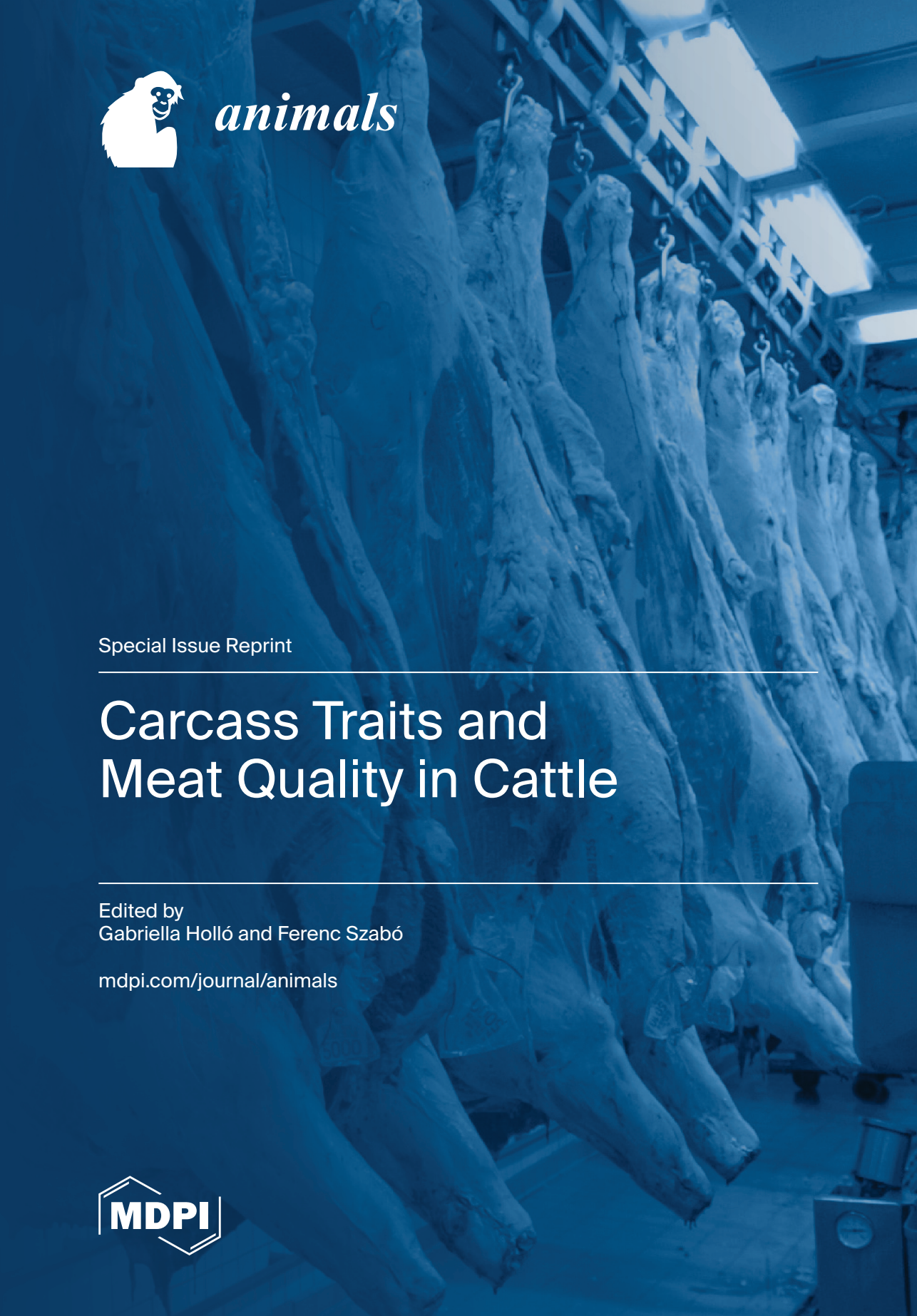

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Special Issue Reprint

Carcass Traits and Meat Quality in Cattle

Edited by
Gabriella Holló and Ferenc Szabó

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Guest Editors

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About the Editors

Gabriella Holló

Gabriella Holló, PhD, is an agricultural engineer and currently an Assistant Professor at Kaposvár Campus of the Hungarian University of Agriculture and Life Sciences (MATE, Gödöllő). She has been a participant in several national projects, such as the "Development of innovative breeding technology with modern biotechnical (embryo transfer), biotechnological and feeding solutions to improve the efficiency of milk and meat production" and "Improvement of the industrial milk production technologies supported by molecular biological tools widely accepted from nutritional physiology point of view". She coordinated the "Healthy beef—the effects of breed and nutrition on fatty acid composition of beef" and "Improvement of beef quality and milk composition by molecular and imaging techniques" projects. Her current research focuses on precision cattle breeding with a primary focus on sustainable dairy and beef production to achieve higher yields and quality, while minimizing environmental emissions. She has published 90 peer-reviewed articles, 3 books, 13 book chapters, 75 conference papers, and 121 abstracts. Her Scopus Citation Index is 278, and her H-index is 10. She is a member of the Association of Pro Scientia Gold Medallist, Regional Committee of Hungarian Academy of Sciences, and president of the Committee of Agricultural Sciences of the Regional Committee of Hungarian Academy of Sciences (Pécs).

Ferenc Szabó

Ferenc Szabó, DSc, graduated in agricultural sciences and worked in animal husbandry, mainly cattle breeding, on a relatively large Hungarian farm. Later, he was appointed to the Department of Animal Husbandry at the University, where he taught and researched. He obtained his PhD and DSc degrees and became a Full Professor. So far, 11 of his PhD students have graduated under his supervision. He has edited and partly written six textbooks on animal science, animal husbandry, and beef cattle breeding. His research has mainly been focused on the field of beef cattle breeding, beef cattle husbandry, and dairy cattle herds, especially through population genetic analyses. He has published more than 600 papers, mainly scientific, especially with co-authors, and has reviewed and edited numerous publications. Formerly, he has fulfilled the roles of Head of Department, Head of PhD School, Associate Dean, and Dean. He has worked as a visiting scientist in the USA and was a member of the International Committee for Animal Recording Working Group. Currently he is Professor Emeritus at the Hungarian University of Agricultural and Life Sciences and Széchenyi István University in Hungary.

Preface

Beef, which can come from both the dairy and beef sectors, is a high-quality animal product that provides essential amino acids, minerals (such as iron and zinc), and vitamins, as well as anti-inflammatory conjugated linoleic acid, that humans need. Consumers are demanding more safe, nutritious, and palatable beef produced by sustainable and acceptable methods. Experience of flavor, tenderness and juiciness will be most important in shaping quality experience, satisfaction, and future purchases. In recent years, the entire beef production chain has faced significant challenges, from cattle breeding, rearing fattening, slaughter and delivery to the consumer. These challenges, whether the beef comes from the dairy or the beef sector, have involved achieving higher yields and quality while minimizing environmental emissions.

These matters are addressed in the Special Issue "Carcass Traits and Meat Quality in Cattle", which was published in MDPI's journal *Animals* from April 2024 to November 2024.

Twenty-two manuscripts were submitted for consideration for this Special Issue, and they were subjected to the journal's rigorous peer-review process. In total, eleven papers (ten research papers and one review) were accepted for publication and inclusion in this Special Issue.

This Special Issue welcomed researchers from Brazil, China, Hungary, Ireland, Mexico, Pakistan, Poland, Spain, South Africa, Uruguay, and the USA.

We provide a brief overview of the papers included in this Special Issue to allow readers to familiarize themselves become acquainted with the contributions close to their research interests.

The papers are listed below:

In Contribution 1, the authors evaluated the effect of five myostatin alleles on muscularity and on the calving ease, birth weight and 205-day weaning weight of weaned calves in the Hungarian Charolaise population.

Contribution 2 compared the nutritional value comparison of meat from the four most popular cattle breeds (Polish Holstein-Friesian, Limousine, Hereford and Charolaise) in Poland.

Contribution 3 characterized pasture grazing as predominant in the beef production system in Uruguay, in addition to in other systems, where steers are now increasingly fed cereals to reduce the production time. This has implications for meat quality, so meat from both systems (pasture and concentrate) was compared under chilled retail conditions.

Contribution 4 reviewed fossil shell flour as a feed additive as a novel feeding strategy, but to date, there is little to no scientific evidence to support its efficacy in sustainable beef production and in response to climate change and/or carbon footprints.

Contribution 5 evaluated the effect of the β–adrenergic agonist zilpaterol hydrochloride (ZH) on skeletal muscle growth, feedlot performance and carcass yield in British (B) and British × Continental (BC) steers, as well as marbling score and beef tenderness.

In Contribution 6, the beef quality of South African feedlot bulls was studied over two ageing periods. Animals were fed supplemented with two commercially available zilpaterol hydrochloride (ZH) β-adrenergic agonists during the finishing period.

Contribution 7 aimed to study the carcass traits and the technological and sensory quality of meat from an autochthonous Spanish cattle breed (Lidia). This pasture-based system is linked to the conservation of biodiversity and sustainable agricultural practices, and plays an important role in ecosystem conservation and rural development; however,

Contribution 8 investigated four single-nucleotide polymorphisms (SNPs) of bovine Stearoyl-CoA desaturase-1 (SCD1) to explore the correlation between carcass, meat quality,

adipogenic traits, fatty acid composition, and milk production traits in Chinese Simmental steers and in Holstein cows.

Contribution 9 defined the effects of premortem stress on beef quality of castrated Holstein calves subjected to an adrenocorticotropic hormone (ACTH) challenge to emulate a stress response.

In Contribution 10, the authors conducted Brazilian experiments with different genotypes (Angus, Nellore, Charolaise) of beef heifers finished in feedlots. In addition, their diets were supplemented with different sources of nonprotein nitrogen (NPN) to evaluate carcass and meat characteristics, such as carcass weight, fat content in the carcass and in meat, and the fatty acid profile of the meat.

Contribution 11 investigated the effects of genotype (Bos indicus x Bos taurus, Bos taurus), the age of an animal, and duration of maturation on the meat quality characteristics of four muscles (Psoas major, Longissimus thoracis, Longissimus lumborum, and Gluteus Medius) from bulls bred in Pakistan.

Overall, this Special Issue, "Carcass Traits and Meat Quality in Cattle", identifies several potential research opportunities and future directions:

- Among the five examined single-nucleotide polymorphisms (SNPs) investigated, Q204X of the bovine MSTN had a significant effect on the 205-day weaning weight and all measured muscle traits in the Hungarian Charolaise population. It would be advisable to pay more attention to this allele in the breeding program, considering its side effect on calving difficulty.

- Breed significantly influences the antioxidant potential of beef. Polish beef breeds had higher levels of total antioxidant status and degree of antioxidant potential than Polish Holstein-Friesian (HH) beef. The Limousine (LM) breed had the highest concentrations of anserine, taurine, and creatine, while the Charolaise (CH) breed had the highest levels of carnosine and coenzyme Q10. In addition, LM, CH, and HH had significantly higher levels of conjugated linoleic acid content.

- Uruguayan meat from pasture-fed steers showed lower levels of lipid and protein oxidation during the display period due to the higher levels of antioxidants, such as β-carotene and α-tocopherol. In addition, meat from pasture-raised steers was healthier for consumers in terms of its fatty acid composition and lower intramuscular fat content; it was more stable during retail refrigeration, potentially allowing for a longer shelf-life; and it was healthier for consumers compared to meat from concentrate-fed steers.

- The use of fossil shell flour (FSF) in beef production will be very challenging due to the large amount of feed required and the unknown nutritional value. Although FSF is known to be completely safe and non-toxic, we should take into consideration the interaction between the mineral content of FSF animal's diet. The inclusion of 4% FSF is suggested due to increased N retention and to aid in environmental pollution control, as well as influence enteric methane emissions.

- Supplementation with Zilpaterol hydrochloride (ZH) may be justified if market signals demand leaner, higher-yielding carcasses. Conversely, marketing channels that value consumer palatability may consider limiting the use of ZH due to negative effects on tenderness and other sensory traits of beef from steers.

- Bulls fed β-adrenergic agonists containing zilpaterol hydrochloride (ZH) for 30 showed a significant decrease in meat tenderness due to increased WBSF values. Dietary supplementation

with ZH in feedlot diets during the finishing period did not affect the meat quality differently during extended aging compared to the negative controls. Although meat tenderness was initially negatively affected by ZH supplementation, it improved significantly over extended postmortem aging. ZH supplementation per se did not affect postmortem meat quality more than untreated bulls, but extended aging may have compromised beef quality if it exceeded 56 days.

- Age at slaughter in Lidia females had a significant influence on carcass traits and meat quality. Increasing age at slaughter influenced the morphometric and color variables of carcass muscle and subcutaneous fat. The tissular composition of cull cows showed higher lean, fat and bone proportions and a higher percentage of fat. Cull cows presented higher values in flavor intensity, juiciness, overall tenderness, and overall acceptability. Although the Lidia females were small to medium-sized, with a lower carcass weight compared to other breeds, their sensory characteristics suggested a positive potential for consumption.

- The four SNPs of the SCD1 gene were associated with carcass traits, meat quality, adipogenic traits, and fatty acid composition in Chinese Simmental steers. There was also a modest effect on milk production traits such as average milk yield and milk fat content in Holstein cows. Furthermore, the importance of the well-known SCD1 gene marker in bovine breed improvement is underscored.

- Time of harvest and cortisol response following a stressful event affected beef quality. The time of harvest and the level of stress during a stressful event did impact the quality of beef in different ways. Generally, animals that were harvested 12 h after the ACTH challenge increased the quality of beef due to the differences related to improved steak color, lower steak pH, decreased oxidation, and decreased miofibrillar fragmentation index.

- The meat compositions for heifers from different genetic groups differed only in terms of fat content. The Charolais × Nellore heifers had lower concentrations of SFAs in meat and fat, giving a better fatty acid profile for this crossbreed. Regardless of the genetic group, the proportion of PUFAs and the PUFA/SFA ratios were lower in the subcutaneous fat than in that extracted from the meat.

- Humped bulls showed better color and oxidative stability, whereas humpless bulls showed better instrumental tenderness and sensory characteristics. The tenderloin and rib-eye muscles improved in color, tenderness, and sensory traits with up to seven days of aging. The sirloin and rump muscles continued to improve for up to 14 days. The tenderloin was the most tender and palatable, while the sirloin had the best color and oxidative stability. The results highlighted the importance of applying muscle-specific aging strategies to improve meat quality in both humped and humpless bulls.

We believe that this Special Issue will broaden our knowledge of the role of beef production and beef quality.

We are pleased to say that this Special Issue has been viewed more than fifteen thousand times, demonstrating both the interest of researchers in the beef quality and slaughter value of cattle and the importance of open access research.

The Guest Editors would like to express their gratitude to all the authors for their high-quality contributions to this Special Issue, as well as to the reviewers for their professional and valuable

comments, which helped the authors to significantly improve the final quality of the submitted manuscripts.

We are also pleased to acknowledge the cooperation of the administrative staff of MDPI in the realization of this project. Special thanks are due to the Managing Editor of this Special Issue, Dr Nicolas Gai, for his excellent collaboration and valuable assistance.

Gabriella Holló and Ferenc Szabó
Guest Editors

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Article

Relationship between Some *Myostatin* Variants and Meat Production Related Calving, Weaning and Muscularity Traits in Charolais Cattle

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Simple Summary: The objective of this study was to evaluate the effect of different *myostatin* alleles on muscularity of four body parts and overall muscularity, and, moreover, on calving ease, birth weight and 205-day weaning weight of weaned calves in the Hungarian Charolais population. Five *myostatin* alleles of 2046 calves were involved in the study. Among the *myostatin* alleles, the effect of *Q204X* was statistically proved ($p < 0.01$ and $p < 0.05$) on the 205-day weaning weight, muscle score of back, muscle score of thigh, loin thickness score and overall muscle development percentage. It would be advisable to pay more attention to this allele in the breeding program.

Abstract: The slaughter value of live cattle can be assessed during visual conformation scoring, as well as by examining different molecular genetic information, e.g., the *myostatin* gene, which can be responsible for muscle development. In this study, the *F94L*, *Q204X*, *nt267*, *nt324* and *nt414* alleles of the *myostatin* gene (*MSTN*) were examined in relation to birth weight (BIW), calving ease (CAE), 205-day weaning weight (CWW), muscle score of shoulder (MSS), muscle score of back (MSB), muscle score of thigh (MST), roundness score of thigh (RST), loin thickness score (LTS), and overall muscle development percentage (OMP) of Charolais weaned calves in Hungary. Multi-trait analysis of variance (GLM) and weighted linear regression analysis were used to process the data. Calves carrying the *Q204X* allele in the heterozygous form achieved approximately 0.14 points higher MSB, MST and LTS, and 1.2% higher OMP, and gained 8.56 kg more CWW than their counterparts not carrying the allele ($p < 0.05$). As for the *F94L* allele, there was a difference of 4.08 kg in CWW of the heterozygous animals, but this difference could not be proved statistically. The other alleles had no significant effect on the evaluated traits.

Keywords: *myostatin* alleles; *Q204X*; *F94L*; muscularity scores; calving and weaning traits

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1. Introduction

The value of slaughter animals, that is, the carcass composition and meat quality of meat-producing farm animals, such as slaughter cattle, can be reliably evaluated with post-slaughter muscle and fat measurements and laboratory tests. In beef production, however, slaughterhouse evaluation and laboratory meat quality testing are often impossible in the trade, as the animals are marketed on a live basis. Despite failing the mentioned objective evaluation possibilities, both the sellers and the buyers must be able to appraise, visually or in other ways, the meat production value of these animals.

The meat production value of slaughter animals can be evaluated with a high degree of accuracy based on several seen, measured and estimated conformation traits. A large

number of research results from literary sources support the fact that the age, weight, sex, conformation, condition, muscle mass and shape of live animals provide reliable information about their meat production; however, some environmental factors can also play an important role [1]. The mentioned traits can be easily assessed by visual scoring. At the same time, some major genes or quantitative trait loci (QTL) have been identified related to meet quantity and quality [2,3]. The latter situation gives us the opportunity to perform tests on live animals, as DNA can be isolated from blood or other tissue, and the gene or gene variants affecting meat production can be detected. Such tests can be carried out early, before slaughter of animals at a young age.

An indicator of slaughter value could be *myostatin*, which is an extracellular cytokine mostly expressed in skeletal muscles and known to play a crucial role in the negative regulation of muscle mass [4,5].

Sellick et al. [6] studying the different variants of *MSTN* found that *F94L* was the only polymorphism consistently related to increased muscling. Wiener et al. [7] found that the *myostatin* allele with the 11-bp deletion (MH) segregating in the South Devon breed affected several traits related to beef production. The MH allele was associated with heavier calves at birth but slower growth, leading to lighter adult animals. Allais et al. [8] found the superiority of carcass traits of calves carrying one copy of the mutated allele (*Q204X* or *nt821*) over noncarrier animals was approximately +1 SD in the Charolais and Limousin breeds but was not significant in the Blonde d'Aquitaine. In the Charolais breed, for which the frequency was the greatest (7%), young bulls carrying the *Q204X* mutation presented a carcass with less fat, less intramuscular fat and collagen contents, and a clearer and more tender meat than those of homozygous-normal cattle. Hales et al. [9] reported that the average daily gain measured in Limousin heifers across the whole study (121 days) was greater with two copies of the *F94L* (homozygous) variant. According to Ceccobelli et al. [10], the heterozygous *MSTN* in Marchigiana bulls showed slight superiority in the carcass weight (heterozygote 426 kg and normal 405 kg) and meat quality parameters, although not always with statistical significance.

Looking at the relevant literature, even though there are many research results available on the effect of *myostatin* on meat production in cattle, especially in double-muscled cattle [11,12], relatively less is known about the effect of certain alleles in the Charolais breed. Based on previous data [13–15], it seems that there are significant differences between the phenotypic performance of individuals carrying and not carrying the *myostatin* alleles [16]. According to our opinion, this information is very important for improving performance, quality and genetic traits of the Hungarian Charolais population.

To our knowledge, phenotypic characteristics of calves related to *MSTN* alleles in the Hungarian Charolais population, even certain allele variants in the Charolais breed, has not been studied so far.

The objective of the present study was to evaluate some *myostatin* alleles such as *F94L* and *Q204X* and others (*nt267*, *nt324* and *nt414*) on birth weight, calving ease, 205-day weaning weight and muscle score of some body parts (shoulder, back, thigh and loin), and overall muscularity showing muscle development and trend of these traits in the Charolais beef cattle population in Hungary.

2. Materials and Methods

2.1. The Database

Data processed during the work were collected from the pedigree database, the National Association of Hungarian Charolais Cattle Breeders. The available and evaluated initial database contained pedigree, weaning, conformation traits and molecular genetic information. In the study, there were 2046 EU-registered weaned Charolais calves (688 male and 1358 female) born between 2015 and 2021.

2.2. The Studied Traits

During the study, the birth weight of calves (BIW), calving ease of dams (CAE), 205-day weaning weight of calves (CWW), muscle score of shoulder (MSS), muscle score of back (MSB), muscle score of thigh (MST), roundness score of thigh (RST), loin thickness score (LTS) and overall muscle development percentage (OMP) as phenotypic traits of weaned calves were evaluated in relation to *MSTN* mutations.

The conformation traits were scored at the weaning. The scoring of the mentioned body parts was carried out according to the Conformation Scoring Guideline of the National Association of Hungarian Charolais Cattle Breeders [17]. Each animal for each trait was scored from 1 to 10 points depending on the mass and shape of the muscles. However, the values of the OMP were calculated as the sum of the scores of each body part and the ratio of the maximum possible total score in per cent as follows:

$$OMP = (MSS + MSB + MST + RST + 2 \times LTS) / 60 \quad (1)$$

The calving ease of cows was scored as follows: normal light calving = 1, calving with assistance = 2 and difficult calving = 3.

2.3. The Molecular Genetic Informations

The molecular genetic information of the 2046 weaned calves was determined with the Weatherbys Scientific Bovine VersaSNP 50K chip. The description of the method and the possibilities of interpreting the results are described in detail by [18].

The genetic database contained information on 117 different alleles. In the course of this study, five relevant alleles of the gene encoding the *myostatin* protein (growth differentiation factor 8; GDF8), *F94L*, *Q204X*, *nt267*, *nt324* and *nt414* were examined [19,20]. Based on the available information [21–23], it seems that these alleles can have a significant impact on muscle growth, including the development of muscularity. In each case, it was indicated in the database whether the individuals carry the *F94L*, *Q204X*, *nt267*, *nt324* and *nt414* alleles in the homozygous or heterozygous form, or not. The distribution of these alleles by sex of calves is shown in Table 1.

Table 1. Occurrence of *myostatin* alleles in the examined population.

Myostatin Allele	Genotype	Male Calves	Female Calves	Total
		Number of Animals		
F94L	Noncarrier	651	1282	1933
	Heterozygous	37	76	113
	Homozygous	0	0	0
Q204X	Noncarrier	606	1185	1791
	Heterozygous	82	173	255
	Homozygous	0	0	0
nt267	Noncarrier	633	1318	1981
	Heterozygous	25	40	65
	Homozygous	0	0	0
nt324	Noncarrier	547	1060	1607
	Heterozygous	132	277	409
	Homozygous	9	21	30
nt414	Noncarrier	357	705	1062
	Heterozygous	277	548	825
	Homozygous	54	105	159
Total		688	1358	2046

2.4. The Effect of Different Factors

Before evaluating the database, the basic statistical parameters of the examined traits (mean, standard deviation, CV%, etc.) were calculated. The Kolmogorov–Smirnov test was used to check the normality of the data, and Levene's test was used to check the homogeneity of the variances (Table 2).

Table 2. Basic statistics of the examined traits (number of animals for each trait 2046).

Trait	Mean	SD	CV%	Min	Max	Norm *	Hom #
BIW (kg)	43.63	5.99	13.74	21	70	0.07	0.11
CAE (score)	1.16	0.45	38.55	1	3	0.51	0.00
CWW (kg)	258.15	44.30	17.16	125	404	0.03	0.00
MSS (score)	5.54	1.10	19.91	2	9	0.18	0.06
MSB (score)	5.13	1.05	20.39	2	8	0.19	0.02
MST (score)	5.36	1.16	21.71	2	10	0.17	0.27
RST (score)	5.35	1.12	21.01	2	9	0.18	0.33
LTS (score)	5.26	1.07	20.45	2	9	0.18	0.13
OMP (%)	53.15	9.62	18.10	20	87	0.05	0.04

BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight; MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage. * Normality test: if $p > 0.05$, the normal distribution is confirmed; # homogeneity test: if $p > 0.05$, the homogeneity is confirmed.

To evaluate the database, the multifactor analysis of variance (general linear model) was applied [24]. During this work, the birth year and sex of the calves, as well as the genotype determined on the basis of the *myostatin* alleles (mentioned above), were incorporated into the model as fixed effects [16]. The nine examined traits were treated separately from each other, and in all nine cases separated models were performed. The general formula of the models used was as follows:

$$\hat{y}_{hijklmn} = \mu + Y_h + S_i + F_j + Q_k + N_l + M_m + T_n + e_{hijklmn} \quad (2)$$

where $\hat{y}_{hijklmn}$ = trait of a weaned calf of "h" year, "i" sex, "j" *F94L*, "k" *Q204X*, "l" *nt267*, "m" *nt324* and "n" *nt414* genotypes; μ = average of all observations; Y_h = effect of birth year of calves; S_i = effect of sex of calves; F_j = effect of *F94L* allele; Q_k = effect of *Q204X* allele; N_l = effect of *nt324* allele; M_m = effect of *nt324* allele; T_n = effect of *nt414* allele; and $e_{hijklmn}$ = random error [10].

2.5. Estimation of Phenotypic Trends and Phenotypic Correlations

For all nine traits, the data of the calves born in the same year were analyzed and averaged by year. Weighted one-way linear regression analysis was used to estimate the phenotypic trends. The dependent variable was the evaluated trait, the birth year of calves was considered as an independent variable, and the weight was the number of individuals per year.

Among the nine evaluated traits, Pearson's phenotypic correlation values (r) were also determined.

2.6. The Used Softwares

The data were prepared using Microsoft Excel 2003 and Word 2003. The evaluation of the database was performed with the statistical software package SPSS 27.0 [25].

3. Results

For all traits, the influence of the sex and birth year of the calf was statistically verifiable ($p < 0.01$) and played a decisive role (62.27–96.74%) in the development of the phenotype (Table 3). The effect of the year of birth of the calves on the tested traits was also significant ($p < 0.01$). Among the *myostatin* alleles, the effect of *Q204X* was statistically proved ($p < 0.01$

and $p < 0.05$) on the traits CWW, MSB, MST, LTS and OMP. The other alleles had no effect on the evaluated weaning and muscularity traits.

Table 3. Effect of the examined factors on the calving, weaning and the muscularity traits.

Factors	Traits								
	BIW	**CAE**	**CWW**	**MSS**	**MSB**	**MST**	**RST**	**LTS**	**OMP**
					p				
Birth year of calves	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Sex of calves	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
F94L	NS	NS	NS	NS	NS	NS	NS	NS	NS
Q204X	NS	NS	<0.01	NS	<0.05	<0.05	NS	<0.05	<0.05
nt267	NS	NS	NS	NS	NS	NS	NS	NS	NS
nt324	NS	NS	NS	NS	NS	NS	NS	NS	NS
nt414	NS	NS	NS	NS	NS	NS	NS	NS	NS
Factors	The ratio of the examined factors in phenotype (%)								
Birth year of calves	8.53	19.19	3.84	1.95	1.26	1.52	6.63	2.44	1.97
Sex of calves	90.37	62.27	87.90	96.18	96.74	94.43	92.32	95.53	96.49
F94L	0.24	1.39	0.68	0.12	0.39	0.01	0.21	0.50	0.24
Q204X	0.00	2.05	5.29	0.63	1.04	1.79	0.04	1.07	0.81
nt267	0.01	6.12	0.03	0.10	0.03	1.30	0.07	0.10	0.16
nt324	0.07	1.02	1.17	0.35	0.06	0.24	0.07	0.08	0.03
nt414	0.16	4.54	0.38	0.40	0.21	0.19	0.28	0.03	0.07
Error	0.62	3.42	0.71	0.27	0.27	0.52	0.38	0.25	0.23
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight; MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage.

The adjusted overall mean values (±SE) of the examined traits was as follows (Tables 4 and 5): BIW 43.65 ± 0.63 kg, CAE 1.12 ± 0.05 points, CWW 269.07 ± 4.73 kg, MSS 5.90 ± 0.11 points, MSB 5.39 ± 0.11 points, MST 5.65 ± 0.12 points, RST 5.54 ± 0.12 points, LTS 5.52 ± 0.11 points and OMP 55.86 ± 0.96%.

Table 4. The effect of different factors on the calving and weaning traits.

Factors	N	Calving and Weaning Traits		
		BIW (kg)	**CAE (Score)**	**CWW (kg)**
Adjusted overall mean (±SE)	2046	43.65 ± 0.63	1.12 ± 0.05	269.07 ± 4.73
		Deviation from the overall mean		
Birth year of calves				
– 2015	195	−0.98	+0.16	−6.02
– 2016	51	−0.37	−0.10	−9.20
– 2017	139	−2.36	−0.02	−4.12
– 2018	296	+0.46	+0.00	−2.01
– 2019	540	−0.06	+0.04	+4.67
– 2020	597	+0.76	-0.02	+6.93
– 2021	228	+2.54	−0.05	+9.74

Table 4. *Cont.*

Factors	N	Calving and Weaning Traits		
		BIW (kg)	**CAE (Score)**	**CWW (kg)**
Adjusted overall mean (±SE)	2046	43.65 ± 0.63	1.12 ± 0.05	269.07 ± 4.73
		Deviation from the overall mean		
Sex of calves				
– male	688	+1.67	+0.05	+11.54
– female	1358	−1.67	−0.05	−11.54
F94L				
–noncarrier	1933	+0.17	+0.01	−2.04
– heterozygous	113	−0.17	−0.01	+2.04
Q204X				
– noncarrier	1791	−0.01	−0.01	−4.28
– heterozygous	255	+0.01	+0.01	+4.28
nt267				
– noncarrier	1981	−0.05	+0.04	−0.54
– heterozygous	65	+0.05	−0.04	+0.54
nt324				
– noncarrier	1607	+0.08	+0.00	−4.58
– heterozygous	409	−0.07	−0.02	−1.27
– homozygous	30	+0.00	+0.02	+5.85
nt414				
– noncarrier	1062	+0.13	+0.02	−0.67
– heterozygous	825	+0.11	+0.02	−1.57
– homozygous	159	−0.24	−0.04	+2.25

BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight.

