-related blood variables in cattle. **Meat Science**, v. 139, p. 228–230, 1 maio 2018.

LU, X. et al. Effect of oxygen concentration in modified atmosphere packaging on color changes of the M. longissimus thoraces et lumborum from dark cutting beef carcasses. **Meat Science**, v. 161, p. 107999, 1 mar. 2020.

MACH, N. et al. Association between animal, transportation, slaughterhouse practices, and meat pH in beef. **Meat Science**, v. 78, n. 3, p. 232–238, 1 mar. 2008.

MAHMOOD, S. et al. Can potential for dark cutting be predicted by phenotype? Relationship between sex, carcass characteristics, and the incidence of dark cutting beef. **Canadian Journal of Animal Science**, v. 96, n. 1, p. 19–31, 24 mar. 2016a.

MAHMOOD, S. et al. Relationship between phenotype, carcass characteristics and the incidence of dark cutting in heifers. **Meat Science**, v. 121, p. 261–271, 1 nov. 2016b.

MAHMOOD, S. et al. Understanding the quality of typical and atypical dark cutting beef from heifers and steers. **Meat Science**, v. 133, p. 75–85, 1 nov. 2017.

MAHMOOD, S. et al. Proteomics of dark cutting longissimus thoracis muscle from heifer and steer carcasses. **Meat Science**, v. 137, p. 47–57, 1 mar. 2018.

MANCINI, R. A.; HUNT, M. C. Current research in meat color. **Meat Science**, v. 71, n. 1, p. 100–121, 1 set. 2005.

MARTELLO, L. S. et al. Infrared thermography as a tool to evaluate body surface temperature and its relationship with feed efficiency in Bos indicus cattle in tropical conditions. **International Journal of Biometeorology**, v. 60, n. 1, p. 173–181, 1 jan. 2016.

MCCARTHY, S. N. et al. Evaluation of beef eating quality by Irish consumers. **Meat Science**, v. 132, p. 118–124, 1 out. 2017.

MCGILCHRIST, P. et al. Beef carcasses with larger eye muscle areas, lower ossification scores and improved nutrition have a lower incidence of dark cutting. **Meat Science**, v. 92, n. 4, p. 474–480, 1 dez. 2012.

MCGILCHRIST, P. et al. The incidence of dark cutting in southern Australian beef production systems fluctuates between months. **Animal Production Science**, v. 54, n. 10, p. 1765–1769, 2014.

MCKEITH, R. O. et al. Mitochondrial abundance and efficiency contribute to lean color of dark cutting beef. **Meat Science**, v. 116, p. 165–173, 1 jun. 2016.

MCPHAIL, N. G. et al. Factors influencing the occurrence of high ultimate pH in three muscles of lamb carcasses in Australia. **Animal Production Science**, v. 54, n. 10, p. 1853–1859, 19 ago. 2014.

MEAT & LIVESTOCK AUSTRALIA. **Annual Report 2016-17**. Disponível em: <https://www.mla.com.au/about-mla/how-we-are-governed/Planning-reporting/annual-reporting/annual-report-2016-17/>. Acesso em: 16 mar. 2023.

MILLER, M. **Dark, Firm and Dry Beef**. Disponível em: <https://fyi.extension.wisc.edu/wbic/files/2011/04/Dark-Firm-and-Dry-Beef.pdf>. Acesso em: 14 mar. 2023.

MINKA, N. S.; AYO, J. O. Physiological responses of food animals to road transportation stress. **African Journal of Biotechnology**, v. 9, n. 40, p. 6601–6613, 16 ago. 2010.

MOHAN RAJ, A. B. et al. Effect of mixing male sex types of cattle on their meat quality and stress-related parameters. **Meat Science**, v. 32, n. 4, p. 367–386, 1 jan. 1992.

MONTANHOLI, Y. R. et al. On the determination of residual feed intake and associations of infrared thermography with efficiency and ultrasound traits in beef bulls. **Livestock Science**, v. 125, n. 1, p. 22–30, 1 out. 2009.

MONTANHOLI, Y. R. et al. Assessing feed efficiency in beef steers through feeding behavior, infrared thermography and glucocorticoids. **Animal**, v. 4, n. 5, p. 692–701, 1 jan. 2010.

MOORE, M. C. et al. National Beef Quality Audit–2011: In-plant survey of targeted carcass characteristics related to quality, quantity, value, and marketing of fed steers and heifers,. **Journal of Animal Science**, v. 90, n. 13, p. 5143–5151, 1 dez. 2012.

MOTA-ROJAS, D. et al. Clinical Applications and Factors Involved in Validating Thermal Windows Used in Infrared Thermography in Cattle and River Buffalo to Assess Health and Productivity. **Animals 2021, Vol. 11, Page 2247**, v. 11, n. 8, p. 2247, 30 jul. 2021.

MUCHENJE, V. et al. Relationship between pre-slaughter stress responsiveness and beef quality in three cattle breeds. **Meat Science**, v. 81, n. 4, p. 653–657, 1 abr. 2009.

MUELLER, L. F. et al. Gender status effect on carcass and meat quality traits of feedlot Angus × Nellore cattle. **Animal Science Journal**, v. 90, n. 8, p. 1078–1089, 1 ago. 2019.

MUIR, P. D.; DEAKER, J. M.; BOWN, M. D. Effects of forage- and grain-based feeding systems on beef quality: A review. **New Zealand Journal of Agricultural Research**, v. 41,

n. 4, p. 623–635, 1998.

NELIS, J. L. D. et al. Biomarkers and biosensors for the diagnosis of noncompliant pH, dark cutting beef predisposition, and welfare in cattle. **Comprehensive Reviews in Food Science and Food Safety**, v. 21, n. 3, p. 2391–2432, 1 maio 2022.

NEWCOMBE, M. A. et al. “Eat like a man”. A social constructionist analysis of the role of food in men’s lives. **Appetite**, v. 59, n. 2, p. 391–398, 1 out. 2012.

NIELSEN, B. L.; DYBKJR, L.; HERSKIN, M. S. Road transport of farm animals: effects of journey duration on animal welfare. **Animal**, v. 5, n. 3, p. 415–427, 1 jan. 2011.

O’LOUGHLIN, A. et al. Biomarker responses to weaning stress in beef calves. **Research in Veterinary Science**, v. 97, n. 2, p. 458–463, 1 out. 2014.

ÖZDEMIR, S.; EKIZ, E. E.; EKIZ, B. Effect of lairage duration on cattle behaviors and stockperson actions in the slaughter corridor in Simmental and Swiss Brown breeds. **Tropical Animal Health and Production**, v. 54, n. 2, p. 1–7, 1 abr. 2022.

PAGE, J. K.; WULF, D. M.; SCHWOTZER, T. R. A survey of beef muscle color and pH. **Journal of Animal Science**, v. 79, n. 3, p. 678–687, 1 mar. 2001.

PEDREIRA, C.; SILVA, L.; ALONSO, M. **Use of grazed pastures in the brazilian livestock industry: a brief overview**. (R. Carnevalli et al., Eds.) Forages in Warm Climates. **Anais**...2015. Disponível em: <https://www.researchgate.net/profile/Roberta-Carnevalli-2/publication/280612162_Silvopastoral_systems_with_Tifton-85_grass_and_tropical_fruit_trees/links/55bea9a908aec0e5f445e28f/Silvopastoral-systems-with-Tifton-85-grass-and-tropical-fruit-trees.pdf#page=7>. Acesso em: 1 mar. 2023

PICARD, B. et al. Inverse relationships between biomarkers and beef tenderness according to contractile and metabolic properties of the muscle. **Journal of Agricultural and Food Chemistry**, v. 62, n. 40, p. 9808–9818, 8 out. 2014.

PICARD, B.; GAGAOUA, M.; GAGAOUA, M. Muscle Fiber Properties in Cattle and Their

Relationships with Meat Qualities: An Overview. **Journal of Agricultural and Food Chemistry**, v. 68, n. 22, p. 6021–6039, 3 jun. 2020.

PIGHIN, D. G. et al. Relationship between changes in core body temperature in lambs and post-slaughter muscle glycogen content and dark-cutting. **Animal Production Science**, v. 54, n. 4, p. 459–463, 15 maio 2013.

POLETI, M. D. et al. Label-free quantitative proteomic analysis reveals muscle contraction and metabolism proteins linked to ultimate pH in bovine skeletal muscle. **Meat Science**, v. 145, p. 209–219, 1 nov. 2018.

POLKINGHORNE, R. et al. Development of a commercial system to apply the Meat Standards Australia grading model to optimise the return on eating quality in a beef supply chain. **Australian Journal of Experimental Agriculture**, v. 48, n. 11, p. 1451–1458, 16 out. 2008.

PONNAMPALAM, E. N. et al. Causes and Contributing Factors to “Dark Cutting” Meat: Current Trends and Future Directions: A Review. **Comprehensive Reviews in Food Science and Food Safety**, v. 16, n. 3, p. 400–430, 1 maio 2017.

PULFORD, D. J. et al. The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. **Meat Science**, v. 79, n. 4, p. 623–630, 1 ago. 2008.

PURCHAS, R. W. An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. **Meat Science**, v. 27, n. 2, p. 129–140, 1 jan. 1990.

PURCHAS, R. W.; AUNGSUPAKORN, R. Further investigations into the relationship between ultimate pH and tenderness for beef samples from bulls and steers. **Meat Science**, v. 34, n. 2, p. 163–178, 1 jan. 1993.

PURSLOW, P. P. et al. Variations in meat colour due to factors other than myoglobin chemistry; a synthesis of recent findings (invited review). **Meat Science**, v. 159, p. 107941, 1 jan. 2020.

RAMANATHAN, R. et al. Effects of 4-hydroxy-2-nonenal on beef heart mitochondrial ultrastructure, oxygen consumption, and metmyoglobin reduction. **Meat Science**, v. 90, n. 3, p. 564–571, 1 mar. 2012.

RAMANATHAN, R. et al. Mitochondrial functionality and beef colour: A review of recent research. **South African Journal of Animal Science**, v. 49, n. 1, p. 9–19, 1 abr. 2019.

RAMANATHAN, R. et al. Recent Updates in Meat Color Research: Integrating Traditional and High-Throughput Approaches. **Meat and Muscle Biology**, v. 4, n. 2, p. 1–24, 28 jul. 2020.

REALINI, C. E. et al. Relative importance of cues underlying Spanish consumers' beef choice and segmentation, and consumer liking of beef enriched with n-3 and CLA fatty acids. **Food Quality and Preference**, v. 33, p. 74–85, 1 abr. 2014.

REIS, M. M.; ROSENVOLD, K. Early on-line classification of beef carcasses based on ultimate pH by near infrared spectroscopy. **Meat Science**, v. 96, n. 2, p. 862–869, 1 fev. 2014.

RENERRE, M. Factors involved in the discoloration of beef meat. **International Journal of Food Science & Technology**, v. 25, n. 6, p. 613–630, 1 dez. 1990.

RIBEIRO, C. C. DE S. et al. New alternatives for improving and assessing the color of dark–cutting beef – a review. **Scientia Agricola**, v. 79, n. 1, p. 1–16, 18 jan. 2021.

ROSA, A. et al. Incidence of DFD meat on Brazilian beef cuts. **Meat Science**, v. 112, p. 132–133, fev. 2016.

RUSSELL, E. et al. Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. **Psychoneuroendocrinology**, v. 37, n. 5, p. 589–601, 1 maio 2012.

SANTANA, M. L. et al. Structure and genetic diversity of Brazilian Zebu cattle breeds assessed by pedigree analysis. **Livestock Science**, v. 187, p. 6–15, 1 maio 2016.

SANTOS, D. et al. The most important attributes of beef sensory quality and production variables that can affect it: A review. **Livestock Science**, v. 250, p. 104573, 1 ago. 2021.

SCHAEFER, A. et al. 497 The automated and real time use of infrared thermography in the detection and correction of DFD and fevers in cattle. **Journal of Animal Science**, v. 96, n. suppl_3, p. 275–275, 7 dez. 2018.

SCHEFFLER, T. L. et al. Mitochondria influence postmortem metabolism and pH in an in vitro model. **Meat Science**, v. 110, p. 118–125, 1 dez. 2015.

SCHEFFLER, T. L.; GERRARD, D. E. Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. **Meat Science**, v. 77, n. 1, p. 7–16, 1 set. 2007.

SEPULVEDA, C. A. et al. Cooking Method and USDA Quality Grade Affect Consumer Palatability and Flavor of Beef Strip Loin Steaks. **Meat and Muscle Biology**, v. 3, n. 1, 1 out. 2019.

SHACKELFORD, S. D. et al. Effect of biological type of cattle on the incidence of the dark, firm, and dry condition in the longissimus muscle. **Journal of Animal Science**, v. 72, n. 2, p. 337–343, 1 fev. 1994.

SHAW, F. D.; TUME, R. K. The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents—A review of recent work. **Meat Science**, v. 32, n. 3, p. 311–329, 1 jan. 1992.

SILVA, J. A.; PATARATA, L.; MARTINS, C. Influence of ultimate pH on bovine meat tenderness during ageing. **Meat Science**, v. 52, n. 4, p. 453–459, 1 ago. 1999.

STEEL, C. C. et al. Abattoir Factors Influencing the Incidence of Dark Cutting in Australian Grain-Fed Beef. **Animals 2021, Vol. 11, Page 474**, v. 11, n. 2, p. 474, 10 fev. 2021.

SUMAN, S. P.; JOSEPH, P. Myoglobin Chemistry and Meat Color. **Annual Review of Food Science and Technology** , v. 4, p. 79–99, 2013.

SUTHERLAND, M. A. et al. Evaluation of infrared thermography as a non-invasive method of measuring the autonomic nervous response in sheep. **PLOS ONE**, v. 15, n. 5, p. e0233558, 1 maio 2020.

TADICH, N. et al. Effects of transport and lairage time on some blood constituents of Friesian-cross steers in Chile. **Livestock Production Science**, v. 93, n. 3, p. 223–233, 1 maio 2005.

TANG, F. et al. Non-Invasive Glucose Measurement by Use of Metabolic Heat Conformation Method. **Sensors 2008, Vol. 8, Pages 3335-3344**, v. 8, n. 5, p. 3335–3344, 21 maio 2008.

TANG, J. et al. Postmortem Oxygen Consumption by Mitochondria and Its Effects on Myoglobin Form and Stability. **Journal of Agricultural and Food Chemistry**, v. 53, n. 4, p. 1223–1230, 23 fev. 2005.

TANG, J.; FAUSTMAN, C.; HOAGLAND, T. A. Krzywicki revisited: Equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. **Journal of Food Science**, v. 69, n. 9, 2004.

TARRANT, P. V. Animal Behaviour and Environment in the Dark-Cutting Condition in Beef - A Review. **Irish Journal of Food Science and Technology**, v. 13, n. 1, p. 1–21, 1989.

TARRANT, P. V.; SHERINGTON, J. An investigation of ultimate pH in the muscles of commercial beef carcasses. **Meat Science**, v. 4, n. 4, p. 287–297, 1980.

TARUMÁN, J. A. et al. Risk Factors for Bruises and High Muscle pH in Lamb Carcasses of Tierra Del Fuego, Chilean Patagonia. **Open Access Library Journal**, v. 5, n. 1, p. 1–11, 4 jan. 2018.

TEKE, B. et al. Effects of different lairage times after long distance transportation on carcass and meat quality characteristics of Hungarian Simmental bulls. **Meat Science**, v. 96, n. 1, p. 224–229, 1 jan. 2014.

TERLOUW, C. E. M. et al. Understanding the Determination of Meat Quality Using

Biochemical Characteristics of the Muscle: Stress at Slaughter and Other Missing Keys. **Foods 2021, Vol. 10, Page 84**, v. 10, n. 1, p. 84, 4 jan. 2021.

TESTA, M. L. et al. Color and Marbling as Predictors of Meat Quality Perception of Argentinian Consumers. **Foods 2021, Vol. 10, Page 1465**, v. 10, n. 7, p. 1465, 24 jun. 2021.

TORRES-BURGOS, Y. I. et al. pH Variability and its Relationship with Sarcomere Length and Free Calcium in Beef from Commercial Cattle in Puerto Rico. **Meat and Muscle Biology**, v. 3, n. 2, 30 nov. 2019.

VESTERGAARD, M.; OKSBJERG, N.; HENCKEL, P. Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. **Meat Science**, v. 54, n. 2, p. 177–185, 1 fev. 2000.

VIERCK, K. R. et al. Marbling Texture's Effects on Beef Palatability. **Meat and Muscle Biology**, v. 2, n. 1, 30 abr. 2018.

VILJOEN, H. F.; DE KOCK, H. L.; WEBB, E. C. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. **Meat Science**, v. 61, p. 181–185, 2002a.

VILJOEN, H. F.; DE KOCK, H. L.; WEBB, E. C. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. **Meat Science**, v. 61, n. 2, p. 181–185, 1 jun. 2002b.

WARNER, R. D. et al. Acute stress induced by the preslaughter use of electric prodders causes tougher beef meat. **Australian Journal of Experimental Agriculture**, v. 47, n. 7, p. 782–788, 2 jul. 2007.

WARNER, R. D. et al. Factors influencing the incidence of high rigor temperature in beef carcasses in Australia. **Animal Production Science**, v. 54, n. 4, p. 363–374, 28 fev. 2014.

WARNER, R. D. et al. Meat tenderness: advances in biology, biochemistry, molecular mechanisms and new technologies. **Meat Science**, v. 185, p. 108657, 1 mar. 2022.

WARRIS, P. D. The extraction of haem pigments from fresh meat. **International Journal of**

Food Science & Technology, v. 14, n. 1, p. 75–80, 1 fev. 1979.

WARRISS, P. D. The handling of cattle pre-slaughter and its effects on carcass and meat quality. **Applied Animal Behaviour Science**, v. 28, n. 1–2, p. 171–186, 1 nov. 1990.

WATANABE, A.; DALY, C. C.; DEVINE, C. E. The effects of the ultimate pH of meat on tenderness changes during ageing. **Meat Science**, v. 42, n. 1, p. 67–78, 1 jan. 1996.

WEAVER, A. D.; BOWKER, B. C.; GERRARD, D. E. Sarcomere length influences μ-calpain-mediated proteolysis of bovine myofibrils. **Journal of Animal Science**, v. 87, n. 6, p. 2096–2103, 1 jun. 2009.

WERNER, M. et al. Effects of a long distance transport and subsequent recovery in recently weaned crossbred beef calves in Southern Chile. **Livestock Science**, v. 152, n. 1, p. 42–46, 1 mar. 2013.

WHEELER, T. L. et al. Characterization of biological types of cattle (Cycle V): carcass traits and longissimus palatability. **Journal of Animal Science**, v. 79, n. 5, p. 1209–1222, 1 maio 2001.

WHEELER, T. L. et al. Characterization of biological types of cattle (Cycle VI): Carcass, yield, and longissimus palatability traits. **Journal of Animal Science**, v. 82, n. 4, p. 1177–1189, 1 abr. 2004.

WHEELER, T. L. et al. Characterization of biological types of cattle (Cycle VIII): Carcass, yield, and longissimus palatability traits. **Journal of Animal Science**, v. 88, n. 9, p. 3070–3083, 1 set. 2010.

WHEELER, T. L.; SHACKELFORD, S. D.; KOOHMARAIE, M. Cooking and palatability traits of beef longissimus steaks cooked with a belt grill or an open hearth electric broiler. **Journal of Animal Science**, v. 76, n. 11, p. 2805–2810, 1 nov. 1998.

WULF, D. M. et al. Genetic influences on beef longissimus palatability in charolais- and limousin-sired steers and heifers. **Journal of Animal Science**, v. 74, n. 10, p. 2394–2405, 1 out. 1996.

XIAO, L. et al. A Review of Previous Studies on DFD Beef. **Food Science**, v. 36, n. 19, p. 271, 15 out. 2015.

YANG, X. et al. Investigation of the physicochemical, bacteriological, and sensory quality of beef steaks held under modified atmosphere packaging and representative of different ultimate pH values. **Meat Science**, v. 174, p. 108416, 1 abr. 2021.

ZHANG, Y. et al. Determination of a pH threshold for dark cutting beef based on visual evaluation by Asian consumers. **Meat Science**, v. 172, p. 108347, 1 fev. 2021.

6 ARTIGO B – SENSORY QUALITY OF BEEF WITH DIFFERENT ULTIMATE PH VALUES - A BRAZILIAN PERSPECTIVE

Esta seção é a versão que será submetida para publicação e segue as normas da revista Meat Science.

ARTIGO 2

Sensory quality of beef with different ultimate pH values - a Brazilian perspective

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Abstract

This study evaluated Brazilian consumer perceptions of beef with different pH values at 48 h post-mortem (pHu) through sensory analysis. A total of 138 consumers evaluated raw and grilled steaks. The steaks were divided according to their pHu (normal pH < 5.8, atypical darkness, firmness, and dryness [DFD] > 5.8 pH < 6, and typical DFD pH ≥ 6). There was no difference in the visual evaluation of raw steaks or purchase intention. Evaluation of the grilled steaks showed that consumers preferred typical DFD steaks in terms of tenderness, and there was a tendency for the same behavior in terms of juiciness. No differences were observed in other evaluated parameters. Cluster analysis identified three consumer segments for visual evaluation, indicating a preference for steaks with higher pHu in terms of freshness. Additionally, four segments were identified for evaluation, with some groups expressing a preference for higher pHu meat in terms of freshness, appearance, tenderness, and overall acceptability. The developed regression models for overall acceptability and purchase intention exhibited favorable adjustment indices, with r^2 values of 0.86 and 0.57, respectively, for raw steaks and 0.90 for grilled steaks for in overall liking. Regression models indicated a strong influence of color and appearance of freshness, in addition to tenderness and juiciness, on the overall liking scores of consumers. These results indicate that Brazilian consumers do not dislike dark cutting and, despite differentiating their greater tenderness, do not show a preference between the different pHu values.

Keywords: Atypical DFD; Consumer acceptance; Dark cutting; DFD; Nellore bulls

1. Introduction

Meat consumption is heavily influenced by various factors such as sensory quality, tenderness, juiciness, flavor, and appearance (WHEELER; SHACKELFORD; KOOHMARAIE, 1998). Among these attributes, color plays a crucial role in determining meat freshness and influences consumer purchasing decisions (FONT-I-FURNOLS; GUERRERO, 2014). The occurrence of the darkness, firmness, and dryness (DFD) anomaly associated with preslaughter stress alters the final pH (pHu) of meat, resulting in a darker color (HOLDSTOCK et al., 2014; MAHMOOD et al., 2017). However, the research findings on consumer preferences have diverged. Some studies have suggested a preference for brighter red meat over darker meat (GRAYSON et al., 2016a; LEIGHTON et al., 2023; PONNAMPALAM et al., 2017), whereas others argue in favor of darker red meat (YANG et al., 2021).

Consumer judgments are formed during two critical moments, namely at the point of purchase in the store and after preparation at home (SANTOS et al., 2021), which significantly impact future buying decisions (BELLO ACEBRÓN; CALVO DOPICO, 2000). Understanding the factors that contribute to a positive consumer experience is vital to guide subsequent steps in the production chain. To gain a comprehensive and reliable understanding, studies involving consumers in diverse settings are essential because relying solely on assessments by trained individuals may provide limited insights (LEIGHTON et al., 2023). This study aimed to examine whether Brazilian beef consumers can perceive differences in steaks with varying pHu ranges and to assess their acceptance of these visual and palatable characteristics through evaluations of raw and grilled steaks.

2. Material and Methods

This research was part of a larger project that was submitted to the Ethics Committee for

Research Involving Human Beings of the State University of Londrina (CEP-UEL) and was approved under number 4.528.778.

2.1 Sample collection

A total of 69 meat samples were randomly selected from Nellore bulls aged 24–38 months from a larger sample pool (n = 113) for the experiment. Sample collection took place over three consecutive days, in a slaughterhouse with federal inspection. The carcasses were kept for 48 h at chilling temperature (4 ºC). Carcasses were selected based on their pHu values, which were measured using a Hanna HI98163® pH meter (Hanna Instruments Inc., Barueri, Brazil) previously calibrated at room temperature with commercial standard solutions of pH 4 and 7, temperature was measured simultaneously with pH assessment. Carcasses with pH < 5.8 were categorized as normal (normal, n = 23), those with pH ≥ 5.8 and < 6.0 were classified as intermediate pHu (atypical DFD, n = 23), and carcasses with pH > 6.0 were designated as high pHu (typical DFD, n = 23). The left half-carcasses were divided at the 12th rib to extract approximately 15 cm samples (m. *Longissimus thoracis*) for subsequent analyses. From this portion, two steaks were sliced at a thickness of 2.52 cm for sensory evaluation. These steaks were carefully placed in polyamide/polyethylene bags, vacuum-sealed, and frozen at -20 °C until further analysis.

2.2 Consumer sensory evaluation

A total of 138 consumers were carefully selected based on their responses to a prescreening assessment, making them eligible to participate in the study. The selected individuals were required to be aged ≥18 years, regular consumers of beef with a frequency of at least once weekly, and preferred steaks cooked between medium rare and medium well. Following the pre-screening process, the participants were asked to provide additional information regarding their gender and age to ensure a balanced representation within these categories. Each evaluation session consisted of six participants who assessed one steak from each treatment group (three steaks overall). A total of 23 steaks from each treatment group were evaluated. Each session lasted approximately 30 min. The evaluation process was extended over 4 d, consecutively, with a minimum of four sessions daily. To mitigate potential bias, the order of sample presentation was randomized and balanced between all participants using William's Latin square design.

2.2.1 Raw steak evaluation

For visual analysis, the steaks were thawed for 24 h at 4 °C. After thawing, the steaks were placed in a Styrofoam mold and covered with plastic film for 1 h at 4 °C for oxygenation. Before being evaluated, the steaks were uncovered, and excess liquid was removed. Each steak was assigned a random three-digit code. Consumers were asked regarding the color, appearance, freshness, and overall liking of the steaks (1 = extremely disliked, 9 = extremely liked) using a 9-point hedonic scale with a 5-point hedonic scale (1 = definitely would not purchase, 5 = would definitely buy).

2.2.2 Grilled steak evaluation

To prepare the grilled meat samples for consumer evaluation, frozen steaks were thawed at 4 °C for 24 h. Once thawed, the steaks were allowed to reach room temperature, ensuring an internal temperature of 4 °C. Subsequently, the steaks were grilled using an electric grill until they reached an internal temperature of 71 °C. Individual temperatures were monitored using a dedicated thermometer for each steak. After grilling, the steaks were carefully sliced into 1.27 × 2.5 cm pieces, deliberately avoiding areas with connective tissue or fat. The steak pieces were then placed in white plastic cups, labeled with unique random three-digit codes, and used for evaluation. Additionally, each consumer was provided with a glass of water and unsalted biscuits to cleanse their palate between the samples. It is important to note that the samples were prepared before the consumer evaluation. Consumers were asked regarding the tenderness, flavor, juiciness, and overall liking of the steaks (1 = dislike extremely, 9 = like extremely) using a 9-point hedonic scale and regarding purchase intention using a 5-point hedonic scale (1 = definitely would not purchase, 5 = would definitely buy).

2.4 Shear force

The samples were weighed and thawed at 2–4 °C for 24 h. A thermocouple connected to a digital thermometer was inserted into the center of the samples to monitor the internal temperature. The steaks were roasted in a preheated oven using a thermostat. Once the internal temperature reached 40 °C, the steaks were turned over and cooked until they reached 71 °C. After chilling, the samples were refrigerated and weighed, and the cooking loss was calculated. Cylinders measuring 1.27 cm in diameter were extracted using a punch attached to an industrial drill. The cylinders were sheared using a Brookfield CT-3 Texture Analyzer, and eight repeated measurements were captured per steak to increase precision. The results were obtained by averaging the measured values.

2.5 Statistical analysis

The effect of pHu on consumer ratings of raw and grilled steaks was determined using one-way analysis of variance (ANOVA). Differences between means were identified using Tukey's test with statistical significance set at $P < 0.05$, and trending toward statistical significance set at $P < 0.10$. Agglomerative hierarchical cluster analysis considering Euclidean distances was performed using Ward's method. This method combines factor analysis and classification techniques to identify market segments and drivers of preferences within these segments. The effect of pHu on the frequency of consumer responses was determined using chi-square analysis. Multiple linear regression analysis was performed to predict the overall liking and purchase intention for raw and grilled steaks. The model included the fixed effect of pHu groups and the random effects of session, consumer day analyses and day of collection. All analyses were performed using the Jamovi® (The Jamovi Project, version 2.2) statistical software.

3. Results and Discussion

3.1Demographic profile of consumers

Table 1 presents the demographic profiles of the consumers involved in this study. The sensory panel comprised a similar representation of women (48%) and men (52%). Most participants (92%) resided in households with three or more individuals and had a monthly income exceeding two minimum wages, with a substantial portion (33%) having an income surpassing five minimum wages. These income levels can be attributed to the age and educational background of the participants, as approximately 45% were ≥36 years, and 53% had completed undergraduate or graduate studies. A significant proportion (64%) of the participants consumed beef more than seven times weekly, which is notably higher than the consumption patterns in other countries. For instance, Zhang et al. (2021) reported that 14.76% of Asian participants consumed meat twice weekly or more, whereas Sepulveda, Garmyn, Legako, and Miller (2019), in a study conducted in the United States, determined that 24% of participants consumed meat more than seven times weekly. Hierarchical cluster analysis revealed three distinct consumer segments for evaluating raw steaks and four consumer segments for evaluating grilled steaks (Table 1). Notably, there were no significant differences ($P > 0.05$) in sociodemographic characteristics or consumption habits among the raw steak segments. For the grilled steaks, the only disparity observed was in terms of sex, specifically between Clusters 1 (predominantly men) and 2 (predominantly women) (P = 0.05).

Table 1. Socio-demographic data and consumption habits of participants in the sensory evaluation of beef steaks by final pH (%).

Variable	Total	Raw steak			Grilled steak			
		Cluster 1	Cluster 2	Cluster 3	Cluster 1	Cluster 2	Cluster 3	Cluster 4
	n = 138	n = 27	n = 73	n = 38	n = 40	n = 41	n = 40	n = 17
Gender								
Female	48	54	45.9	50	42.5	57.5	45	52.9
Male	52	46	54.1	50	57.5	42.5	55	47.1
Household size								
1 Person	4	3.8	4.1	5.3	5	-	10	-
2 people	4	19.2	27	23.7	27.5	26.8	22.5	17.6
3 people	24	23.1	27	10.5	22.5	19.5	17.5	35.3
4 people	23	46.2	33.7	39.4	32.5	44	37.5	35.3
5 people	37	7.7	4.1	13.2	5	7.3	10	5.9
> 5 people	8	-	4.1	7.9	7.5	2.4	2.5	5.9
Age group (years)								
18-25	39	34.6	43.2	39.5	42.5	39	32.5	58.8
26-35	16	15.4	17.6	15.8	22.5	19.5	12.5	6
36-45	19	15.4	14.8	26.3	15	19.5	20	17.7
46-55	10	19.2	8.1	2.6	7.5	9.8	12.5	-
> 55	16	15.4	16.2	15.8	12.5	12.2	22.5	17.7
Monthly household income (R$)[1]								
≤ 2,420	6	7.7	4.1	7.9	8	2.4	10	-
2,421 - 4,850	46	38.5	47.3	51.7	51	39	47.5	53
4,850 - 7,270	15	19.2	13.5	18.4	18	17.1	10	23.5
> 7,270	33	34.6	35.1	22	23	41.5	32.5	23.5
Level of education								
Non-elementary school graduate	3	7.7	6.8	5.3	2.5	4.9	12.5	5.9
Elementary school graduate	7	7.7	1.4	2.6	-	2.4	5	5.9
Non-high school graduate	22	3.9	1.4	10.5	7.5	-	7.5	-
High school graduate	4	27.7	21.6	18.4	20	24.4	20	23.5
Non-college graduate	10	33.8	32.4	34.2	35	26.8	27.5	52.9
College Graduate	31	7.7	13.5	7.9	15	12.2	7.5	5.9
Postgraduate	22	11.5	22.9	21.1	17.5	29.3	20	5.9
Weekly beef consumption								
1 to 3 times	6	23.1	32.4	33.9	20	25	33	31
4 to 6 times	30	57.7	40.5	53	55	49	54	47
7 to 10 times	48	15.4	20.3	10.5	23	19	6	18
11 or more times	16	3.8	6.8	2.6	2	6	7	4

[1] Considering a monthly salary of R$1,212.00, around U$230.00.