Table 5. The effect of different factors on the muscularity traits.

Factors	N	Muscularity Traits					
		MSS (Score)	**MSB (Score)**	**MST (Score)**	**RST (Score)**	**LTS (Score)**	**OMP (%)**
Adjusted overall mean (±SE)	2046	5.90 ± 0.11	5.39 ± 0.11	5.65 ± 0.12	5.54 ± 0.12	5.52 ± 0.11	55.86 ± 0.96
		Deviation from the overall mean					
Birth year of calves							
– 2015	195	+0.13	+0.15	+0.04	−0.19	+0.12	+0.61
– 2016	51	+0.06	+0.14	-0.25	+0.00	+0.20	+0.57
– 2017	139	+0.19	+0.06	+0.06	+0.41	+0.07	+1.44
– 2018	296	+0.09	+0.01	+0.22	+0.36	+0.08	+1.40
– 2019	540	−0.02	-0.03	-0.04	+0.03	+0.03	+0.00
– 2020	597	−0.21	−0.18	−0.05	−0.24	−0.28	−2.06
– 2021	228	−0.24	−0.16	+0.02	−0.36	−0.22	−1.97

Table 5. *Cont.*

Factors	N	Muscularity Traits					
		MSS (Score)	MSB (Score)	MST (Score)	RST (Score)	LTS (Score)	OMP (%)
Sex of calves							
– male	688	+0.47	+0.44	+0.35	+0.41	+0.47	+4.34
– female	1358	−0.47	−0.44	−0.35	−0.41	−0.47	−4.34
F94L							
– noncarrier	1933	+0.03	+0.06	−0.01	+0.04	+0.07	+0.43
– heterozygous	113	−0.03	−0.06	+0.01	−0.04	−0.07	−0.43
Q204X							
– noncarrier	1791	−0.06	−0.07	−0.07	−0.01	−0.07	−0.60
– heterozygous	255	+0.06	+0.07	+0.07	+0.01	+0.07	+0.60
nt267							
– noncarrier	1981	−0.04	−0.02	−0.11	−0.03	−0.04	−0.46
– heterozygous	65	+0.04	+0.02	+0.11	+0.03	+0.04	+0.46
nt324							
– noncarrier	1607	−0.11	−0.04	+0.00	+0.02	+0.00	−0.23
– heterozygous	409	−0.07	−0.01	−0.06	+0.05	−0.04	−0.29
– homozygous	30	+0.17	+0.05	+0.06	−0.06	+0.04	+0.52
nt414							
– non carrier	1062	−0.06	−0.03	−0.03	−0.05	+0.00	−0.27
– heterozygous	825	+0.03	+0.03	−0.03	−0.01	−0.02	−0.01
– homozygous	159	+0.03	+0.00	+0.05	+0.06	+0.01	+0.28

MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage.

Regarding CWW, the calves carrying the *Q204X* allele in the heterozygous form in the studied population gained 8.56 kg more weight than their counterparts not carrying the allele. From the point of view of the *F94L* allele, there was a difference of 4.08 kg in favor of the heterozygous individuals, but this difference could not be verified statistically. The weight of the individuals carrying the *nt324* and *nt414* alleles in the homozygous form was higher (10.43 kg and 2.92 kg, respectively) than the noncarriers, but these differences were not significant either.

Regarding the muscularity scores, it could be established that calves carrying the *Q204X* allele in the heterozygous form achieved approximately a 0.14 point higher MSB, MST and LTS, and a 1.2% higher OMP than their noncarrying partners. Despite the fact that the *F94L* allele had no statistically verifiable effect on muscularity parameters, it was striking that noncarrier calves showed higher values in almost all muscularity scores than heterozygous carriers. In the case of the *nt267* allele, the muscularity score of the heterozygous calves was higher—although not significantly—than that of the noncarrier individuals, and in the case of the *nt324* and *nt414* alleles even more so in the homozygous carriers.

In the case of all traits, we observed considerable differences between the individuals born in different years. This was also supported by the results of the phenotypic trend calculation (Table 6), according to which six of the nine examined traits were statistically reliable ($p < 0.05$ and $p < 0.01$), and fairly well matched ($R^2 = 0.57-0.93$) regression functions were obtained. In the case of BIW and CWW, the slope of the straight lines (b) was in a positive increasing direction, while in the case of the other traits it was in a negative

decreasing direction. Here it must be noted that, in the case of muscularity parameters, the annual decrease is very small, typically −0.05 or −0.07 points/year.

Table 6. The phenotypic trend of the estimated traits.

Traits	Slope (bX)			Intercept (a)			Fitting	
	b	SE	*p*	a	SE	*p*	R^2	*p*
BIW (kg)	+0.54	0.20	<0.05	−1042.52	4407.67	<0.05	0.59	<0.05
CAE (score)	−0.01	0.02	NS	29.82	31.44	NS	0.14	NS
CWW (kg)	+3.18	0.44	<0.01	−6146.81	885.23	<0.01	0.91	<0.01
MSS (score)	−0.06	0.02	<0.05	134.90	38.18	<0.05	0.70	<0.05
MSB (score)	−0.06	0.01	<0.01	122.69	14.19	<0.01	0.93	<0.01
MST (score)	+0.01	0.03	NS	−16.01	59.19	NS	0.03	NS
RST (score)	−0.05	0.06	NS	103.80	115.26	NS	0.13	NS
LTS (score)	−0.07	0.02	<0.05	150.65	36.77	<0.01	0.76	<0.05
OMP (%)	−0.51	0.20	<0.05	1077.82	401.49	<0.05	0.57	<0.05

BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight; MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage.

Based on the obtained phenotypic correlation values (Table 7), it could be established that the calving and weaning traits did not show a close relationship with each other or with the muscularity traits (r = 0.00−0.24). On the other hand, there was a close (r = 0.61−0.92) and statistically reliable ($p < 0.01$) correlation between the muscularity scores.

Table 7. Phenotypic correlation values between the estimated traits.

r	CAE	CWW	MSS	MSB	MST	RST	LTS	OMP
BIW	* 0.13	* 0.24	* 0.13	* 0.15	* 0.08	* 0.13	* 0.13	* 0.14
CAE		0.00	* 0.09	* 0.09	0.04	* 0.08	* 0.09	* 0.09
CWW			* 0.21	* 0.20	* 0.17	* 0.24	* 0.21	* 0.24
MSS				* 0.86	* 0.61	* 0.68	* 0.80	* 0.90
MSB					* 0.63	* 0.66	* 0.82	* 0.91
MST						* 0.67	* 0.62	* 0.79
RST							* 0.65	* 0.82
LTS								* 0.92

* $p < 0.01$; BIW = birth weight; CAE = calving ease; CWW = 205-day weaning weight; MSS = muscle score of shoulder; MSB = muscle score of back; MST = muscle score of thigh; RST = roundness score of thigh; LTS = loin thickness score; OMP = overall muscle development percentage.

4. Discussion

The *myostatin* gene (*MSTN*) or sometimes called growth and differentiation factor 8 (*GDF8*) is a major negative regulator of skeletal muscle mass and differentiation, but *MSTN* also exists in smooth muscles [26]. In addition to muscle tissue, the influence of the *MSTN* on bone development has been established [27]. Moreover, *MSTN* causes a variety of metabolic changes affecting glucose and lipid metabolism and total bile acid content [28], as well as resulting in changes in semen characteristics [29]. An association was observed between the mutation in *MSTN* and susceptibility to a skin disease [30].

It is well known that there are several mutations in the coding region that have been detected as disruptive mutations (deletions, insertions and nucleotide substitutions) and they are thought to inhibit the function of the *MSTN* protein and are strongly associated with the double-muscling phenotype [22,31].

The *F94L* allele, a missense variant, was characterized by the substitution of cytosine by adenine at the nucleotide position of 282 in exon 1, which led to causing substitutions of leucine (Leu) for phenylalanine (Phe) at the 94th amino acid in the *MSTN* gene. Interestingly, the *F94L* mutation was not considered to cause a loss of *MSTN* function, which led an intermediate muscling in Charolais cattle [22].

The *Q204X* allele of this gene is a disruptive variant, and heterozygous carriers in the Charolais population had a greater mean carcass weight and conformation estimated breeding values (EBVs) [32], but this allele also caused calving difficulties and fertility problems [33].

We are interested in further three silent mutations, i.e., the polymorphisms of the *myostatin* gene caused by the *nt267*, *nt324* and *nt414 MSTN* mutations.

In our study, during the evaluation of the effect of *myostatin* alleles, *Q204X* was statistically proved to have an effect on the 205-day weaning weight, muscle score of back, muscle score of tight, loin thickness score and muscle development percentage. Calves carrying the *Q204X* allele in the heterozygous form in the studied population were heavier than those not carrying this allele. However, animals carrying the *F94L* allele in the heterozygous form were also heavier, but the difference was not significant. The weaning weights of calves carrying the *nt324* and *nt414* alleles in the homozygous form were higher than the noncarriers, but these differences were not significant either.

Similar to the results of our work, several previous sources [8,21,34] contain information on the statistically verifiable effect of the *Q204X* allele on meat production related traits. Contrary to our results, several previous studies [6,16] found the effect of the F94L allele to be significant on some muscularity-related parameters. Among the alleles belonging to the "small" *myostatin* group, we only found information on the effect of the double-muscled related allele *nt821* in existing sources [31,35,36]; however, this allele did not have an effect in the tested Charolais stock. The genetic structure of the *nt267*, *nt324* and *nt414* alleles was previously described by Dunner et al. [21], but no literature data were found on their effect on the phenotypic results.

The results of our work are similar to the findings of Casas et al. [12], according to which *myostatin* alleles in heterozygous form can have a favorable effect on weaning traits. Contrary to the results of Allais et al. [8], we could not detect the effect of the *Q204X* allele on birth weight in the examined Charolais herd. Similar to the results of Esmailizadeh et al. [22], the effect of the *F94L* allele on birth and weaning traits was not found to be significant. Our results are in line with Zhao et al.'s [29] findings: the *MSTN*-gene-edited Chinese Yellow cattle had improved growth traits compared with wild-type counterparts; however, the birth weight yielded no significant difference among groups, but, with increasing month age, the weight gain rate of *MSTN*-gene-edited cattle was significantly higher.

In this study, the weaning weight of Charolais calves were similar to the data found in most of the relevant literary sources [37–39].

The *MSTN* polymorphisms have negative effects on their reproductive traits, for example, calving difficulties (dystocia) [27]. First, Arthur et al. [40] studied Charolais cross animals and reported a higher incidence of dystocia, which was associated with phenotypically muscular calves. Moreover, the height, width and area of pelvic opening in homozygous dams were significantly smaller compared with normal dams. As previously established [41], Charolais heterozygous calves were slightly heavier at birth, with no association with calving ease.

On the basis of the calving ease score observed during our work, it seems that there was fewer difficult calving in the studied herd than what was found in the literature [42,43] in the case of the Charolais breed. It can be explained by the fact that our calves, heterozygous for the double-muscle gene, are superior to normal cattle in terms of meat production traits and do not have calving problems.

We found very little information available in the literature about the conformation of Charolais calves related to their muscularity. Arango et al. [44] and Vallée et al. [45] published data on purebred and crossbred Charolais herds, but, due to the different methodology, we did not have the opportunity to compare them with our results.

A better muscular conformation in heterozygote (*E291X* variant) carcasses of Marchigiana bulls [10] reflected our statement about the muscularity score of the heterozygous calves in the cases of the *nt267*, *nt324* and *nt414* alleles. As previously stated by Ceccobelli

et al. [10], the greater muscularity of heterozygous animals compared with normal ones could be a starting point to improving productive efficiency in beef cattle. Regarding these *MSTN* gene polymorphisms in Charolais cattle, to our knowledge, no such data exist in the literature.

It seems that *MSTN* calves had significant improvement in muscularity traits, as previously described in *MSTN*-gene-edited cattle [29]. Recently, Gaina and Amalo [46] found two SNPs (*c424* and *c467*) of the *MSTN* gene in (Bos indicus) cattle, which are associated with phenotypes of wither height, heart girth and hip height, but not with body weight or body length.

The differences by birth year and sex of calves in weaning weight obtained during our work are very well known in the literature [47,48]. However, we did not find any data for this kind of evaluation of the muscularity parameters of Charolais calves.

Similar to our results, Gutiérrez et al. [49] and Chud et al. [50] did not find a close correlation between BIW, CAE and CWW traits in the case of the Asturiana de los Valles breed of cattle, and in the case of the Nellore breed.

5. Conclusions

Since *Q204X* had the greatest effect on calving, weaning and muscularity-related traits, we think it would be advisable to pay attention to this allele in the breeding strategy, to increase the proportion of carriers from generation to generation. It would be advisable to repeat this test periodically, because, based on literature data too, it seems that the allele in its homozygous form could cause calving difficulties.

Based on the results, the favorable effect of the *F94L* allele was not detectable in our study, contrary to some literary reports, which could be a consequence of the proportion of animals carrying the allele (about 5.5%) being very small in the studied population. On the other hand, based on previous studies, the better phenotypic performance of individuals carrying the allele was more evident in the fattening and slaughter traits.

The proportion of calves carrying the *nt324* and *nt414* alleles was quite high (21.5% and 48.1%, respectively) in the examined Charolais population. However, in the literature, there was very little information about their effect on phenotypic performance. Based on our results, it seems that homozygous carrier individuals may have better growth performance-related traits than noncarrier individuals. Therefore, it would be advisable to pay more attention to this allele.

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Relationship between Beef Quality and Bull Breed

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Simple Summary: The aim of this study was to determine and compare the nutritional value of meat from the four most popular cattle breeds in Poland. A study comparing the nutritional value and quality of beef from the Polish Holstein-Friesian (PHF) dairy breed with that from the Limousine (LM), Hereford (HH) and Charolaise (CH) beef breeds found that beef from beef breeds had higher levels of total antioxidant status (TAS) and degree of antioxidant potential (DAP) than PHF beef. The LM breed had the highest concentration of DAP, anserine, taurine, and creatine, while CH had the highest levels of TAS, carnosine, and coenzyme Q10. In addition, LM, CH, and HH had significantly higher levels of C18:2 *cis*-9, *trans*-11. The breed significantly influences the antioxidant potential of beef.

Abstract: The beef industry in Poland heavily relies on the Polish Holstein-Friesian (PHF) breed, known for its primary use in dairy production, but which also contributes significantly to the beef supply. In contrast, the Limousine (LM), Hereford (HH), and Charolaise (CH) breeds have gained popularity due to their ideal specialized characteristics for beef production. As PHF continues to dominate the beef market, a thorough comparison of its beef quality and nutritional attributes with the three most popular beef breeds in Poland is essential. This study aims to address this knowledge gap by conducting a rigorous comparison. The experiment was carried out on the beef from 67 bulls kept in a free-stall system with standardized feeding. The highest total antioxidant status (TAS) was found in CH and was 147.5% higher than that in PHF. Also, compared with PHF, a large difference of 70% was observed in LM, while in HH it was only 6.25%. For degree of antioxidant potential (DAP), the highest concentration was found in LM, while CH had a slightly lower score than LM. PHF had the lowest scores for each of the analyzed parameters of protein fraction. For anserine, taurine, creatinine, and creatine content, the highest results were found for LM. For carnosine and coenzyme Q10, the highest values were found for CH. Overall, these results highlight the impact of maturity and breed on carcass composition and quality. Late-maturing breeds, such as LM and CH, tend to exhibit leaner carcasses with superior fatty acid profiles and antioxidant properties. This knowledge is valuable for producers, enabling them to make informed decisions regarding breed selection and production strategies to meet specific market demands for beef with the desired composition and quality.

Keywords: cattle; beef; bioactive components; fatty acid profiles; antioxidants

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1. Introduction

Cattle production in Poland is focused on milk production [1]. This means that breeding work carried out over the years has focused on improving animals for milk, especially in the Polish Holstein-Friesian (PHF) breed, and has ignored issues to do with the meat quality of these animals; this has been influenced by the lack of tradition related to beef consumption [2]. According to Statistics Poland (GUS), in 2021 there were 6,378,742 head of cattle, including 2,289,025 cows [3]. The number of beef cows under performance

evaluation was determined by the Polish Federation of Cattle Breeders and Dairy Farmers (PZHiPBM), and on 31 December 2021 this figure was 21,840 head of cattle [4]. It was established that the average number of cows per farm was 21.8 head, and the number of herds registered with the PZHiPBM was 1147. The Polish Federation of Cattle Breeders and Dairy Farmers reported that there were 704,506 head of PHFs under dairy performance evaluation in 2021, which accounted for 88.76% of all dairy cows under evaluation in Poland and 11.04% of the total number of cattle [5]. The number of herds containing cows under performance evaluation in 2021 was 18,559. Thus, the proportion of beef cattle in Poland is dramatically low compared with the dominant dairy cattle population. This is due to milk production intensification, which results in dairy breeds dominating in beef production. In 2021, 1,866,484 bovine animals were slaughtered in Poland, including 52,053 calves, 310,881 heifers, 555,220 cows, and 948,330 bullocks and bulls [3], resulting in 553,706 tons in carcasses post-slaughter warm weight. Of the total amount of cattle in the country, 93% are dairy, so special attention should be paid to the quality and nutrient content indicators of the meat being produced, and crossbreeding should be considered as a way to improve these indicators [6]. Based on data since 2012, changes in the population of the most popular breeds have been observed. The changes in the Polish Holstein-Friesian (PHF), Limousine (LM), Charolaise (CH), and Hereford (HH) populations are shown in Table 1.

Table 1. Population changes of PHF, LM, CH, and HH [4].

	No. of Heads in 2012	No. of Heads in 2021	Population Change
PHF	597,715	704,506	106,791 (+17.86%)
Limousine	11,879	13,948	+2069 (+17.4%)
Charolaise	2265	1571	−694 (−30.6%)
Hereford	743	1409	+666 (+89.6%)

The characteristics of cattle production in Poland during the 2012–2021 period did not change significantly. Dairy production continued to be the dominant production sector. During this period, an increase of 17.86% in the country's PHF herd, 17.4% in the LM herd, and 89.6% in the HH herd was observed, while the CH herd decreased by 30.6%, which is related to the higher price prosperity obtained for LM. The increases in the LM and HH herds are too low to be able to compensate for PHF beef production, which is the main contributor, so it is important to analyze and compare these breeds to determine their quality and health-promoting differences.

In order to study the influence of genotype on the formation of meat quality and its nutritional value, this study included bulls from the dominant dairy breed in Poland, the Polish Holstein-Friesian, and the three most popular beef cattle breeds, Limousine, Charolaise, and Hereford. The selection of breeds was made on the basis of available data on the proportion of breeds within the structure of beef production in Poland. The PHF breed is characterized by high milk yield, but in meat production it is characterized by poorer feed utilization and higher collagen content in muscles, which negatively affects consumption quality. Dairy cattle account for 93% of cattle heads, while the remaining 7% are made up of other cattle, including beef breeds, of which there are 15 registered in Poland. Of the beef breeds, about 70% are LM, 12% are CH, and 6% are HH. The cattle breeds used in the study make it possible to determine the quality of the meat produced in the country and to observe any differences due to the course of breeding work in Poland.

Beef is of worldwide importance as a source of protein fraction bioactive components such as anserine, carnosine, taurine, coenzyme Q10, creatine and creatinine, polyunsaturated fatty acids (FAs), fat-soluble vitamins, and of high-biological-value protein. All these

elements have beneficial effects on human health [6]. Anserine and carnosine demonstrate antioxidant activity and inhibit the formation of carbonyl groups of proteins, which, by their actions, can prevent many diseases, including Alzheimer's disease, atherosclerosis, and diabetes [7]. They also exhibit chelating and anti-glycation effects [8]. In the case the ω-3 family of fatty acids, C18:3 n-3, C20:5 n-3, C22:6 n-3, and C18:2 n-6, as well as C18:2 *cis*-9 *trans*-11, have anti-carcinogenic properties and antioxidant properties that help build a balance between oxidants and antioxidants. Fat-soluble vitamins also reduce the effects of free radicals [9]. β-Carotene and α-retinol affect the differentiation of epithelial cells in the gastrointestinal tract, urinary tract, respiratory tract, and organs for vision, and are essential for the biosynthesis of fat from sugars, as well as for catalyzing the oxidation of unsaturated fatty acids. α-Tocopherol has strong antioxidant properties and protects the body from degenerative diseases [9].

The production of high-quality culinary beef is still rare on Polish farms. Crossbreeding with beef breeds could be used to improve quality parameters and nutritional value, which would improve meat quality over a relatively short period of time, as well as feed conversion and growth rates [10]. Testing the concentrations of valuable nutrients will allow the necessary directions for animal breeding to be determined. Improving the analyzed parameters in the case of the PHF breed will benefit consumer health, improve meat quality through increased proportions of antioxidant substances [11], reduce the risk of animal diseases, and improve the export value of the raw material [12]. The aim of this study was to compare the beef quality and nutritional value of the dominant dairy breed and the three most popular beef breeds in Poland. The Polish Holstein-Friesian (PHF) breed is the main source of beef produced in the country. Limousine (LM), Hereford (HH), and Charolaise (CH) are the most popular beef breeds in Poland. A comparison of the four breeds in terms of beef quality will answer whether this is a good solution, especially when it comes to the formation of the level of bioactive components in muscle tissues. The PHF breed, which is the main source of the beef produced in Poland, was taken as the reference. The aim of this study was to determine and compare the nutritional value of meat from the four most popular cattle breeds in Poland

2. Materials and Methods

The experiment was conducted on 67 bulls from four breeds: Limousine, Hereford, Charolaise, and Polish Holstein-Friesian (PHF). Live weight and daily gain parameters were standardized at 605 days of age (Table 2).

Table 2. Bull characteristics on the day of slaughter (standardized at 605 days).

	Number (*n*)	Standardized Live Weight (kg)	Carcass Weight (kg)	Standardized Daily Gains (kg)	Daily Carcass Gains (kg)
PHF	16	536	317	0.82	0.53
Limousine	18	694	413	1.08	0.68
Charolaise	17	689	416	1.06	0.67
Hereford	16	669	390	1.04	0.66
p-value		*0.001*	*0.001*	*0.001*	*0.001*
SEM		4.251	8.213	0.054	0.012

The characteristics of the feed characteristics are shown in Table 3. The bulls were kept in a free-stall system in accordance with the minimum standards for the maintenance of cattle (Journal of Laws No. 167/position 1629 of 2003, as amended).

Table 3. Characteristics of the research material.

Composition	Value
Maize silage (%)	68
Barley (%)	29
Supplements (%)	3
Nutritional value	
Dry matter (%)	54
Protein (g/kg)	128
NEm (Mcal/kg)	1.77
Neg (Mcal/kg)	1.15
NDF (g/kg)	343
ADF (g/kg)	194
Crude fat (g/kg)	19

The animals were slaughtered at 21–24 months of age, and the carcass weight was recorded before slaughter (selected groups in the same day). The carcasses were then cooled for 24 h at 2–4 °C, after which 300 g of semimembranosus muscles was sampled parallel to the muscle axis. During the fattening period, all the bulls received the same TMR ration, *ad libitum*, balanced according to National Research Council recommendations for beef cattle.

2.1. Analytical Methods

Beef samples were chopped, then placed in a blender and ground until homogeneous. These was later analyzed using a near-infrared spectrophotometer. The basic chemical composition of the meat was determined using a Food Scan™ analyzer.

Meat fat extraction was performed using the Folch method [13]. Fatty acid methylation was performed according to the EN ISO 5509 [14] transesterification method. The functional fatty acid content was determined using an Agilent 7890A GC gas chromatograph and a Varian Select FAME column according to Solarczyk et al. [6].

The measurement of the fat-soluble vitamin content was performed using an Agilent 1100 RP-HPLC instrument and a ZORBAX Eclipse XDB column according to the methodology of Puppel et al. [15].

The measurement of the bioactive component of the protein fraction content was performed using an RP-HPLC Agilent 1100 instrument and a Jupiter 5u C18 300A column according to the methodology of Łukasiewicz et al. [8].

The determination of MDA (malondialdehyde) was carried out using a Tecan NanoQuant Infinite M200 PRO analyzer (Tecan Austria GmbH, Grödig, Austria) according to the methodology of Kapusta et al. [16].

The cholesterol determination was achieved using an Agilent 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) and a BP-5 column according to the methodology of Kapusta et al. [16].

DAP (degree of antioxidant protection) was calculated from the molar ratio between antioxidants and oxidants according to Pizzoferrato et al. [17]:

$$DAP = \frac{\sum_{i=1}^{n} AC_i \left(n^{\circ}\ moles\right)}{OT\ \left(n^{\circ} moles\right)}$$

Total antioxidant potential (TAS) according to RANDOX application.

Incubation of ABTS® with peroxidase (methemoglobin) leads to the formation of radical cation ABTS + +. This substance is blue-green and can be detected at 600 nm.

Antioxidants present in the sample reduce the formation of blue-green color in proportion to their concentration.

$$HX\text{-}Fe^{III} + H_2O_2 \rightarrow X\text{-}[Fe^{IV} = 0] + H_2O$$

$$ABTS^{®} + X\text{-}[Fe^{IV} = 0] \rightarrow ABTS^{®+} + HX\text{-}Fe^{III}$$

where HX-Fe^{III}—metmyoglobin, *X*-[Fe^{IV} = 0], ABTS®—2,2-azino-di [3-ethylbenzothiazolin-osulfonate] (RANDOX materials). U/L defines the concentration of TAS.

2.2. Statistical Analysis

The data obtained were subjected to analysis of variance using ANOVA. The distribution of bioactive components was checked using the Shapiro–Wilk test. All tests were performed using the IBM SPSS 23 (2023) package [18]. Data were presented as least-squares means with standard error of the mean.

The following statistical model was used:

$$Yijk = \mu + Ai + eij$$

where *Yijk*—value of the tested trait; μ—mean; A*i*—effect of the *i*-th breed (*i* = 1–4); *eij*—standard error.

3. Results

For the most part, the results in Table 4 show significant differences between the protein, crude fat, and collagen content of muscle. The highest proportion of protein was determined for the LM breed, and was 22.32% higher than that of the PHF breed, which had the lowest protein content. Protein is the most valuable nutrient, so the highest possible results should be expected in resource-consuming meat production.

Table 4. The effect of breed on the formation of the basic chemical composition in *Semimembranosus muscles*. In parentheses are % of variation in relation to PHF. Means (in column) marked with the same letters differ significantly at: lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$.

	Protein [g/100 g]	Crude Fat [g/100 g]	Collagen [mg/100 g]
PHF	19.40 ^{A,B,C}	2.95 ^{A,B}	592.24 ^{A,B,C}
Limousine	23.73 ^{A,d} (+22.32%)	2.16 ^{A,C} (−26.78%)	549.84 ^{A,d} (−7.16%)
Charolaise	22.05 ^{B} (+13.66%)	2.26 ^{B,D} (−23.39%)	542.52 ^{B,e,} (−8.40%)
Hereford	21.21 ^{C,d} (+9.33%)	3.01 ^{C,D} (+2.04%)	552.62 ^{C,d,e} (−6.69%)
SEM	1.236	0.441	7.625

The fatty acid profile showed significantly better results for LM, CH, and HH compared with PHF in the C18:2 *cis*-9, *trans*-11 group, which is a potent antioxidant and may have anti-carcinogenic effects (Table 5). The results of the fatty acid profile were higher for HH (40.15%) and CH (64.86%). These are important differences that should be taken into consideration in consumer decisions for their health-promoting properties [19]. The greatest differences in the analyzed acids were observed in the C22:6 and C18:1 content in the CH group compared with PHF, with differences of 100% and 96.39%, respectively. Fatty acid content in the PHF breed was no higher compared to the rest. These results indicate that beef breeds have a very significant advantage over dairy breeds.

Table 5. The effect of breed on the formation of functional fatty acid levels in *Semimembranosus muscles*. In parentheses are % of variation in relation to PHF. Means (in column) marked with the same letters differ significantly at: lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$.

[g/100 g]	C18:1 *trans*-11	C18:2 n-6	C18:2 *cis*-9, *trans*-11	C18:3 n-3	C20:5 n-3	C22:6 n-3
PHF	0.83 ^{A,B,C}	8.24 ^{A,B,C}	2.59 ^{A,B,C}	0.49 ^{A,B,C}	0.42 ^{A,B,C}	0.07 ^{A,B,C}
Limousine	1.39 ^{A,D,E} (+67.45%)	12.19 ^{A,d,E} (+47.94%)	4.03 ^{A,d,E} (+55.60%)	0.74 ^{A,D} (+51.2%)	0.71 ^{A,D} (+69.05%)	0.11 ^{A,d} (+57.14%)
Charolaise	1.63 ^{B,D,F} (+96.39%)	13.45 ^{B,d,F} (+63.23%)	4.27 ^{B,d,F} (+64.86%)	0.71 ^{B,E} (+44.90%)	0.74 ^{B,E} (+76.19%)	0.14 ^{B,d,E} (+100%)
Hereford	1.26 ^{C,E,F} (+51.80%)	10.38 ^{C,E,F} (+25.97%)	3.63 ^{C,E,F} (+40.15%)	0.66 ^{C,D,E} (+34.70%)	0.65 ^{C,D,E} (+54.76%)	0.09 ^{C,D,E} (+28.57%)
SEM	0.233	0.478	0.119	0.017	0.041	0.011

β-Carotene and α-retinol, which function as vitamin A, showed high variability across breeds (Table 6). The lowest results were observed for PHF in each group. The smallest difference was found between HH and PHF for α-retinol, with a variation of 3.03%, and the highest was between LM and PHF for β-carotene, at 80%. Large differences were observed in the α-tocopherol group, which is a type of vitamin E with strong antioxidant properties. CH had a 93.17% higher result relative to PHF, while LM showed the smallest difference, but still high, at 72.67% relative to PHF (Table 6). In most of the analyzed cases, the differences were significant and reached dozens of percentage points, and in some cases exceeded 90%, which indicates the significantly higher amounts of vitamin content in the meat of beef breeds compared with that of the dairy PHF breed.