3.2 Acceptability of raw steak

The evaluation results of the visual preference of raw steaks from carcasses with different pHu values are presented in Table 2. There was no significant difference in consumer evaluation of the evaluated parameters, and the chi-square test did not show differences between the frequencies of rating scores. All treatments had positive rating scores regarding color acceptability, freshness appearance, and overall liking, with >63% of ratings scoring > 6 on the hedonic scale for color and appearance of freshness, and 59% for overall liking (Figure 1), indicating that they liked the steaks at least moderately. Concerning purchase intention, ≥63% of the scores given indicated that the participants would "probably buy" or "definitely buy."

Prior studies conducted in various countries (CARPENTER; CORNFORTH; WHITTIER, 2001; VILJOEN; DE KOCK; WEBB, 2002a) have consistently demonstrated a heightened consumer preference for bright red meat, which is indicative of meat with a final pH value of <5.8, which is categorized as normal in this study. Surprisingly, contrary to our initial expectations, consumers did not express a dislike for darker; instead, they exhibited positive acceptance of both color groups, without any discernible derogatory distinctions based on color variations.

In a recent study with Argentine consumers, Testa et al. (2021) reported that color perception and marbling are influenced by sociodemographic characteristics and purchasing habits, suggesting the potential for stratifying consumer groups based on their perceptions. Similarly, Zhang et al. (2021) corroborated the influence of sociodemographics and determined that Asian consumers exhibit a preference for darker meat. In contrast, Canadian consumers, as revealed in a study assessing the reclassification of cuts with a dark appearance, awarded higher scores to meat with a brighter red color. This finding poses a potential challenge in replacing DFD meat in the Canadian market (LEIGHTON et al., 2023).

Table 2. Sensory consumer evaluation of raw beef steaks with different pHu values using a hedonic scale.

Attribute	**Normal (pHu < 5.8)**	**Atypical DFD (pHu 5.8 – < 6)**	**Typical DFD (pHu ≥ 6)**
Color acceptability [1]			
Total	6.8	7.1	7.2
Cluster 1	7.0	7.1	7.5
Cluster 2	6.79	7.0	7.0
Cluster 3	7.0	7.5	7.7
Freshness appearance [1]			
Total	6.6	6.7	6.9
Cluster 1	7.0^{AB}	7.0^{AB}	6.9
Cluster 2	6.2^{bB}	6.6^{abB}	7.0^{a}
Cluster 3	7.6^{A}	7.5^{A}	7.6
Overall liking [1]			
Total	6.5	6.7	7.0
Cluster 1	6.9^{AB}	6.9^{AB}	7.2^{AB}
Cluster 2	6.1^{Bb}	7.0^{aB}	7.0^{aB}
Cluster 3	7.1^{A}	7.4^{A}	7.7^{A}
Purchase intent [2]			
Total	3.7	3.7	4.0
Cluster 1	3.6^{CAB}	3.5	4.0^{AB}
Cluster 2	3.4^{Bb}	3.9^{a}	3.9^{aB}
Cluster 3	4.3^{A}	4.1	4.4^{A}

Different letters (a-c) in the same column indicate significant differences (*P* < 0.01). [1] Acceptability scores: 1 = dislike extremely, 9 = like extremely. [2] Purchase intent: 1 = definitely would not purchase, 5 = definitely would purchase.

Cluster 2 stood out as the only group in which a variation in the perception of steak freshness was observed. These consumers preferred steaks from the typical DFD group, whereas they did not detect disparities among the higher pHu groups, and there were no distinctions between the atypical DFD and normal groups. Furthermore, when assessing overall liking, consumers in Cluster 2 preferred steaks from the higher pHu group (typical and atypical

DFD) to steaks from the normal pHu group. Consumers from Cluster 2 also showed greater purchase intention for steaks from the higher pHu groups than for steaks from the normal pHu group.

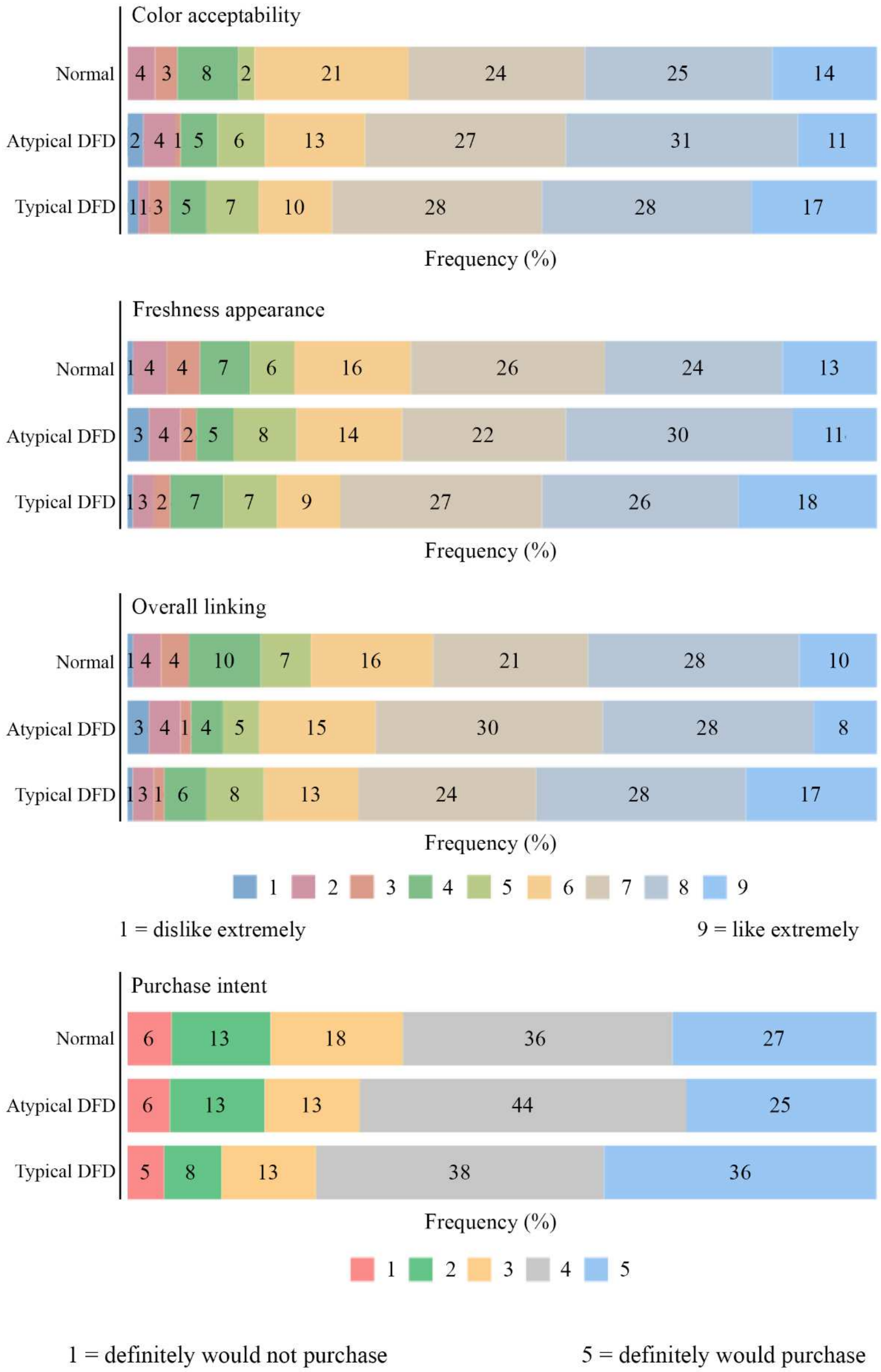

Figure 2. Frequencies of consumer evaluation for raw steaks with different pHu.

The absence of inter-cluster differences implies homogeneity among consumers within each segment in terms of sociodemographic characteristics and consumption habits. This lack of variability poses a challenge to identifying the precise factors that influence sensory preferences within each cluster. However, despite the uniformity within clusters, the observed distinctions between clusters were significant, indicating the presence of distinct consumer groups with specific sensory preferences. These inter-cluster differences may be attributed to unexplored factors in this study, such as the prior food experiences of consumers, individual preferences, or genetic variations in sensory perception.

3.3 Acceptability of grilled steak

We found that consumers identified differences between treatments in assessing tenderness ($P < 0.05$) and tendency for juiciness ($P = 0.056$) when assessing grilled steak preferences (Table 3). There was a greater consumer preference for typical DFD steaks, which were considered more tender than normal and atypical DFD steaks. There were no significant differences in the evaluation of flavor, overall liking, or purchase intent.

After cluster analysis, four consumer segments were identified. However, it is notable that no differences ($P > 0.05$) were found within the clusters in terms of sociodemographic characteristics and consumption habits. This suggests that the sensory preferences identified in the different clusters cannot be attributed to sociodemographic factors, and that other unexamined variables in this study should be considered.

Consumers in Cluster 1 rated steaks from the typical and atypical DFD groups higher in terms of tenderness, juiciness, flavor, overall liking, and purchase intent than those in the normal group. No differences were observed between the atypical DFD and normal groups.

However, consumers in Cluster 2 rated only steaks with higher pHu (typical DFD) values as significantly higher in tenderness and did not perceive differences in juiciness, flavor, or purchase intent among the groups. In terms of overall liking, they exhibited a stronger preference for steaks from the typical and atypical DFD groups than those from the normal group, with no difference between the atypical DFD and normal groups. Cluster 3 displayed similar behavior to that of Cluster 1, and consumers in Cluster 4 observed no differences in the evaluation of steaks among the groups.

When evaluating the frequency of scores given by consumers (Figure 2), we determined that there was also a difference in the overall liking of the samples ($P = 0.036$), with the typical DFD group being evaluated as the most liked by the panel, with 58% of scores above 6 compared to 41% and 48% of the normal and atypical DFD groups, respectively. There was a tendency ($P = 0.082$) for a difference in the preference of the steaks for flavor acceptability, indicating a certain similarity between the typical and atypical DFD steaks, both of which had 53% of scores above 6, with the atypical DFD group having 28% of scores of 8 compared to 21% and 18% of the normal and typical DFD groups, respectively.

The results of the consumer perception of steak tenderness are in line with the results of previous studies, which also reported that meat with a higher pHu has higher levels of tenderness that is perceptible to trained consumers (GRAYSON et al., 2016a; YANG et al., 2021) or untrained consumers, as in our study. Similar findings were reported by Leighton et al. (2023), in addition to greater juiciness, which is also in line with the trend shown in our study. The greater tenderness of meat with high pHu is due to faster myofibrillar degradation catalyzed by μ-calpain, through which a more neutral environment is activated more quickly, as explained by Lomiwes et al. (2014).

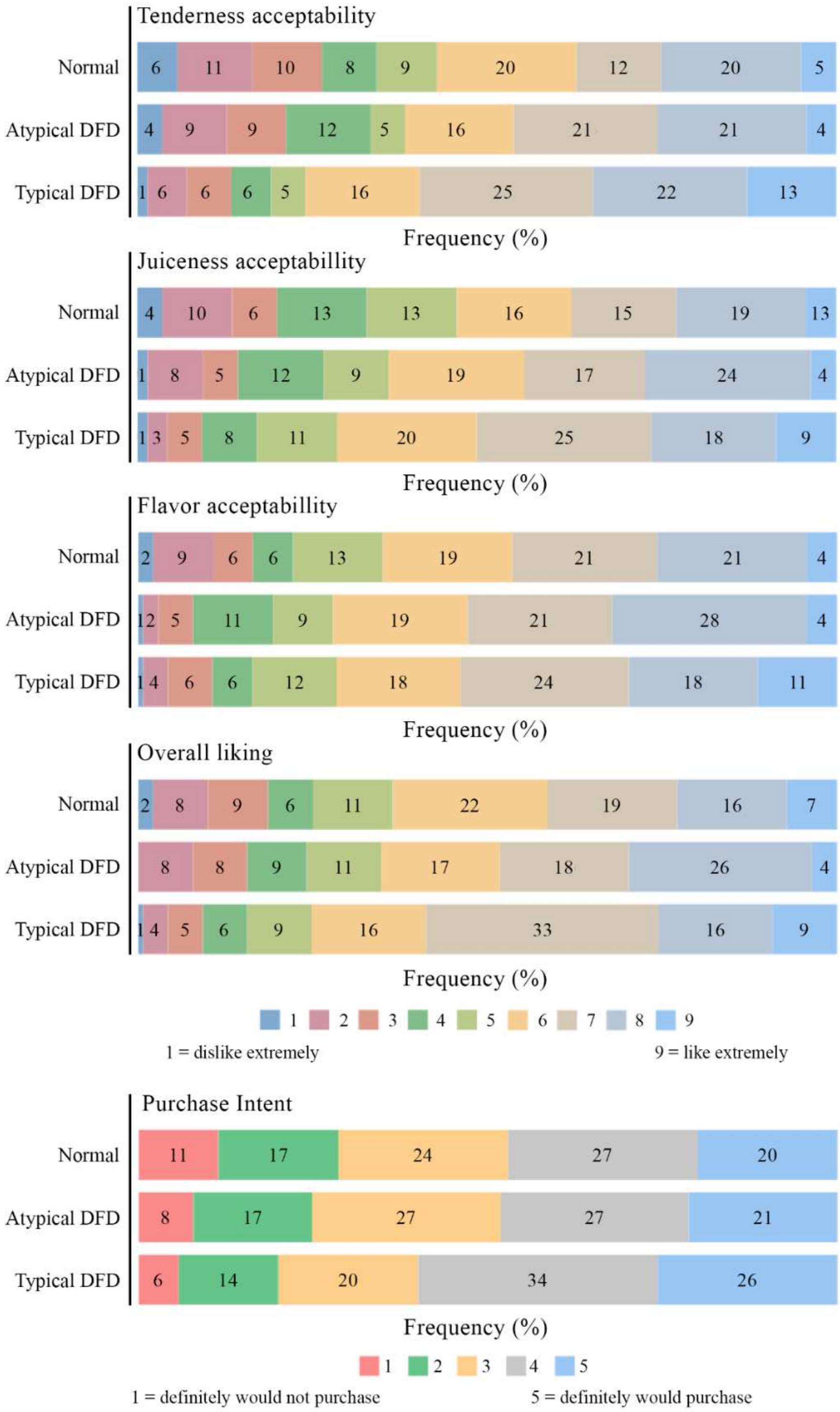

Figure 3. Frequencies of consumer evaluation for grilled steaks from Nellore Bull's carcasses with different pHu.

In addition, the greater sensation of juiciness can be explained by the characteristic of DFD meat to have a greater capacity to retain water; during cooking, it also loses less water, which directly influences the sensory experience of the consumer (Dansfield, 1981; Purchas &

Aungsupakorn, 1993). However, Grayson et al. (2016) reported that this small difference in DFD does not have a notable impact on the consumer palatable experience. The results of this study reaffirm that even with differences in tenderness and possibly also in juiciness, consumers did not have negative sentiments toward any of the groups in terms of overall liking and purchase intention, as shown by the mostly positive scores given.

3.4 Regressions models

The sensory parameters, color and appearance of freshness, and overall liking were regressed in models of visual preference for overall liking and purchase intent (Table 4). In Models 1–4, overall liking was used as an independent variable, and in Models 5–9, purchase intent was used as an independent variable, with the other parameters serving as dependent variables.

In Models 1 and 2, the color and freshness variables, respectively, were used as the only covariates in the overall liking regression model and were responsible for explaining 80% and 82%, respectively, of the overall liking for the steaks. Furthermore, 86% of the overall liking variation can be explained using multiple regression Model 3, through the equation 0.24 + 0.45 color + 0.49 freshness. By analyzing the standardized equation, we obtained 0.42 color + 0.51 freshness, indicating that for every 1 unit change in freshness, the impact is the greater than color in overall liking.

When evaluating the results related to purchase intention, overall liking exhibited the highest adjusted R^2 value, and the other parameters had nearly the same adjusted R^2 values. When analyzing consumer purchase intentions considering all the parameters evaluated, we have obtained the equation 0.28 + 0.10 color + 0.04 freshness + 0.56 overall liking (adjusted R^2 = 0.57).

The models presented in Table 5 show the results of the regression of the sensory parameters of tenderness, juiciness, and flavor on consumer overall liking. For the construction of Models

10–14, overall liking was considered an independent variable and tenderness, juiciness, and flavor were the dependent variables. Considering the separate variables (Models 10–12), tenderness presented the highest R^2, juiciness showed intermediate R^2, and flavor exhibited the lowest R^2; however, all three presented high R^2 values close to 1. Analyzing all the parameters together showed that Model 13 explains 90% of the variation in consumer overall liking, with tenderness being the most important variable.

In a study conducted in Europe evaluating an Australian methodology, the equation explained 94% of the relationship between overall liking with tenderness, juiciness, and flavor: overall liking = 0.3T + 0.1 J + 0.6F (Liu, 2020). The primary driver of consumer satisfaction is flavor, as suggested by Goodson et al. (2002), as 75% of the variation in beef appreciation was attributed to taste preferences.

Meat color preferences may be influenced by previous experiences and habits, as suggested by Santos et al. (2021). The perception of intrinsic quality cues such as appearance, color, freshness, and visible fat, as well as extrinsic quality cues such as price, origin/brand information, and presentation, varies across regions, educational levels, occupations, and other sociodemographic characteristics (DE MORENO et al., 2020).

Consumers rely on both intrinsic and extrinsic cues to form a visual impression that shapes their expectations of quality and impacts their purchasing choices (Santos et al., 2021). Meat color is a crucial indicator of freshness, taste, and texture (Henchion et al., 2017). Leighton et al. (2023) conducted a comprehensive study in Canada encompassing visual evaluations of both raw and grilled steak, where the acceptability of raw meat color was determined to be the key factor explaining the high degree of variation in raw purchase intention (R^2 adj = 57%, $P < 0.001$), aligning with the outcomes observed in the present study.

Regression methods have also been found to influence flavor and overall liking of beef and sometimes suggest that overall liking and flavor are more subjective and prone to variation based on consumer perception, whereas tenderness and juiciness are relatively more consistent (LIU et al., 2020). This is consistent with the findings of the present study, in which tenderness and juiciness were more indicative of overall liking than flavor.

Table 4. Multiple regression of sensory parameters on overall liking of raw steaks from beef carcasses with different pHu.

Overall liking	Intercept	Color (Coefficient)	Freshness (Coefficient)	R^2	Adjusted R^2	RMSE	P
Model 1	0.330	0.938***	-	0.84	0.80	0.39	< 0.01
Model 2	0.781	-	0.851***	0.86	0.82	0.37	< 0.01
Model 3	0.239	0.449***	0.491***	0.94	0.86	0.33	< 0.01
Model 4: Standardized	-	0.424	0.512	0.94	0.86	0.33	< 0.01

Purchase Intent	Intercept	Color (Coefficient)	Freshness (Coefficient)	Overall liking (Coefficient)	R^2	Adjusted R^2	RMSE	P
Model 5	0.420	0.467***	-	-	0.60	0.49	0.42	< 0.01
Model 6	0.660	-	0.420***	-	0.60	0.50	0.43	< 0.01
Model 7	0.399	-	-	0.473***	0.58	0.57	0.36	< 0.01
Model 8	0.283	0.096	0.043	0.350*	0.65	0.57	0.35	< 0.01
Model 9: Standardized	-	0.146	0.071	0.561	0.66	0.57	0.35	< 0.01

* *P* < 0.05, ** *P* < 0.01, *** *P* <.001.

Table 5. Multiple regression of sensory parameters on overall liking of grilled steaks from beef carcasses with different pHu.

Overall liking		Tenderness	Juiciness	Flavor	R^2	Adjusted R^2	RMSE	P
	Intercept		Coefficient					
Model 10	1.817	0.760***	-	-	0.85	0.82	0.38	< 0.01
Model 11	0.702	-	0.905***	-	0.83	0.80	0.40	< 0.01
Model 12	0.650	-	-	0.914***	0.81	0.76	0.44	< 0.01
Model 13	0.457	0.313***	0.332***	0.330***	0.92	0.90	0.29	< 0.01
Model 14: Standardized	-	0.386	0.339	0.326	0.92	0.90	0.29	< 0.01

* *P* < 0.05, ** *P* < 0.01, * *P* <.001.

4. Conclusions

The present study provides novel insights into the perception of DFD beef among Brazilian consumers. The results revealed that consumers do not devalue dark-colored fresh meat and perceive it as more tender when grilled. Surprisingly, meat color did not affect purchase intention. These findings underscore the significance of incorporating sensory preferences in addition to sociodemographic characteristics when formulating marketing strategies and developing food products.

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References

APAOBLAZA, A. et al. Muscle from grass- and grain-fed cattle differs energetically. **Meat Science**, v. 161, p. 107996, 1 mar. 2020.

APPLE, J. K. et al. Lactic acid enhancement can improve the fresh and cooked color of dark-cutting beef. **Journal of Animal Science**, v. 89, n. 12, p. 4207–4220, 1 dez. 2011.

BAIRD, M. F. et al. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. **Journal of Nutrition and Metabolism**, v. 2012, 2012.

BARÓN, C. L. C. et al. Influence of ultimate pH on biochemistry and quality of Longissimus lumborum steaks from Nellore bulls during ageing. **International Journal of Food Science & Technology**, v. 56, n. 7, p. 3333–3343, 1 jul. 2021.

BARRETO, C. D. et al. Infrared thermography for evaluation of the environmental thermal comfort for livestock. **International Journal of Biometeorology**, v. 64, n. 5, p. 881–888, 1 maio 2020.

BEKHIT, A. A. et al. Exogenous Proteases for Meat Tenderization. **Critical Reviews in Food Science and Nutrition**, v. 54, n. 8, p. 1012–1031, 2014.

BEKHIT, A. E. D.; FAUSTMAN, C. Metmyoglobin reducing activity. **Meat Science**, v. 71, n. 3, p. 407–439, 1 nov. 2005.

BELLO ACEBRÓN, L.; CALVO DOPICO, D. The importance of intrinsic and extrinsic cues to expected and experienced quality: an empirical application for beef. **Food Quality and Preference**, v. 11, n. 3, p. 229–238, 1 maio 2000.

BENDALL, J. R.; TAYLOR, A. A. Consumption of oxygen by the muscles of beef animals and related species. II. Consumption of oxygen by post-rigor muscle. **Journal of the Science of Food and Agriculture**, v. 23, n. 6, p. 707–719, 1 jun. 1972.

BETHANCOURT-GARCIA, J. A. et al. Pre-slaughter factors affecting the incidence of severe bruising in cattle carcasses. **Livestock Science**, v. 222, p. 41–48, 1 abr. 2019.

BORGOGNO, M. et al. The role of product familiarity and consumer involvement on liking and perceptions of fresh meat. **Food Quality and Preference**, v. 44, p. 139–147, 1 set. 2015.

BRAGA, J. S. et al. **Ebook transporte legal bovinos — Ministério da Agricultura e Pecuária**. 1. ed. Jaboticabal: [s.n.].

BRANCACCIO, P.; MAFFULLI, N.; LIMONGELLI, F. M. Creatine kinase monitoring in sport medicine. **British Medical Bulletin**, v. 81–82, n. 1, p. 209–230, 1 jan. 2007.

BRANDT, P.; AASLYNG, M. D. Welfare measurements of finishing pigs on the day of slaughter: A review. **Meat Science**, v. 103, p. 13–23, 1 maio 2015.

CARPENTER, C. E.; CORNFORTH, D. P.; WHITTIER, D. Consumer preferences for beef color and packaging did not affect eating satisfaction. **Meat Science**, v. 57, n. 4, p. 359–363, 1 abr. 2001.

CHULAYO, A. Y.; BRADLEY, G.; MUCHENJE, V. Effects of transport distance, lairage time and stunning efficiency on cortisol, glucose, HSPA1A and how they relate with meat quality in cattle. **Meat Science**, v. 117, p. 89–96, 1 jul. 2016.

CHULAYO, A. Y.; MUCHENJE, V. Activities of some stress enzymes as indicators of slaughter cattle welfare and their relationship with physico-chemical characteristics of beef. **animal**, v. 11, n. 9, p. 1645–1652, 1 set. 2017.

CLABORN, S. W. et al. CONSUMER EVALUATION OF THE PALATABILITY OF USDA SELECT, USDA CHOICE AND CERTIFIED ANGUS BEEF STRIP LOIN STEAKS FROM RETAIL MARKETS IN LUBBOCK, TEXAS, U.S.A. **Journal of Food Quality**, v. 34, n. 6, p. 425–434, 1 dez. 2011.

COCKRAM, M. S.; CORLEY, K. T. T. Effect of pre-slaughter handling on the behaviour and blood composition of beef cattle. **British Veterinary Journal**, v. 147, n. 5, p. 444–454, 1 set. 1991.

COOK, N. et al. Assessing Consistency in Radiated Thermal Output of Beef Steers by Infrared Thermography. **Journal of Imaging 2016, Vol. 2, Page 21**, v. 2, n. 3, p. 21, 11 jul. 2016.

CORCORAN, K. et al. **Current consumer attitudes towards lamb and beef in Europe**. Zaragoza: CIHEAM, 2001.

CORRÊA, A. M. **Termografia infravermelha para a avaliação de carcaça e qualidade de carne bovina**. [s.l.] Universidade Federal de Viçosa, 28 mar. 2019.

CUTHBERTSON, H. et al. Using infrared thermography on farm of origin to predict meat quality and physiological response in cattle (Bos Taurus) exposed to transport and marketing. **Meat Science**, v. 169, p. 108173, 1 nov. 2020.

DE FRESLON, I. et al. Caracterización de la reactividad conductual frente al manejo y su relación con el Cortisol sanguíneo, contusiones y pH de la carne en novillos. **Archivos de medicina veterinaria**, v. 46, n. 2, p. 229–237, 2014.

DE LA FUENTE, J. et al. Physiological response and carcass and meat quality of suckling lambs in relation to transport time and stocking density during transport by road. **Animal**, v. 4, n. 2, p. 250–258, 1 jan. 2010.

DE MORENO, L. A. et al. Attitudinal Determinants of Beef Consumption in Venezuela: A Retrospective Survey. **Foods 2020, Vol. 9, Page 202**, v. 9, n. 2, p. 202, 16 fev. 2020.

DEVINE, C. E. et al. Pre-slaughter stress arising from on-farm handling and its interactions with electrical stimulation on tenderness of lambs. **Meat Science**, v. 73, n. 2, p. 304–312, 1 jun. 2006.

DU, Y. et al. Effect of pre-slaughter conditions on beef quality. **Scientia Agricultura Sinica**, v. 42, n. 10, p. 3625–3632, 2009.

DUNNE, P. G.; MONAHAN, F. J.; MOLONEY, A. P. Current perspectives on the darker beef often reported from extensively-managed cattle: Does physical activity play a significant role? **Livestock Science**, v. 142, n. 1–3, p. 1–22, 1 dez. 2011.

EGAN, A. F.; FERGUSON, D. M.; THOMPSON, J. M. Consumer sensory requirements for beef and their implications for the Australian beef industry. **Australian Journal of Experimental Agriculture**, v. 41, n. 7, p. 855–859, 2001.

EGBERT, W. R.; CORNFORTH, D. P. Factors Influencing Color of Dark Cutting Beef Muscle. **Journal of Food Science**, v. 51, n. 1, p. 57–59, 1 jan. 1986.

EKIZ, B. et al. Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs. **Meat Science**, v. 90, n. 4, p. 967–976, 1 abr. 2012.

ENGLAND, E. M. et al. Excess glycogen does not resolve high ultimate pH of oxidative muscle. **Meat Science**, v. 114, p. 95–102, 1 abr. 2016.

ENGLISH, A. R. et al. Effects of aging on the fundamental color chemistry of dark-cutting beef. **Journal of Animal Science**, v. 94, n. 9, p. 4040–4048, 1 set. 2016.

FAUCITANO, L. et al. Shelf life of pork from five different quality classes. **Meat Science**, v. 84, n. 3, p. 466–469, 1 mar. 2010.

FERGUSON, D. M.; WARNER, R. D. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? **Meat Science**, v. 80, n. 1, p. 12–19, 1 set. 2008.

FINK, J.; SCHOENFELD, B. J.; NAKAZATO, K. The role of hormones in muscle hypertrophy. **The Physician and Sportsmedicine**, v. 46, n. 1, p. 129–134, 2 jan. 2017.

FONT-I-FURNOLS, M.; GUERRERO, L. Consumer preference, behavior and perception about meat and meat products: An overview. **Meat Science**, v. 98, n. 3, p. 361–371, 1 nov. 2014.

FOURY, A. et al. Alternative rearing systems in pigs: consequences on stress indicators at slaughter and meat quality. **animal**, v. 5, n. 10, p. 1620–1625, out. 2011.

GAGAOUA, M. et al. Clustering of sensory eating qualities of beef: Consistencies and differences within carcass, muscle, animal characteristics and rearing factors. 2018.

GAGAOUA, M. et al. Dark-cutting beef: A brief review and an integromics meta-analysis at the proteome level to decipher the underlying pathways. **Meat Science**, v. 181, p. 108611, 1 nov. 2021.

GALLO, C. Carnes de corte oscuro en bovinos (dark cutting beef). **Revista Americarne para América Latina y El Caribe (español-inglés)**, v. 3, p. 10, 2004.

GARCÍA-TORRES, S. et al. Assessment of Stress by Serum Biomarkers in Calves and Their Relationship to Ultimate pH as an Indicator of Meat Quality. **Animals 2021, Vol. 11, Page 2291**, v. 11, n. 8, p. 2291, 3 ago. 2021.

GAŠPERLIN, L.; ŽLENDER, B.; ABRAM, V. Colour of normal and high pH beef heated to different temperatures as related to oxygenation. **Meat Science**, v. 54, n. 4, p. 391–398, 1 abr. 2000.

GERSONY, J. T. et al. Scaling Thermal Properties from the Leaf to the Canopy in the

Alaskan Arctic Tundra. **https://doi.org/10.1657/AAAR0016-013**, v. 48, n. 4, p. 739–754, 1 nov. 2018.

GOODSON, K. J. et al. Beef customer satisfaction: factors affecting consumer evaluations of clod steaks. **Journal of Animal Science**, v. 80, n. 2, p. 401–408, 1 fev. 2002.

GOTOH, T. et al. Meat produced by Japanese Black cattle and Wagyu. **Animal Frontiers**, v. 4, n. 4, p. 46–54, 1 out. 2014.

GRAYSON, A. L. et al. The effects of degree of dark cutting on tenderness and sensory attributes of beef,. **Journal of Animal Science**, v. 94, n. 6, p. 2583–2591, 1 jun. 2016a.

GRAYSON, A. L. et al. The effects of degree of dark cutting on tenderness and sensory attributes of beef. **Journal of Animal Science**, v. 94, n. 6, p. 2583–2591, 1 jun. 2016b.

GREBITUS, C. et al. Fresh Meat Packaging: Consumer Acceptance of Modified Atmosphere Packaging including Carbon Monoxide. **Journal of Food Protection**, v. 76, n. 1, p. 99–107, 1 jan. 2013.

GREBITUS, C.; JENSEN, H. H.; ROOSEN, J. US and German consumer preferences for ground beef packaged under a modified atmosphere – Different regulations, different behaviour? **Food Policy**, v. 40, p. 109–118, 1 jun. 2013.

GREGORY, N. G. Animal welfare at markets and during transport and slaughter. **Meat Science**, v. 80, n. 1, p. 2–11, 1 set. 2008.

GRUNERT, K. G.; BREDAHL, L.; BRUNSØ, K. Consumer perception of meat quality and implications for product development in the meat sector—a review. **Meat Science**, v. 66, n. 2, p. 259–272, 1 fev. 2004.

HENCHION, M. M.; MCCARTHY, M.; RESCONI, V. C. Beef quality attributes: A systematic review of consumer perspectives. **Meat Science**, v. 128, p. 1–7, 1 jun. 2017.