Table 6. The effect of breed on the formation of fat-soluble vitamin levels in *Semimembranosus muscles*. In parentheses are % of variation in relation to PHF. Means (in column) marked with the same letters differ significantly at: lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$.

[μg/g]	β-Carotene	α-Retinol	α-Tocopherol
PHF	0.20 ^{A,B,C}	0.66 ^{A,B,C}	1.61 ^{A,B,C}
Limousine	0.36 ^{A,D} (+80%)	0.81 ^{A,D} (+22.73%)	2.78 ^{A,D,E} (+72.67%)
Charolaise	0.33 ^{B,E} (+65%)	0.79 ^{B,E} (+19.70%)	3.11 ^{B,D,f} (+93.17%)
Hereford	0.21 ^{C,D,E} (+5%)	0.68 ^{C,D,E} (+3.03%)	3.08 ^{C,E,f} (+91.30%)
SEM	0.012	0.078	0.113

The highest total antioxidant status (TAS) was found in CH and was 147.5% higher than PHF. This is a very large difference, and indicates that the meat of this breed has significantly greater antioxidant properties compared with that of the other breeds (Table 7). Also, compared with PHF, a large difference of 70% was observed in LM, while in HH it was only 6.25%. For the degree of antioxidant potential (DAP), the highest concentration was found in LM, while CH had a slightly lower score than LM. Both the TAS and DAP indexes indicate antioxidant properties, and it is desirable to have them at their highest possible concentrations. In the case of malondialdehyde (MDA), the lower the score, the better. The lowest MDA content was found in CH, with LM being only slightly worse, while in HH the result was significantly worse. For all three analyzed elements, PHF had the worst results, indicating that it had lower quality and lower nutritional value parameters (Table 7).

Table 7. The effect of breed on the formation of TAS, DAP, and MDA levels in *Semimembranosus muscles*. In parentheses are % of variation in relation to PHF. Means (in column) marked with the same letters differ significantly at: lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$.

	TAS [mmol/L]	DAP	MDA [mM/g]
PHF	0.80 ^{A,B,C}	5.12×10^{-3} ^{A,B,C}	3.30 ^{A,B,C}
Limousine	1.36 ^{A,D,E} (+70%)	8.10×10^{-3} ^{A,d,E} (+58.20%)	1.24 ^{A,D} (−62.42%)
Charolaise	1.98 ^{B,D,F} (+147.5%)	7.80×10^{-3} ^{B,d,F} (+52.34%)	1.20 ^{B,E} (−63.64%)
Hereford	0.85 ^{C,E,F} (+6.25%)	6.25×10^{-3} ^{C,E,F} (+22.07%)	2.45 ^{C,D,E} (−25.76%)
SEM	0.011	0.013	0.013

The analysis of the bioactive content of the protein fraction was in line with previous results. The higher the index of each parameter, the higher the nutritional value of the meat. PHF had the lowest scores for each of the analyzed parameters (Table 8). For anserine, taurine, creatinine, and creatine content, the highest results were found for LM. For carnosine and coenzyme Q10, the highest values were found for CH. In the HH group, all analyzed elements were at higher levels than the PHF group, but they were not as high as they were in the LM and CH groups (Table 8).

Table 8. The influence of breed on bioactive protein fraction levels in *Semimembranosus muscles*. In parentheses are % of variation in relation to PHF. Means (in column) marked with the same letters differ significantly at: lowercase letters, $p \leq 0.05$; uppercase letters, $p \leq 0.01$.

[mg/100 g]	Anserine	Carnosine	Taurine	Coenzyme Q10	Creatinine	Creatine
PHF	61.22 ^{A,B,C}	387.30 ^{A,B,C}	34.28 ^{A,B,C}	1.87 ^{A,B,C}	4.12 ^{A,B,C}	396.96 ^{A,B,C}
Limousine	74.08 ^{A,d,E} (+21.00%)	431.53 ^{A,D,E} (+11.42%)	43.49 ^{A,d,E} (+26.87%)	2.33 ^{A,d,e} (+24.60%)	5.61 ^{A,d,E} (+36.17%)	422.66 ^{A,d,E} (+6.47%)
Charolaise	72.52 ^{B,d,F} (+18.46%)	445.36 ^{B,D,F} (+14.99%)	42.14 ^{B,d,F} (+22.92%)	2.54 ^{B,d,F} (+35.82%)	5.44 ^{B,d,F} (+32.04%)	418.22 ^{B,d,F} (+5.36%)
Hereford	69.29 ^{C,E,F} (+13.18%)	419.59 ^{C,E,F} (+8.34%)	37.31 ^{C,E,F} (+8.84%)	2.08 ^{C,e,F} (+11.23%)	4.85 ^{C,E,F} (+17.72%)	411.05 ^{C,E,F} (+3.55%)
SEM	0.752	1.114	0.442	0.022	0.073	0.812

4. Discussion

Long et al. [20] conducted research revealing that optimal slaughter ages and weights vary significantly depending on the rate of maturity, which is characterized by the accumulation of fat during the "finishing" period. The average fattening time has been standardized at 605 days. PHF gained 0.82 kg per day, LM 1.08 kg, CH 1.06, and HH 1.04 kg (Table 2). Sakowski et al. [21], Kayar and İnal [22], and Pogorzelska et al. [23] reported similar trends in fattening rates and fattening results for LM, CH, and HH. Southgate et al. [24] conducted a study comparing breeds slaughtered at the same carcass fat cover. They found that Canadian Holsteins required approximately an additional 65 and 45 days to reach slaughter in a 16-month and 24-month production system, respectively, compared with either British Friesian or Charolais × Friesian steers. The Netto daily gains (daily carcass gains, Table 2) of PHF were 0.53 kg, 0.68 kg for LM, 0.67 kg for CH, and 0.66 for HH; this stays in agreement with McGee et al. [25] and indicates the benefits of CH steers compared with HF steers in fattening. Those results show the lowest daily gains and daily carcass gains for PHF achieved on the same feeding.

The growth ability of cattle is influenced by various factors, including breed, genetic predisposition, nutrition, microclimatic conditions, farm or breeding conditions, and the month of birth [26]. Daily weight gains can be visualized using a growth curve, which exhibits a sigmoid character [27]. Differences in growth ability exist not only among different breeds but also among individuals within the same breed, emphasizing the impact of body frame on slaughter age and carcass weight [28]. The length of the fattening period significantly affects growth parameters and carcass quality. Ustuner et al. [29] confirmed that both the initial weight at the start of fattening and the timing for the end of the fattening period are crucial for the final meat production performance. Carcass weights were 536 kg, 694 kg, 689 kg, and 669 kg for PHF, Limousine, Charolaise, and Herford breeds, respectively (Table 2). The differences in standardized live weight among these breeds can be attributed to various factors, including genetic characteristics and growth rates. Albertí et al. [30] reported that LM and CH breeds had higher carcass yields than Angus and HH. However, HH had the highest slaughter weight. Each breed has its own genetic potential for growth and carcass development, which influences the final carcass weight attained. Abramowicz et al. [31] highlighted that fat accumulation occurs after the relative growth of muscle decreases, while bone growth continues to decrease. This suggests that as animals mature, there is a shift in nutrient allocation towards fat deposition. The growth rate of fatty tissues can vary depending on their location and the stage of growth [32]. This indicates that different fat depots may exhibit different growth patterns and rates. In the study by Berg et al. [33], the carcass composition of seven different beef breeds was compared. The breeds exhibited variations in the muscle, fat, and bone composition of the carcasses. Notably, larger-framed breeds such as Chiannia and Blonde d'Aquataine resulted in carcasses with less fat compared with Danish Red and Hereford at a standard carcass weight. This suggests that breed-specific characteristics play a significant role in determining carcass composition.

Augustini et al. [34] demonstrated that the percentage of carcass meat and the proportion of beef cuts undergo changes during the growth of cattle. As animals mature, each tissue reaches its growth maximum at different stages, resulting in alterations in carcass tissue composition. Irshad et al. [35] highlighted that late-maturing cattle breeds exhibit slower physiological development compared with early-maturing breeds. This slower development is associated with a higher growth potential and slower fat accretion. Late-maturing breeds tend to show a preference for leaner carcasses, as they exhibit faster growth rates and more efficient conversion of high-energy feed into carcass weight. Van der Westhuizen [36] supported the notion that late maturity in cattle leads to increased growth of leaner carcasses. The delayed maturity allows for faster growth rates and improved conversion of feed into carcass weight [6]. This suggests that late-maturing breeds may have advantages in terms of producing leaner beef. The highest proportion of fat was determined for the HH breed, and was 2.04% higher than that of the PHF breed (Table 4). The Limousine breed had the highest average standardized live weight of 694 kg, followed closely by the Charolais breed with 689 kg. These breeds are known for their excellent muscling and growth potential, which may contribute to their higher carcass weights. The Hereford breed had a slightly lower average standardized live weight of 669 kg (Table 4). Collectively, these studies emphasize the dynamic nature of tissue development during cattle growth and the impact of maturity on carcass composition. Kempster et al. [37] highlight that when cattle of diverse breeds are compared at the same age and under similar management conditions, there will be variations in carcass weight. These differences are influenced by various factors, including the breed-specific growth curves and the range of target weights for each breed.

The results of the basic chemical composition, functional fatty acids, bioactive components of the protein fraction, fat-soluble vitamins, and oxidative and antioxidant potential indicate that PHF beef will have a significantly lower palatability and nutritional quality. Concerning fat content, LM and CH had significantly lower percentages (−26.78% and −23.39%, respectively) than the PHF breed. In the realm of cattle genetics and fat parti-

tioning, Kempster et al. [37] conducted a seminal study demonstrating the significance of genetic variation in the distribution of fat between different depots. The findings of this study underscore the role of genetics in shaping fat deposition patterns in cattle. Additionally, Casasús et al. [38] reported that such genetic variation in fat partitioning persists even under similar nutritional conditions, further supporting the influence of genetic factors on fat distribution. Low fat content is characteristic for consumer needs in many developed countries, despite its lower palatability [39]. Only HH had a higher fat content, which was minimal at 2.04%. As the dominant breed in beef production worldwide, HH is characterized by high palatability because of its higher intramuscular fat content. The crude fat content positively influences the juiciness of the meat as intramuscular fat, but not as cover fat around the meat [40,41]. Additionally, Diler et al. [42] reported that muscle and fat type are essential sources of variations in the textural characteristics, sensory panel attributes, and fatty acid profile of meat from Holstein-Friesian bulls. The highest collagen content was found in PHF—in culinary beef production, this indicator should be as low as possible due to its negative effect on meat tenderness [43]. Increased collagen content also negatively affects meat quality [44,45], and significantly higher proportions of it were found in PHF. Compared with the beef breeds HH, CH, and LM, the dominant PHF breed significantly stands out in terms of its lower nutritional value and quality. Meat quality might be improved by crossbreeding with beef breeds [6,46]. This is a solution that will help make progress in shaping the quality of the beef produced in Poland and positively influence its health-promoting properties.

The hormonal and metabolic distinctions between beef cattle and dairy breeds play a crucial role in determining their respective fat deposition tendencies. As elucidated by Kempster et al. [37], beef cattle exhibit a remarkable ability to convert nutrients predominantly into proteins. In contrast, dairy breeds, due to their unique hormonal and metabolic status, tend to deposit more intra-abdominal fat. The influence of breed and feed type shape the basic chemical composition and nutritional value of beef. The positive effect of the fatty acids' improvement has been confirmed in a number of studies concerning the Limousine [47,48], Polish Holstein-Friesian [49,50], Charolaise [51,52], and Hereford breeds [53,54]. The authors of the studies demonstrated there is a variability in the fatty acid profile, indicating a benefit in nutritional value from the use of meat from breeds with better monounsaturated and polyunsaturated FA profiles. However, Sobczuk-Szul et al. [55] reported that intramuscular fat had a higher MUFA concentration (46.2%) than visceral fat (36.7%). Appropriate breed selection decreases the saturated FA content and improves the ratio of n-3 and n-6 fatty acids [21]. Barton et al. [56] indicated similar trends in the basic chemical composition of meat and the FA profile of LM and CH. Gregory et al. [57] pointed out that breed is an important factor in shaping slaughter performance. Comparative studies on the fattening value of PHF, LM, CH, and HH confirmed these reports [58–60]. Additionally, Sargentini et al. [61] and Humada et al. [62] reported that total SFA content did not significantly alter with increasing slaughter age. Breed has a significant effect on the fatty acid profile and how fat is deposited with age due to differences in intramuscular fat [47]. The proportion of unsaturated fatty acids in intramuscular fat increases with age [63,64]. By 18 months of age, meat palatability increases [65], accompanied by changes in soluble collagen structure [66]. The mechanism of these changes is not fully understood [67], but they are observable over the life of the cattle. Cattle slaughtered at the same weight, but of different breeds, can be characterized by different intramuscular fat content, which is deposited after the muscles' growth phase, indicating different rates of breed development [68]. Research by Nürnberg et al. [69] on the dairy German Holstein and Belgian Blue breeds indicate relatively small modifications in the proportions of saturated fatty acids and n-3 between 18 and 25 months of cattle age, which may suggest a limited effect of age on the fatty acid profile after 18 months.

Fat-soluble vitamins are an essential part of the human diet, which can be supplemented with beef that is rich in vitamins E and A. There are a number of properties of vitamins E and A, from protective effects on lipids [70] to improved health in people with

Alzheimer's [71] and cancer [72], but we can still observe deficiencies of those vitamins in human diets [73]. It seems reasonable to raise vitamin E levels in animals to benefit both animal welfare and the diets of meat consumers. The results in Table 6 show that breed has a significant effect on the formation of levels of β-carotene, α-retinol, and α-tocopherol, which may suggest the need for further research on the concentrations of these vitamins in beef meat. The results obtained in this study indicate that there are significantly higher concentrations of these vitamins in LM and CH relative to PHF, and, in the case of HH, that α-tocopherol was clearly higher than in PHF. This allows us to conclude that the LM and CH breeds are significantly more nutritious than PHF. In addition, vitamin ratios can be further improved by pasture grazing, which beef breeds are predisposed to utilize effectively [74]. The effect of age on vitamin E content is described in the study by Warren et al. [75]. In the case of Holstein-Friesian and Angus breeds, the differences in concentration of animals uniformly fed vitamin E concentrations on the example of Longissimus muscle at 14, 19, and 24 months were 1.3, 1.4, and 1.6 μg/g, respectively.

TAS results for LM and CH were characterized by high values; however, CH had the highest score. For DAP, the results for LM and CH were similar. Skaperda et al. [76] indicated increased antioxidant potential, which can be further increased by utilizing pasture grazing [77]. In contrast to TAS and DAP, where higher values benefit animal health, MDA content should be as low as possible [78]. In this case, LM, CH, and HH showed significantly lower values than PHF. Elevated MDA levels can negatively affect beef quality [79], so nutritional supplements containing selenium are used to improve this situation [79,80].

Anserine β-Alanyl-3-methyl-L-histidine is a dipeptide, a methyl-carnosine derivative. It consists of β-alanine and L-(*N*-methyl) histidine. With carnosine, it exhibits antioxidant activity, and high concentrations are found in skeletal muscle. The concentration is influenced by breed, sex, age, environment, and type of muscle [8]. Carnosine decreases MDA concentrations [81], reduces cellular aging processes [82], and can inhibit metmyoglobin formation [83]. The largest difference relative to PHF was found for anserine in LM and was 21%, while the smallest difference was between PHF and HH for carnosine and was 8.34% in favor of HH. The study by Watanabe et al. [84] shows that between 15 and 25 months of age there can be significant changes in the content of anserine and taurine in the *Longissimus dorsi muscle*. Such a relationship was not observed for carnosine. However, the authors indicate a significant effect of breed on each of the three and a variation in concentration in individual carcass elements [85].

Taurine is a 2-aminoethylsulfonic acid and is responsible for maintaining adequate leukocyte levels; its lack negatively affects the ability of neutrophils to oxygen burst and carry out phagocytosis. It also reduces the effects of oxidative stress and pro-inflammatory changes [86], and is involved in free radical neutralization, membrane stabilization, the formation of bile acid salts, and the maintenance of calcium homeostasis [87]. In this study, taurine was shown to have increased concentrations in all the beef breeds relative to PHF, reaching as high as 26.87% in LM. Taurine is also important for reducing nervous tension and improving mental performance by increasing glial cell metabolism [88]. It also has lipids, protective properties [89], and extends the shelf-life of beef [90].

Meat is a rich source of coenzyme Q10 [91]. It is an important component in the oxidative phosphorylation process. It converts energy from carbohydrates and fatty acids into ATP [92] and is part of the mitochondrial electron transport chain [93]. A lack of coenzyme Q10 results in the insufficient production of high-energy compounds [94]. A reduced form of coenzyme Q10 called ubiquinone is able to renew vitamin E [95]. The largest differences, when compared with PHF, were found in CH (35.82%), while in LM the variation was 24.60%, and that in HH was 11.23%.

Creatinine is a creatine derivative formed during metabolic processes as an endogenous metabolite of the non-enzymatic breakdown of creatine phosphate. Creatinine is used to store and transfer energy in muscle cells and tendons. It is synthesized in the body; however, it must also be partially supplied via the human diet from food [96,97]. All the

beef breeds in the study contained higher concentrations of creatinine relative to PHF: 36.17% for LM, 32.04% for CH, and 17.72% for HH.

Creatine is synthesized from arginine, methionine, and glycine [98]. It is responsible for providing the energy in skeletal muscles, especially muscles characterized by a high energy transfer to ADP in muscle cells [99]. Creatine is converted to creatinine through the non-enzymatic processes of creatine phosphate breakdown. Its concentration in the samples showed less variation across all the breeds than did creatinine. It was higher than PHF by only 6.47% for LM, 5.36% for CH, and 3.55% for HH.

The quality of beef is significantly influenced by functional type and breed. Beef production in Poland is based on dairy or dual-purpose cattle herds. Intensive feeding and extended finishing time can favorably affect the yield and quality of beef produced. It is important to increase the intensity and method of feeding in the final fattening stage [100]. The high growth potential of the animal at a young age is inhibited when the animal reaches somatic maturity [101]. Late-maturing animals are effective for intensive fattening. The growth phase, where most fat is deposited, is postponed, so they can be fattened to high body weights without a decrease in carcass quality. Tissue development occurs through the expansion of the skeleton, followed by muscle and fat in the last stage. Age affects nutritional value, so slaughtering rules have been adopted to help maintain quality beef [102]. All animals were fed the same diet and slaughtered at around 20 months of age, which allowed the results to be used for comparison after standardization due to the effect of age on shaping beef quality and composition. From birth to physical maturity, the rate of muscle growth is higher than the rate of fat deposition and influence on fatty acid composition [103]. The effect of age on the development of the fatty acid profile is important and should be taken into account during the current work on beef quality. It also affects the vitamin content of the meat [58] and is an important element in the formation of beef quality [21]. This also affects the bioactive protein fraction [84]. Therefore, it is important to take into account the age of the animals in comparisons for different breeds and feeding systems. The age of the animal is an important factor shaping meat quality. With age, the fat content and size of fat cells change [69,104].

Each cattle breed possesses unique genetic characteristics that influence their growth potential and carcass development. As cattle mature, there is a shift in nutrient allocation, with a decrease in the relative growth of muscle and bone and an increase in fat accumulation. This phenomenon implies that as animals reach their mature stages, they tend to deposit more fat, contributing to the final carcass weight attained. The allocation of nutrients towards fat deposition during the finishing period is essential for meat production and quality. The balance between muscle and fat development can impact the meat's marbling, tenderness, and flavor. Understanding breed-specific growth patterns and nutrient partitioning is crucial for the effective management and optimization of cattle production.

5. Conclusions

Overall, these results highlight the impact of maturity and breed on carcass composition and quality. Late-maturing breeds, such as LM and CH, tend to exhibit leaner carcasses with superior fatty acid profiles and antioxidant properties. The fatty acid profile showed significantly better results for LM and CH compared with HH and PHF in the C18:2 *cis*-9, *trans*-11 group, which is a potent antioxidant and may have anti-carcinogenic effects. Large differences were observed in the α-tocopherol group, which is a type of vitamin E with strong antioxidant properties. CH had a 93.17% higher result relative to PHF, while LM showed the smallest difference, but still high, at 72.67% relative to PHF. The highest TAS was found in CH and was 147.5% higher than PHF. Also, compared with PHF, a large difference of 70% was observed in LM, while in HH it was only 6.25%. For DAP, the highest concentration was found in LM, while CH had a slightly lower score than LM. Both the TAS and DAP indexes indicate antioxidant properties, and it is desirable to have them at their highest possible concentrations. The lowest MDA content was found in CH, with LM being only slightly worse, while in HH the result was significantly worse. PHF had the

lowest scores for each of the analyzed parameters of protein fraction. For anserine, taurine, creatinine, and creatine content, the highest results were found for LM. For carnosine and coenzyme Q10, the highest values were found for CH. In the HH group, all analyzed elements were at higher levels than the PHF group, but they were not as high as they were in the LM and CH groups.

Collectively, these studies emphasize the dynamic nature of tissue development during cattle growth and the impact of maturity on carcass composition. Late-maturing breeds exhibit a different growth pattern, with slower fat deposition and a preference for leaner carcasses. Carcass composition is a result of the interplay between genetic factors, growth patterns, and management practices. Different cattle breeds exhibit distinct growth trajectories and rates of fat deposition, which ultimately affect the composition of their carcasses. Additionally, the target weights set for each breed based on market preferences and production goals also play a role in determining the composition of the final carcass. This knowledge has implications for breed selection, production strategies, and meeting specific market demands for beef with desired composition and quality.

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Article

The Oxidative and Color Stability of Beef from Steers Fed Pasture or Concentrate during Retail Display

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Simple Summary: In Uruguay and some other countries in the region, where pasture grazing has always been predominant in beef production, steers are now increasingly fed grains to shorten the production time. This has implications for meat quality, so meat from both systems (pasture and concentrate) was compared under refrigerated retail conditions in this study. Meat from pasture-fed steers exhibited lower levels of lipid and protein oxidation during the display period, likely due to the higher levels of antioxidants, such as β-carotene and α-tocopherol, found in this investigation. In addition, meat from pasture-raised steers was healthier for consumers in terms of its fatty acid composition and lower intramuscular fat content. In conclusion, meat from pasture-fed steers was more stable during retail refrigeration, possibly allowing for a longer shelf-life, and was healthier for consumers compared to meat from concentrate-fed steers.

Abstract: Beef production in Uruguay is based on pasture (~85%) or concentrate (~15%), resulting in differences in meat quality. The objective of this study was to compare the oxidative stability and color of beef from these two systems during refrigerated retail display. For these purposes, the *Semimembranosus* muscle was removed from ten Aberdeen Angus steers raised and fed on pasture (130 days prior to slaughter) and from another ten steers fed concentrate (100 days prior to slaughter), sliced. The muscles were placed in a refrigerated showcase for 3, 6, and 9 days. The contents of β-carotene, α-tocopherol, and fatty acids were determined before the meat was placed on display. Lipid and protein oxidation, color, and heme iron content were determined before and during display. The meat from pasture-fed steers had a lower intramuscular fat content (1.78 ± 0.15 vs. 4.52 ± 0.46), lower levels of monounsaturated fatty acids, a lower n-6/n-3 ratio, less lipid and protein oxidation, lower L* and a* values, and higher levels of α-linolenic acid, DHA, total n-3, β-carotene, and α-tocopherol. In conclusion, the meat from pasture-fed steers was more stable during retail display from an oxidative point of view, which may be due to its higher levels of antioxidant compounds such as β-carotene and α-tocopherol and had a healthier fatty acid profile for consumers.

Keywords: lipid oxidation; protein oxidation; β-carotene; α-tocopherol; fatty acid; heme iron; beef; pasture; concentrate

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1. Introduction

Oxidative processes are major non-microbiological factors involved in the deterioration of the quality of meat during refrigerated storage [1]. Indeed, oxidation induces modifications in muscle lipids and proteins and therefore affects the organoleptic and nutritional properties of meat and meat products. The oxidation of myoglobin and lipids reduces the color and flavor acceptability of fresh meat during refrigerated storage [2,3]. In fact, color is an important parameter for consumers that has a substantial influence on acceptability and purchasing decisions at retail points [4]; a bright red color in beef is indicative of freshness [5]. When lipid peroxidation propagates in membranes, it promotes the oxidation

of myoglobin, resulting in color deterioration and the formation of rancid odors and other off flavors in fresh meat [6]. This is why the beef industry is concerned with the shelf life of products [7]. The stability of meat with respect to oxidation is the result of a balance between prooxidants and antioxidants [1]. Transition metals, specifically iron, are considered strong pro-oxidant species [8]. Dietary antioxidants can be delivered to the muscle where, together with endogenous defense systems, they counteract the action of prooxidants [9]. However, endogenous antioxidant concentrations depend on the animal species, muscle type, and diet [10–12]. As shown in other works [9,13,14], dietary treatments may directly affect the balance between antioxidant and prooxidant components in muscle.

The beef cattle production systems in Uruguay rely almost exclusively (~85%) on grazed pastures. However, more recently, intensive beef production systems have received an increased amount of interest from some beef producers. These two systems produce meat with different antioxidant, prooxidant, and fatty acid contents and compositions [9,15,16]. Fresh herbage is rich in antioxidants such as tocopherols, carotenoids, ascorbic acid, and phenolic compounds [12], and grains contain polyphenols like proanthocyanidins and phytic acid [17]. Indeed, tocopherols and β-carotene constitute the main lipid-soluble free radical scavengers in meat. Tocopherols are a group of plant phenolic isomers deposited from dietary sources [18], and several studies indicate that α-tocopherol delays the oxidation of myoglobin and extends color stability in retail beef [19–21]. In addition, β-carotene quenches sites localized within the hydrophobic regions of biological membranes in contrast with the scavenging activity of α-tocopherol, which operates close to the membrane's surface [22].

Thus, the aim of this investigation was to study the oxidative and color stability of beef during refrigerated retail display, simulating typical commercial conditions and assessing the α-tocopherol, β-carotene, and fatty acid contents prior to the display, utilizing meat from animals produced in pastures and in concentrate finishing systems.

2. Materials and Methods

2.1. Muscle Samples

The *Semimembranosus* muscle was selected from twenty young Aberdeen Angus steers (two–teeth) aged from 26 to 30 months on the same day in a commercial slaughterhouse named Frigorífico Breeders & Packers of Uruguay (BPU), which is located in the city of Durazno. All the steers were raised and sacrificed according to the good animal welfare practices approved by the Honorary Committee for Animal Experimentation (CHEA, protocol ID 702, approved on 14 February 2020) and came from commercial operations run by the Breeders Society of Aberdeen Angus of Uruguay (SCAAU).

Ten animals with an average final live weight of 495.8 kg were reared under characteristic Uruguayan conditions based on the exploitation of natural resources with traditional extensive grazing. They grazed on natural pastures (gramineae and legumes) and were finished (130 days before slaughter) on an improved pasture (comprising 40% natural and 60% cultivated grass) consisting of tall fescue (*Festuca arundinacea*), white clover (*Trifolium repens*), and birdsfoot trefoil (*Lotus subbiflorus* cv. El Rincón).

The other ten animals, with an average final live weight of 498.2 kg, came from an intensive production farm that was registered and authorized and which followed the requirements for exportation to the European Union (EU), which are stated in the European Commission Regulation (Number 481/2012). Quota 481 requires that at least 100 days prior to slaughter, animals receive the concentrate (which comprises 62% concentrated or cereal coproducts in the form of dry matter, at least 12.26 MJ/kg of dry matter, and at least 1.4% of body weight in a dry-matter-based form). The diet usually consists of whole plant sorghum silage (comprising 34% dry matter), wet grain sorghum (74% dry matter) and wet grain corn silage (70% dry matter), sunflower pellets (26% protein), and a mineral–vitamin premix, urea, and ionophores. A complete diet contains approximately 12% crude protein. The animals were expected to consume about 15.7 kg/animal/day of the complete diet (10.4 kg of dry matter/animal/day).

According to the official classification system of INACUR-Uruguay, carcass conformation and fatness were classified as A and 2, respectively, for both feeding systems [23]. The carcasses were kept refrigerated at 1–2 °C for 36 h post mortem, and the *Semimembranosus* muscle was then sampled, vacuum-packaged, and maintained at 1–2 °C in the meat processing plant. This muscle forms part of a cut (inside round) that is commonly seen in the form of steaks in refrigerated showcases at retail points and is highly consumed in Uruguay because it is lean and more affordable for consumers. Once in the laboratory, the muscle samples were cut into slices which weighed 100 g and were 1 cm thick; the color of the samples was tested, and some of the samples (day 0) were immediately frozen at −18 °C until further determinations were made. The rest of the samples were placed on food-grade polyfoam trays and wrapped with a food-grade, oxygen-permeable polyvinyl chloride film (density: 1.39 g/cm^3; O_2 permeability: cm^3 mm/m^2 day atm). Trays containing 3 slices of meat from each animal were placed in a commercial refrigerated showcase (CE, SS1500 model; 1.25 m tall, 90 cm wide, and 1.50 m long) at 2–8 °C with artificial light (120 cm, 18 W, 2700 Kelvin, and 1700 lm).

After each display time was completed (3, 6, and 9 days), a sample was tested for color, immediately vacuum-packaged (105 μm, Lacor Menaje Profesional S.L., LR69454, Bergara, Spain), and frozen at −18 °C until further determinations were made (n = 10 for each feeding system and display time).

2.2. Determination of Lipid Oxidation

The degree of lipid oxidation in the meat was determined on day 0 and after 3, 6, and 9 days of display. Samples of 5 g of frozen meat were homogenized in a Waring blender with 100 mL of extraction buffer (containing 0.15 M of KCl, 0.02 M of EDTA, and 0.30 M of BHT) at 12,000 rpm for 1 min. Part of the homogenate was frozen for carbonyl and protein content assays, and part was used for the TBARS (thiobarbituric acid reactive species) test. The TBARS procedure for the determination of lipid oxidation was followed according to [24,25], as described in [15]. The results were expressed as mg of malondialdehyde/kg of fresh meat.

2.3. Determination of Protein Oxidation

The level of protein oxidation was determined in the meat on day 0 and after 3, 6, and 9 days of display, following the carbonyl protein assay according to [26], as described in [15]. The results were expressed as nmoles of dinitrophenylhydrazine/mg of protein. The protein content was determined at 280 nm in the extraction buffer using bovine serum albumin (BSA) from Sigma chemicals Co. (St. Louis, MI, USA) as a protein standard, as described in [27].

2.4. B-Carotene and α-Tocopherol Contents

Both compounds were extracted from the meat on day 0, following [28], Ref. [18] for carotenoids, and [29] for tocopherol, with some modifications. Briefly, 0.5 g of frozen meat was homogenized with 11 mL of methanol: THF (1:1) in an Ultra-Turrax IKA T18 basic for 30 s at 12,000 rpm. Then, centrifugation was carried out at 12,000 rpm and 4 °C for 1 min. The supernatant was kept at −80 °C, and prior to measurement, an aliquot of 1 mL was taken and evaporated using N_2. The pellet was resuspended in 0.2 mL of the same extraction solution, and 20 μL was injected into the HPLC (Thermo Fisher Scientific Inc., Thermo Separation Products, Spectral Series P100, Waltham, MA, USA) with a UV2000 detector. The β-carotene content was measured at 450 nm and the α-tocopherol content was measured at 290 nm via a C30 thermostatted column at 30 °C. The mobile phase used was ethanol: methanol: THF (75:20:5), HPLC grade, with a flux of 0.5 mL/min. Calibration curves were produced using β-carotene (Sigma Aldrich Co., C9750, St. Louis, MO, USA) and DL-α-tocopherol (Sigma Aldrich Co., T3251, St. Louis, MO, USA) standards. The results were expressed as μg/g fresh meat.

2.5. Determination of Fat Content and Fatty Acid Composition

The total intramuscular fat was extracted from the meat on day 0, following the chloroform: methanol (2:1; *v:v*) procedure described in [30]. An extract containing approximately 40 mg of fat was methylated following the method described in [31], and a fatty acid analysis was performed via gas chromatography, using a Clarus 500 (Perkin Elmer Instruments Inc., Shelton, CT, USA) split/splitless instrument equipped with a 100 m CPSIL 88 capillary column. Hydrogen was the carrier gas (flow rate: 1 mL/min), and an FID detector was used. Fatty acids (FAMEs) were determined by comparing the retention times to fatty acid standards.

2.6. Color Measurement

Color parameters were measured in the meat on day 0 and after 3, 6, and 9 days of display, using a Minolta CR-10 colorimeter (Konica Minolta Inc., Tokyo, Japan) with a D65 standard illuminant, a observer angle of 10°, and an 8 mm aperture. The CIE L*a*b* system provided the values of three color components: L* (0: black to 100: white component; lightness), a* (+ red to − green component), and b* (+ yellow to − blue component) [32]. All the samples were allowed to bloom for 10 min before the measurements were taken. The hue angle (tone) was calculated as $\tan^{-1}$ (b*/a*), and chroma (saturation) was calculated as $[(a^*)^2 + (b^*)^2]^{1/2}$.

2.7. Heme Iron Content

The total heme pigments in the meat were determined on day 0 and after 3, 6, and 9 days of display as hemin after their extraction with an acidified acetone solution, as described in [33–35]. The heme iron content was calculated using the factor 0.0882 μg of iron/μg of hematin [33], and the results were expressed as ppm. All samples were assayed in duplicate.

2.8. Statistical Analysis

The data regarding the TBARS, carbonyls, β-carotene, α-tocopherol, total fat content, fatty acid composition, color parameters, and heme iron content in the meat were reported as means ± the standard error of the mean (SEM). The experimental unit was muscle (coming from ten animals in each system, n = 10). Data regarding lipid and protein oxidation, color, and heme iron content were analyzed using a repeated-measures ANOVA, taking the feeding system and display days as fixed effects. In addition, a one-way ANOVA followed by the Tukey–Kramer multiple comparisons test was used to compare feeding the systems on each day of the display. A one-way ANOVA followed by the Tukey–Kramer multiple comparisons was also used to compare the days of display in each feeding system. Data regarding the intramuscular fat, fatty acid, α-tocopherol, and β-carotene contents were analyzed using a one-way ANOVA. The statistical analysis was performed using NCSS 2007 software (NCSS, 329 North 1000 East, Kaysville, UT, USA), and the level of significance was established at $p < 0.05$.

3. Results

3.1. Lipid and Protein Oxidation

The levels of lipid oxidation and protein oxidation were lower (main effect of $p < 0.0001$ and $p < 0.01$, respectively) in meat from pasture-fed Aberdeen Angus steers compared with meat from concentrate-fed Aberdeen Angus steers (Figure 1).

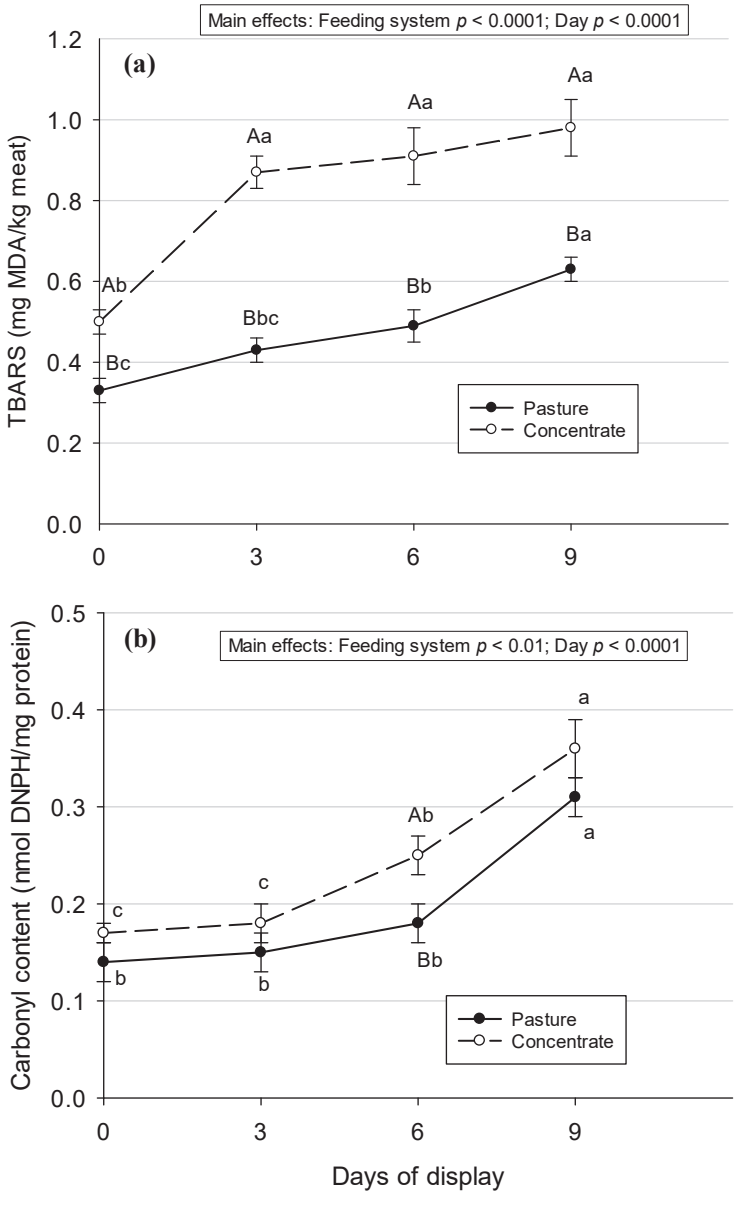

Figure 1. Lipid oxidation (**a**) and protein oxidation (**b**) before and during retail display in *Semimembranosus* muscles from Aberdeen Angus steers fed pasture or concentrate. Values are means ± SEM (n = 10). Different capital letters show significant differences between feeding systems on each day of the display ($p < 0.05$). Different lowercase letters show significant differences between days of display in each feeding system ($p < 0.05$).

When the display day effect was considered, an increment in both lipid and protein oxidation ($p < 0.0001$ for both) with the time of the display was found. When the evolution of lipid oxidation was analyzed day by day (Figure 1a), a significant increment in TBARS can be seen at the beginning of the display (day 0 vs. day 3) in meat from concentrate-fed animals and at days 6 and 9 in meat from pasture-fed animals. In addition, the carbonyl

content (Figure 1b) increased significantly from day 3 to day 6, from day 6 to day 9 of the display in the meat from concentrate-fed animals, and from day 6 to day 9 in the meat from pasture-fed animals.

3.2. β-Carotene and α-Tocopherol Contents

As observed in Figure 2, the α-tocopherol content in the meat measured before the display was almost twofold greater ($p < 0.05$) in the meat from pasture-fed animals (3.7 ± 0.5 μg/g fresh meat) than in the meat from concentrate-fed animals (1.9 ± 0.3 μg/g fresh meat). Regarding the β-carotene content measured before the display, it was six times higher (1.73 ± 0.18 μg/g fresh meat) in the meat from pasture-fed Aberdeen Angus steers than in the meat from concentrate-fed steers (0.27 ± 0.04 μg/g fresh meat) ($p < 0.0001$).

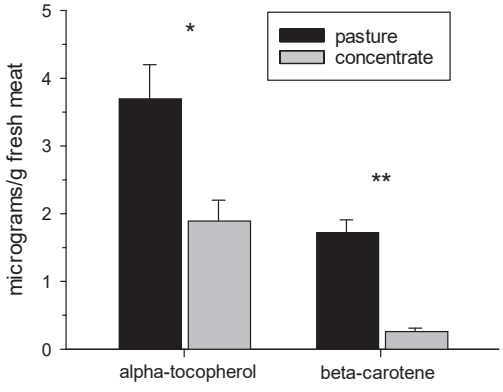

Figure 2. α-tocopherol and β-carotene contents (μg/g fresh meat) in *Semimembranosus* muscles from pasture- or concentrate-fed Aberdeen Angus steers prior to being placed in a retail display. Values are means ± SEM (n = 6). Significant differences between feeding systems are indicated as * ($p < 0.05$) or ** ($p < 0.0001$).

3.3. Lipid Content and Fatty Acid Composition

The meat from pasture-fed Aberdeen Angus steers presented a lower intramuscular fat content (Table 1) than the meat from concentrate-fed Aberdeen Angus steers ($p < 0.0001$).

Regarding the fatty acid composition of the intramuscular fat measured prior to the display, no differences between feeding systems were found in the even-chain saturated fatty acids (SFAs) 12:0, 14:0, 16:0, and 18:0, nor in the MUFAs 14:1, 16:1, 17:1, 18:1, and 20:1. The contents of the n-6 PUFAs 18:2 and 20:4, the n-3 PUFAs EPA and DPA, and CLA in the intramuscular fat were also not significantly different between feeding systems (Table 1). However, higher contents of α-linolenic acid (C18:3), C20:3, and DHA, which are n-3 PUFAs, were observed in the meat from pasture-fed animals compared with the meat from concentrate-fed animals (Table 1). In particular, the α-linolenic acid content was 3.4-fold greater, and the DHA content was 5-fold greater.

Regarding the total SFA, total PUFA, and total n-6 contents, no differences between feeding systems were observed (Table 1). Despite this, the total MUFA content was lower ($p < 0.05$), and the total n-3 content was higher ($p < 0.001$) in the meat from the pasture systems. In addition, the n-6/n-3 ratio was lower ($p < 0.05$) in the meat from pasture-fed steers (Table 1). No differences were found between feeding systems in the PUFA/SFA ratio.

Table 1. Total intramuscular fat content (%) and fatty acid composition (g/100 g fatty acids) in *Semimembranosus* muscles of pasture- or concentrate-fed Aberdeen Angus steers prior to retail display.

	Pasture	**Concentrate**	***p*-Value**
% Fat	1.78 ± 0.15b	4.52 ± 0.46a	<0.0001
C12:0	0.13 ± 0.04	0.08 ± 0.03	NS
C14:0	3.46 ± 0.71	3.10 ± 0.85	NS
C15:0i	0.25 ± 0.04a	0.12 ± 0.03b	0.01
C15:0ai	0.26 ± 0.03	0.17 ± 0.04	NS
C14:1	0.60 ± 0.12	0.59 ± 0.17	NS
C15:0	0.72 ± 0.10	0.51 ± 0.11	NS
C16:0i	0.20 ± 0.03a	0.10 ± 0.01b	<0.05
C16:0	31.85 ± 2.71	28.75 ± 2.45	NS
C16:1	4.16 ± 0.38	4.22 ± 0.35	NS
C17:0	1.10 ± 0.04b	1.27 ± 0.05a	0.01
C17:1	0.74 ± 0.24	0.94 ± 0.07	NS
C18:0	12.12 ± 1.25	12.53 ± 1.08	NS
C18:1	34.46 ± 2.91	41.53 ± 2.57	NS
C18:2 n-6	2.84 ± 0.36	3.07 ± 0.22	NS
C20:1	0.13 ± 0.04	0.21 ± 0.08	NS
C18:3 n-3	0.61 ± 0.04a	0.18 ± 0.03b	0.001
CLA	0.31 ± 0.03	0.31 ± 0.09	NS
C20:3 n-3	0.14 ± 0.04a	0.03 ± 0.01b	0.01
C20:3 n-6	0.34 ± 0.10a	0.11 ± 0.02b	0.05
C20:4 n-6	0.30 ± 0.12	0.21 ± 0.06	NS
EPA n-3	0.04 ± 0.05	0.01 ± 0.01	NS
DPA n-3	0.13 ± 0.08	0.04 ± 0.02	NS
DHA n-3	0.41 ± 0.06a	0.08 ± 0.07b	<0.01
Others	4.73 ± 1.37	1.92 ± 0.41	.....
SFA	50.09 ± 2.54	46.64 ± 2.39	NS
MUFA	40.08 ± 2.68b	47.49 ± 2.19a	<0.05
PUFA	5.13 ± 0.62	4.02 ± 0.49	NS
Σn-6	3.79 ± 0.41	3.69 ± 0.37	NS
Σn-3	1.33 ± 0.24a	0.33 ± 0.13b	<0.001
n-6/n-3	2.85 ± 0.35b	11.18 ± 3.96a	<0.05
PUFA/SFA	0.10 ± 0.02	0.09 ± 0.02	NS

Values are means ± SEM (n = 10). Different letters in the same row show significant differences between feeding systems ($p < 0.05$). CLA: conjugated linoleic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; NS: not significant. i: iso; ai: anteiso.

3.4. Color

When evaluating the main effects for the color parameters, a feeding system effect was found for L* ($p < 0.01$) and a* ($p < 0.05$) in which the meat from pasture-fed animals showed lower values (Figure 3). This translates into darker and less red meat compared with the concentrate-produced meat. However, no feeding system effect was found for the b* parameter (Figure 3c) in the present study.

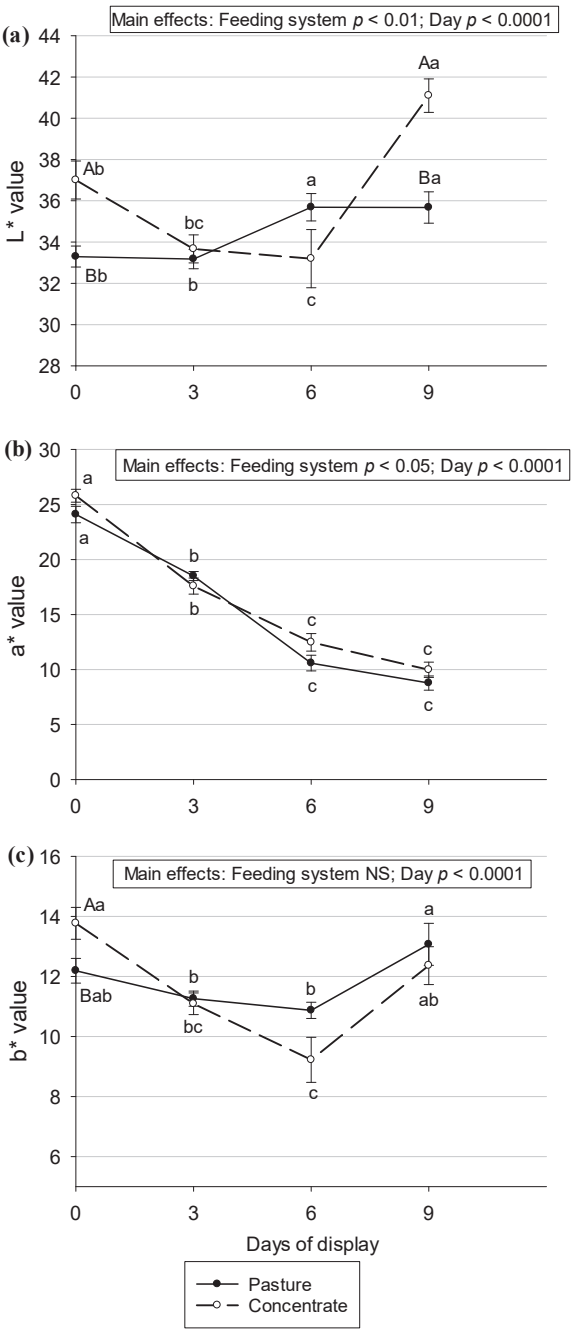

Figure 3. Color parameters, (**a**) L* value, (**b**) a* value, and (**c**) b* value, before and during retail display in *Semimembranosus* muscle of pasture- or concentrate-fed Aberdeen Angus steers. Values are means ± SEM (n = 10). Different capital letters show significant differences between feeding systems on each day of the display ($p < 0.05$). Different lowercase letters show significant differences between days of display in each feeding system ($p < 0.05$). NS: not significant.

When the display day effect was evaluated, an effect was found for the three parameters ($p < 0.0001$ in all cases) (Figure 3). The L* value was higher on day 9 compared with the other days of the display, so the meat became brighter with time (Figure 3a). In meat from pasture-fed steers, L* values were relatively constant; only a significant increase was observed from day 3 to day 6. In the meat from concentrate-fed steers, L* values decreased from day 0 until day 6 and then increased until day 9. When observing L* values on each day of the display, differences between feeding systems can be seen at the beginning (day 0) and at the end of the display (day 9) in which the meat from concentrate-fed animals was brighter. In addition to this, a decrease in the a* value was observed (Figure 3b) in meat from both systems, so the meat lost redness with days on display, and the b* value was higher at the beginning and at the end of the display (Figure 3c).

Associated with the a* and b* parameters are the hue (°) and chroma values. In the present study, a feeding system effect was observed for hue ($p < 0.05$) in which the meat from pasture-fed animals had a higher tone value than the meat from concentrate-fed animals (Figure 4a). A display day effect was observed for hue ($p < 0.0001$) in which the values were lower on days 0 and 3 than on day 6, which were lower than on day 9. A significant increase in the hue value was observed on day 3 in the meat from pasture-fed steers and on day 6 in the meat from concentrate-fed steers. On day 6, we can see that the tone is significantly different between the meats ($p < 0.05$, pasture > feedlot).

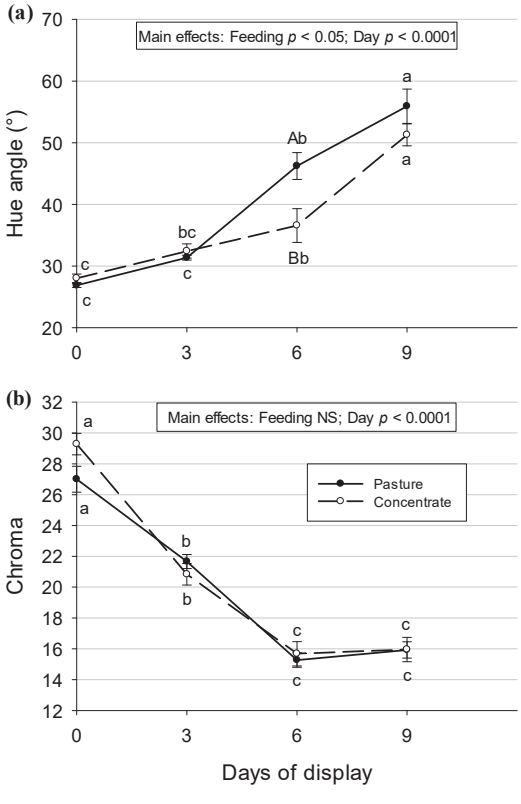

Figure 4. (**a**) Hue angle (°) and (**b**) chroma values before and during retail display in *Semimembranosus* muscle of pasture- or concentrate-fed Aberdeen Angus steers. Values are means ± SEM (n = 10). Different capital letters show significant differences between feeding systems on each day of the display ($p < 0.05$). Different lowercase letters show significant differences between days of display in each feeding system ($p < 0.05$). NS: not significant.

No feeding system effect was found for chroma (saturation), but a display day effect was observed ($p < 0.0001$). The color saturation diminished from the fresh meat (day 0) until day 6 of display and then remained steady until the end (day 9). As can be seen in Figure 4b, the color saturation diminishes with the length of display time and at the same rate in the meat from concentrate- and pasture-fed steers.

3.5. Heme Iron Content

The heme iron content results did not show any statistical difference between the feeding systems (pasture vs. concentrate) or between the days of the display (Figure 5).

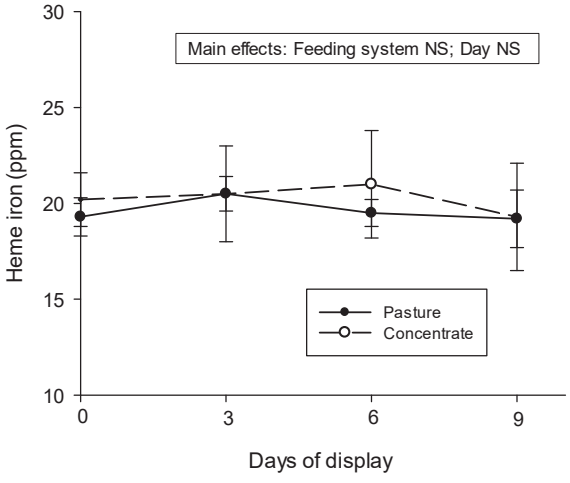

Figure 5. Heme iron content (ppm) in *Semimembranosus* muscle from pasture- or concentrate-fed Aberdeen Angus steers before and during the retail display. Values are means ± SEM (n = 6).

4. Discussion

The results found for lipid oxidation in the current study, in which the meat from pasture-fed Aberdeen Angus steers presented lower levels of TBARS compared with the meat from concentrate-fed Aberdeen Angus steers during refrigerated storage, agreed with the results of other publications. For example, the results in Ref. [36], in which differences in strip loins from Angus and Angus–Hereford cross steers were assessed between conventional grain-finished beef and beef fed with grass for 25 months, and the results in Ref. [37], in which both loin steaks from Tudanca bulls were assessed over 6 days of refrigerated retail display and [8] the *Longissimus thoracis* muscle from British and Zebu cross steers were assessed over 13 days of refrigerated storage. There are a few studies concerning protein oxidation in meat, and our results agree with the results in [4] in which *Psoas major* steaks from crossbreed steers were assessed over 9 days in retail display and meat from pasture-fed animals presented lower levels of protein carbonyls compared with meat from concentrate-fed animals. All these results indicate that beef coming from pasture systems has greater oxidative stability under the conditions of refrigerated retail display than beef coming from concentrate feeding systems. This can be explained by the higher contents of α-tocopherol and β-carotene found in the meat before the refrigerated retail display in the present investigation. It is known that α-tocopherol and β-carotene provide antioxidant protection in meat, as observed in previous investigations [38,39]. Vitamin E (α-tocopherol) protects cells against the effects of free radicals and may also block the formation of nitrosamines [19]. In addition, carotenes can quench singlet oxygen and scavenge toxic free radicals, preventing or reducing damage to living cells. They can also react chemically with free radicals, and the system of conjugated double bonds is directly destroyed. Due to their long conjugated chains, carotenoids are highly reactive [40].

As the PUFA contents did not differ between the feeding systems, they could not explain the higher levels of lipid oxidation in the meat from concentrate-fed animals due to the greater contents of PUFAs. It is relevant to mention that the lipids containing PUFAs are particularly susceptible to free radical attack. It is also important to consider that the meat from concentrate-fed steers had a higher intramuscular fat content, which may have caused greater levels of lipid oxidation in the meat.

The increments observed in lipid and protein oxidation with the display time were in accordance with other research [4,41–43] and could be explained by the presence of oxygen in the air and the artificial light of the showcase, which favor oxidative processes [44]. However, despite the increment of lipid oxidation during the time of display, the maximum TBARS values reached in the meat from both feeding systems are lower than those proposed as a threshold of acceptability by consumers, 2 mg MDA/kg meat [45]. Following the results obtained for lipid and protein oxidation day by day during the retail display in both feeding systems, the oxidation processes started to increase earlier in the meat from concentrate-fed Aberdeen Angus steers than in the meat from pasture-fed Aberdeen Angus steers. A similar pattern was reported in [37] in loin steaks from Tudanca bulls over 6 days in a refrigerated retail display and in [4] for *Psoas major* steaks from crossbreed steers over 9 days in a refrigerated retail display. These results could be explained by the higher levels of antioxidant protection from α-tocopherol and β-carotene present in the meat from pasture-fed animals, which can delay the oxidation processes in this meat.

Furthermore, the higher content of β-carotene found in the meat from pasture-fed steers agrees with other publications [4,46–48] and may occur due to higher concentrations of β-carotene in pastures, legumes, and other green plants, while seeds and whole-plant silage have low vitamin contents [49]. In addition, the higher content of α-tocopherol found in the meat from pasture-fed steers is supported by numerous publications [4,8,18,37,46,48,50,51] and can be explained by the greater concentration of α-tocopherol in green leaf tissues compared with grains [26]. As was mentioned in [52], desirable levels of α-tocopherol in muscle must be above 3.5 μg/g to minimize lipid oxidation processes in meat. Therefore, this threshold level was exceeded in the meat from pasture-fed steers (3.7 ± 0.5 μg/g fresh meat) but was not reached in the meat from concentrate-fed steers (1.9 ± 0.3 μg/g fresh meat). It is important to note that this is the first time that β-carotene has been measured in meat produced in Uruguay and the second time α-tocopherol has been measured in meat produced in Uruguay.

When observing the intramuscular fat content results, it is logical to think that the animals raised in an extensive system with large surfaces of pasture where they can move freely would deposit lower amounts of intramuscular fat in contrast to animals raised in intensive systems, which confine the animals to a limited area [19]. There are several studies [15,16,37,46,48,53–55] that are in accordance with our result.

Regarding the fatty acid composition measured in the meat prior to its display, we can highlight higher contents of α-linolenic acid and DHA in the meat from pasture-fed animals on pasture because it they have positive health aspects for meat consumers as both fatty acids have antithrombogenic properties and can prevent cardiovascular diseases [56]. Similar results regarding the n-3 content in the intramuscular fat of beef were found in other national [15,16,51] and international [57] research studies. As DHA is the result of elongase activity, in the ruminant that receives n-3, it is logical to expect a higher content of DHA in meat from pasture-fed animals. It is likely that elongase enzymes with n-3 substrates are more active in ruminants in a pasture system, which justifies the formation of DHA from α-linolenic acid. These elongases may have been stimulated by clover-based pastures [58].

Despite not finding differences in PUFA contents in the present investigation, higher concentrations of PUFAs were observed in pasture-fed animals compared to grain-finished animals in several studies [46,50,51,59,60]. Concerning the PUFA/SFA ratio in meat from both feeding systems in the present study (0.10 ± 0.02 in pasture and 0.09 ± 0.02 in concentrate), both were under the recommendations (0.45) from [61]. The PUFA/SFA ratio is nowadays recommended to be above 0.4 or 0.5 to prevent an excess of SFA, which has a

negative effect on the level of plasmatic LDL cholesterol, and an excess of PUFAs, some of which are precursors of clotting agents and are involved in the etiology of some cancers [62]. In addition, the higher contents of total MUFAs found in the meat from the concentrate system is in accordance with other publications [7,46,51,59,63,64] and could be due to the incidence of ingredients in the concentrates, which provide different composition profiles and are of interest for human nutrition.

Furthermore, the greater n-3 contents and the lower n-6/n-3 ratio found in the meat from the pasture-fed steers pasture are desirable because the balance between n-6 and n-3 PUFAs is an important determinant in decreasing the risk of cardiovascular diseases and in the prevention of atherosclerosis [65]. Indeed, pastures such as gramineae and legumes contain mainly n-3 fatty acids and in general, pasture-fed animals tend to accumulate more n-3 fatty acids [19,37], while the concentrate, which contained wet grain sorghum, wet grain corn silage, and sunflower pellets in the present investigation, all of which are cereals and asteracea, contain mainly n-6 fatty acids. These results were also observed in other research studies [46,60,66,67].

Indeed, the value observed for the n-6/n-3 ratio in the meat from the concentrate-fed steers (11.18 $\pm$ 3.96) was higher than the recommendations (4/1 or 5/1) in [61] and exceeded a value of 10, which is not recommended for the consumer's health. Concerning the lipid content and fatty acid composition results, we can conclude that the meat from pasture-fed Aberdeen Angus steers is apparently more beneficial for the consumer's health than the meat from animals in a concentrate system.