HOLDSTOCK, J. et al. The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade.

Meat Science, v. 98, n. 4, p. 842–849, 1 dez. 2014.

HOLMAN, B. W. B. et al. The identification of dark cutting beef carcasses in Australia, using Nix Pro Color Sensor™ colour measures, and their relationship to bolar blade, striploin and topside quality traits. **Meat Science**, v. 148, p. 50–54, 1 fev. 2019.

HOPKINS, D. L. et al. The effect of pH decline rate on the meat and eating quality of beef carcasses. **Animal Production Science**, v. 54, n. 4, p. 407–413, 20 fev. 2014.

HORCADA, A. et al. Short communication: Using infrared ocular thermography as a tool to predict meat quality from lean cattle breeds prior to slaughter: Exploratory trial. **Spanish Journal of Agricultural Research**, v. 17, n. 4, p. e06SC01-e06SC01, 2019.

HUGHES, J. et al. **Improving beef colour at grading-Final report**. Disponível em: <https://www.ampc.com.au/getmedia/fb3a8f45-75c0-4791-acef-0da992fb4e3c/AMPC_ImprovingBeefColourAtGrading_FinalReport.pdf?ext=.pdf>. Acesso em: 14 mar. 2023a.

HUGHES, J. et al. High pH in beef longissimus thoracis reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. **Food Research International**, v. 101, p. 228–238, 1 nov. 2017b.

HUGHES, J. et al. Differences in light scattering between pale and dark beef longissimus thoracis muscles are primarily caused by differences in the myofilament lattice, myofibril and muscle fibre transverse spacings. **Meat Science**, v. 149, p. 96–106, 1 mar. 2019.

HUGHES, J. M. et al. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. **Meat Science**, v. 98, n. 3, p. 520–532, 1 nov. 2014.

HUGHES, J. M. et al. Meat color is determined not only by chromatic heme pigments but also by the physical structure and achromatic light scattering properties of the muscle. **Comprehensive Reviews in Food Science and Food Safety**, v. 19, n. 1, p. 44–63, 1 jan. 2020.

HUGHES, J. M.; KEARNEY, G.; WARNER, R. D. Improving beef meat colour scores at carcass grading. **Animal Production Science**, v. 54, n. 4, p. 422–429, 21 fev. 2014.

I. CAPPELLOZZA, B.; S. MARQUES, R. Effects of Pre-Slaughter Stress on Meat Characteristics and Consumer Experience. **Meat and Nutrition**, 28 jul. 2021.

IJAZ, M. et al. Association between meat color of DFD beef and other quality attributes. **Meat Science**, v. 161, 1 mar. 2020.

IJAZ, M. et al. Changes in postmortem metabolites profile of atypical and typical DFD beef. **Meat Science**, v. 193, p. 108922, 1 nov. 2022.

IMMONEN, K. et al. Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. **Meat Science**, v. 55, n. 1, p. 25–31, 1 maio 2000.

JEONG, D. W. et al. Correlations of trained panel sensory values of cooked pork with fatty acid composition, muscle fiber type, and pork quality characteristics in Berkshire pigs. **Meat Science**, v. 86, n. 3, p. 607–615, 1 nov. 2010.

JEREMIAH, L. E.; TONG, A. K. W.; GIBSON, L. L. The usefulness of muscle color and pH for segregating beef carcasses into tenderness groups. **Meat Science**, v. 30, n. 2, p. 97–114, 1 jan. 1991.

JOHNSON, E. O. et al. Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. **Neuroscience & Biobehavioral Reviews**, v. 16, n. 2, p. 115–130, 1 jun. 1992.

KARVATTE, N. et al. Infrared thermography for microclimate assessment in agroforestry systems. **Science of The Total Environment**, v. 731, p. 139252, 20 ago. 2020.

KENNY, F. J.; TARRANT, P. V. The behaviour of young Friesian bulls during social re-grouping at an abattoir. Influence of an overhead electrified wire grid. **Applied Animal Behaviour Science**, v. 18, n. 3–4, p. 233–246, 1 out. 1987.

KILLINGER, K. M. et al. Consumer visual preference and value for beef steaks differing in marbling level and color. **Journal of Animal Science**, v. 82, n. 11, p. 3288–3293, 1 nov. 2004.

KIM, Y. et al. Canopy skin temperature variations in relation to climate, soil temperature, and carbon flux at a ponderosa pine forest in central Oregon. **Agricultural and Forest Meteorology**, v. 226–227, p. 161–173, 15 out. 2016.

KIYIMBA, F. et al. Changes in glycolytic and mitochondrial protein profiles regulates postmortem muscle acidification and oxygen consumption in dark-cutting beef. **Journal of Proteomics**, v. 232, p. 104016, 10 fev. 2021.

KOCH, R. M.; DIKEMAN, M. E.; CROUSE, J. D. Characterization of Biological Types of Cattle (Cycle III).III. Carcass Composition, Quality and Palatability. **Journal of Animal Science**, v. 54, n. 1, p. 35–45, 1 jan. 1982.

KOMATSU, T. et al. Effects of genetic and environmental factors on muscle glycogen content in Japanese Black cattle. **Animal Science Journal**, v. 85, n. 8, p. 793–798, 1 ago. 2014.

KOU, H. X. et al. Automated measurement of cattle surface temperature and its correlation with rectal temperature. **PLOS ONE**, v. 12, n. 4, p. e0175377, 1 abr. 2017.

KREIKEMEIER, K. K.; UNRUH, J. A.; ECK, T. P. Factors affecting the occurrence of dark-cutting beef and selected carcass traits in finished beef cattle. **Journal of Animal Science**, v. 76, n. 2, p. 388–395, 1 fev. 1998.

LAHUCKY, R. et al. Effect of preslaughter handling on muscle glycogen level and selected meat quality traits in beef. **Meat Science**, v. 50, n. 3, p. 389–393, 1 nov. 1998.

LAWRENCE, T. L. J.; FOWLER, V. R.; NOVAKOFSKI, J. E. **Growth of farm animals**. 3. ed. [s.l.] CABI, 2012.

LEIGHTON, P. L. A. et al. Towards improving classification of Canadian dark-cutting beef carcasses: Consumer sensory evaluation. **Meat Science**, v. 195, 1 jan. 2023.

LI, M. et al. Comparative profiling of sarcoplasmic phosphoproteins in ovine muscle with different color stability. **Food Chemistry**, v. 240, p. 104–111, 1 fev. 2018.

LIU, J. et al. Contributions of tenderness, juiciness and flavor liking to overall liking of beef in Europe. **Meat Science**, v. 168, p. 108190, 1 out. 2020.

LOMIWES, D. et al. Small heat shock proteins and toughness in intermediate pHu beef. **Meat Science**, v. 95, n. 3, p. 472–479, 1 nov. 2013.

LOMIWES, D. et al. The development of meat tenderness is likely to be compartmentalised by ultimate pH. **Meat Science**, v. 96, n. 1, p. 646–651, jan. 2014.

LÓPEZ-CAMPOS, Ó. et al. Packaging for retail appearance improvement of dark cutting beef. **Meat Science**, v. 1, n. 96, p. 121, jan. 2014.

LOSADA-ESPINOSA, N. et al. Pre-slaughter cattle welfare indicators for use in commercial abattoirs with voluntary monitoring systems: A systematic review. **Meat Science**, v. 138, p. 34–48, 1 abr. 2018.

LOWE, G. et al. Infrared Thermography—A Non-Invasive Method of Measuring Respiration Rate in Calves. **Animals 2019, Vol. 9, Page 535**, v. 9, n. 8, p. 535, 7 ago. 2019.

LU, X. et al. Association of ultimate pH and stress-related blood variables in cattle. **Meat Science**, v. 139, p. 228–230, 1 maio 2018.

LU, X. et al. Effect of oxygen concentration in modified atmosphere packaging on color changes of the M. longissimus thoraces et lumborum from dark cutting beef carcasses. **Meat Science**, v. 161, p. 107999, 1 mar. 2020.

MACH, N. et al. Association between animal, transportation, slaughterhouse practices, and meat pH in beef. **Meat Science**, v. 78, n. 3, p. 232–238, 1 mar. 2008.

MAHMOOD, S. et al. Can potential for dark cutting be predicted by phenotype? Relationship between sex, carcass characteristics, and the incidence of dark cutting beef. **Canadian Journal of Animal Science**, v. 96, n. 1, p. 19–31, 24 mar. 2016a.

MAHMOOD, S. et al. Relationship between phenotype, carcass characteristics and the incidence of dark cutting in heifers. **Meat Science**, v. 121, p. 261–271, 1 nov. 2016b.

MAHMOOD, S. et al. Understanding the quality of typical and atypical dark cutting beef from heifers and steers. **Meat Science**, v. 133, p. 75–85, 1 nov. 2017.

MAHMOOD, S. et al. Proteomics of dark cutting longissimus thoracis muscle from heifer and steer carcasses. **Meat Science**, v. 137, p. 47–57, 1 mar. 2018.

MANCINI, R. A.; HUNT, M. C. Current research in meat color. **Meat Science**, v. 71, n. 1, p. 100–121, 1 set. 2005.

MARTELLO, L. S. et al. Infrared thermography as a tool to evaluate body surface temperature and its relationship with feed efficiency in Bos indicus cattle in tropical conditions. **International Journal of Biometeorology**, v. 60, n. 1, p. 173–181, 1 jan. 2016.

MCCARTHY, S. N. et al. Evaluation of beef eating quality by Irish consumers. **Meat Science**, v. 132, p. 118–124, 1 out. 2017.

MCGILCHRIST, P. et al. Beef carcasses with larger eye muscle areas, lower ossification scores and improved nutrition have a lower incidence of dark cutting. **Meat Science**, v. 92, n. 4, p. 474–480, 1 dez. 2012.

MCGILCHRIST, P. et al. The incidence of dark cutting in southern Australian beef production systems fluctuates between months. **Animal Production Science**, v. 54, n. 10, p. 1765–1769, 2014.

MCKEITH, R. O. et al. Mitochondrial abundance and efficiency contribute to lean color of dark cutting beef. **Meat Science**, v. 116, p. 165–173, 1 jun. 2016.

MCPHAIL, N. G. et al. Factors influencing the occurrence of high ultimate pH in three muscles of lamb carcasses in Australia. **Animal Production Science**, v. 54, n. 10, p. 1853–1859, 19 ago. 2014.

MEAT & LIVESTOCK AUSTRALIA. **Annual Report 2016-17**. Disponível em:

<https://www.mla.com.au/about-mla/how-we-are-governed/Planning-reporting/annual-reporting/annual-report-2016-17/>. Acesso em: 16 mar. 2023.

MILLER, M. **Dark, Firm and Dry Beef**. Disponível em: <https://fyi.extension.wisc.edu/wbic/files/2011/04/Dark-Firm-and-Dry-Beef.pdf>. Acesso em: 14 mar. 2023.

MINKA, N. S.; AYO, J. O. Physiological responses of food animals to road transportation stress. **African Journal of Biotechnology**, v. 9, n. 40, p. 6601–6613, 16 ago. 2010.

MOHAN RAJ, A. B. et al. Effect of mixing male sex types of cattle on their meat quality and stress-related parameters. **Meat Science**, v. 32, n. 4, p. 367–386, 1 jan. 1992.

MONTANHOLI, Y. R. et al. On the determination of residual feed intake and associations of infrared thermography with efficiency and ultrasound traits in beef bulls. **Livestock Science**, v. 125, n. 1, p. 22–30, 1 out. 2009.

MONTANHOLI, Y. R. et al. Assessing feed efficiency in beef steers through feeding behavior, infrared thermography and glucocorticoids. **Animal**, v. 4, n. 5, p. 692–701, 1 jan. 2010.

MOORE, M. C. et al. National Beef Quality Audit–2011: In-plant survey of targeted carcass characteristics related to quality, quantity, value, and marketing of fed steers and heifers,. **Journal of Animal Science**, v. 90, n. 13, p. 5143–5151, 1 dez. 2012.

MOTA-ROJAS, D. et al. Clinical Applications and Factors Involved in Validating Thermal Windows Used in Infrared Thermography in Cattle and River Buffalo to Assess Health and Productivity. **Animals 2021, Vol. 11, Page 2247**, v. 11, n. 8, p. 2247, 30 jul. 2021.

MUCHENJE, V. et al. Relationship between pre-slaughter stress responsiveness and beef quality in three cattle breeds. **Meat Science**, v. 81, n. 4, p. 653–657, 1 abr. 2009.

MUELLER, L. F. et al. Gender status effect on carcass and meat quality traits of feedlot Angus × Nellore cattle. **Animal Science Journal**, v. 90, n. 8, p. 1078–1089, 1 ago. 2019.

MUIR, P. D.; DEAKER, J. M.; BOWN, M. D. Effects of forage- and grain-based feeding systems on beef quality: A review. **New Zealand Journal of Agricultural Research**, v. 41, n. 4, p. 623–635, 1998.

NELIS, J. L. D. et al. Biomarkers and biosensors for the diagnosis of noncompliant pH, dark cutting beef predisposition, and welfare in cattle. **Comprehensive Reviews in Food Science and Food Safety**, v. 21, n. 3, p. 2391–2432, 1 maio 2022.

NEWCOMBE, M. A. et al. “Eat like a man”. A social constructionist analysis of the role of food in men’s lives. **Appetite**, v. 59, n. 2, p. 391–398, 1 out. 2012.

NIELSEN, B. L.; DYBKJR, L.; HERSKIN, M. S. Road transport of farm animals: effects of journey duration on animal welfare. **Animal**, v. 5, n. 3, p. 415–427, 1 jan. 2011.

O’LOUGHLIN, A. et al. Biomarker responses to weaning stress in beef calves. **Research in Veterinary Science**, v. 97, n. 2, p. 458–463, 1 out. 2014.

ÖZDEMIR, S.; EKIZ, E. E.; EKIZ, B. Effect of lairage duration on cattle behaviors and stockperson actions in the slaughter corridor in Simmental and Swiss Brown breeds. **Tropical Animal Health and Production**, v. 54, n. 2, p. 1–7, 1 abr. 2022.

PAGE, J. K.; WULF, D. M.; SCHWOTZER, T. R. A survey of beef muscle color and pH. **Journal of Animal Science**, v. 79, n. 3, p. 678–687, 1 mar. 2001.

PEDREIRA, C.; SILVA, L.; ALONSO, M. **Use of grazed pastures in the brazilian livestock industry: a brief overview**. (R. Carnevalli et al., Eds.) Forages in Warm Climates. **Anais**...2015. Disponível em: <https://www.researchgate.net/profile/Roberta-Carnevalli-2/publication/280612162_Silvopastoral_systems_with_Tifton-85_grass_and_tropical_fruit_trees/links/55bea9a908aec0e5f445e28f/Silvopastoral-systems-with-Tifton-85-grass-and-tropical-fruit-trees.pdf#page=7>. Acesso em: 1 mar. 2023

PICARD, B. et al. Inverse relationships between biomarkers and beef tenderness according to contractile and metabolic properties of the muscle. **Journal of Agricultural and Food**

Chemistry, v. 62, n. 40, p. 9808–9818, 8 out. 2014.

PICARD, B.; GAGAOUA, M.; GAGAOUA, M. Muscle Fiber Properties in Cattle and Their Relationships with Meat Qualities: An Overview. **Journal of Agricultural and Food Chemistry**, v. 68, n. 22, p. 6021–6039, 3 jun. 2020.

PIGHIN, D. G. et al. Relationship between changes in core body temperature in lambs and post-slaughter muscle glycogen content and dark-cutting. **Animal Production Science**, v. 54, n. 4, p. 459–463, 15 maio 2013.

POLETI, M. D. et al. Label-free quantitative proteomic analysis reveals muscle contraction and metabolism proteins linked to ultimate pH in bovine skeletal muscle. **Meat Science**, v. 145, p. 209–219, 1 nov. 2018.

POLKINGHORNE, R. et al. Development of a commercial system to apply the Meat Standards Australia grading model to optimise the return on eating quality in a beef supply chain. **Australian Journal of Experimental Agriculture**, v. 48, n. 11, p. 1451–1458, 16 out. 2008.