When considering color results in the meat in the current investigation, it was observed that the beef from animals finished on pasture was darker (L* = 34.5 $\pm$ 2.2) compared with those finished on grains (L* = 36.2 $\pm$ 4.4). Similar results were found in other research studies [4,36,48,68–71]. This darker color was explained [69] by a higher myoglobin content in the pasture-fed animals. In our case, this cannot be the explanation because no differences in heme iron content were observed between the systems heme iron content were observed. Another hypothesis is that the animals produced via an extensive system perform more physical activity compared with animals in a confined system [72]. Physical exercise elevates the concentration of reactive oxygen species (ROS) in vivo, causing oxidative damage to tissues, including muscle. During the conversion of muscle into meat, biochemical events favor oxidation, and the intermediate free radicals that are generated are similar to ROS and contribute to oxymyoglobin oxidation, causing a loss of color in the meat [73]. Another hypothesis is that animals produced on pastures are older than those in the concentrate system, which is reflected in darker meat, but in the present study, the age difference between the systems was 30 days to reach similar pre-slaughter weights.

The greater redness observed in the meat from the concentrate-fed animals was in opposition to what was found by the authors of [8] in *Longissimus thoracis* muscle from British and Zebu cross steers at days 10 and 13 of refrigerated storage, the authors of Ref. [4] in *Psoas major* steaks from crossbreed steers at 7 days of retail display, and the authors of [74] in *Longissimus thoracis et lumborum* muscle of steers over 10 days of display. However, the authors of [51,75] did not find a feeding system effect (pasture vs. grain) in the a* parameter in the *Longissimus thoracis et lumborum* muscle of steers. In general, consumers prefer a bright red color in beef because it indicates freshness. This determines purchasing decisions at retail points, so the results of the present research favor meat from concentrate-fed animals. Hence, the fact that no differences were found for heme iron content between meat from different feeding systems in the current investigation cannot explain the differences in redness. Regarding the heme iron content results found in other research studies, no differences between feeding systems were observed in *Longissimus thoracis et lumborum* and *Psoas major* muscles in the Charolais breed and crossbreed (British $\times$ Indicus), respectively [4,75].

Yellowness in meat, which is indicated by the b* parameter, presented no feeding system effect. This was in accordance with the results of [51], in which the *Longissimus thoracis et lumborum* muscle of Hereford steers was investigated, the results in Ref. [76], in which the

Longissimus thoracis et lumborum muscle of beef was investigated, and the results in [4], in which the *Psoas major* of steers was investigated. However, in [8], steaks from pasture-fed steers were significantly yellower when compared to grain steaks.

During the time of display, some variations can be seen in the L*, a*, and b* parameters. As seen in Figure 3a, lightness is more stable in the *Semimembranosus* muscles from pasture-produced Aberdeen Angus steers than in the muscles from concentrate-fed steers during the refrigerated display because fewer variations can be seen. The decline in the a* value observed in meat from both systems indicates a loss of redness, which indicates myoglobin oxidation and was also observed in several publications [4,8,41,43,73,75,77] in beef under retail display conditions. Even though a decrease in heme iron content in the meat was expected during the nine days of display in a refrigerated showcase because meat loses fluids and consequently heme iron and myoglobin [77], the heme iron content did not show differences between the days of the display in the present research. When comparing our b* results with other works, the authors of Ref. [75] found that b* values remained stable during the first 2 days and decreased after 6 days of storage, and in [4], in animals from pasture and concentrate systems, this decrease was observed on day 6 of display but the values stayed the same until day 9. In addition, a decrease in b value was also observed in meat with increasing days of storage [8]. These variations in a* and b* values found in the current research during the time of display were reflected in variations in the tone (hue angle) and saturation (chroma) of the meat's color. The hue angle is a good indicator of the stability of meat in retail display [37]. It seems that the loss of redness in meat during the time of display influences the tone, which changed to an orange tone, indicated by a hue value near 50, at the end of the display. Color saturation, which diminishes with the time of display, also seems to be affected by the loss of redness. Indeed, a decrease in chroma under retail display conditions is expected to occur and could be considered an indicator of metmyoglobin accumulation on the surface of the meat [37]. In [42], a decrease in chroma values during refrigerated storage was also observed in the *Longissimus lumborum* muscle of beef.

5. Conclusions

When the *Semimembranosus* muscle was submitted to a refrigerated retail display process which simulated commercial conditions, the meat from pasture-fed Aberdeen Angus steers was more stable from an oxidative perspective, and the oxidation processes started later than in the meat from concentrate-fed steers. This fact occurred in lipid oxidation, even though the meat from pasture-fed animals presented higher contents of two fatty acids which are very sensitive to oxidation, as α-linolenic acid and DHA. Therefore, this result could probably be explained by the higher contents of antioxidant compounds found in the meat from pasture-fed steers, such as β-carotene and α-tocopherol, both of which were determined in the present investigation. This allows the meat from animals that are pasture-fed to achieve a longer shelf life. In addition, pasture feeding may improve the lipid profile of the beef for consumers, highlighting the greater n-3 content in intramuscular fat. Concerning color stability in the *Semimembranosus* muscle during the display time, the concentrate system seems to be better because the meat had a higher redness value, which is preferred by consumers at retail points, and presented a significant increment in tone afterward than the meat from pasture-fed animals. This study generates new information about the oxidative and color stability of meat under retail display conditions in another muscle which is not studied as frequently; this muscle is frequently consumed as steak in South America and is the muscle from which carpaccio, an export product of Uruguay, is made.

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Data Availability Statement: The data presented in this study are part of the PhD thesis of Alejandra Terevinto within the framework of a Doctorate in Agricultural Sciences (Facultad de Agronomía, Universidad de la República, Uruguay) and are available at: https://hdl.handle.net/20.500.12008/29294 (accessed on 24 July 2023).

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Review

Knowledge Gaps on the Utilization of Fossil Shell Flour in Beef Production: A Review

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Simple Summary: With the increase in the number of people, there is also an increase in the demand for livestock products, including good-quality beef. However, beef production is faced with the challenges of climate change while it also contributes to climate change. Although several strategies have been in place to address this synergy, they have constraints that do not enable them to be used resourcefully and by all types of farmers. There is a need, therefore, for new strategies that will address this synergy while ensuring that beef production will be sustainable, and quality will be preserved and/or improved. Fossil shell flour is one such strategy because it is cheap, readily available, eco-friendly, and can be used by all types of farmers. However, it has not been explored enough in research, and as a result, there is little to no scientific evidence to support its efficiency in sustainable beef production. There is, therefore, a need to address this research gap as a step towards sustainable and eco-friendly beef production.

Abstract: Population growth in many countries results in increased demand for livestock production and quality products. However, beef production represents a complex global sustainability challenge, including meeting the increasing demand and the need to respond to climate change and/or greenhouse gas emissions. Several feed resources and techniques have been used but have some constraints that limit their efficient utilization which include being product-specific, not universally applicable, and sometimes compromising the quality of meat. This evokes a need for novel techniques that will provide sustainable beef production and mitigate the carbon footprint of beef while not compromising beef quality. Fossil shell flour (FSF) is a natural additive with the potential to supplement traditional crops in beef cattle rations in response to this complex global challenge as it is cheap, readily available, and eco-friendly. However, it has not gained much attention from scientists, researchers, and farmers, and its use has not yet been adopted in most countries. This review seeks to identify knowledge or research gaps on the utilization of fossil shell flour in beef cattle production, with respect to climate change, carcass, and meat quality. Addressing these research gaps would be a step forward in developing sustainable and eco-friendly beef production.

Keywords: beef quality; climate change; natural feed additives

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1. Introduction

Population growth and the enrichment of many countries are increasing the demand for increased livestock production and quality products [1]. Worldwide meat production has increased from 317 million metric tons to approximately 350.5 million metric tons from 2016 to 2023 [2] and is forecasted to marginally increase to a further 364 million tons [3]. This was accompanied by an increase in demand for higher-quality products [4,5]. Beef and veal have the third largest production volume, behind poultry and pork and ahead of sheep [6]. However, beef production represents a complex global sustainability challenge, including the need to meet the increasing demand and the need to respond to climate change and/or environmental footprints. Of all agricultural products, beef

production requires the most land and water, and its production contributes the highest amount of greenhouse gas (GHG) emissions. Thus, the consumption of beef continues to pose several threats to the environment. On the other hand, the ability to predict the sensory and nutritional properties of meat according to production factors has become a major objective of the supply chain [7]. This evokes a need for sustainable beef production with the exploration of novel feed resources that will provide mitigation strategies for environmental impacts while not compromising beef quality.

The feasibility of using alternative feeds for ruminants depends, among other things, on the feed value of novel feeds, animal production responses, and feed costs compared to conventional feeds [8]. Several feed resources which include crop residues, Agro-industrial by-products, and non-conventional feedstuffs are already in use, but some of the constraints limiting the efficient utilization of these feed resources include low nutritive content, their conservation is challenging, some have antinutritional components, production often seasonal, and processing may be difficult.

A novel or under-explored feed additive like fossil shell flour (FSF) has the potential to supplement traditional crops in beef cattle rations, responding to environmental footprints while providing sustainable production and not compromising product quality [9]. Fossil shell flour, commonly known as diatomaceous earth (DE) is the remains of microscopic single-celled plants (Phytoplankton) called diatoms found in oceans and lakes in many parts of the world [9]. These remains have long been mined from underwater beds or ancient dried lake bottoms for decades and have numerous industrial applications.

Diatomaceous earth is mined, milled, and processed into various types for several uses. There are two main types of diatomaceous earth: food grade and filter/non-food grade [9]. Unlike the filter grade, the food-grade DE is commonly used in agricultural and food industries since it is considered safe for both humans and animals [9,10]. Fossil shell flour is non-toxic, cheap, and readily available in huge quantities in many countries [9]. This makes affordability and availability two of the greatest advantages of FSF for its use by any farmer [9]. In livestock production, FSF has recently been modified as an additive for several uses. It has been used as a feed additive, growth promoter, mycotoxin binder, water purifier, vaccine adjuvant, in inert dust applications in stored-pest management, a pesticide, and a natural source of silicon and anthelmintic [9]. Although FSF is beginning to gain interest in livestock production, more so in sheep, broilers, and layers, the use of FSF in cattle production remains unexplored.

The objective of this review is to explore knowledge gaps on the utilization of FSF in beef production. The use of FSF is evaluated based on sustainable beef production, beef quality, and requirements to respond to climate change and/or carbon footprints.

2. Fossil Shell Flour as a Feed Additive

The quality, flavor, and composition of beef change with the composition of the cattle's diet [11]. The plane of nutrition has been found to influence meat quality due to its regulatory effect on the biological processes in muscle and in fat deposition [12–14]. Likewise, the type of forage fed to cattle affects both the carcass and meat quality characteristics of beef. Thus, recently beef quality including its fatty acid composition has been the focus of interest of many customers and researchers [15]. In most tropical countries, livestock is mainly reared under extensive production systems, where they mostly depend on natural pastures for their nutrients [16]. This type of production has the disadvantage of the scarcity of forage during the dry season, resulting in animals consuming a greater quantity of low-quality forage [17] and less palatable species resulting in an approximately 50% reduction in live weight gained during the wet season [18]. Likewise, feedlot cattle are also affected by climate change through impacts on forage and crop-based feeds. As a result, several feed additives have been used to supplement poor-quality feeds for livestock. These include antibiotics, probiotics, prebiotics, enzymes, antioxidants, mycotoxin binders, organic acids, beta-agonists, hormones, defaunation agents, herbal feed additives, and essential oils, which are mostly chemical-based [19]. It has, however, been indicated that, because of their chemical and

physical characteristics, some of these feed additives could decrease feed intake due to a decline in liking and appetite of the consumed feed [20,21]. Also, recently, the use of these chemical-based feed additives has created concerns about chemical residues in meat and other animal products [22,23]. There is also an alarming ecological risk that is increasing with the accumulation of veterinary antibiotic residue in animal manure [24].

Due to the possible risks of chemical-based feed additives, there has been a rising interest in natural growth promoters (NGPs). Production systems have their interest inclined toward various plants and plant extracts, enzymes, organic acids, and oils as possible NGPs that are eco-friendly [25]. However, one of the major constraints in using these NGPs is the time and cost involved in their harvesting [9]. Nonetheless, one NGP that could substitute chemical-based feed additives, boost feed intake, and be useful as a cost-effective, readily available, healthy, and eco-friendly feed additive is fossil shell flour. Fossil shell flour is a natural, organic, silicon-rich substance that occurs as a soft sedimentary rock made up of fossilized relics of diatoms. It has important physical and chemical characteristics enabling its use as a feed additive, with mineral constituents that include Copper (30 mg/kg), Sodium (923 mg/kg), Zinc (118 mg/kg), Iron (79.55 mg/kg), Boron, 23 mg/kg, Magnesium (69 mg/kg), Vanadium (438 mg/kg), Calcium (0.22%), Potassium (0.08%), Magnesium (0.11%), Sulfate (0.062%), and Aluminum (0.065%) [26–28]. Although there is little to no information on the nutritive value of FSF, its richness in trace elements such as Zn, S, Cu, and Fe qualifies it as a possible solution to there being low levels of these minerals, especially in semi-arid regions, resulting in low growth rates and poor quality of livestock. Moreover, since it supplies more than 14 trace elements and other elements that are usually not available in abundance in most field crops [28], it may be used to correct nutritional mineral imbalances in livestock. A review by Ikusika et al. [9] details the physical and chemical properties of FSF as well as its uses in the animal industry and other human activities; readers are, therefore, encouraged to refer to this article.

Table 1 further summarizes some studies that have been conducted to research using FSF as a feed additive. Although there was no significant impact of fossil shell flour on poultry, the studies in Table 1 indicate that fossil shell flour influences growth performance parameters, diet digestibility, feeding behavior, feed acceptability/preference, and body condition score, with each improving with increased inclusion levels of FSF up to 6% or 60 g FSF/kg in sheep. In the study by Adeyemo [27], the authors have attributed the efficacy of the broilers to convert nutrients from feed into body tissue to the fact that fossil shell flour inclusion in animal diets daily tends to keep the animals free of parasites (particularly, worms) and toxic chemicals so they can reap maximum benefits from the feed and water they consume. However, it is not clear which compound in FSF is directly related to this phenomenon. The authors also reported an imbalance between calcium and other minerals in the diet, although there was a concomitant increase in the phosphorus content up to 1.5% inclusion of fossil shell powder, after which the phosphorus level dropped. The study by Ikusika [29,30] attributed the improved feeding behavior and/or acceptability of feed by rams to the rich Sodium, Calcium, Potassium, and Magnesium contents in FSF which improves the taste and aroma of the diets. The studies in Table 1 have, therefore, attributed the different effects of FSF on different animal parameters to its mineral content; however, there is no clear indication of the specific contribution of each element and which compound led to a specific result.

Table 1. Some studies that were conducted on fossil shell flour.

Author and Year of Publication	Brief Methodology	Species	Country	Summary of Findings
Ikusika [29]	Twenty-four Dohne Merino rams were completely randomized and individually housed in pens for 90 days. Four different supplementation levels of FSF (0, 20, 40, and 60g/kg) were considered treatments for the rams.	Sheep	South Africa	The average daily feed intake, body condition score, average daily weight gain (g), and coefficient of preference (CoP was evaluated as the proportion of diet consumed by an individual to all the diets' standard intake) were significantly higher in rams supplemented with 60 g FSF/kg than the other treatments. The order of preference of diets supplemented with FSF in feed intake by Dohne Merino rams was: 60 g FSF/kg > 40 g FSF/kg > 20 g FSF/kg > 0 g FSF/kg.
Ikusika [30]	Twenty-four wethers, weighing 20 ± 1.5 kg on average were fed dietary food-grade fossil shell flour in a completely randomized design of four treatments with six wethers in each treatment. The wethers were fed a basal diet without FSF addition (control, 0%), or with the addition of FSF (2%, 4%, or 6%) into the diet for 105 days.	Sheep	South Africa	Using fossil shell flour supplementation in the diets (2%, 4%, and 6%) improved dry matter intake, average daily weight gain, and body condition score as well as influenced feed preference and wool production and quality of Dohne–merino wethers.
Emeruwa [31]	Sixteen rams (18.5 ± 1.05 kg) were allotted to four treatments: Tl (0% FSF), T2 (2% FSF), T3 (4% FSF), and T4 (6% FSF) in a twelve-week growth study.	Sheep	Nigeria	The inclusion of 2.0% fossil shell flour in the diet of West-African dwarf sheep improved dry matter intake and reduced weight loss during lactation, while the inclusion of 4.0% enhanced the daily weight gain.
Adeyemo [27]	A total of 120-day-old broiler chicks were used for the experiment and randomly allotted to 5 treatments (T1—0.9%, T2—1.2%, T3—1.5%, T4—1.8%, and T5—0% inclusion levels, respectively).	Broiler chickens	Nigeria	Fossil shell inclusion had no significant influence on feed intake and feed conversion ratio but had a significant impact on weight gain. Values for feed intake and feed conversion ratio showed no significant differences ($p > 0.05$) among the treatment means. Results showed that for feed intake, there were no significant differences ($p > 0.05$) observed, and all treatments had the same mean value. For feed: gain ratio, T4 had the highest value (2.91) while T1 had the lowest value (2.31). Weight gain, however, showed significant differences ($p < 0.05$) between T1 and T4 (1.30 and 1.03, respectively). Results for the finisher phase showed no significant differences ($p > 0.05$) and were observed for weight gain, feed intake, and feed–gain ratio. Values for feed–gain ratio showed that T4 had the highest value (3.10) while the control (T5) had the lowest value (2.06). However, T2 (4.39) and T5 (3.62) had the highest and lowest values, respectively for feed intake. Weight gain values showed that T1 (1.78) and T4 (1.36) had the highest and lowest values, respectively.

3. The Potential of FSF in Enhancing Carcass and Meat Quality in Beef

Recently, global meat consumption has been increasing along with concerns about food quality [32]. To meet this demand, growth promoters like antibiotics have been used; however, their use has been limited and/or banned in many countries due to the development of bacterial resistance that has alarmed the livestock sector [33]. As a result, natural additives have been opted for as a solution, as they have shown great potential to replace antibiotics by enhancing animal performance without changing or improving the meat quality [34–36]. However, these natural additives contain several compounds [37] that can be absorbed in the gut without being degraded and losing their main properties in the rumen, thus their properties like antioxidant activity may be transferred to the animals' meat [35]. Consequently, this may have negative effects on the nutritional and sensory properties of meat depending on the antioxidant types. This, therefore, evokes a need for a natural feed additive that will enhance animal performance without negatively affecting the quality of the final product, i.e., meat, but will instead maintain or improve the quality and its safety for human consumption. Fossil shell flour is one such alternative.

The physical and chemical properties of FSF enabled it to be acknowledged as a natural animal health and sustenance product. Previous research has indicated that the inclusion of FSF in animal diets did not adversely affect the lean mass percentage of animals [38], improved the average body weight gain of cockerels [27,28] and sheep [29,30], the growth rate of piglets [39], and the body condition scores of sheep [30]. Among these growth parameters influenced by FSF, the growth rate has an impact on carcass traits, muscle and fat deposition, and meat quality attributes and appears to be the main factor contributing to the chromatic qualities of beef [40]. Although meat color and quality are not well correlated [41], beef color is the most important attribute for consumers as they use it to gauge the quality of fresh meat at retail points. However, 15% of retail beef cuts fail to meet the expectations associated with bright cherry-red lean color [42,43]. Thus, since most additives have been used yet there is still persistence in beef color not meeting expectations associated with a bright cherry-red lean color, this leaves room for research that will explore novel feed additives. Thus, it is important to test the effect of FSF-enhanced growth rate on beef color to respond to this knowledge gap.

Furthermore, other growth-related attributes like body weight gains and body condition scores are positively related to some carcass and meat quality characteristics. In a study by Apple et al. [44], the body condition of culled beef cows at slaughter influenced carcass quality and cutability characteristics, and further had an impact on the subprimal cut yields. Moreover, research has also produced various results on the link between carcass weight and meat quality based on different experimental conditions [45], with some authors reporting advantages while others have reported the negative influence of heavier carcasses on meat quality [46]. Among other factors that are attributed to heavier carcasses, are growth steroidal enhancers [47,48]. Although the knowledge of animal responses towards current growth enhancers is common, recent reports on currently used natural feed additives have shown that they can enhance animal performance without changing or improving meat quality [34–36]. Thus, variability and inconsistency in bovine carcasses and meat are still high [7]. Recent reports have shown that fossil shell flour can also be used as a growth enhancer [9,49]; however, nutritional manipulation using FSF to enhance growth parameters that are positively related to some carcass and meat quality characteristics remains unexplored. Addressing this knowledge gap may also provide solutions to the inconsistency in bovine carcasses and meat quality.

4. The Potential of FSF in Mitigating the Negative Climate Effects of Beef Production

The increasing demand for livestock products including beef due to the increasing population requires an increase in livestock production. On the other hand, the potential impacts of climate change on current livestock systems, while livestock production is also a contributing factor to climate change worldwide, are a major concern. Therefore, the interaction between increasing livestock production and ongoing climate change makes it

challenging to increase production while lowering climate impacts [50]. One of the largest negative effects of climate change on meat production is heat stress. Meat production is affected by heat stress for all major commercial livestock types [51]. To decrease metabolic heat production, animals tend to reduce feed intake as an adaptive response to chronic heat stress [52], which consequently has implications on carcass deposition, carcass yield, and intramuscular fat content [53]. Heat-stressed ruminants exhibit reduced body size, carcass weight, fat thickness, and lower meat quality [54–56].

There is a lot of research that has focused on mitigation and adaptation strategies for climate change, which include, among others, adequate shade and water provision, sprinklers, and air conditioners. However, these strategies are not universally applicable, some are limited by dietary needs for specific productions, and availability, and they are too costly, and/or resource-intensive to be afforded by all types of farmers. Hence there is a need for alternative management practices that can be universally applicable and reduce heat load without affecting animal performance. One such product is fossil shell flour, which is readily available, cheap, environmentally friendly, and can be used by all farmers.

Recent research by Mwanda [57] that investigated the effects of fossil shell flour supplementation on heat tolerance of Dohne–Merino rams showed that water and feed intake increase with increasing levels of FSF, while the physiological parameters (skin temperature, rectal temperature, respiratory rate, and pulse rate) declined as the levels of FSF increased. The authors concluded that fossil shell flour could be used as a supplement in Dohne–Merino rams' diet to mitigate heat stress and promote the overall productivity of the sheep. Furthermore, a study by Kellaway and Colditz [58] indicated that Friesians responded to heat stress by decreasing N retention while nitrogen losses were evident through urinary excretions. A study by Ikusika et al. [59] also investigated the effect of varying inclusion levels of fossil shell flour on growth performance, water intake, digestibility, and N Retention in Dohne–Merino Wethers. The study showed that a 4% inclusion rate of FSF will give the best improvement in growth performance, diet digestibility, and N retention of Dohne–Merino sheep. The authors further indicated that the addition of FSF in the diets of sheep is a safe natural additive that can help to reduce environmental pollution by reducing fecal and urinary N excretion. Nitrogen is an essential nutrient critical for the productivity of ruminants, if it is, therefore, excreted in excess, it becomes an important environmental pollutant contributing to climate change. So, ruminant feed manipulation using FSF as an additive may increase N retention and alleviate environmental pollution caused by urinary N excretions [60]. Although conclusions cannot be drawn on the impacts of FSF on climate change-induced heat stress based on limited scientific evidence/data, feed manipulation using fossil shell flour to mitigate heat stress and alleviate climate change effects needs to be explored more for sustainable beef production.

5. Limitations of the Use of Fossil Shell Flour in Beef Cattle Production

i. Due to the large body size and large amount of feed needed in beef production, the use of FSF will be very challenging, as large amounts of FSF will be needed. This will have a great effect on mining areas due to the demand for fossils and the deleterious impact on the environment and climate change.
ii. Although FSF is known to be completely safe and non-toxic, there may be toxicity that may be associated with the interaction between the mineral content of FSF and that of animal diets, especially when it comes to heavy metals. For instance, FSF is suggested to have a concentration of 79.55 mg/kg of iron, while the daily dietary requirement in cattle is 50 mg of iron per kg of feed, Thus, possible interactions between fossil shell flour mineral contents and basal mineral contents of animal diets remain a research gap that needs to be further explored.
iii. The feasibility of using alternative feeds for ruminants depends among others on the nutritive value, so since the nutritive value of fossil shell flour is also unknown, it is often difficult to determine the impact of its constituents on certain results in many

studies. This may result in challenges in mixing well-balanced ratios of the essential nutrients in livestock feeds.

iv. There is a dearth of information or studies to validate the safety of fossil shell flour and recommend safer and/or optimum inclusion levels for specific production purposes and species. This may result in farmers being reluctant to use FSF, especially small-scale farmers.

v. Although FSF inclusion of 4% is suggested to increase N retention and aid in environmental pollution control, nutritional measures using FSF to reduce N excretion affect enteric methane emissions. For instance, a study by Ikusika [61] indicated that 4% and 6% inclusion of FSF in sheep diets increased enteric methane emissions. Thus, at certain inclusion levels, FSF can harm the environment.

Figure 1 (see Figure 1 below) depicts a solution approach to the limitations.

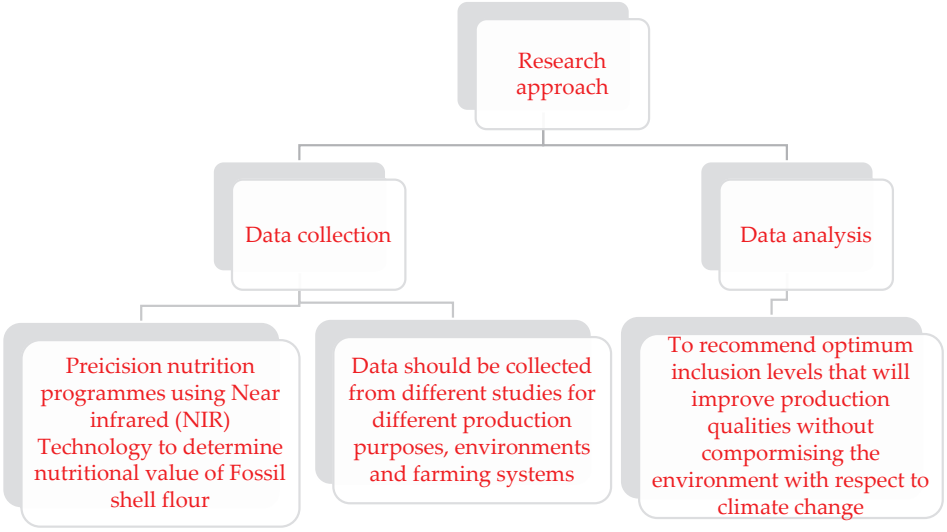

Figure 1. Limitations' solution approach.

6. Conclusions and Recommendations

The need to meet the increasing demand for quality beef while responding to climate change represents a complex global challenge that needs novel, universally applicable, and sustainable interventions. Several novel and underexplored feed additives like fossil shell flour have the potential to supplement traditional crops in beef cattle rations to respond to this complex challenge. Fossil shell flour has not gained much attention from scientists and farmers, particularly small-scale farmers, and its use has not yet been adopted in most countries. The few studies that have been conducted on fossil shell flour have focused on small stock with an emphasis on growth performance. There is a dearth of information on the potential of using fossil shell flour in beef production. Research is needed to identify the potential of FSF in improving beef production, enhancing carcass and meat quality, and mitigating climate change effects in the context of all types of farmers and different countries. Addressing these research gaps would be a step forward in developing sustainable beef production.

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The Effects of Different Zilpaterol Hydrochloride Feed Supplements and Extended Aging Periods on the Meat Quality of Feedlot Bulls

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Simple Summary: The aim of this study was to evaluate the effects of β-adrenergic agonist feed additives, namely two commercially available types of zilpaterol hydrochloride (ZH) molecules denoted as ZH-A and ZH-B, on the beef quality of feedlot bulls over extended aging periods. The research stems from concerns that zilpaterol hydrochloride (ZH) may compromise beef quality during exportation by boat as an alternative to the exportation of live animals. Typical South African feedlot bulls were fed a finisher ration supplemented with either ZH-A or ZH-B (both at 105 g ZH/ton) or a negative control (CT) diet during the finishing period. ZH supplementation decreased meat tenderness of both ZH-A and ZH-B treatments compared to the CT when compared at 7, 14, 28, 56 and 120 days of post-mortem aging. ZH supplementation had no effect on meat quality characteristics during post-mortem aging when compared to the CT. The duration of the post-mortem aging period significantly influenced all meat quality characteristics that were investigated, showing improvements in meat tenderness, a decrease in meat colour and an increase in drip and cooking losses from day 56 to 120.

Abstract: This study researched the effects of two commercially available zilpaterol hydrochloride (ZH) β-adrenergic agonists, denoted as ZH-A and ZH-B, on the meat quality characteristics of typical South African feedlot bulls (taurine × indicus composites), over extended aging periods of up to 120 days. The effects of ZH were studied to address concerns about the possible adverse effects of ZH on beef quality following extended aging, which typically occurs during the exportation of beef by boat. The completely randomized control study consisted of 3 homogenous experimental groups, with 3 replicates per treatment and 50 bulls per replicate = 450 animals. Treatments were a negative control (CT) with no ZH supplementation added to the basal diet or a basal diet supplemented with either zilpaterol hydrochloride A (ZH-A) or zilpaterol hydrochloride B (ZH-B), both at 105 g ZH/ton, fed from the first day of the finishing period (D_0) for 30 days. Subsamples were collected from 38 random carcasses from each treatment for proximate analysis and meat quality analysis using Longissimus dorsi samples. ZH supplementation decreased meat tenderness (Warner–Bratzler shear force values (WBSF)) of bulls fed ZH-A or ZH-B, compared to those fed the CT diet ($p < 0.05$; $\eta^2 = 0.24$). The WBSF values of both ZH treatments were about 0.5–0.8 kg higher during the aging periods compared to the CT, but ZH did not affect post-mortem meat aging or meat quality characteristics differently compared to the CT. Post-mortem aging per se influenced all meat quality characteristics investigated ($p < 0.001$; $\eta^2 > 0.30$), showing improvements in WBSF, a decrease in meat colour and an increase in drip and cooking losses. L*-values increased from 3 to 56 days of aging and then decreased to day 120 ($p < 0.001$; $\eta^2 = 032$). Chroma values decreased from day 3 to day 120 ($p < 0.001$; $\eta^2 = 0.50$). Hue° decreased from day 3 to day 7 and stabilized until day 120 ($p < 0.001$; $\eta^2 = 0.40$). Moisture and cooking loss (CL) increased to 56 days and then decreased to 120 days.

Keywords: β-adrenergic agonist; confined feeding; beef; tenderness; colour; drip loss

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1. Introduction

There are growing concerns about the projected world population growth [1] and the sustainability of agriculture in terms of its environmental impact, the use of natural resources and the ethics of animal source food production [2,3]. The demand for food is projected to increase markedly [4], with subsequent effects on employment rates, job creation, cost of living and the socioeconomic welfare of citizens [2,5,6]. There is growing pressure on low- and middle-income countries (LMICs) to be more sustainable, but improvements in crop production are generally limited by climatic conditions, access to natural resources or geography. In contrast, livestock farming is one of a few sustainable and economically viable options to produce nutrient-dense foods, compared to vegan/vegetarian alternatives [2,5,6].

A growing number of consumers have a negative perception of livestock production, but in most countries, animal agriculture is making concerted efforts to minimize the environmental impact and improve resource use to ensure sustainability and to gain consumer support for animal source foods [3,6]. With better production management and the use of growth-enhancing technologies, fewer animals are required to produce the same amount of kg product, which also has welfare benefits [3,4]. The use of feeding strategies and exogenous growth-enhancing molecules improves production efficiency and can reduce the environmental impact of animal agriculture [4,7–10]. Currently, countries such as the European Union (EU) and Russia have legislation in place (e.g., 96/22/EC) that ban the importation of products that were produced using growth-enhancing molecules such as β-adrenergic agonists (βAs) and steroidal growth implants [11,12]. The South African beef industry currently uses such molecules to supply its own demand for beef, and exports to neighbouring countries and China are increasing [5].

Food distribution via live animal exportation is also attracting attention due to animal welfare concerns. The National Council of the Society for the Prevention of Cruelty to Animals (NSPCA) and consumers in South Africa are concerned about the exportation of live animals, especially following recent incidents like the exportation of about 80,000 live sheep from the Eastern Cape, South Africa to Kuwait, Western Asia via boat in 2019. This led to high animal, product and financial losses and raised concerns about the ethics of live animal exports. The export of carcasses and selected meat cuts is a more practical and ethical approach. Unfortunately, concerns were raised about the effects of exogenous growth-enhancing molecules such as zilpaterol hydrochloride on beef quality after extended aging periods, such as during transportation by boat.

The objective of the present study was to investigate the effects of two commercially used zilpaterol hydrochloride β-adrenergic agonists, denoted as ZH-A and ZH-B (both registered for use in South Africa), compared to a negative control treatment on beef quality and shelf life (from 24 h to 120 days post-mortem, including a short retail display). Meat tenderness, drip loss, cooking loss, colour stability and shelf life are important quality attributes of beef [13,14], which may discourage the exportation of beef if compromised.

2. Materials and Methods

2.1. Ethical Approval and Treatments

Ethical approval for this research was obtained from the Research Ethics Committee of the University of Pretoria with the ethics approval number NAS390/2019. The current research formed part of a larger research study on the effects of beta-adrenergic agonist feed additives on the growth performance, carcass and meat quality of typical South African feedlot cattle. The present experiment focused specifically on the effects of the feed supplementation with two different types of commercially available zilpaterol hydrochloride (ZH) molecules (denoted as ZH-A and ZGH-B) during the finishing period of typical feedlot bulls on the meat quality characteristics and shelf life of meat up to 120 days post-mortem. The active ingredient of both beta-adrenergic agonists is zilpaterol hydrochloride, and both were supplemented in the feed during the last 30 days of the finishing period.

2.2. Experimental Design and Animals

The experimental design was a completely randomized control study, consisting of 3 experimental groups, 3 replicates per treatment group and 50 bulls per replicate ($3 \times 3 \times 50$ factorial design). The research study was conducted at a large commercial beef feedlot located in Gauteng, South Africa. A total of 450 homogenous intact bulls were randomly selected from a total of 2000 typical intact feedlot bulls made available for this study. Composite-type bulls of medium size and maturity (e.g., Bonsmara crosses) were used. The cattle were representative of typical feedlot cattle in South Africa, which were all identified with durable Allflex® (MSD Animal Health, Somerset West, South Africa) ear tags. The average initial mass of the bulls was ca. 400 kg when the experiment commenced (D_0).

All bulls were processed and managed similarly to limit variation. This included preventative treatments and vaccinations for internal and external parasites, injecting vitamin supplements and immunization against Clostridial and viral diseases, including respiratory diseases such as infectious bovine rhinotracheitis (IBR). During processing, all cattle received an initial hormonal growth ear implant, i.e., Ralgro® (MSD Animal Health, Somerset West, South Africa) and were then re-implanted with Revalor H® (MSD Animal Health, Somerset West, South Africa) after 45 days. Revalor H® was then effective for another 60–70 days. All bulls were housed in large soil-surfaced floor pens (ca. 50 m^2 standing space/bull) and fed the same starter and grower diet after an adaption period of 14 days. Fourteen days prior to the normal finishing period (D-14), the cattle were weighed using a heavy-duty Richter electronic weight beam scale and randomly allocated to different treatments to commence (D_0) the finishing period.

2.3. Allocation of Experimental Treatments

The treatments were administered from D_0 for 30 days during the normal finishing period, followed by a 3-day compulsory ZH-withdrawal period before slaughter (D_{33}), to comply with regulations of the use of β-adrenergic agonists in South Africa. The treatments were administered to the experimental groups during the feedlot finishing period, i.e., from D_0 to D_{30}. The treatments were a negative control (CT) which received no zilpaterol hydrochloride (ZH) supplementation, the second was ZH-A feed supplementation (ZH-A at 105 g ZH/ton) and the third was ZH-B feed supplementation (ZH-B at 105 g ZH/ton).

2.4. Feeding and β-Agonist Feed Supplementation

Following standard feedlot operating procedures, the animals were fed in bulk feed troughs that ensured adequate feeding space per animal (ca. 30 cm feeding space/bull). The animals received a standard, balanced, dry feedlot concentrate ration ad libitum that provided 10.5 MJ ME/kg DM energy during the finishing phase of feeding. The daily allotment of feed for each pen was estimated and recorded via bunk-reading the feed consumed from that which was provided. The expected or predicted feed intake was calculated using an estimate of roughly 3% of the live weight (kg) being consumed per day for live performance analyses. The animals had ad libitum access to good-quality water. Supplements were mixed into the 75% DM content feed rations at a standard recommended concentration of 105 g of ZH/ton. Every batch of finishing ration was sampled and bio-assayed to confirm the β-agonist concentrations.

2.5. Slaughter, Meat Sample Collection and Storage

After a feedlot finishing period of 30 days and a 3-day compulsory withdrawal period, the final mass (D_{33} mass) of the bulls was recorded, and all the bulls were loaded and transported to the commercial abattoir in Gauteng, South Africa. After 24 h of lairage, the animals were slaughtered in the conventional way (e.g., slaughter after stunning). This involved the mechanical stunning of bulls using a pneumatic stunner (captive bolt), followed by suspension from their hocks and exsanguination. The carcasses were electri-

cally stimulated (110 V, 17 Hz, 5 m/s; Jarvis Product Corporation, Alberton, South Africa, Pty Ltd.) during hoisting, followed by evisceration and skinning [12,15].

Of the 450 carcasses, 38 were randomly selected per experimental group for meat quality analyses (38 × 3 treatments = 114 carcasses). The carcasses were placed in a chiller (2–4 °C) at approximately 45 min post-mortem. Muscle samples were collected from the carcasses after chilling for 24 h. Samples were excided from the Longissimus dorsi muscle (LD; muscle generally used for meat science studies) between the 9th and 12th ribs on the left side of each carcass [12,15]. Muscle samples were labelled, vacuum packed and stored at −1 to 0 °C for different aging periods (7, 14, 28, 56 and 120 days) until meat quality analyses were performed.

2.6. Meat Quality Analyses

The following meat quality evaluations were performed after LD samples were thawed at 4 °C for 36 h after aging periods of 7, 14, 28, 56 and 120 days. Meat samples were placed at 25 °C (room temperature) for 20 min to bloom (i.e., turning bright red due to oxygen exposure) for colour measurements using a colorimeter—Hunter Lab ColorFlex EZ (Model 45/0 LAV, Hunter Laboratory Associates, Inc., Reston, VA, USA) [16]. According to the manufacturer's guidelines, the ColorFlex EZ was calibrated immediately before readings against black and white tiles. L* (lightness), a* (redness) and b* (yellowness) values were determined utilizing a standard observation made at 10° using illuminant D65 (with an accuracy of ΔE* < 0.15 CIE L*a*b* (Avg) on BCRA II Tile Set). Three random readings were taken to obtain a mean value recorded. Chroma (red colour intensity) and hue angle (meat discoloration) values were calculated [16] as follows:

$$Chroma\ (C^*) = (a^{*2} + b^{*2})^{1/2}$$

$$Hue\ angle\ (h^\circ) = tan - 1\ ((b^*)/(a^*)).$$

Radiant values were converted to degrees using multiplication by 57.2958. Moisture (drip) loss was calculated as the percentage mass of fluid that was lost from meat samples during storage, and cooking loss was determined as previously described [17]. Meat samples were weighed and then placed in a plastic bag to be cooked in a water bath at 80 °C until the internal temperature of meat samples was 70 °C. The cooked samples were then chilled overnight at 4 °C, blotted dry and weighed to calculate the weight loss after cooking. Cooking loss was calculated as a percentage of the initial mass by (mass loss after cooking)/(initial sample mass) × 100%. Meat tenderness was tested on the same samples used for CL measurements via a shear force (WBSF) test performed with an Instron apparatus equipped with a Warner–Bratzler shear blade (G-R Elec. Mfg. Co., Manhattan, KS, USA). The WBSF measurement was used as a physical indicator of meat tenderness instead of a perceived sensory panel score. Core meat samples (2.54 × 10 cm) were excised from the cooked meat samples to measure the mean peak force value (kg) to cut the meat perpendicularly to the muscle fibres. The mean WBSF was derived from five peak force recordings.

2.7. Statistical Analyses of Data

Data were recorded in Excel and then imported into to IMB SPPS Statistics, Version 28, 2022 (New York, NY, USA). ZH supplementation and differences between the two ZH molecules were investigated using General Linear Model (GLM) analyses, and significant differences were recorded at $p < 0.05$. Because the number of bulls differed marginally between the replicates and experimental groups (a bull jumped the fence on D_0 and remained there), Bonferroni's multiple range test was used to analyze the differences between least squares means in an unbalanced experimental design for both pooled and pairwise comparisons between treatments. The analysis of the effects of extended post-mortem aging on beef tenderness and colour parameters of ZH and control groups were

carried out via Multinomial Logistic regression analyses, with the aging period and ZH treatment as factors, and each meat quality attribute as reference categories.

3. Results

The growth and carcass results of the feedlot bulls supplemented with zilpaterol hydrochloride (ZH) compared to the negative control (CT) treatment are summarized in Table 1. The average mass of bulls at the start of the finishing phase (D_0) was similar due to randomization, but slaughter mass was higher ($p < 0.05$; Table 1) for the bulls supplemented with ZH compared to the bulls not supplemented. Similarly, ZH supplementation increased the carcass mass ($p < 0.01$) and dressing percentage compared to CT. The percentage of carcass mass loss at 24 h post-mortem was higher ($p < 0.01$) for the carcasses from the ZH treatment compared to those from CT, probably due to the lower subcutaneous fat thickness ($p < 0.02$) of the ZH carcasses.

Table 1. Effects of feed supplementation with zilpaterol hydrochloride (ZH) during the finishing period compared to negative control (CT) treatment on the growth and carcass characteristics of composite-type feedlot bulls.

Growth Characteristic	Control (CT) Mean (Std Error)	Zilpaterol (ZH) Mean (Std Error)	p = F
D_0 mass (kg)	397.97 (1.843)	397.58 (1.303)	0.86
Slaughter mass (kg)	420.83 [a] (1.267)	425.95 [b] (0.896)	0.05
Cold carcass mass (kg)	249.40 [a] (0.903)	256.52 [b] (0.638)	0.01
Carcass mass loss %	2.43 [a] (0.091)	2.74 [b] (0.065)	0.01
Dressing %	59.23 [a] (0.180)	60.23 [b] (0.127)	0.01
SC fat (mm)	5.77 [a] (0.158)	5.31 [b] (0.112)	0.02

[a,b] Means with different superscript letters differ ($p < 0.01$); D_0 mass—mass at start of finishing period; SC fat—Subcutaneous fat thickness over 13th thoracic vertebrae, 5 cm from the medial line. ZH—Zilpaterol hydrochloride treatment (pooled).

The effects of feed supplementation with either ZH-A or ZH-B, compared to CT, on meat quality attributes of the composite-type feedlot bulls of medium maturity during the finisher period are presented in Table 2.

Table 2. Effects of the supplementation of feedlot bulls during the finisher phase with β-adrenergic agonists ZH-A, ZH-B or negative control (CT) on meat quality attributes.

Meat Quality Variable	Control (CT) Mean (Std Error) (n = 162)	ZH-A Mean (Std Error) (n = 172)	ZH-B Mean (Std Error) (n = 166)	p = F
WBSF_N	4.79 (0.110) [a]	5.65 (0.105) [b]	5.61 (0.121) [b]	0.001
Colour L*	40.15 (0.296)	40.93 (0.283)	40.72 (0.289)	0.147
Colour a*	15.30 (0.170)	15.01 (0.163)	15.12 (0.166)	0.471
Colour b*	4.99 (0.163)	4.85 (0.156)	5.03 (0.159)	0.687
Hue°	18.60 (0.524)	18.30 (0.467)	17.62 (0.477)	0.567
Chroma	16.18 (0.196)	15.87 (0.188)	16.02 (0.191)	0.517
H_2O-loss (%)	7.59 (0.203)	7.74 (0.194)	7.89 (0.198)	0.564
Cooking loss (%)	23.57 (0.275) [x]	22.67 (0.263) [y]	22.97 (0.268) [xy]	0.057

[a,b] Means with different superscript letters differ ($p < 0.01$); [x,y] Means with different superscript letters tend to differ ($p < 0.1$).

There was no difference between the two types of zilpaterol hydrochloride products, namely ZH-A, compared to ZH-B on the meat quality attributes of LD meat samples. However, LD samples from the bulls supplemented with either ZH-A or ZH-B (collectively referred to as ZH) had higher shear force values ($p < 0.001$; Table 2) and were therefore less tender compared to those from the control treatments. The colour attributes of the

meat samples did not differ between the CT and ZH groups, but cooking loss tended to be lower ($p = 0.057$) in samples from the ZH-A group compared to the control group. There were no significant differences in meat quality characteristics between ZH-A vs. ZH-B, as determined at all of the post-mortem aging periods of 7, 14, 28, 56 or 120 days in this study, indicating similar meat colour, moisture, cooking losses and WBSF (Table 2). For this reason, the effects of extended post-mortem aging periods of the CT compared to pooled ZH treatments on meat quality parameters are presented in Table 3.

Table 3. Effects of extended meat aging periods (7-, 14-, 28-, 56- and 120 days) and zilpaterol hydrochloride treatment (ZH~ZH-A + ZH-B pooled), compared to negative controls on the meat quality attributes of Longissimus dorsi samples from feedlot bulls.

Meat Quality Variable		Control Treatment Mean (Std. Error)	ZH Treatment Mean (Std. Error)
WBSF # (kg)	7	6.62 (0.278) ^{a}	7.12 (0.186) ^{a}
	14	5.57 (0.188) ^{b,A}	6.43 (0.132) ^{b,B}
	28	4.70 (0.188) ^{c,A}	6.07 (0.130) ^{b,B}
	56	3.80 (0.188) ^{d,A}	4.50 (0.132) ^{c,B}
	120	4.25 (0.191) ^{cd,A}	4.77 (0.131) ^{c,B}
Colour L*	7	39.26 (0.891) ^{a}	40.15 (0.586) ^{a}
	14	40.72 (0.586) ^{b}	41.43 (0.414) ^{ab}
	28	42.29 (0.594) ^{b}	42.67 (0.409) ^{bc}
	56	42.80 (0.586) ^{b}	43.62 (0.412) ^{c}
	120	35.68 (0.594) ^{c}	36.27 (0.409) ^{d}
Colour a*	7	14.99 (0.512) ^{a}	15.17 (0.337) ^{ab}
	14	16.22 (0.337) ^{a}	16.47 (0.238) ^{b}
	28	15.97 (0.342) ^{ab}	15.57 (0.235) ^{ab}
	56	16.75 (0.337) ^{b}	15.63 (0.237) ^{ab}
	120	12.58 (0.342) ^{c}	12.49 (0.235) ^{c}
Colour b*	7	3.13 (0.489) ^{a}	3.86 (0.322) ^{a}
	14	5.70 (0.322) ^{b}	5.48 (0.228) ^{b}
	28	5.36 (0.326) ^{bc}	5.13 (0.225) ^{bc}
	56	6.39 (0.322) ^{b}	5.68 (0.226) ^{b}
	120	4.36 (0.326) ^{ac}	4.52 (0.225) ^{ac}
Chroma	7	15.38 (0.590) ^{a}	15.77 (0.388) ^{a}
	14	17.32 (0.388) ^{ab}	17.44 (0.274) ^{b}
	28	16.91 (0.393) ^{ab}	16.49 (0.271) ^{ab}
	56	17.97 (0.388) ^{b,A}	16.71 (0.273) ^{ab,B}
	120	13.33 (0.393) ^{c}	13.32 (0.271) ^{c}
Hue°	7	11.29 (1.462) ^{a}	13.17 (0.978) ^{a}
	14	20.10 (0.991) ^{b,C}	17.87 (0.696) ^{b,D}
	28	18.07 (0.991) ^{b}	17.50 (0.687) ^{b}
	56	20.49 (0.991) ^{b}	19.49 (0.696) ^{b}
	120	19.12 (1.004) ^{b}	19.38 (0.691) ^{b}
H_2O_loss (%)	7	4.88 (0.609) ^{a}	4.81 (0.400) ^{a}
	14	6.13 (0.400) ^{ab}	6.73 (0.283) ^{b}
	28	7.02 (0.406) ^{b}	7.46 (0.279) ^{b}
	56	10.69 (0.400) ^{c}	10.86 (0.281) ^{c}
	120	9.22 (0.406) ^{c}	9.22 (0.279) ^{d}
Cooking loss (%)	7	4.88 (0.826) ^{a}	4.81 (0.543) ^{a}
	14	28.10 (0.543) ^{b}	27.75 (0.384) ^{b}
	28	30.57 (0.551) ^{b}	29.85 (0.379) ^{c}
	56	30.62 (0.543) ^{b}	29.80 (0.381) ^{c}
	120	22.79 (0.551) ^{c}	21.89 (0.379) ^{d}

^{a,b,c,d} Means with different superscript letters in the same column (between aging periods) differ ($p < 0.05$); ^{A,B} Means in rows (between treatments) differed ($p < 0.01$); ^{C,D} Means in rows (between treatments) tended to differ ($p < 0.1$); # WBSF—Warner–Bratzler shear force (kg).

ZH treatments mainly affected meat tenderness, while aging periods affected beef colour, moisture and cooking losses (Table 3). Comparisons between WBSF shear force between ZH and CT were small at 7 days of aging but differed at extended aging for 14, 28,

56 and 120 days ($p < 0.01$). Although shear force differed at 120 days of aging ($p < 0.05$), the tenderness from all treatments was best at about 56 days of aging. WBSF values decreased, and therefore the tenderness of the LD samples improved with increasing aging periods from 7 to 120 days post-mortem, but extended aging did not completely negate the meat toughing effects of ZH supplementation in this study. Fortunately, meat samples from all treatments were classified as tender in terms of the tenderness threshold of about 7 kg or 70 N, which occurred after 7 days of aging [18].

No significant differences in any of the meat colour parameters tested, nor for moisture loss of samples from CT and ZH groups, were observed at any of the aging periods tested. Post-mortem aging period influenced ($p < 0.01$) meat colour parameters, moisture and cooking losses, with marginal differences between CT and ZH treatment groups but with similar trends over time.

4. Discussion

Zilpaterol hydrochloride (ZH) supplementations (ZH-A or ZH-B) in the feed during the finisher period increased slaughter mass, cold carcass mass and dressing percentage of feedlot bulls compared to the negative control groups (CT), which agrees with previous studies [7–9,15,18–25]. Subcutaneous carcass fat thickness was reduced in ZH-supplemented bulls, which resulted in higher carcass weight losses post-mortem compared to the CT. Similar findings were reported in previous studies, indicating that zilpaterol hydrochloride supplementation reduces carcass fat content in intensively fed cattle [7–9,15,18–25].

No differences were observed between the two ZH supplementation groups (i.e., ZH-A vs. ZH-B; $p > 0.40$) for any of the meat quality characteristics. A previous study also found no significant meat quality differences; although some trends in chroma and hue angle were observed [8], those results were in zebu cattle, while the present study was in taurine x zebu composites. Overall, ZH supplementation increased WBSF values (hence decreased tenderness) as tested at all aging days, compared to samples from the CT treatment ($p < 0.05$; $\eta^2 = 0.24$).

In one previous study [18], ZH supplementation increased WBSF values by 22% in feedlot steers compared to negative controls. In studies where WBSF was measured over 21 days, ZH supplementation increased WBSF values compared to untreated controls [19,20]. Several previous studies [9,12,19,20,23,24] indicated that post-mortem aging significantly improved tenderness by decreasing WBSF. It was also reported [25] that the increase in WBSF in ZH-supplemented cattle is due mostly to muscle hypertrophy and that longer aging periods may be required to ensure acceptable meat tenderness.

For this study, extending the aging period of LD samples from feedlot bulls to 120 days post-mortem had a large, significant effect ($p < 0.01$) on all meat quality characteristics measured. Although the aging of the meat samples significantly decreased WBSF values for both CT and ZH, the aging effect size for WBSF of samples from ZH was smaller compared to that from the CT treatments ($\eta^2 = 0.18$ vs. 33). The regression equations for WBSF values over extended post-mortem aging periods for samples from ZH and CT, are presented in Figure 1. Although these regression equations indicate a decrease in WBSF over extended post-mortem aging periods, WBSF decreased faster in CT samples and differed ($p < 0.05$) compared to ZH samples from 14 days post-mortem onwards.

No significant differences were observed between ZH-A and ZH-B for L*, a*, b*, chroma or hue$^\circ$ values of beef samples during specific aging periods. These observations generally agree with previous research [7]. However, L*-values (brightness) were similar, but a* (redness) and b* (yellowness) values were lower for all treatments compared to the previous study on the same molecules [7], which may reflect breed type and dietary effects like the type of roughage used [26]. Although no differences were observed in meat colour attributes between ZH and CT, apart from chroma at 56 days ($p < 0.01$) and a trend for hue$^\circ$ at 14 days ($p < 0.1$) of aging, the multinomial logistic regression analyses of WBSF and colour attributes over post-mortem aging days, revealed differences ($p < 0.01$) between the CT and ZH treatments.

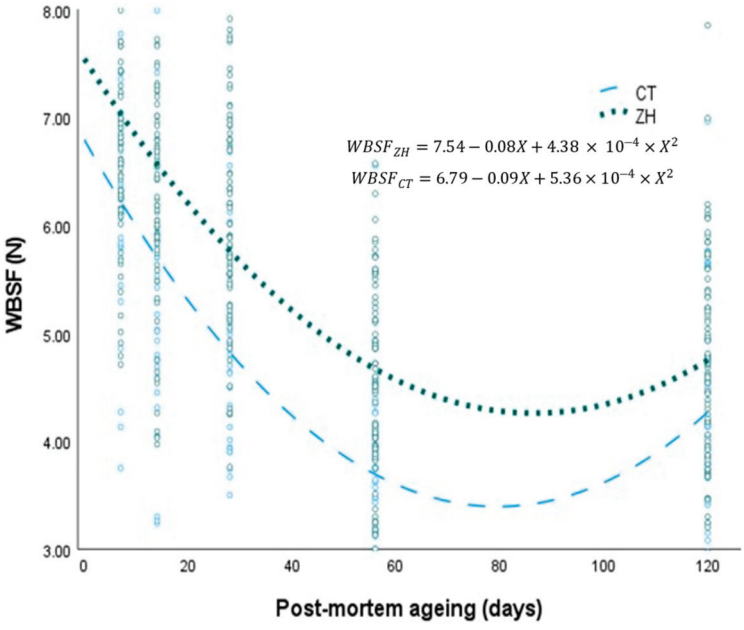

Figure 1. Effects of post-mortem aging on beef tenderness (WBSF) of feedlot bulls supplemented with zilpaterol hydrochloride (ZH) compared to negative controls (CT), (°° Data points).

The multinomial logistic regressions for WBSF and colour attributes over extended aging periods, as affected by CT vs. ZH treatments, are presented in Figure 2a–f. These regression curves reflect lower values for WBSF and meat brightness for CT over extended aging periods, while meat redness (a*), yellowness (b*), hue°, and colour saturation (chroma) reflect lower curves for samples from ZH. So, although the multinomial logistic regressions confirm the beneficial effects of aging on WBSF, lower a*, b* and red colour intensity in ZH-supplemented bulls was more apparent, while the risk of meat discoloration (higher hue°) was higher for CT bulls with extended aging.

No differences were observed in moisture losses between CT and ZH treatments, while a trend for lower cooking losses ($p = 0.057$) was observed for samples from ZH. Both CT and ZH treatments had the highest moisture losses at 56 and 120 days of aging ($p < 0.01$), while cooking losses were highest at 28 and 56 days of aging. Moisture losses over extended aging periods were due to the combined effects of muscle fibre structure damage (via protein degradation), which releases intracellular fluids and chromatic pigments (mainly myoglobin) in the moisture losses [27].

Previous studies [18,19] reported no ZH-supplementation effects on cooking losses, but those studies only include sampling up to 21 days post-mortem. Structural muscle damage over extended aging periods may influence moisture and cooking losses, water-holding capacity and WBSF values [27]. In the present study, moisture loss explained a significant portion of the variation in WBSF ($R^2 = 0.15$, $p < 0.01$) for samples of both CT and ZH treatments.

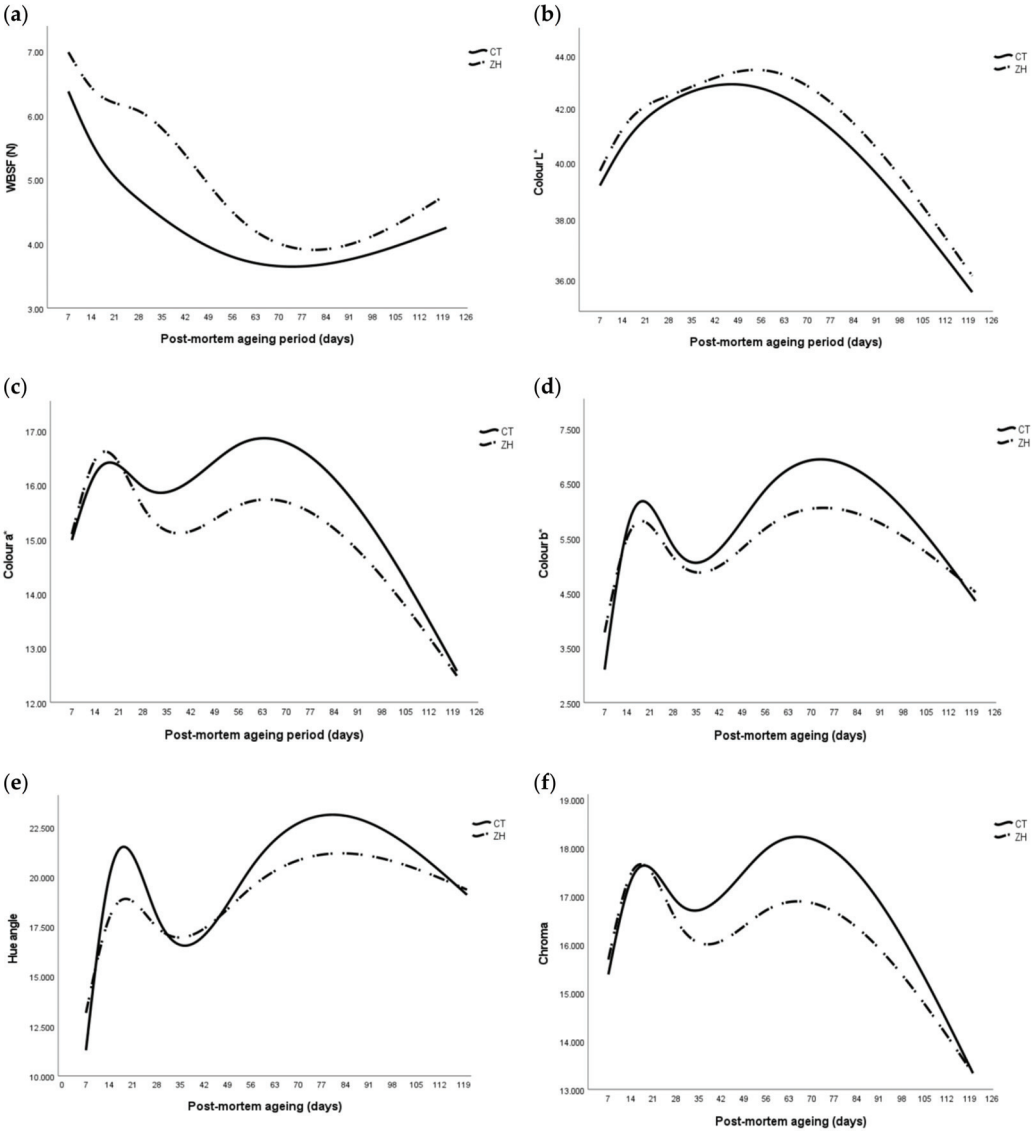

Figure 2. Multinomial logistic regression analyses for beef (**a**) WBSF and colour attributes (**b**) L*, (**c**) a*, (**d**) b*, (**e**) hue° and (**f**) chroma over extended aging periods for samples of bulls supplemented with zilpaterol hydrochloride compared to controls.