PONNAMPALAM, E. N. et al. Causes and Contributing Factors to “Dark Cutting” Meat: Current Trends and Future Directions: A Review. **Comprehensive Reviews in Food Science and Food Safety**, v. 16, n. 3, p. 400–430, 1 maio 2017.

PULFORD, D. J. et al. The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. **Meat Science**, v. 79, n. 4, p. 623–630, 1 ago. 2008.

PURCHAS, R. W. An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. **Meat Science**, v. 27, n. 2, p. 129–140, 1 jan. 1990.

PURCHAS, R. W.; AUNGSUPAKORN, R. Further investigations into the relationship between ultimate pH and tenderness for beef samples from bulls and steers. **Meat Science**, v. 34, n. 2, p. 163–178, 1 jan. 1993.

PURSLOW, P. P. et al. Variations in meat colour due to factors other than myoglobin chemistry; a synthesis of recent findings (invited review). **Meat Science**, v. 159, p. 107941, 1 jan. 2020.

RAMANATHAN, R. et al. Effects of 4-hydroxy-2-nonenal on beef heart mitochondrial ultrastructure, oxygen consumption, and metmyoglobin reduction. **Meat Science**, v. 90, n. 3, p. 564–571, 1 mar. 2012.

RAMANATHAN, R. et al. Mitochondrial functionality and beef colour: A review of recent research. **South African Journal of Animal Science**, v. 49, n. 1, p. 9–19, 1 abr. 2019.

RAMANATHAN, R. et al. Recent Updates in Meat Color Research: Integrating Traditional and High-Throughput Approaches. **Meat and Muscle Biology**, v. 4, n. 2, p. 1–24, 28 jul. 2020.

REALINI, C. E. et al. Relative importance of cues underlying Spanish consumers' beef choice and segmentation, and consumer liking of beef enriched with n-3 and CLA fatty acids. **Food Quality and Preference**, v. 33, p. 74–85, 1 abr. 2014.

REIS, M. M.; ROSENVOLD, K. Early on-line classification of beef carcasses based on ultimate pH by near infrared spectroscopy. **Meat Science**, v. 96, n. 2, p. 862–869, 1 fev. 2014.

RENERRE, M. Factors involved in the discoloration of beef meat. **International Journal of Food Science & Technology**, v. 25, n. 6, p. 613–630, 1 dez. 1990.

RIBEIRO, C. C. DE S. et al. New alternatives for improving and assessing the color of dark–cutting beef – a review. **Scientia Agricola**, v. 79, n. 1, p. 1–16, 18 jan. 2021.

ROSA, A. et al. Incidence of DFD meat on Brazilian beef cuts. **Meat Science**, v. 112, p. 132–133, fev. 2016.

RUSSELL, E. et al. Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. **Psychoneuroendocrinology**, v. 37, n. 5, p. 589–601, 1 maio 2012.

SANTANA, M. L. et al. Structure and genetic diversity of Brazilian Zebu cattle breeds assessed by pedigree analysis. **Livestock Science**, v. 187, p. 6–15, 1 maio 2016.

SANTOS, D. et al. The most important attributes of beef sensory quality and production variables that can affect it: A review. **Livestock Science**, v. 250, p. 104573, 1 ago. 2021.

SCHAEFER, A. et al. 497 The automated and real time use of infrared thermography in the detection and correction of DFD and fevers in cattle. **Journal of Animal Science**, v. 96, n. suppl_3, p. 275–275, 7 dez. 2018.

SCHEFFLER, T. L. et al. Mitochondria influence postmortem metabolism and pH in an in vitro model. **Meat Science**, v. 110, p. 118–125, 1 dez. 2015.

SCHEFFLER, T. L.; GERRARD, D. E. Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. **Meat Science**, v. 77, n. 1, p. 7–16, 1 set. 2007.

SEPULVEDA, C. A. et al. Cooking Method and USDA Quality Grade Affect Consumer Palatability and Flavor of Beef Strip Loin Steaks. **Meat and Muscle Biology**, v. 3, n. 1, 1 out. 2019.

SHACKELFORD, S. D. et al. Effect of biological type of cattle on the incidence of the dark, firm, and dry condition in the longissimus muscle. **Journal of Animal Science**, v. 72, n. 2, p. 337–343, 1 fev. 1994.

SHAW, F. D.; TUME, R. K. The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents—A review of recent work. **Meat Science**, v. 32, n. 3, p. 311–329, 1 jan. 1992.

SILVA, J. A.; PATARATA, L.; MARTINS, C. Influence of ultimate pH on bovine meat tenderness during ageing. **Meat Science**, v. 52, n. 4, p. 453–459, 1 ago. 1999.

STEEL, C. C. et al. Abattoir Factors Influencing the Incidence of Dark Cutting in Australian Grain-Fed Beef. **Animals 2021, Vol. 11, Page 474**, v. 11, n. 2, p. 474, 10 fev. 2021.

SUMAN, S. P.; JOSEPH, P. Myoglobin Chemistry and Meat Color. **Annual Review of Food Science and Technology** , v. 4, p. 79–99, 2013.

SUTHERLAND, M. A. et al. Evaluation of infrared thermography as a non-invasive method of measuring the autonomic nervous response in sheep. **PLOS ONE**, v. 15, n. 5, p. e0233558, 1 maio 2020.

TADICH, N. et al. Effects of transport and lairage time on some blood constituents of Friesian-cross steers in Chile. **Livestock Production Science**, v. 93, n. 3, p. 223–233, 1 maio 2005.

TANG, F. et al. Non-Invasive Glucose Measurement by Use of Metabolic Heat Conformation Method. **Sensors 2008, Vol. 8, Pages 3335-3344**, v. 8, n. 5, p. 3335–3344, 21 maio 2008.

TANG, J. et al. Postmortem Oxygen Consumption by Mitochondria and Its Effects on Myoglobin Form and Stability. **Journal of Agricultural and Food Chemistry**, v. 53, n. 4, p. 1223–1230, 23 fev. 2005.

TANG, J.; FAUSTMAN, C.; HOAGLAND, T. A. Krzywicki revisited: Equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. **Journal of Food Science**, v. 69, n. 9, 2004.

TARRANT, P. V. Animal Behaviour and Environment in the Dark-Cutting Condition in Beef - A Review. **Irish Journal of Food Science and Technology**, v. 13, n. 1, p. 1–21, 1989.

TARRANT, P. V.; SHERINGTON, J. An investigation of ultimate pH in the muscles of commercial beef carcasses. **Meat Science**, v. 4, n. 4, p. 287–297, 1980.

TARUMÁN, J. A. et al. Risk Factors for Bruises and High Muscle pH in Lamb Carcasses of Tierra Del Fuego, Chilean Patagonia. **Open Access Library Journal**, v. 5, n. 1, p. 1–11, 4 jan. 2018.

TEKE, B. et al. Effects of different lairage times after long distance transportation on carcass and meat quality characteristics of Hungarian Simmental bulls. **Meat Science**, v. 96, n. 1, p.

224–229, 1 jan. 2014.

TERLOUW, C. E. M. et al. Understanding the Determination of Meat Quality Using Biochemical Characteristics of the Muscle: Stress at Slaughter and Other Missing Keys. **Foods 2021, Vol. 10, Page 84**, v. 10, n. 1, p. 84, 4 jan. 2021.

TESTA, M. L. et al. Color and Marbling as Predictors of Meat Quality Perception of Argentinian Consumers. **Foods 2021, Vol. 10, Page 1465**, v. 10, n. 7, p. 1465, 24 jun. 2021.

TORRES-BURGOS, Y. I. et al. pH Variability and its Relationship with Sarcomere Length and Free Calcium in Beef from Commercial Cattle in Puerto Rico. **Meat and Muscle Biology**, v. 3, n. 2, 30 nov. 2019.

VESTERGAARD, M.; OKSBJERG, N.; HENCKEL, P. Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. **Meat Science**, v. 54, n. 2, p. 177–185, 1 fev. 2000.

VIERCK, K. R. et al. Marbling Texture's Effects on Beef Palatability. **Meat and Muscle Biology**, v. 2, n. 1, 30 abr. 2018.

VILJOEN, H. F.; DE KOCK, H. L.; WEBB, E. C. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. **Meat Science**, v. 61, p. 181–185, 2002a.

VILJOEN, H. F.; DE KOCK, H. L.; WEBB, E. C. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. **Meat Science**, v. 61, n. 2, p. 181–185, 1 jun. 2002b.

WARNER, R. D. et al. Acute stress induced by the preslaughter use of electric prodders causes tougher beef meat. **Australian Journal of Experimental Agriculture**, v. 47, n. 7, p. 782–788, 2 jul. 2007.

WARNER, R. D. et al. Factors influencing the incidence of high rigor temperature in beef carcasses in Australia. **Animal Production Science**, v. 54, n. 4, p. 363–374, 28 fev. 2014.

WARNER, R. D. et al. Meat tenderness: advances in biology, biochemistry, molecular mechanisms and new technologies. **Meat Science**, v. 185, p. 108657, 1 mar. 2022.

WARRIS, P. D. The extraction of haem pigments from fresh meat. **International Journal of Food Science & Technology**, v. 14, n. 1, p. 75–80, 1 fev. 1979.

WARRISS, P. D. The handling of cattle pre-slaughter and its effects on carcass and meat quality. **Applied Animal Behaviour Science**, v. 28, n. 1–2, p. 171–186, 1 nov. 1990.

WATANABE, A.; DALY, C. C.; DEVINE, C. E. The effects of the ultimate pH of meat on tenderness changes during ageing. **Meat Science**, v. 42, n. 1, p. 67–78, 1 jan. 1996.

WEAVER, A. D.; BOWKER, B. C.; GERRARD, D. E. Sarcomere length influences μ-calpain-mediated proteolysis of bovine myofibrils. **Journal of Animal Science**, v. 87, n. 6, p. 2096–2103, 1 jun. 2009.

WERNER, M. et al. Effects of a long distance transport and subsequent recovery in recently weaned crossbred beef calves in Southern Chile. **Livestock Science**, v. 152, n. 1, p. 42–46, 1 mar. 2013.

WHEELER, T. L. et al. Characterization of biological types of cattle (Cycle V): carcass traits and longissimus palatability. **Journal of Animal Science**, v. 79, n. 5, p. 1209–1222, 1 maio 2001.

WHEELER, T. L. et al. Characterization of biological types of cattle (Cycle VI): Carcass, yield, and longissimus palatability traits. **Journal of Animal Science**, v. 82, n. 4, p. 1177–1189, 1 abr. 2004.

WHEELER, T. L. et al. Characterization of biological types of cattle (Cycle VIII): Carcass, yield, and longissimus palatability traits. **Journal of Animal Science**, v. 88, n. 9, p. 3070–3083, 1 set. 2010.

WHEELER, T. L.; SHACKELFORD, S. D.; KOOHMARAIE, M. Cooking and palatability traits of beef longissimus steaks cooked with a belt grill or an open hearth electric broiler. **Journal of Animal Science**, v. 76, n. 11, p. 2805–2810, 1 nov. 1998.

WULF, D. M. et al. Genetic influences on beef longissimus palatability in charolais- and limousin-sired steers and heifers. **Journal of Animal Science**, v. 74, n. 10, p. 2394–2405, 1 out. 1996.

XIAO, L. et al. A Review of Previous Studies on DFD Beef. **Food Science**, v. 36, n. 19, p. 271, 15 out. 2015.

YANG, X. et al. Investigation of the physicochemical, bacteriological, and sensory quality of beef steaks held under modified atmosphere packaging and representative of different ultimate pH values. **Meat Science**, v. 174, p. 108416, 1 abr. 2021.

ZHANG, Y. et al. Determination of a pH threshold for dark cutting beef based on visual evaluation by Asian consumers. **Meat Science**, v. 172, p. 108347, 1 fev. 2021.

ARTIGO C - NON-INVASIVE MEAT QUALITY ASSESSMENT: EXPLORING THE POTENTIAL OF OCULAR INFRARED THERMOGRAPHY TO PREDICT ULTIMATE PH IN NELLORE BEEF CATTLE

Esta seção é a versão que será submetida para publicação e segue as normas da revista Meat Science.

Non-invasive meat quality assessment: exploring the potential of ocular infrared thermography to predict ultimate pH in Nellore beef cattle

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Abstract

This study investigated the use of infrared thermography (IRT) to identify the dark, firm, and dry (DFD) phenomenon in Brazilian beef, which is a significant concern for the industry because of its inferior quality and reduced shelf life. This study examined 113 Nelore bulls and analyzed their minimum and maximum ocular temperatures using IRT. Correlations were established between the final pH of the meat, color measurements, and selected blood biomarkers (lactate, cortisol, lactate dehydrogenase [LDH], and creatine kinase [CK]) to explore the potential relationships among these variables. Our regression analysis demonstrated significant correlations between infrared thermography (IRT) and various predictors. IRT max alone showed a notable R-squared (R^2) value of 0.75, indicating a strong relationship with the outcome variable. Combining IRT max with different variables, such as Glucose and Lactate, resulted in improved R^2 values, reaching up to 0.80. These findings underscore the potential of IRT as a valuable tool for predictive modeling in our study.

Keywords: Blood parameters; DFD; Ocular temperature; Stress

1. Introduction

Brazil is one of the largest beef producers and exporters worldwide, with a beef cattle population of approximately 232 million heads in 2021, according to the Brazilian Institute of Geography and Statistics (IBGE). Beef cattle production is distributed throughout the country, with the highest concentrations in the central-western, southeastern, and southern regions. The primary beef cattle breeds in Brazil are Zebu breeds, which are adapted to the tropical climate and are disease resistant. The most common Zebu breeds are Nelore, Guzerá, Brahman, and Tabapuã (SANTANA et al., 2016). However, there are also some European breeds, such as Angus, Hereford, and Charolais, which are mainly used for crossbreeding to improve meat quality.

The beef cattle production system in Brazil is mostly based on grazing systems that make use of the vast areas of pastureland available in the country (PEDREIRA; SILVA; ALONSO, 2015). Beef cattle are typically raised in expansive systems, where they have access to large areas of pasture and natural resources such as water and shade. Production data on the Brazilian beef cattle industry are noteworthy, with Brazil being the largest beef exporter worldwide. In 2021, Brazil exported approximately 2.2 million tons of beef, generating approximately US$ 9.5 billion in revenue. In addition, the Brazilian beef industry employs approximately 7 million individuals, making it a crucial sector for the economy of the country (ABIEC 2022).

However, one of the main problems affecting this industry is the incidence of dark, firm, and dry (DFD) meat, which causes a dark color, firm texture, and reduced water-holding capacity. DFD meat is caused by pre-slaughter stress, which leads to a rapid depletion of glycogen stores in the muscles and an accumulation of lactic acid, and these changes are caused by a series of pre-harvest, harvest, and post-harvest factors, including stress, genetics, and handling practices (PONNAMPALAM et al., 2017).

The incidence of DFD in Brazilian beef is a significant concern for the industry, as it can lead to significant economic losses owing to its inferior quality and reduced shelf life (LÓPEZ-CAMPOS et al., 2014). Furthermore, DFD meat does not meet consumer expectations in terms of sensory properties, such as tenderness, juiciness, and flavor (BEKHIT; FAUSTMAN, 2005; TARRANT; SHERINGTON, 1980; VILJOEN; DE KOCK; WEBB, 2002b). Therefore, the development of noninvasive and rapid methods for identifying DFD meat is essential for the industry to maintain competitiveness in the global market. One potential tool for identifying DFD meat is infrared thermography (IRT), a noninvasive technique that measures the surface temperature of objects. IRT has been used for various applications (KOU et al., 2017; LOWE et al., 2019; MARTELLO et al., 2016; MOTA-ROJAS et al., 2021; SUTHERLAND et al., 2020). The use of IRT for the identification of DFD meat has shown promising results in recent studies (HORCADA et al., 2019), because this technique can quickly and accurately identify anomalies. Thus, the potential use of IRT as a tool for identifying DFD meat in beef carcasses holds significant interest to the industry. If successful, this method can provide a rapid and non-invasive means of identifying DFD meat, enabling the industry to reduce economic losses and improve product quality.

Despite its potential for meat quality evaluation, the use of IRT for identifying DFD meat in the meat industry has not yet been widely adopted. Further research is required to understand the relationship between surface temperature and meat quality parameters in DFD meat, as well as to develop practical and effective applications of this technique. Therefore, the objective of this study was to investigate the use of IRT to identify DFD anomalies based on ocular temperature.

2. Materials and Methods

2.1 Location and climate characterization

This study was conducted in March 2022 in a commercial slaughterhouse under federal inspection in Nova Andradina, Mato Grosso do Sul, Brazil. The area is located at latitude 22° 14' 6" South, longitude 53° 19' 54" West at an altitude of 401 meters. In the city the average temperature is 23.9 °C and the average annual rainfall is 1455 (CLIMATE DATA, 2022). All procedures followed the current rules applied to establishments under federal inspection.

2.2 Collection of IRT images

The eye temperatures of the animals were measured at the slaughterhouse inside a stunning box without interfering with the standard procedure of the industry. Image acquisition was performed by positioning the animal's head on a head support before stunning, which was performed using a pistol with a captive dart activated by compressed air using the percussive penetrative method.

IRT images were obtained using a Flir T440 portable infrared camera (FLIR Systems Inc., Wilsonville, OR, USA) with a 640 × 480 pixel detector, equipped with a 15° × 11° lens (40 mm), thermal sensitivity of <40 mK (<0.04 °C at 30 °C ambient temperature), temperature range pf -20–350 °C, in automatic focus adjustment option, and operated by a trained technician. The adopted emissivity was 0.98, as suggested in mammalian studies (Hoffmann et al., 2013, Bernard, Staffa, Mornstein, & Bourek, 2013; Hoffmann et al., 2013; Kastberger & Stachl, 2003).

The right eye of each animal was framed at a 90º angle at 60 cm. Two to three images were captured per animal to ensure clarity for image analysis, and the eyes of the animals were open. The IRT images were analyzed using FLIR TOOLS® software (Teledyne FLIR LLC, Wilsonville, Oregon, USA) to obtain the maximum temperature (°C) within the area of the posterior medial palpebral border, lower lid, and lacrimal caruncle (Fig. 1).

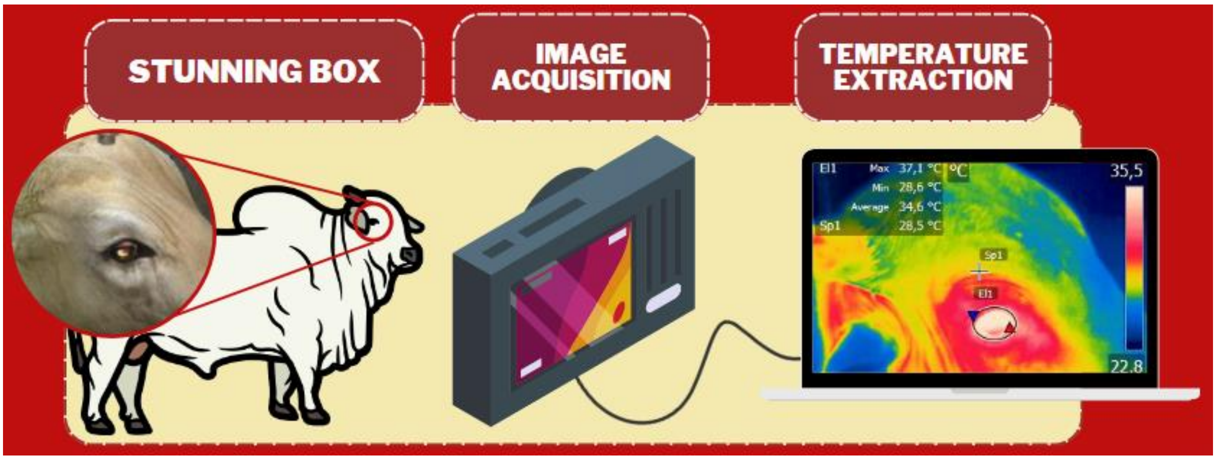

Figure 1. Process of acquiring animals' ocular temperature using infrared thermography in the stunning box at slaughterhouse.

To avoid interference of ambient temperature and humidity in measuring the ocular temperature of the animals, the temperature and humidity of the environment were measured throughout the collection period using a data logger model HOBO ware® (Sigma Sensors, São José dos Campos, São Paulo, Brazil) fixed on the platform attached to the stunning box. The equipment was programmed for automatic data acquisition, recording readings every 5 min, and averages were generated daily during image acquisition.

2.3 Slaughter and meat quality

A total of 113 male Nelore bulls (aged 22–38 months) were used in this study and slaughtered according to current federal inspection standards. The final pH (pHu) was measured twice between the 12th and 13th ribs in the longissimus thoracis muscle until stabilization at the exit of the sanitary maturation chamber (24–48 h *post-mortem*) using a professional pH meter Hanna HI98163® (Hanna Instruments Inc., Barueri, Brazil) previously calibrated with the commercial standard pH 4 and pH solutions.

Color measurements of the steaks were recorded after 30 min of oxygen exposure at 4 °C (blooming time). The color was measured using the CIELab system of the CR-10® colorimeter (Konica Minolta, Inc., São Paulo, Brazil) illuminant D65, with an observation angle of 10° and aperture of 8.0 mm. Readings were performed thrice on the surface of the sample of LT muscle. An average of three measurements was generated for each variable (L*, a*, and b*), where L* = lightness, a* = redness, and b* = yellowness.

2.4 Blood analyses

Blood samples were collected from the animals during bleeding by cutting the large vessels and stored in 10-mL tubes (BD Vacutainers®, Curitiba, Paraná, Brazil). Sodium fluoride was added to measure lactate and cortisol levels, with an anticoagulant to measure creatine kinase (CK) and lactate dehydrogenase (LDH) levels. The samples were immediately centrifuged at 3000 rpm for 15 min to obtain plasma and to prevent any metabolic activity that may interfere with the results. The serum was collected and divided into aliquots, and both plasma and serum were stored at -20 °C for subsequent analysis.

Serum and plasma levels were determined in duplicate using commercial kits. Glucose, CK, LDH, and lactate were analyzed using the Dimension® Xpand Plus device (Siemens Healthcare Diagnostics Inc., USA) with commercial kits Gluc Ver Flex®, Ck Flex®, LDI Flex®, and Dimension LA®, respectively. Cortisol concentrations in plasma samples were measured using a commercial cortisol enzyme-linked immunosorbent assay (ELISA) kit (Jiaxing Korain Biotech Co., Zhejiang, China). Absorbance was determined using an ELISA reader (iMark™ Microplate Absorbance Spectrophotometer, Bio-Rad Laboratories Inc.) at 450 nm absorbance.

2.5 Statistical analyses

Data were analyzed using the Jamovi® program (The Jamovi Project, version 2.2) and the statistical significance of results were set at $P < 0.05$. Linear and multiple regression models were performed to establish the relationships between the minimum and maximum ocular IRT images, blood parameters, and meat quality to predict the pHu of the carcasses, with pHu as the dependent variable and day of collection and batch as random effects, and the other variables (lactate, glucose, CK, LDH, cortisol, *L*, a*,* and *b**) inserted into the models as covariates.

3. Results and discussion

The average dry bulb temperature (DBT, ºC) measured was 26.1 ± 0.31 and relative humidity (RH%) was 77.7 ± 0.57%, and there were no significant changes in these parameters during data collection. Descriptive statistics of blood variables (lactate, glucose, CK, LDH, and cortisol) related to animal stress, and physicochemical variables related to meat quality (carcass pHu, color, and myoglobin complex) and IRT are presented in Table 1.

The association between the maximum ocular IRT and pHu showed that as the temperature of the animals increased in the pre-stunning period, the carcasses of these animals had a greater chance of presenting a dark cut, thus corroborating the results of when the carcasses were separated based on pHu. The same was observed with luminosity; as the temperature of the animals before stunning increased, the carcasses of these animals had a greater chance of being darker, due to the lower value of L*.

IRT of the ocular temperature and ultimate pH (pHu) are used as indicators of stress levels in animals prior to slaughter and meat quality, respectively. Our findings align with the results obtained when we categorized the carcasses by pHu. The observed relationship between maximum ocular IRT and pHu suggests that as the pre-stunning period's animal temperature rises, there is an increased likelihood of these carcasses exhibiting dark cuts. The time lapse between these two measurements can lead to changes in the physiological conditions of the animal as well as changes in the pH and other biochemical properties of the meat. Ocular IRT is typically measured prior to slaughter, while ultimate pH is measured post-slaughter. The time lapse between these two measurements can lead to changes in the physiological conditions of the animal, as well as changes in the pH and other biochemical properties of the meat.

Table 1. Descriptive statistic of beef quality attributes and blood biochemical parameters in Nellore carcasses.

Variables	Mean	SE	Median	Minimum	Maximum
DBT (ºC)	26.1	0.308	25.7	23.2	30.7
RH (%)	77.7	0.565	77.8	68.5	86.2
IRTmax (ºC)	35.733	0.096	35.7	34.4	37.4
IRTmin (ºC)	28.439	0.115	28.4	25.8	31.3
Carcass pHu	5.88	0.018	5.830	5.5800	6.530
Lactate (mmol/L)	6.807	0.243	6.300	3.2000	14.500
Glucose (mmol/L)	111.38	2.044	108.500	72	172
CK (U/L)	804.29	52.487	698.500	294	3019
LDH (U/L)	1431.56	14.432	1426.000	1139	1802
Cortisol (nmol/L)	23.12	1.087	21.635	13.3083	46.703
L*	35.415	0.163	35.317	31.2667	39.300
a*	10.135	0.219	10.183	5.3000	15.833
b*	12.228	0.107	12.267	9.8333	14.533

DBT = dry bulb temperature. RH = relative humidity. IRT min = Minimum Temperature. IRT max = Maximum temperature. pHu = final pH. CK = creatine kinase; LDH = lactate dehydrogenase.

Linear and multiple regression analyses were used to understand the associations between the variables to predict pHu. The results demonstrated a strong relationship between the IRT measurements and the final pH, indicating the potential of IRT as a useful predictive tool (Table 2).

Table 2. Linear and Multiple regression of ocular IRT and beef quality traits and blood parameters to predict pHu of Nellore beef carcasses.

Predictors	R^2	R^2 adjusted	RMSE	P
IRT max	0.75	-	0.08	< 0.001
IRT max + Glucose	0.77	0.76	0.11	<0.001
IRT max + Lactate	0.80	0.79	0.11	<0.001
IRT max + *a**	0.76	0.75	0.11	<0.001
IRT max + *b**	0.76	0.75	0.11	<0.001

IRT max = temperature maximum. *a** = redness. *b** = yellowness.

The R^2 for IRT max was 0.75, signifying a significant correlation with the final pH of carcasses. The RMSE was 0.08, indicating good prediction accuracy. When IRT max was combined with the "Glucose" variable, we observed an improvement in predictive capability. The R^2 increased to 0.77, indicating an even stronger correlation. The adjusted R^2 also increased to 0.76. The RMSE remained at 0.11, maintaining prediction precision. The combination of IRT max with "Lactate" demonstrated an even better performance. The R^2 reached 0.80, indicating a very strong correlation between the variables. The adjusted R^2 was 0.79, confirming the robustness of the relationship. The RMSE remained at 0.11, maintaining prediction accuracy.

Combinations of IRT max with "a*" and "b*" also produced consistent results. Both combinations showed an R^2 of 0.76 and an adjusted R^2 of 0.75. These results suggest that IRT, especially when combined with variables such as Glucose and Lactate, can be a promising tool in predicting the final pH of bovine carcasses. The strong correlation and accuracy of the models highlight the viability of this approach as a potentially useful method in the meat industry. The models developed with average IRT and minimum IRT were statistically significant, but the variables in question did not show statistical significance and were therefore removed, along with other variables (L*, CK, LDH, cortisol).

Cuthbertson et al. (2020) reported no correlation between these variables but attributed this effect to the possible time difference between the measurements recorded, the IRT being conducted on the farm days or weeks before slaughter, and blood analyses being performed at different times. However, this phenomenon was not observed in the present study. The animals underwent the same treatment before slaughter, and they were maintained under the same climatic conditions and with the acquisition of images being captured seconds before the animals were slaughtered, while the blood was collected for analysis.

The results of this study reveal that IRT max is a promising tool for predicting the final pH of bovine carcasses. The significant and positive R^2, along with a low, indicates that IRT max, on its own, exhibits a strong correlation with the final pH, showcasing its predictive capability. We observed an improvement in predictive ability when IRT max was combined with additional variables such as glucose and lactate. The increase in R^2 and adjusted R^2 in these combinations suggests that the inclusion of these variables enhances prediction accuracy. The ability to predict the final pH of carcasses is critical for meat quality and can be beneficial for optimizing production processes and carcass selection. However, it's important to note that these results are promising, and further research is necessary to validate these findings under different conditions and in various animal populations.

4 Conclusion

Using animal ocular temperature data obtained through IRT, it was not possible to establish a strong relationship between blood parameters and meat quality. This study suggests that IRT, especially when combined with relevant blood variables, can be a valuable tool in predicting the final pH of bovine carcasses. A strong relationship IRT and glucose and lactate parameters or those related to meat color (a* and b*) were found in this preliminary study. Future studies can explore acquiring IRT images at different stages of the production process to determine the optimal timing for IRT acquisition. Increasing the number of animals used to test this technology for this purpose may also advance our understanding.

References

7 CONSIDERAÇÕES FINAIS

As causas bioquímicas da aparência da carne DFD ou carne de corte escuro estão relacionadas às mudanças que ocorrem no tecido muscular após o abate do animal. Essas mudanças são influenciadas por uma série de fatores, incluindo genética, nutrição, manejo e o próprio processo de abate e tudo que o antecede. No geral, as causas da carne DFD são complexas e multifatoriais.

A IRT tem sido usada para detectar sinais precoces de doenças em bovinos, como mastite, claudicação e infecções respiratórias. Também tem sido usada para avaliar os efeitos de fatores ambientais, como estresse por calor e estresse por frio, em bovinos. Uma vantagem do IRT é que ela permite a mensuração da temperatura corporal sem a necessidade de contato direto com o animal, o que pode reduzir o estresse e o risco de lesões tanto para o animal quanto para a pessoa que faz a mensuração. Além disso, a IRT pode fornecer um método rápido e eficiente para a triagem de muitos animais.

Estudos futuros podem explorar o uso da termografia, para avaliar a relação entre a temperatura ocular e a qualidade da carne em diferentes momentos do processamento das carcaças. Além disso, investigar o impacto das práticas de manejo pré-abate, como transporte e manuseio nos currais, na IRT ocular e na qualidade da carne pode auxiliar os resultados obtidos neste estudo.

ANEXOS

Parecer do Comitê de ética em Pesquisa envolvendo Seres Humanos

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Estratégias para identificar a anomalia DFD em carcaças bovinas.

Pesquisador: ana maria bridi **Área Temática:**
Versão: 3
CAAE: 39688920.9.0000.5231
Instituição Proponente:CCA - Departamento de Zootecnia
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.528.778

Apresentação do Projeto:
As informações elencadas foram retiradas do arquivo Informações Básicas da Pesquisa “PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO _1655866" de 28.01.2021.
Casos de estresse prolongado levam ao esgotamento das reservas de glicogênio muscular e a baixa produção de ácido lático na glicólise anaeróbica. O pH que deveria ser de 5,8, estabiliza acima de 6,0, caracterizando a carne DFD (Dry, firm and dark). Na indústria a carne é classificada pelo pH, entretanto, não é possível classificar a carne durante o processamento com a agilidade desejada. A espectroscopia por infravermelho próximo (NIRS) e a termografia de infravermelho (IRT) tem provado ser ferramentas eficientes para a avaliação de atributos de qualidade em carnes e produtos cárneos. O objetivo deste estudo é analisar o uso do NIRS e IRT na identificação da anomalia DFD. Para isso, será preciso identificar quais as diferenças entre carnes com pH 5,9 e igual ou maior a 6 por meio de análises físico-químicas, testar se a anomalia DFD pode ser identificada no sangue e no músculo semimembranosus, além de testar se a identificação por meio do NIRS pode ser calibrada por biomarcadores proteômicos. Por fim, analisar se o consumidor identifica esta carne por meio de um questionário. Como resultados, esperamos que as carcaças possam ser classificadas durante a sangria e ser correlacionadas com mensurações térmicas dos animais nos currais de espera. Além disso, identificar qual a percepção dos consumidores com relação a esta carne no Brasil.