5. Conclusions

It was demonstrated that bulls fed the β-adrenergic agonists ZH-A or ZH-B containing zilpaterol hydrochloride (ZH) for 30 days significantly decreased meat tenderness due to increased WBSF values. Dietary supplementation with ZH in feedlot diets during the finishing period did not affect the meat quality differently during extended aging compared to the negative controls. No significant differences in meat quality were observed between ZH-A and ZH-B. The extended aging of meat samples significantly influenced all meat quality characteristics of feedlot bulls in all experimental groups. Although meat tenderness was initially negatively affected by ZH supplementation, it improved significantly over

extended post-mortem aging. ZH supplementation per se does not affect post-mortem meat quality more than untreated bulls, but extended aging may compromise beef quality if it exceeds 56 days.

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Article

Effects of Zilpaterol Hydrochloride Supplementation on Growth Performance, Carcass Characteristics and Meat Quality for Steers Differing in Breed Type

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Simple Summary: The β–adrenergic agonist zilpaterol hydrochloride (ZH) enhances skeletal muscle growth in beef cattle, resulting in a shift in the composition of gain. This results in improvements in feedlot performance and carcass yield but has been reported to decrease marbling score and beef tenderness. British (B) and British × Continental (BC) cattle possess inherent differences in muscle and fat composition and may respond differently to the growth-promoting mechanism of ZH. Therefore, the objective of this study was to determine the effects of ZH on growth performance, carcass characteristics and meat quality for B and BC steers. Steers with B (n = 76) or BC (n = 57) backgrounds were assigned to a control (no ZH) or ZH-supplemented treatment group. Cumulative growth performance was evaluated. Steers were subjected to ultrasound immediately before ZH supplementation and following withdrawal of ZH to evaluate changes in body composition. After harvest, carcass data were recorded, and muscle samples were collected to determine impacts on meat quality. Inclusion of ZH largely did not impact composition of gain and meat quality characteristics of B and BC cattle. Supplementation with ZH did improve loin muscle area and yield grade for steers but increased Warner–Bratzler shear force, indicating tougher steaks.

Abstract: To determine the effects of zilpaterol hydrochloride (ZH) on growth performance, carcass characteristics and meat quality for steers differing in breed type, steers with British (B; n = 76) or British × Continental (BC; n = 57) backgrounds were allocated to a randomized incomplete block design with a 2 × 2 treatment structure. Pens within each block × breed type were randomly assigned to either ZH (8.3 mg/kg of DM; fed for 20 d before slaughter, followed by a 3-day withdrawal) or control (CON; 0 mg/kg ZH). Steers were subjected to ultrasound immediately before ZH inclusion and following withdrawal to determine the influence of ZH on changes in longissimus muscle area (LMA), fat thickness and percent intramuscular fat (IMF). Carcass data were collected, and the longissimus lumborum was collected for analysis of tenderness, moisture percentage, crude fat content, collagen content, postmortem proteolysis and sensory attributes. The ZH × breed type interaction did not influence ($p > 0.05$) the feedlot performance, carcass or meat quality attribute traits evaluated, with the exception of moisture percentage. Responses among breed types were as expected for B vs. BC cattle types. Supplementation with ZH improved ($p < 0.05$) LMA and yield grade but increased Warner–Bratzler shear force.

Keywords: beef; beta-agonist; breed; carcass; meat quality; zilpaterol hydrochloride

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1. Introduction

The inclusion of zilpaterol hydrochloride (ZH, Zilmax®, Merck Animal Health, Summit, NJ, USA) in beef finishing diets has shown dramatic effects on skeletal muscle growth,

shifting the composition of gain from fat to lean and resulting in improvements in average daily gain (ADG) and gain-to-feed (G:F) efficiency in the feedlot as well as increases in dressing percentage, hot carcass weight (HCW) and the cutability of the carcass [1,2]. However, research has also revealed that this product has a negative impact on tenderness, which can lead to consumer dissatisfaction and potentially impact beef demand [3–5]. To optimize the use of ZH to balance both yield and palatability characteristics, it is necessary to understand how ZH mechanistically functions across varied groups of cattle, feeding strategies and management conditions.

British and British × Continental cattle have inherent differences in muscle and adipose composition that could result in different responses to ZH. Thus, it is necessary to determine how differing breed types respond to the growth-promoting mechanism of ZH. Furthermore, it is also important to determine the impact of this potentially divergent response to growth on tenderness, as the pathways through which this enhanced muscle growth is believed to be accomplished greatly affect postmortem aging [2,6], which impacts beef tenderness. Additionally, collagen content and quality are key regulators for beef tenderness [7] and could be affected by ZH due to the rapid increase in muscle growth from feeding the beta-agonist.

Much work has focused on the effects of feeding ZH to a single 'type' of cattle (e.g., [3,8–10]), while research comparing the impact of feeding ZH to divergent 'breed types' with the aim of optimizing management strategies is limited. Therefore, our hypothesis was that the inclusion of ZH would differentially impact the composition of gain in British versus British × Continental cattle, thereby influencing the mechanisms that regulate beef tenderness. The objective of this study was to determine the effect of ZH on growth performance, carcass characteristics and meat quality for cattle with variations in breed type.

2. Materials and Methods

2.1. Animals

The South Dakota State University (SDSU) Institutional Animal Care and Use Committee approved all procedures involving animals (Approval Number 13-028E). Cows at the SDSU Antelope Research station, with primarily Angus genetics, were artificially inseminated by 1 of 2 bulls. Bulls were either 100% Angus or 50% Angus × 50% Simmental from a common Angus sire. Clean-up bulls that were 100% Angus from the same common sire were used for 60 days post-artificial insemination. Therefore, all progeny were grandsons of a common sire that was a trait leader for carcass characteristics in the Angus breed. Steer progeny (n = 133) were transported as one group after weaning (average age at weaning = 184 d) to the University of Nebraska-Lincoln Panhandle Research and Extension Center Feedlot. All steers were fed a common 60% roughage and 40% concentrate [dry matter (DM) basis] backgrounding ration for 45 days prior to the start of the project. At the start of the trial, all steers were fed a 45% roughage and 55% concentrate (DM basis) ration and were stepped up using three rations over a 63-day period to reach the final ration of 16% roughage and 84% concentrate (DM basis; Table 1). All steers remained on this ration until marketed. All steers were implanted with 36 mg zeranol (Ralgro®, Merck Animal Health, Summit, NJ, USA) at day −2 of trial initiation. All steers were re-implanted at day 70 with 200 mg trenbolone acetate and 40 mg estradiol (Revalor®-XS, Merck Animal Health, Summit, NJ, USA).

Table 1. Finishing diet composition.

Ingredient	Diet DM, %
Dry-rolled corn	64.0
Wet distillers grains with solubles	15.0
Corn silage	15.0
Liquid supplement [1,2]	6.0
Nutrient composition, %	
Starch	51.40
NDF	15.46
CP	12.96
Fat	3.44
Ca	0.60
K	0.55
P	0.34
Mg	0.15
S	0.15

[1] Liquid supplement contained 0.6% urea, 1.6% Ca, 0.3% salt, 0.02% potassium chloride; supplement also formulated to provide a dietary DM inclusion of 0.3% salt, 60 mg/kg of Fe, 40 mg/kg of Mg, 25 mg/kg of Mn, 10 mg/kg of Cu, 1 mg/kg of I, 0.15 mg/kg of Se, 1.5 IU/g of vitamin A, 0.15 IU of vitamin D, 8.81 IU/kg of vitamin E. [2] Rumensin (30 g/ton) and Tylan (9 g/ton) were added via micromachine.

2.2. Experimental Design and Treatments

Steers representing British (B, Angus sired, n = 76) and British × Continental (BC, Angus × Simmental sired, n = 57) breed types were allocated to a randomized incomplete block design with 4 blocks of B and 3 blocks of BC. Treatments were arranged as a 2 × 2 factorial with 2 sire breeds and finishing diets fed with (ZH) or without (CON) ZH (8.3 mg/kg of DM; Zilmax®, Merck Animal Health, Summit, NJ, USA) for 20 days prior to slaughter as per product label instructions. Initial body weight (BW) were 272.2 ± 18.9 kg and 277.3 ± 18.9 kg (least squares means ± SEM) for B and BC, respectively. Using initial BW as the blocking factor, steers were stratified by initial BW into 3 blocks of 4 pens and 1 block of 2 pens of B only (9 or 10 head per pen) with ZH treatment randomly assigned within each breed type × block combination. The experimental design resulted in 4 BW blocks (3 complete and 1 incomplete, with only 2 pens for B represented), 7 pen replicates per ZH treatment, 8 pen replicates for B, 6 pen replicates for BC, 4 pen replicates for each ZH × B treatment combination, and 3 pen replicates for each ZH × BC combination. Following a 3-day withdrawal from ZH as per product label instructions, steers were marketed in 2 groups (153 days and 182 days on feed) when their 12th rib backfat thickness was estimated to average 1.0 cm. All steers in blocks 3 and 4 (heaviest initial BW blocks) were marketed in the first group (n = 76), and all steers in blocks 1 and 2 (lightest initial BW blocks) were marketed in the second group (n = 57).

2.3. Ultrasound Measurements

Real-time ultrasound measurements were collected and interpreted by the National CUP lab (Ames, IA) to determine 12th rib subcutaneous fat thickness, percent intramuscular fat (IMF) and LM area (LMA) for each steer using an Aloka 500V instrument (Aloka, Wallingford, CT, USA) equipped with a 17 cm linear array and 3.5 MHz transducer. Initial ultrasound measurements were collected 4 days prior to ZH inclusion, and final measurements were collected on the morning of harvest. Body weights were also collected at these time points. Differences (final–initial) in fat thickness, IMF and LMA were calculated to evaluate changes in carcass composition during the ZH feeding period.

2.4. Carcass Data and Sample Collection

Final BW was measured when steers were subjected to ultrasound on the morning of slaughter (final BW was reduced by 4% to represent a standard shrink). Steers were transported approximately 198 km to a commercial packing plant, where they were slaughtered under standard procedures. Steers were tracked through the harvest floor

to maintain animal identification. Hot carcass weight for each individual carcass was recorded. Longissimus muscle area, 12th rib backfat and percentage KPH were recorded by university-trained personnel. Marbling score and United States Department of Agriculture (USDA) Quality Grade were assigned by a USDA grader. Hot carcass weight, LMA, 12th rib backfat and KPH were then used to calculate USDA Yield Grade for each individual carcass [11].

Following carcass chilling (approximately 48 h), carcasses were ribbed between the 12th and 13th ribs on the right side and the exposed longissimus dorsi was allowed to bloom for approximately 30 min before objective color (L*, a* and b*) measurements were recorded. A Minolta colorimeter (model CR-310; Minolta Corp., Ramsey, MJ; 50 mm diameter measuring space and D65 illuminant) was used to obtain measurements from two locations on the right longissimus dorsi (medial and lateral) and averaged for each carcass. The right longissimus lumborum was collected from each carcass and transported under refrigeration (2.2 °C) to the SDSU Meat Laboratory in Brookings, SD. At approximately 72 h postmortem, samples were trimmed to 0.64 cm of external fat. The connective tissue, gluteus medius and multifidus dorsi were removed so that only the longissimus muscle remained. Beef samples were then immediately cut into 0.6 cm slices or 2.54 cm steaks. A 0.6 cm slice was removed from the anterior face of the longissimus lumborum, aged for 3 days and assigned for determination of moisture and crude fat percentage. Nine steaks (2.54 cm) were then fabricated with steaks 1, 3, 5, 7 and 9 being assigned for determination of Warner–Bratzler shear force (WBSF) and percent cook loss at 3, 7, 14, 21 and 28 days of aging, respectively. Steaks 2, 4 and 6 were utilized for sensory evaluation by a trained sensory panel at 3, 14 and 28 days of aging, respectively. Steak 9 was assigned for collagen evaluation and was aged for 14 days to determine collagen content and solubility. Following fabrication of steaks, the next five 0.6 cm slices were assigned for determination of troponin-T proteolysis at 3, 7, 14, 21 and 28 days of aging, respectively. All samples were vacuum-sealed and aged in the absence of light at 2–3 °C and were immediately frozen (−20 °C) after each specified aging period and checked regularly for seal integrity until thawed for specific analyses.

2.5. Moisture and Crude Fat Percentage

Moisture percentage and ether extractable fat percentage for the longissimus muscle were determined according to procedures described by Mohrhauser et al. [12]. Steaks were thawed slightly and trimmed of external fat and additional muscles; then, samples were minced, immersed in liquid nitrogen and powdered using a commercial blender. Duplicate powdered samples (5 g) were weighed into dried tins, covered with dried filter papers and dried in an oven at 101 °C for 24 h. Dried samples were then reweighed after cooling. Moisture content was calculated as the difference between pre- and post-drying sample weights and expressed as a percentage of the pre-drying sample weight. Dried samples were extracted with petroleum ether in a side-arm Soxhlet extractor (Thermo Fischer Scientific, Rockville, MD, USA) for 60 h followed by drying at room temperature and subsequent drying in an oven at 101 °C for 24 h. Dried, extracted samples were cooled then reweighed. Crude fat was calculated as the difference between pre- and post-extraction sample weight and expressed as a percentage of the pre-extraction sample weight.

2.6. Warner–Bratzler Shear Force and Cook Loss

To determine objective tenderness, steaks were thawed for 24 h at 4 °C for shear force evaluation. All steaks were weighed prior to cooking on an electric clam-shell grill (George Forman Grilling Machine, Model GRV-120 GM, Lake Forest, IL, USA) to a target internal peak temperature of 71 °C. Peak internal cooked temperature measurements were recorded for each steak using a handheld thermometer (Thermoworks, Super-Fast® Pocket Thermometer Model: RT600C, Lindon, UT, USA) placed near the geometric center of the steak. After cooking, all steaks were allowed to cool to room temperature before they were reweighed to determine cook loss. Cook loss was reported as a percentage of the raw

weight using the following equation: [(raw weight − cooked weight)/raw weight] × 100. Cooked steaks were cooled for 24 h at 4 °C before removing 6 cores (1.27 cm in diameter) parallel to the muscle fiber orientation [13]. A single peak shear force measurement was obtained for each core using a Warner–Bratzler shear machine (G-R Electric Manufacturing Company, Manhattan, KS, USA). Shear force values of each core (n = 6 per steak) were averaged to determine the peak shear force value for each steak.

2.7. Collagen Content and Solubility

To determine if ZH differentially impacted growth and tenderness parameters for different breed types of cattle through alterations in connective tissue, 14-day aged steaks from 2 heads per pen (n = 28) closest to pen average for WBSF were selected and analyzed for collagen content and solubility. Total intramuscular collagen and percent soluble collagen of muscle samples were determined using a modified procedure of Hill et al. [14], as described by Gerrard et al. [15].

2.8. Postmortem Proteolysis

To determine if ZH differentially impacted growth and tenderness parameters for different breed types of cattle through changes in proteolysis, longissimus muscle samples were selected from slices that had been aged for 3, 14 or 21 days from 2 heads per pen closest to pen average for WBSF. Following the assigned aging period, each sample was frozen until preparation for SDS-PAGE and Western blotting. Immunoreactive intact troponin T (a classical marker of postmortem proteolysis) was identified and quantified as a measure of the extent of postmortem proteolysis as described by Mohrhauser et al. [16], with slight modifications. The abundance of troponin T is expressed relative to the abundance of actin and normalized back to two common molecular weight standards (Bio Rad #161-0374 Precision Plus Protein Dual Color Standards Bands 50 and 37 kD).

2.9. Trained Sensory Panel

Eight sensory panelists were trained to evaluate meat quality attributes for longissimus lumborum steaks according to the American Meat Science Association training guidelines appropriate for the study [13]. Steak samples were evaluated for juiciness (1 = extremely dry; 18 = extremely juicy), tenderness (1 = extremely tough; 18 = extremely tender) and beef flavor (1= extremely bland; 18 = extremely intense) on an eight-point hedonic scale. Steaks were cooked on an electric clamshell grill (George Forman Grilling Machine, Model GRV-120 GM, Lake Forest, IL, USA) to an internal temperature of 71 °C. After cooking, steaks were rested for five minutes and then cut into 2.5 × 1 × 1 cm samples. Two cubes were placed into a prelabeled plastic cup, covered with a plastic lid to retain heat and moisture and held in a warming oven (Metro HM2000, Wilkes-Barre, PA, USA) at 60 °C until served. Evaluations were performed according to American Meat Science Association guidelines [13]. Sample evaluations were alternated by treatment to reduce first and last order bias. Samples were served to panelists in a randomized fashion, and panelists were isolated in individual booths, under red lights to limit observation of visual differences.

2.10. Statistical Analysis

Continuous response variables, including growth, measured carcass traits and meat quality measurements were analyzed in a 2 × 2 factorial treatment structure in a randomized incomplete block design using the mixed procedure of SAS version 9.3 (SAS Institute, Cary, NC, USA). Pens served as the experimental units and were included as a random effect. Breed type, ZH treatment and their interaction were included as fixed effects. The Kenward–Roger option was used to calculate denominator degrees of freedom. Peak internal cooked temperature was included as a covariate for WBSF, cook loss and sensory scores. Least squares means were calculated and separated by F-tests for fixed effects. The breed type × ZH interaction was not significant ($p > 0.05$) for any response variable except steak moisture percentage ($p = 0.0496$). For this interaction, lsmeans were calculated and

separated using least significant differences (PDIFF) with a Tukey adjustment. As the Quality and Yield Grade classifications for each carcass conform to binomial distributions, the proportions (number graded in the class divided by number in the pen) for carcasses in each grade classification were analyzed as binomial distributions in the GLIMMIX procedure of SAS using the same model as above. Proteolysis and WBSF values were analyzed using day of aging as a repeated measures using the mixed procedure of SAS. Least square means for the aging effect were calculated and separated using the Tukey adjustment. The aging effect did not interact with treatments for any response variable ($p > 0.05$). Reponses were considered significant at $p \leq 0.05$, and tendencies were considered at $p > 0.05$ to $p \leq 0.10$.

3. Results

3.1. Feedlot Performance and Carcass Traits

The ZH × breed type interaction did not influence ($p > 0.05$) any of the feedlot performance or carcass traits evaluated in this study (Table 2).

Table 2. Least square means for performance and carcass traits as affected by main effects of breed type [1] and zilpaterol hydrochloride (ZH) [2] supplementation.

	Breed Type				ZH, mg/kg of DM				ZH × Breed
Item	**B**	**BC**	**SEM [3]**	***p* > F [4]**	**0**	**8.3**	**SEM [3]**	***p* > F [4]**	***p* > F [4,5]**
Final body weight, kg	580	588	14.65	0.453	578	590	14.03	0.282	0.703
Cumulative average daily gain, kg	1.76	1.79	0.04	0.521	1.75	1.81	0.03	0.222	0.543
Dry matter intake, kg·hd^{-1}·d^{-1}	10.0	10.1	0.58	0.574	10.1	10.0	0.58	0.423	0.143
Gain:Feed	0.18	0.18	0.02	0.831	0.17	0.18	0.02	0.093	0.371
ZH Supplementation Period									
Fat thickness change [6], cm	0.10	0.07	0.04	0.429	0.11	0.07	0.04	0.330	0.263
Longissimus area change [6], cm^2	2.85	1.58	1.13	0.279	1.53	2.89	1.04	0.221	0.399
Intramuscular fat change [6], %	0.26	0.39	0.20	0.449	0.59	0.06	0.19	0.012	0.196
Period average daily gain, kg	1.04	1.12	0.10	0.556	0.89	1.27	0.10	0.020	0.328
Hot carcass weight, kg	356	362	9.77	0.461	352	366	9.20	0.102	0.768
Fat thickness, cm	1.46	1.30	0.08	0.178	1.42	1.34	0.07	0.433	0.899
Longissimus muscle area, cm^2	88.1	92.0	2.26	0.028	86.3	93.9	2.18	<0.001	0.499
Yield grade [7]	2.94	2.62	0.10	0.033	2.95	2.60	0.09	0.023	0.875
Marbling score [8]	588	488	25.9	<0.001	541	531	24.9	0.575	0.851
USDA Yield Grade [7]									
Yield Grade 2, %	32.4	57.4	7.87	0.044	34.9	54.6	7.66	0.086	0.714
Yield Grade 3, %	68.7	40.2	8.77	0.019	65.0	44.3	8.47	0.059	0.616
USDA Quality Grade									
Prime, %	17.3	11.1	7.41	0.650	14.6	13.3	7.11	0.918	0.918
Upper 2/3 choice, %	68.4	33.1	6.28	0.006	51.5	50.2	6.87	0.895	0.355
Lower 1/3 choice, %	17.6	36.8	6.44	0.076	21.9	30.7	6.34	0.356	0.911
Select, %	15.1	12.7	7.03	0.821	18.3	10.4	7.62	0.517	0.759

[1] Breed type was British (B) or British × Continental (BC); [2] Zilpaterol hydrochloride was fed at 0 (CON) or 8.3 mg/kg (ZH) of DM during the final 20 d of the finishing period; [3] Standard error of the mean; [4] Probability of a greater F; [5] The breed type × ZH interaction did not affect ($p > 0.05$) any traits; [6] Change in ultrasound backfat thickness, LM area and intramuscular fat during the 20 day ZH feeding period; [7] Lower USDA yield grade represents a leaner carcass; [8] 400 = Slight^0; 500 = Small^0; 600 = Modest^0.

Breed type did not affect ($p > 0.05$) final BW, cumulative ADG, DMI or G:F for steers (Table 2). The changes in ultrasound fat thickness, LMA, percent IMF and ADG during the ZH feeding period were not different ($p > 0.05$) between the cattle breed types investigated in this study (Table 2). Carcass evaluation revealed no differences ($p > 0.05$) in HCW or fat thickness between breed types. However, BC had a larger ($p < 0.05$) LMA and improved ($p < 0.05$) Yield Grade compared with B carcasses. The marbling score was increased ($p < 0.001$) in B carcasses compared with BC carcasses (Table 2). British × Continental steers produced a greater proportion ($p < 0.05$) of Yield Grade 2 and fewer Yield Grade 3 ($p < 0.05$) carcasses than B steers (Table 2). However, a greater proportion ($p < 0.01$) of B carcasses were classified as Upper 2/3 Choice. There was no difference between breed types for the number of carcasses graded as Select. However, there was tendency for an

increase ($p < 0.10$) in the proportion of BC carcasses classified in the Lower 1/3 Choice grade compared with B carcasses (Table 2).

Supplementation with ZH did not affect ($p > 0.05$) final BW, overall ADG or DMI over the entire feeding period, but tended to improve ($p < 0.10$) overall G:F (Table 2). However, ADG during the 20 d ZH feeding period was improved ($p < 0.05$) by ZH supplementation. The differences between ultrasound measurements 4 days prior to ZH supplementation and on the day of slaughter revealed ZH-treated cattle did not affect ($p < 0.05$) ultrasound measures for LMA or fat thickness compared with CON, while ZH reduced ($p < 0.05$) ultrasound measures for IMF percentage compared to CON cattle during the supplementation period (Table 2). Supplementation with ZH did not influence HCW ($p > 0.05$). However, the carcasses of ZH-treated steers had greater ($p < 0.001$) LMA and improved ($p < 0.05$) Yield Grades. Despite the greater accretion of IMF during the ZH feeding period, CON carcasses had similar ($p > 0.05$) marbling scores to ZH carcasses. The trend for carcass fat thickness was similar ($p > 0.05$), in agreement with similar changes between treatments in ultrasound fat thickness during the ZH feeding period (Table 2). Additionally, steers supplemented with ZH tended to produce a greater ($p < 0.10$) percentage of Yield Grade 2 carcasses and a lower ($p < 0.10$) percentage of Yield Grade 3 carcasses than CON steers (Table 2). Supplementation with ZH did not affect ($p > 0.10$) the distribution of Quality Grades compared with CON carcasses.

3.2. Meat Quality Traits

Steaks from B carcasses had greater Minolta L* and b* color values ($p < 0.05$) compared to BC steaks, while a* values did not differ ($p > 0.05$) between breed types (Table 3). Steaks from BC carcasses had decreased intramuscular fat percentage ($p < 0.001$) compared to steaks from B carcasses (Table 3). An interaction was observed for breed type × supplementation treatment ($p < 0.05$) for steak moisture percentage. Steaks from the BC × CON treatment had greater ($p < 0.05$) moisture percentages (71.27 ± 0.446%) than steaks from B cattle in either ZH treatment (69.27 ± 0.422% and 70.09 ± 0.422% for B × CON and B × ZH, respectively), with BC × ZH having intermediate and similar ($p > 0.05$) moisture percentages to all other treatments (71.07 ± 0.446%). Breed type did not influence ($p > 0.05$) WBSF values; however, there was a tendency ($p < 0.10$) for increased cook loss for B steaks. Collagen content and solubility did not differ ($p > 0.05$) among breed types. British steers had less ($p < 0.05$) intact troponin T compared to BC steers (Table 3). No differences were detected ($p > 0.05$) among breed types for sensory panel ratings for tenderness, juiciness, beef flavor, off flavor or overall acceptability (Table 3).

Supplementing with ZH did not influence ($p > 0.05$) L* or a* but tended ($p = 0.10$) to lower b* (Table 3). Steaks from cattle supplemented with ZH did not differ ($p > 0.05$) in moisture or fat percentages. Supplementing cattle with ZH increased WBSF values ($p < 0.001$) and percent cook loss ($p < 0.001$). Zilpaterol hydrochloride supplementation did not influence ($p > 0.05$) collagen content and solubility or the degradation of troponin T. (Table 3). Sensory panel scores indicated that tenderness, juiciness and overall acceptability were reduced ($p < 0.001$), and beef flavor and off flavor tended ($p < 0.10$) to be reduced by ZH supplementation.

Aging did not interact ($p > 0.10$) with treatments for WBSF and intact troponin T percentage. However, as expected, WBSF was reduced ($p < 0.001$) as steaks aged (4.55, 3.95, 3.37, 3.16 and 3.04 ± 0.097 kg for 3, 7, 14, 21 and 28 days of aging, respectively). In addition, intact troponin T percent was reduced ($p < 0.001$) with postmortem aging (0.850, 0.342 and 0.221 ± 0.11% at 3, 14 and 21 days, respectively).

Table 3. Least square means for meat quality as affected by main effects of breed type [1] and zilpaterol hydrochloride (ZH) [2] supplementation.

Item	Breed Type				ZH, mg/kg of DM				ZH × Breed
	B	BC	SEM [3]	$p > F$ [4]	0	8.3	SEM [3]	$p > F$ [4]	$p > F$ [4,5]
Minolta color value									
L*	44.6	42.6	0.41	0.004	43.7	43.6	0.35	0.798	0.296
a*	23.0	22.7	0.44	0.134	23.1	22.7	0.43	0.102	0.478
b*	10.3	9.7	0.31	0.015	10.2	9.8	0.30	0.067	0.467
Intramuscular fat content, %	7.60	5.47	0.49	<0.001	6.88	6.18	0.46	0.109	0.142
Warner–Bratzler shear force, kg	3.57	3.66	0.07	0.281	3.04	4.19	0.06	<0.001	0.089
Cook loss, %	21.8	21.3	0.19	0.058	21.1	22.0	0.18	<0.001	0.651
Total collagen content, mg/g [6]	4.5	4.3	0.23	0.558	4.5	4.3	0.20	0.310	0.788
Soluble collagen content, % [7]	17.8	16.3	0.70	0.313	16.8	17.4	0.70	0.809	0.893
Intact troponin T, % [8]	0.37	0.58	0.07	0.032	0.42	0.53	0.07	0.232	0.257
Sensory panel scores [9]									
Tenderness	5.5	5.4	0.02	0.719	6.3	4.6	0.22	<0.001	0.784
Juiciness	5.1	4.7	0.02	0.117	5.5	4.4	0.20	<0.001	0.192
Beef flavor	5.5	5.2	0.02	0.216	5.5	5.1	0.16	0.079	0.923
Off flavor	7.7	7.6	0.01	0.399	7.8	7.6	0.11	0.091	0.990
Overall Acceptability	5.5	5.2	0.02	0.355	6.1	4.6	0.19	<0.001	0.099

[1] Breed type was British (B) or British × Continental (BC); [2] Zilpaterol hydrochloride was fed at 0 (CON) or 8.3 mg/kg (ZH) of DM during the final 20 d of the finishing period; [3] Standard error of the mean; [4] Probability of a greater F; [5] The breed type × ZH interaction did not affect ($p > 0.05$) any traits; [6] Calculated as mg collagen/g wet meat weight; [7] Calculated as a percent of total collagen; [8] Intact Troponin T expressed relative to the abundance of actin and normalized back to two molecular weight standards; [9] Sensory scores using an eight-point hedonic scale for tenderness (8 = extremely tender to 1 = extremely tough), juiciness (8 = extremely juicy to 1 = extremely dry), beef flavor (8 = extremely intense to 1 = non-detectable), off flavor (8 = non-detectable to 1 = extremely intense), overall acceptability (8 = extremely acceptable to 1 = extremely unacceptable).

4. Discussion

Beta-adrenergic agonists commonly elicit compositional changes by increasing muscle synthesis and decreasing adiposity for growing animals [2,17]. Previous research has investigated the impact of ZH on performance and carcass characteristics within similar breed types of cattle such as calf-fed Holsteins [18,19], however it is unknown whether cattle of divergent genetic backgrounds will respond differently to ZH. Therefore, the objective of this study was to determine whether cattle from different breed types would have a differential response to ZH supplementation.