Serão coletadas amostras de carne de bovinos machos não castrados de dois grupos genéticos (Nelore e cruzados) selecionados em uma planta frigorífica com inspeção federal de uma empresa parceira. Para a avaliação da qualidade sensorial da carne DFD as amostras serão maturadas em dois tempos, sete e 21 dias e serão considerados os parâmetros de classe sexual (macho inteiro, macho castrado e fêmea) e grupo genético (Nelore e cruzamento).

Serão utilizados 12 provadores treinados para a realização da análise sensorial que serão divididas em 48 rodadas de seis amostras cada. A análise sensorial será realizada na carne crua e grelhada. Os provadores analisarão parâmetros de odor e sabor da carne (ABNT, 1993). Os provadores treinados selecionados serão treinados durante um período de 3 meses (Dutcosky, 2013). Cada provador receberá uma amostra (de cada animal de cada tratamento) por vez para proceder a análise. Será utilizada duas escalas de intensidade estruturadas, com nove pontos, sendo avaliados os seguintes atributos:

1.maciez (força necessária para comprimir a amostra de carne entre os dentes molares; 1 = muito difícil; 9 = muito mole)

2.maciez inicial (força necessária para mastigar 3 vezes após compressão inicial; 1 = muito resistente; 9 = muito macio)

3.mastigação (energia necessária no 9º mastigar para engolir a uma taxa constante; 1 = muito resistente; 9 = muito macio)

4.taxa de desintegração (número de mastigações necessárias para a amostra se desintegrar durante o processo mastigatório em preparação para engolir; 1 = muito lento; 9 = muito rápido)

5.quantidade de resíduo perceptível (quantidade de tecido conjuntivo restante após a desintegração completa da amostra de carne; 1 = abundante; 9 = nenhum)

6.revestimento bucal (quantidade de óleo e gordura deixada na superfície da boca; 1 = nenhum; 9 = muito alto)

7.suculência (quantidade de umidade liberada após 5 mastigações; 1 = não suculenta; 9 = extremamente suculento)

8.intensidade do sabor (intensidade do sabor da carne após 8 mastigações; 1 = sem sabor da carne; 9 = sabor completo de carne bovina)

9.intensidade de sabor estranho (intensidade de qualquer sabor ou sabor posterior percebido como inadequado para carne cozida; 1 = muito forte; 9 = muito fraco).

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Para a identificação da preferência dos consumidores brasileiros serão analisados 2401 formulários online, divididos por região, sendo 342 no Sul, 1009 no Sudeste, 187 no Centro-oeste, 650 no Nordeste e 212 no Norte. O questionário terá como objetivo verificar se o consumidor de carne bovina brasileira possui conhecimento sobre aspectos qualitativos da carne, perfil de compra e consumo além de saber quanto estaria disposto a pagar a mais pela garantia de padronização do produto ofertado, se este fosse o caso. Por meio da plataforma Google Forms® será enviado de forma a contemplar todas as regiões do país, considerando uma margem de erro de 2 pontos percentuais a 95% de confiabilidade. A avaliação dos bifes será realizada por meio de imagens contendo apenas um bife, onde a variação será apenas em cor e marmoreio, tendo o mesmo tamanho, peso, espessura e refile. Também serão coletadas as seguintes informações: gênero; idade; Escolaridade; Ocupação; Renda;Hábito de compra (em que tipo de estabelecimento); Hábito de consumo (com que frequência); Hábito de cozinhar (se sabe preparar cozinhar e preparar carne); Modo de preparo; Modo que consome (churrasco, dia-a-dia, assado, frita/grelhada, cozida); Satisfação com os produtos comprados rotineiramente; Técnicas utilizadas para melhorar a maciez da carne comprada; É o responsável pela compra da carne em sua família; Sabe o que DFD?; Hábito de armazenamento.

Critérios de Inclusão:

- Para participação no teste sensorial, o avaliador deverá ter o hábito de consumir carne bovina, no mínimouma vez a cada quinze dias e ser capaz de avaliar cor, odor, textura, aceitabilidade, preferência, maciez, suculência, mastigação, taxa de desintegração, quantidade de resíduo, revestimento bucal, intensidade de sabor, intensidade de off flavor.
- Para responder ao questionário online, o participante deverá ser o responsável pela compra da carne naresidência ou dividir essa tarefa de forma igualitária entre os demais moradores. Ter entre 18 e 70 anos e consumir carne com frequência (no mínimo uma vez a cada quinze dias) e não ser empregado do setor da indústria da carne ou área que promova este produto.

Participarão da pesquisa: 2.413 participantes, sendo 2.401 que responderão um questionário online e 12 (doze) que provarão e avaliarão amostras de carnes.

Objetivo da Pesquisa:

As informações elencadas foram retiradas do arquivo Informações Básicas da Pesquisa “PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO _1655866" de 28.01.2021.

Objetivo Primário: Caracterizar e identificar carnes com diferentes valores de pH final.

Objetivos Secundários:
1: Analisar o uso da Espectroscopia de Infravermelho Próximo (NIRS) na identificação da anomalia dry, firm and dark (DFD) durante o processamento de carcaças bovinas de forma não destrutiva e com maior velocidade.
2: Identificar animais que apresentam anomalia DFD na área de descanso e/ou abate por meio de Termografia de Infravermelho.
3: Descrever a preferência visual do consumidor brasileiro de carne bovina.
4: Avaliar a qualidade sensorial de carnes com diferentes valores de pH.

Avaliação dos Riscos e Benefícios:

As informações elencadas foram retiradas do arquivo Informações Básicas da Pesquisa “PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO _1655866" de 28.01.2021.

Quanto aos Riscos a pesquisadora descreve:
Segundo o Ministério da saúde a carne vermelha pode causar problemas de saúde se seu consumo for excessivo. Visto que serão selecionados consumidores frequentes de carne bovina e que a quantidade consumida é pequena (menos de 100 g por avaliador), os riscos de qualquer problema relacionado à saúde são baixos ou inexistentes. Entretanto, no caso de ocorrência de desconforto, crises ou outros tipos de complicações decorrentes dos procedimentos metodológicos adotados, o participante será prontamente atendido e amparado pela pesquisadora, bem como todas as possíveis despesas com exames e/ou remédios serão custeadas pelo projeto. Riscos outros também podem ocorrer quando por quaisquer razões o (a) senhor (a) não se sentir apto (a) para o preenchimento do formulário; ou pelo gasto de tempo ao participar do teste e/ou causar cansaço ou aborrecimento ao participar do teste. Como medidas protetivas, estaremos atentos aos sinais verbais e não verbais de desconforto, limitaremos o acesso aos formulários apenas pelo tempo, quantidade e qualidade das informações específicas para a pesquisa, além de

garantir a não violação e a integridade dos documentos (danos físicos, cópias, rasuras), bem como o acesso aos resultados individuais e coletivos desta pesquisa. Caso ocorra quebra de sigilo, ainda que involuntária e não intencional, informamos que há legislação que inclui uma cláusula genérica sobre indenizações a que o senhor (a) pode achar-se no direito de receber por compensação de danos materiais ou morais decorrentes da pesquisa, inclusive relacionados à quebra de sigilo.

Os benefícios esperados são:

- para o consumidor, informações relacionadas ao consumo de carne bovina e identificação de anomalias nacarne;
- para a indústria, a disponibilidade de tecnologias acessíveis para a identificação de anomalias e maiorvelocidade na análise durante o processo produtivo;
- ambientalmente, a redução de desperdícios decorrentes do processo de classificação ou descarte decarcaças anômalas.

Comentários e Considerações sobre a Pesquisa:

Projeto de pesquisa da Universidade Estadual de Londrina/PR, especificamente do Departamento de Zootecnia. O grupo de pesquisa é formado por cerca de 25 estudantes, dentre eles de Mestrado e Doutorado do Programa de Pós-Graduação em Ciência Animal (CAPES 6) e estudantes de Graduação dos cursos de Medicina Veterinária e Zootecnia. O projeto de pesquisa é constituído por etapas que envolve animais bovinos e seres humanos. Em relação aos seres humanos, a pesquisa terá participantes que farão degustação de carnes (odor e sabor)e participantes que responderão um questionário on line.

Considerações sobre os Termos de apresentação obrigatória:

- Folha de rosto: apresentou. Assinada pela chefia de Departamento de Zootecnia do Centro de CiênciasAgrárias (CCA) da Universidade Estadual de Londrina/PR.

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- TCLE:

1) TCLE: apresentou dois modelos de TCLE, sendo um para participantes avaliadores sensoriais com degustação e outro para participantes da plataforma Google Forms que avaliarão imagens de bifes e responderão outros questionamentos sobre consumo de carnes.

- Projeto de pesquisa detalhado: apresentou

- Instrumentos de coleta de dados: Apresentou.

1) Apresentou questionário, intitulado Projeto Dark Cuer, que será utilizado via Plataforma Google Forms para participantes avaliarem imagens de bifes e outras perguntas

2) Instrumento para avaliadores que analisarão parâmetros de odor e sabor da carne: APRESENTOU- Orçamento: custeio de R$ 2.550,00 com materiais para análises sensoriais e compra de amostras

de carne. Segundo também consta na PB, o projeto será financiado com recursos gerados pelas análises que o Laboratório de análises de carne da UEL realiza para empresas. O pagamento destas análises serão realizadas via ITEDES. Assim, o laboratório tem recursos para compra de reagentes e materiais necessário para a condução do experimento. Como um dos critérios de inclusão para participação no teste sensorial é fazer parte do quadro de funcionários ou estudantes do Centro de Ciências agrárias, não haverá necessidade de pagamento de qualquer valor relacionado a transporte aos participantes do teste.

- Cronograma: Adequado.
- Envio do questionário online: previsto para 01/03/2021.
- Coleta dos dados e amostras de carne: previsto para 01/03/2021.
- Análise sensorial da carne: previsto para 01/05/2021.

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Recomendações:

Prezada pesquisadora, solicitamos que substitua, no início do questionário anexado na Plataforma Brasil "Projeto Dark Cutter" online Google Forms, o TCLE atualizado, corrigido e nesta versão de submissão aprovado por este CEP.

Conclusões ou Pendências e Lista de Inadequações:

Prezada pesquisadora, após análise desta terceira submissão do projeto de pesquisa por este CEP, notificamos a situação das pendências que foram destacadas no parecer anterior:

PENDÊNCIA 1) Projeto de pesquisa: rever, readequar, incluir informações detalhadas na Plataforma Brasil (PB) de acordo com cada objetivo da pesquisa que envolva seres humanos.

a) Incluir os Critérios de inclusão e exclusão dos participantes (degustação e questionário online). ANÁLISE:

PENDÊNCIA ATENDIDA.

b) Deverão estar em consenso todas as informações da PB, do projeto de pesquisa detalhado brochura edos anexos (TCLEs, questionário, roteiro de avaliação, outros documentos apresentados). Quanto aos riscos aos participantes, as informações dos dois TCLES (pesquisa sensorial e pesquisa questionário online) e o campo dos riscos dos participantes da Plataforma Brasil deverão estar em consenso. ANÁLISE: PENDÊNCIA ATENDIDA.

c) Descrever como será a forma de recrutar os participantes, o local de realização da pesquisa e tudo que oparticipante terá que fazer de forma clara e compreensível, principalmente no TCLE.

ANÁLISE:PENDÊNCIA ATENDIDA.

PENDÊNCIA 2)

a) Folha de rosto: providenciar nova folha de rosto com assinatura e carimbo da Chefia Imediata do Departamento de Zootecnia, conforme consta no campo

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Instituição Preponente Unidade/Órgão: CCA – Depto de Zootecnia. A pesquisadora poderá também justificar o motivo pelo qual a Diretora do CCA assinou a folha de rosto que está anexada no PB (por exemplo: conflito de interesse). Providenciar nova folha de rosto ou justificar o motivo pelo qual a Chefia Imediata do Departamento de Zootecnia assinou o documento.
ANÁLISE: PENDÊNCIA ATENDIDA.

PENDÊNCIA 3)

a) Apresentar 02 (dois) TCLE específicos, sendo um para os avaliadores de degustação e outraparaparticipantes que responderão o questionário online. ANÁLISE: PENDÊNCIA ATENDIDA.

b) Atentar para o detalhamento das informações do TCLE. Descrever o local de realização da pesquisa etudo que o participante terá que fazer de forma clara e compreensível, o tempo que durará sua participação (respondendo questionário ou degustação). ANÁLISE: PENDÊNCIA ATENDIDA.

c) Explicitação de possíveis danos/riscos decorrentes da participação na pesquisa, além da apresentaçãodas providências e cautelas a serem empregadas para evitar situações que possam causar danos. Lembramos que toda pesquisa envolve riscos aos participantes e que a assistência de amparo aos participantes frente às intercorrências não poderá onerar o Sistema Único de Saúde (SUS). ANÁLISE:
PENDÊNCIA ATENDIDA.

PENDÊNCIA 4)

a) Instrumento de Coleta de dados: além do questionário que será aplicado via Google Forms, incluir o roteiro de avaliação sensorial de degustação. ANÁLISE: PENDÊNCIA ATENDIDA.

PENDÊNCIA 5)

a) Orçamento: valor mencionado de R$2.550,00 investidos na compra das amostras de carne e material para análise sensorial (copos, pratos, papel alumínio). O projeto será financiado com recursos gerados pelas análises que o Laboratório de análises de carne da UEL realiza para empresas. O pagamento destas análises são realizadas via ITEDES. Como um dos critérios de inclusão para participação no teste sensorial é fazer parte do quadro de funcionários ou estudantes do Centro de Ciências agrárias, não haverá necessidade de pagamento de qualquer valor relacionado a transporte aos participantes do teste. ANÁLISE: PENDÊNCIA ATENDIDA.

PENDÊNCIA 6)

a) Detalhar todas etapas da pesquisa com seres humanos. ANÁLISE: PENDÊNCIA ATENDIDA.

Em síntese, TODAS as pendências descritas foram ATENDIDAS pela pesquisadora.

Considerações Finais a critério do CEP:

Prezado(a) Pesquisador(a),

Este é seu parecer final de aprovação, vinculado ao Comitê de Ética em Pesquisas Envolvendo Seres Humanos da Universidade Estadual de Londrina. É sua responsabilidade apresenta-Lo aos órgãos e/ou instituições pertinentes.

Ressaltamos, para início da pesquisa, as seguintes atribuições do pesquisador, conforme Resolução CNS 466/2012 e 510/2016:

A responsabilidade do pesquisador é indelegável e indeclinável e compreende os aspectos éticos e legais, cabendo-lhe:

- conduzir o processo de Consentimento e de Assentimento Livre e Esclarecido;
- apresentar dados solicitados pelo sistema CEP/CONEP a qualquer momento;
- desenvolver o projeto conforme delineado, justificando, quando ocorridas, a sua mudança ou interrupção;
- elaborar e apresentar os relatórios parciais e final;
- manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por umperíodo mínimo de 5 (cinco) anos após o término da pesquisa;
- encaminhar os resultados da pesquisa para publicação, com os devidos créditos aos pesquisadores epessoal técnico integrante do projeto;
- justificar fundamentadamente, perante o sistema CEP/CONEP, interrupção do projeto ou a não publicaçãodos resultados.

Coordenação CEP/UEL.

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Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1655866.pdf	28/01/2021 17:37:23		Aceito
Recurso Anexado pelo Pesquisador	carta_resposta_pendencias.pdf	28/01/2021 17:36:50	ana maria bridi	Aceito
Folha de Rosto	Termo_Chefe.pdf	20/12/2020 23:05:49	ana maria bridi	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	tcle_sensorial.doc	20/12/2020 22:57:15	ana maria bridi	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	tcle_questionario.doc	20/12/2020 22:57:01	ana maria bridi	Aceito
Outros	Sensorial_Carne_treinados.docx	30/11/2020 15:20:48	ana maria bridi	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_DFD.docx	30/11/2020 15:19:19	ana maria bridi	Aceito
Outros	Questiionario.pdf	30/10/2020 14:45:45	ana maria bridi	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

LONDRINA, 08 de Fevereiro de 2021

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Assinado por:
Adriana Lourenço Soares Russo
(Coordenador(a))

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