The general lack of breed type × ZH treatment interactions suggested that the breed types evaluated in this study did not differentially respond to ZH supplementation. Gruber et al. [20] investigated the effects of ractopamine hydrochloride on feedlot steers of varying genetic backgrounds and reported no interaction between treatment and breed type. Ractopamine hydrochloride functions to increase protein synthesis, while ZH has been shown to both increase protein synthesis and decrease degradation, resulting in increased LMA, decreased fat thickness and higher-yielding carcasses [8]. Although the response to the beta-agonist's main effect differed between the study of Gruber et al. [20] and the current study, the lack of interaction in both studies indicated that steers from different genetic backgrounds respond similarly to beta-agonists, regardless of the mode(s) of action for the specific beta-agonist. McEvers et al. [21] sorted cattle into tenderness genotype groups based on a commercial DNA panel and also detected no ZH × genotype interactions, further supporting the general lack of differential response to beta-agonist supplementation found in the current study. The only breed type × ZH supplementation interaction observed in the current study was in relation to the moisture content of steaks. However, differences among these moisture values are likely not detectable by consumers.

The response by breed types was as expected. Gruber et al. [20] reported similar feedlot performance responses as the current study with British × Continental cattle displaying similar ADG, DMI and G:F results to British cattle when fed diets with or without the beta-agonist ractopamine. However, in Gruber et al. [20], the Continental cross cattle had greater initial and final BWs, which was not the case in the current study

which evaluated the beta-agonist zilpaterol. This difference between studies for BW was further observed for HCW, which was similar among the breed types herein but heavier in Gruber et al. [20]. Gruber et al. [20] and the current study agreed that LMA was greater for British × Continental cattle, which lead to lower USDA Yield Grades, while marbling score was lower for British × Continental cattle. However, Gruber et al. [20] reported that backfat thickness was reduced in Continental crosses, while it was similar to British cattle in the current study. Although, there were slight shifts in percentages of carcasses in each USDA Yield and Quality Grade class, both studies displayed agreement that the incorporation of Continental breeds reduced USDA Yield (a lower grade classification equates to a higher lean yield in carcasses) and Quality Grades. Both studies were in agreement that WBSF was not affected by breed composition. However, sensory panel results differed. The current study indicated that panelists did not detect differences in sensory traits among breed types, while Gruber et al. [22] reported that panelists found British cattle to have improved tenderness, juiciness and beef flavor, with a lower off flavor than British × Continental crosses. Differences among studies may be related to the actual British or Continental breeds used in each study. Overall, the results indicate that the utilization of Continental breeds results in higher-yielding carcasses but reductions in marbling.

In this study, ADG was increased by ZH supplementation during the ZH feeding period, but this did not contribute to an overall improvement in ADG for the entire finishing period. Additionally, final BW and overall DMI were not affected by ZH supplementation, although G:F tended to be improved. Avendaño-Reyes et al. [23] also reported that the inclusion of ZH did not influence DMI but did improve G:F. The inclusion of ZH did not influence HCW in this study, which is similar to the findings of Van Bibber-Krueger et al. [24] and Bloomberg et al. [25]. In contrast, others [8,9,19,21] have reported that feeding ZH increased HCW. The increases in LMA and decreases in Yield Grade by feeding ZH were in agreement with others [8,9,21]. However, the effect of ZH on fat deposition varied among studies. Similar to this study, no difference in fat thickness was reported by McEvers et al. [21], while the fat thickness was reduced by ZH in reports by Scramlin et al. [8] and Rathmann et al. [9]. Furthermore, the marbling score was reduced by ZH in some reports [9,21] but unchanged in others [8], including in the current study. Additionally, the shift to leaner Yield Grades in response to ZH supplementation as found in the current study has been reported by others [9,21]. Similar to this study, others have reported that ZH supplementation did not influence objective color evaluation [25,26]. In agreement with the current study, the Warner–Bratzler shear force has been consistently increased by ZH supplementation [8,10], indicating a toughening effect. However, collagen content and degradation of troponin-T did not differ among treatments, suggesting that differences in tenderness are related to other mechanisms or the degradation of other proteins. Rathmann et al. [10] also reported no differences in collagen content in response to ZH supplementation, while Kellermeier et al. [4] reported no effect of ZH on degradation of desmin. Also in agreement with this study, Garmyn et al. [27] indicated that sensory panels detected decreased tenderness, beef flavor and overall liking in response to ZH supplementation. The only disagreement was that juiciness was decreased in the current study, but no difference in juiciness was detected by the sensory panel reported by Garmyn et al. [27]. The reduction in juiciness detected by the sensory panel for steaks from ZH supplemented cattle in the current study could be related to the increased cook loss detected in the ZH samples.

5. Conclusions

We reject our hypothesis that the inclusion of ZH would differentially impact the composition of gain and meat quality characteristics in British versus British × Continental cattle. This study suggests a consistent response to ZH across the breed types of cattle considered herein. This may allow for the targeted use of ZH to influence economically important carcass traits in response to beef market signals. For example, ZH supplementation may be warranted when market signals demand leaner, higher-yielding carcasses.

Conversely, marketing channels that value consumer palatability may consider limiting ZH utilization due to negative effects on tenderness and other sensory traits. However, further confirmation of this effect across additional breeds would be important before industry adoption.

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Article

Carcass and Meat Quality Traits in Female *Lidia* Cattle Slaughtered at Different Ages

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Simple Summary: The *Lidia* breed is an autochthonous Spanish breed linked to the pasture-based *dehesa* system, where extensive livestock farming is practiced and plays an important role in ecosystem conservation and rural development. This system is linked to the conservation of biodiversity and sustainable agricultural practices. However, it faces important challenges that make it necessary to explore new strategies to help farmers. In this study, we sought to understand the carcass and meat traits (technological and sensorial) as a strategy that would allow it to compete and differentiate itself from other meats. We used 300 *Lidia* females slaughtered at different ages. Age at slaughter influenced meat quality, with particular importance on sensory variables such as flavor, juiciness, overall tenderness and overall acceptability. Technological variables were acceptable and the results of a trained sensory panel pointed to the good qualities of this meat. To our knowledge, this is the first work evaluating carcass traits and the technological and sensory quality of meat with a large sample of animals of the *Lidia* breed.

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Abstract: The aim of this study was to assess the carcass and meat quality of female *Lidia* cattle slaughtered at different ages, in order to deepen our understanding of the breed's unique characteristics. The effect of slaughter age on carcass traits and meat quality attributes of m. *Longissimus* was investigated in *Lidia* heifers (n = 200) and cows (n = 100) reared and finished in an extensive system. The animals were slaughtered at 24–36 months (Heifer I), 36–48 months (Heifer II) or >48 months (Cull cow). The carcasses (~120 kg) presented poor conformation (O, O+) and medium fatness (2, 2+). The dissection of the 6th rib yielded mean values of 58.6%, 14.3% and 24.8% for lean, fat and bone, respectively. The cows had a higher proportion of dissectible fat ($p < 0.05$). Subcutaneous fat was classified as dark and yellowish, and meat (aged for 21 days) as dark (L* = 25.5), reddish (a* = 14.4) and moderately yellowish (b* = 12.9), with acceptable water-holding capacity (TL = 5.34%; DL = 0.97%; PL = 8.9%; CL = 22.1%) and intermediate tenderness (WBSF = 4.6 kg/cm^2). The b* value of meat was higher ($p < 0.05$) in cull cows. The meat of cull cows was more yellowish ($p < 0.05$) and obtained higher scores for flavor ($p < 0.05$), juiciness $p < 0.01$), overall tenderness ($p < 0.001$) and overall acceptance ($p < 0.001$).

Keywords: *Lidia* cattle; carcass traits; meat quality; sensory panel; local breeds

1. Introduction

The *Lidia* cattle breed holds a significant position among Spanish native breeds, due both to its census and geographical distribution [1]. This breed has experienced relative

geographic isolation, resulting in subpopulations or "*encastes*" with notable differentiations [2]. Pelayo et al. [3] identifies the *Lidia* breed as a racial grouping arising from selective pressures for behavioral phenotypes [4].

The farming system of the *Lidia* breed has evolved in pasture-based systems predominantly in the landscapes of Spanish *dehesas* [5]. With grasslands interspersed with oak trees (*Quercus* spp.), these areas offer an ideal environment for the small- to medium-sized *Lidia* breed. From an environmental perspective, breeding *Lidia* cattle in *dehesas* positively impacts biodiversity conservation and animal welfare, promoting sustainable agricultural practices [6]. Additionally, the presence of the *Lidia* breed in rural areas plays a pivotal role in rural development, offering an economic alternative to counteract depopulation and generating sustainable employment [7].

Traditionally reared for bullfighting by emphasizing physical and temperamental traits, the conservation of the *Lidia* breed faces the need to explore new perspectives for its potential survival. In a global context where people demand evolution and sustainability, a transition towards high-quality meat production emerges as a strategic direction. Breeding cattle in Spanish *dehesas*, using natural pastures and agricultural residues, not only improves meat quality but also contributes to addressing existing environmental sustainability issues [8].

This transition aligns with broader trends in the European Union, which recognize meat from native breeds as being of higher quality [9]. The EU currently promotes meat differentiation strategies, endorsing extensive livestock farming and certification systems based on origin and native breeds [10]. In Spain, the 100% Autochthonous Breed Logo serves as a quality mark, highlighting distinctive attributes, environmental relevance, and genetic heritage [11]. Therefore, amid the growing interest in sustainability, biodiversity, agroforestry systems and animal welfare, there is a unique opportunity to position Lidia meat differentially.

Despite the *Lidia* breed significance, an incomplete understanding of the quantitative and qualitative potential of its meat contrasts with the imperative to enhance its economic value. This is particularly crucial for female heifers unsuitable for reproduction and adult cows intended for the meat market, as highlighted by Buxadé [12]. Prior to slaughter, these animals undergo a four-month feed management period, incorporating concentrates and high-quality forage.

In the pursuit of differentiation, meeting consumer expectations for quality becomes essential [13]. Therefore, the aim of this study was to assess the carcass and meat quality of female *Lidia* cattle slaughtered at different ages, in order to deepen our understanding of the breed's unique characteristics. This research intends to develop new insights into livestock adaptability, contributing to a broader knowledge of the diversity and sustainability of farming systems. Additionally, this study sought to provide robust empirical data supporting the potential differentiation and market positioning of *Lidia* meat, aligning with the increasing consumer criteria of sustainability, origin and quality.

2. Materials and Methods

2.1. Animals

This study included 300 carcasses from *Lidia* females collected from ten farms selected to represent existing diversity. Animals were slaughtered at three ages: I (Heifer I): 24–36 months old, II (Heifer II): 36–48 months old, III (Cull cows) > 48 months old [14]. Thirty animals were evaluated on each farm, ten for each slaughter age.

Animals were selected from farms that follow the traditional production system of wild cattle. These breeding conditions are characterized by weaning at 7–8 months of age, open housing throughout the year with very low stocking rates, all-year grazing and supplementation with forage and concentrates in periods of grass scarcity (usually summer and winter). Four months prior to slaughter, the animals were supplemented with 3 kg of concentrate per day. A more detailed description of the production system can be found in the bibliography [15,16].

2.2. Carcass and Meat Quality Analyses

Before slaughter, animals were stunned using captive-bolt, complying with the current European regulations [17]. After slaughter, carcasses were suspended vertically using the Achilles method. At 1 h *post-mortem*, the carcasses were graded for conformation and fatness by trained staff using the EUROP system [18]. Conformation (CS) and fatness (FS) were scored on a 15-point scale (1—very bad conformation; 15—very good conformation; 1—very low fatness; 15—very high fatness, respectively), after which the carcasses were chilled and stored at 4 °C for approximately 24 h. Afterwards, the carcasses were split along the spinal column into two equal parts and the left-half carcass weights were recorded.

The ultimate pH (pH_{24}), meat and subcutaneous fat color and morphological measurements were assessed on the left side of each carcass. The pH_{24} was measured using a Hanna HI9025 portable pH-meter (Hanna Instruments, Laval, QC, Canada) with a penetrating glass electrode on the Longissimus thoracis muscle at the level of the 13th thoracic vertebra of the right side, at right angles to the sagittal plane surface. A Minolta 2600d spectrophotocolorimeter (Konika Minolta, Osaka, Japan), standardized against a white tile (L* = 97.78, a* = 0.19, b* = 1.84), with a D65 illuminant, an angle of 10° and an aperture size of 8 mm, was used to assess the color in CIELab* space of the m. *Rectus abdominis* and subcutaneous fat [19]. Readings for fat color were taken on subcutaneous fat covered with plastic food wrap (calibration was performed using the food wrap to maintain the integrity of the results). Three different locations were scanned and averaged for statistical analyses. Chroma (C*) and hue (h*) were calculated using the mathematical formula described by ISO regulations [19].

Standard measurements were then taken on the left half-carcass [20]. Length, depth of chest, hind-limb length and hind-limb perimeter of the carcasses were recorded. The left half-carcasses were then separated between the 5th and 6th thoracic vertebrae as forequarter and hindquarter. To assess the tissue composition, the 6th rib joint was removed by cutting the length of the bone at the limit of the m. *Serratus dorsalis* [21], which was weighed and dissected into lean, total fat (subcutaneous and intermuscular), bone and waste tissues (blood vessels, tendons).

For the instrumental and sensory analysis, a boneless section of the m. *Longissimus*, including the 12th and 13th thoracic vertebrae, and the m. *Longissimus lumborum* corresponding to the first two lumbar vertebrae, were removed from the left half-carcass. They were then packaged, aged at 4 °C for 21 days and frozen at −20 °C until the required evaluation and analysis. After this process, samples were removed from their bags and dried carefully with blotting paper. The pH and meat color (after 30 min blooming at ambient temperature) values were recorded as previously performed in the fresh carcass.

The water-holding capacity (WHC) was determined as thawing (TL), pressure (PL), drip (DL) and cooking (CL) losses. For TL evaluation, each sample was weighed frozen and thawed after a period of 24 h at 4 °C. The PL was determined following the Grau and Hamm method with the modifications described by Beriain et al. [22], and expressed as the percentage of juice expelled after the compression of 5 g meat samples with 2.25 kg applied for 5 min. The DL was determined by the method described by Honikel et al. [23]: a piece of meat (20 × 20 × 25 mm) devoid of connective tissue and fat was lightly blotted, weighed and suspended in a plastic bottle, ensuring that there was no contact with the walls, and placed in a refrigerator at 4 °C for 24 h [24]. The samples were then lightly blotted and reweighed. The DL was expressed as a percentage of initial weight. The CL was evaluated on meat samples of similar geometry, individually placed in plastic bags in a water bath at 90 °C until the internal temperature reached 70 °C (monitored by thermocouples inserted in the core) and cooled until it had fallen to 4 °C. They were taken from the bags, dried with a blotting paper and weighed. The CL was expressed as the percentage loss related to the initial weight. Then, for Warner–Bratzler shear force assessment, ten cuboid cores (1 cm^2 × 2.5 cm) from each cooked steak were removed parallel to the predominant muscle fiber orientation and sheared using a Texture Analyzer (Model TA.XT-2, Texture Analyzer® Stable Micro Systems, Surrey, UK) equipped with a Warner–Bratzler shear device (25 kg

load cell) and a crosshead speed of 200 mm/min. The down stroke distance was 3 cm (the probe should cut the meat completely). The ten peak shear forces recorded per sub-sample were averaged.

2.3. Sensory Analysis

Sensory analysis of three animals per slaughter age were carried out [25]. A panel composed of sixteen panelists was recruited (8 men and 8 women), with an average age of 37 + 8 years old and previous experience in beef sensory evaluation. Panelists were selected and trained [26] in three sessions with the scale and attributes to utilize. According to Cittadini et al. [27], a control analysis of the trained panel was carried out using the Panel Analysis procedure of the XLSTAT-Sensory software version 2023.1.6.

A total of three sessions were carried out and each panelist tasted three samples of each slaughter age in a randomized order (a total number of nine samples were tasted by each panelist during the three sessions). No information about the experiment was provided before sessions. The sensory traits studied included: color, odor intensity, flavor intensity, juiciness, overall tenderness and overall acceptance [28]. Each variable was scored using a 1-to-9 category scale (low to high intensity, respectively).

This analysis was carried out at the facilities of the University of Córdoba (Spain) in a laboratory equipped in line with ISO standards [29]. Steak samples were thawed overnight prior to the test at 2–4 °C and then taken out, cut to a 2.5 cm thickness and placed in a room until they reached a temperature of 17–19 °C. Meat samples were cooked in an oven (Gastro M6, IberGastro, Lucena, Spain) preheated at 190 °C until an internal temperature of 70 °C was reached, monitored with type K thermocouples (HH374 Omega, Omega Engineering Inc., Norwalk, CT, USA). The samples were trimmed of any external connective tissue, cut into 2 cm side cubes, wrapped individually in coded aluminum foil (three-digit) and placed in hot plates at 50 °C until tasted, for no longer than 15 min. They were then presented together on white plates. The order of tasting was designed and explained to panelists trying to avoid the "first-order carry-over" effect [30]. Unsalted cookies and double-distilled deionized water were provided to clean the palate between samples.

2.4. Statistical Analyses

Firstly, a Kolmogorov–Smirnov test was used to verify normality, a Durbin–Watson test to detect the absence of autocorrelation of the residues and heteroscedasticity was evaluated using the White test [31]. The bivariate association between the carcass and meat traits was explored using Pearson correlations. A mixed model was used (XLSTAT version 2023.1.6) to examine the effect of the slaughter age on carcass and meat quality traits. The slaughter age was introduced as a fixed factor, while the farm was introduced as a random effect. A second linear mixed model was built to evaluate the specific effect of slaughter age on sensorial attributes. In this model, the session and the panelist were included as a random term. The pairwise differences between least-square means were assessed using the Student–Newman–Keuls (SNK) method. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Carcass Traits

The left half-carcass weight (average 59.5 kg) significantly increased with age at slaughter ($p < 0.001$), and similar trends ($p < 0.01$) were observed for carcass measurements and the compactness index (Table 1). Carcasses were classified as O and O+ for conformation (straight to concave profiles, medium muscle development) and as 2 or 2+ for fatness (light fat cover, meat visible almost everywhere), with non-significant differences ($p > 0.05$) between age groups. The sarcopoietic potential of the *encastes* was evident in significant differences found in the hind-limb perimeter ($p < 0.01$) and conformation and fattening scores ($p < 0.05$). Carcasses of Lidia females resulted in short length (114 cm), shallowness

(38.8 cm), short hind-limbs (70.8 cm) and a low compactness index (1.03), similar to others rustic bovine.

Table 1. Effect of age at slaughter (fixed effect) and farm (random effect) on carcass traits of female Lidia breed (mean ± SE [1]).

Variable [2]	Mean ± SE	Age at Slaughter [3]				Farm
		I	II	III	*p*-Value	*p*-Value [4]
LHCW (kg)	59.48 ± 0.93	53.41 ± 2.22 [a]	56.77 ± 1.41 [a]	68.27 ± 1.95 [b]	<0.001	ns
CL (cm)	114.03 ± 0.46	108.59 ± 1.07 [a]	110.75 ± 1.09 [a]	122.76 ± 1.21 [b]	<0.001	ns
DCh (cm)	38.80 ± 0.18	36.79 ± 0.43 [a]	37.86 ± 0.27 [b]	41.77 ± 0.38 [c]	<0.001	ns
HL (cm)	70.84 ± 0.78	67.39 ± 1.80 [a]	70.14 ± 1.14 [a]	74.99 ± 1.59 [b]	<0.01	ns
HP (cm)	78.48 ± 0.76	76.01 ± 1.84 [a]	78.75 ± 1.16 [ab]	80.67 ± 1.61 [b]	<0.01	<0.01
CC (kg/cm)	1.03 ± 0.13	0.98 ± 2.22 [a]	1.02 ± 2.22 [a]	1.11 ± 2.22 [b]	<0.01	ns
CS (1-15)	5.50 ± 0.04	5.52 ± 0.09	5.54 ± 0.06	5.44 ± 0.08	ns	<0.05
FS (1-15)	5.85 ± 0.15	5.86 ± 0.37	5.56 ± 0.23	6.14 ± 0.42	ns	<0.05

[1]: SE = standard error. [2]: LHCW = left half-carcass weight, CL = carcass length, DCh = depth of chest, HL = hind-limb length, HP = hind-limb perimeter, CC = carcass compactness, CS = conformation score, CS = EUROP classification scales for conformation (from P − = 1 to 15 = E +), FS = fat cover classification (from 1 = low to 5 = very high). [3]: Means with different letters (a, b) are significantly different (SNK $p < 0.05$). [4]: ns = non-significant.

The left half-carcass weight showed a positive correlation with carcass length and depth of the chest, as well as with the hind-limb perimeter and carcass compactness. In contrast, it showed a weak correlation with fat cover classification, which also presented a weak correlation with carcass length and depth of the chest. The conformation score showed a weak and positive correlation with the hind-limb perimeter (Table S1, Supplementary Materials).

3.2. pH and Carcass Color

The average pH_{24} was 5.66 for all animals (Table 2); no significant differences were observed ($p > 0.05$) among age groups. Subcutaneous fat was characterized as dark and yellowish (L* = 59.8; b* = 23.5). An age group effect ($p < 0.001$) on a*, b* and C* was evident, with the highest values in older animals. The farm influenced ($p < 0.05$) b*, C*, and h*. Fat L* values decreased with age, though not reaching statistical significance. The m. *Rectus abdominis* appeared dark (L* = 38.5), reddish (a* = 15.9) and slightly yellowish (b* = 15.3), with age significantly influencing a*, b* and C*, although less so ($p < 0.01$) than in subcutaneous fat. The farm also influenced L* and h*.

The values of the colorimetric variables measured in the m. *Rectus abdominis* and subcutaneous fat were highly correlated. Fat L* values showed a weak negative correlation with the left half-carcass weight, carcass length, depth of the chest and the amount of fat in the rib. The hue angle measured in m. *Rectus abdominis* was weakly correlated with the half-carcass weight, carcass length, depth of the chest, carcass compactness and fat cover classification. Carcass length and depth of the chest were positively correlated with a*, b* and C* of the subcutaneous fat, and a* and C* of the m. *Rectus abdominis*. Fat b* and C* values were correlated weakly and negatively with the proportion of subcutaneous fat in the rib, and positively with the proportion of intramuscular fat (Table S1, Supplementary Materials).

3.3. 6th Rib Cut Dissection

Lean constituted the predominant tissue (58.6%), followed by bone (24.8%) and dissectible fat (14.3%), revealing a significant difference in fat percentage among age groups, with higher averages for cull cows ($p < 0.05$) (Table 3). The farm exerted a noteworthy effect ($p < 0.001$) on the tissue composition. Dissection losses were 3.02%, 2.95% and 2.69%, respectively, in groups I, II and III.

Table 2. Effect of age at slaughter (fixed effect) and farm (random effect) on pH and color variables measured on subcutaneous fat and m. *Rectus abdominis* in carcasses of female Lidia breed (mean ± SE [1]).

Variable [2]	Mean ± SE	Age at Slaughter [3]				Farm
		I	II	III	*p*-Value [4]	*p*-Value [4]
pH_{24}	5.66 ± 0.03	5.67 ± 0.06	5.65 ± 0.04	5.66 ± 0.06	ns	<0.05
Subcutaneous fat						
L*	59.77 ± 0.63	62.17 ± 1.51	59.82 ± 0.97	57.33 ± 1.34	ns	ns
a*	8.22 ± 0.48	5.62 ± 1.15 [a]	7.54 ± 0.73 [a]	11.50 ± 1.02 [b]	<0.001	ns
b*	23.48 ± 0.33	19.44 ± 1.74 [a]	20.60 ± 1.12 [a]	30.41 ± 1.54 [b]	<0.001	<0.05
Chroma	25.06 ± 0.37	20.45 ± 1.97 [a]	21.92 ± 1.27 [a]	32.80 ± 1.74 [b]	<0.001	<0.05
Hue angle	72.03 ± 0.80	74.64 ± 1.92	71.41 ± 1.23	70.06 ± 1.69	ns	<0.05
Rectus abdominis						
L*	38.46 ± 0.38	39.03 ± 0.89	38.12 ± 0.57	38.24 ± 0.79	ns	<0.01
a*	15.85 ± 0.37	14.94 ± 0.88 [a]	14.69 ± 0.56 [a]	17.90 ± 0.77 [b]	<0.01	ns
b*	15.03 ± 0.38	13.58 ± 0.89 [a]	15.13 ± 0.57 [ab]	16.37 ± 0.79 [b]	<0.01	ns
Chroma	21.93 ± 0.49	20.09 ± 1.16 [a]	21.36 ± 0.74 [a]	24.35 ± 1.02 [b]	<0.01	ns
Hue angle	44.22 ± 0.53	46.42 ± 1.26	43.50 ± 0.81	42.75 ± 1.11	ns	<0.05

[1]: SEM = standard error. [2]: L* = lightness, a* = redness, b* = yellowness. [3]: Means with different letters (a, b) are significantly different (SNK $p < 0.05$). [4]: ns = non-significant.

Table 3. Effect of age at slaughter (fixed effect) and farm (random effect) on sixth rib joint dissection variables of female Lidia breed (mean ± SE [1]).

Variable [2]	Mean ± SE	Age at Slaughter [3]				Farm
		I	II	III	*p*-Value [4]	*p*-Value [4]
Rib weight (g)	1991.76 ± 40.50	1885.90 ± 96.81 [a]	1930.46 ± 66.14 [a]	2158.92 ± 87.76 [b]	<0.05	ns
SF thickness (cm)	0.20 ± 0.02	0.20 ± 0.04	0.22 ± 0.03	0.19 ± 0.04	ns	ns
Bone + waste (g)	483.05 ± 10.36	460.37 ± 24.65	480.79 ± 16.84	504.98 ± 22.34	ns	<0.01
Bone + waste (%) *	24.81 ± 0.40	24.78 ± 0.95	25.22 ± 0.65	24.43 ± 0.86	ns	<0.001
Lean (g)	1150.20 ± 27.16	1098.13 ± 64.98 [a]	1110.85 ± 44.39 [a]	1233.63 ± 58.91 [b]	<0.05	ns
Lean (%) *	58.64 ± 0.55	59.12 ± 1.25	58.05 ± 0.85	58.77 ± 1.13	ns	<0.000
LT (g)	138.32 ± 3.57	136.96 ± 8.50	139.53 ± 5.80	138.46 ± 7.70	ns	<0.05
LT (%) **	12.02 ± 0.29	12.40 ± 0.40 [b]	12.74 ± 0.27 [b]	10.91 ± 0.36 [a]	<0.05	ns
Fat (g)	312.66 ± 13.71	270.59 ± 26.37 [a]	280.56 ± 18.01 [a]	360.82 ± 23.90 [b]	<0.05	<0.01
Fat (%) *	14.28 ± 0.55	13.31 ± 1.00 [a]	13.26 ± 0.68 [a]	16.25 ± 0.91 [b]	<0.05	<0.000
SF (g)	48.88 ± 4.16	48.57 ± 9.86 [ab]	35.48 ± 6.73 [a]	58.60 ± 8.93 [b]	<0.05	<0.01
SF (%) ***	14.75 ± 0.76	17.14 ± 1.81	12.67 ± 1.24	14.45 ± 1.64	ns	ns
IF (g)	266.77 ± 10.96	222.03 ± 26.37 [a]	243.07 ± 18.01 [a]	299.22 ± 23.90 [b]	<0.05	<0.01
IF (%) ***	85.21 ± 0.76	82.86 ± 1.81	87.24 ± 1.24	85.53 ± 1.64	ns	ns

[1]: SE = standard error. [2]: LT = m. *Longissimus thoracis*, SF = subcutaneous fat, IF = intermuscular fat, * respect to rib weight, ** respect to lean weight, *** respect to fat weight. [3]: Means with different letters (a, b) are significantly different (SNK $p < 0.05$). [4]: ns = non-significant.

Positive correlations were observed between the left half-carcass weight, carcass length and depth of chest with the rub weight and its components, as well as with the conformation score and fat cover classification of the carcass (Table S1, Supplementary Materials).

3.4. Meat Traits

The meat from animals in Group I exhibited a significantly higher pH ($p < 0.05$) compared to the other age groups (Table 4). These differences were also noted in the b* values of the m. *Longissimus thoracis*. Regarding WHC, no significant differences ($p > 0.05$) were observed between age groups at slaughter, while drip losses were influenced ($p < 0.01$) by the farm. The WBSF with a mean value of 4.6 kg/cm^2 remained unaffected by any of the considered factors.

Table 4. Effect of age at slaughter (fixed effect) and farm (random effect) on meat traits from female Lidia breed aged 21 days (mean ± SE [1]).

Variable [2]	Mean ± SE	Age at Slaughter [3]			Farm	
		I	II	III	*p*-Value [4]	*p*-Value [4]
pH	5.73 ± 0.02	5.87 ± 0.06 [b]	5.64 ± 0.04 [a]	5.68 ± 0.06 [a]	<0.01	<0.05
L*	25.49 ± 0.43	24.51 ± 1.02	26.13 ± 0.65	25.83 ± 0.90	ns	ns
a*	14.42 ± 0.33	14.28 ± 0.74	14.64 ± 0.47	13.90 ± 0.66	ns	ns
b*	12.86 ± 0.23	11.86 ± 0.54 [a]	13.69 ± 0.35 [b]	13.02 ± 0.48 [ab]	<0.05	ns
Chroma	19.63 ± 0.34	18.77 ± 0.77	20.13 ± 0.49	19.08 ± 0.68	ns	ns
Hue angle	42.40 ± 0.64	40.38 ± 1.44	43.18 ± 0.93	42.86 ± 1.28	ns	ns
Thawing loss (%)	5.34 ± 0.26	5.45 ± 0.58	4.73 ± 0.37	4.37 ± 0.51	ns	ns
Drip loss (%)	0.97 ± 0.02	0.96 ± 0.13	1.11 ± 0.08	0.85 ± 0.12	ns	<0.01
Pressure loss (%)	8.90 ± 0.23	7.97 ± 0.51	9.21 ± 0.33	9.52 ± 0.45	ns	ns
Cooking loss (%)	22.16 ± 0.40	21.75 ± 0.97	23.12 ± 0.62	21.61 ± 0.88	ns	ns
WBSF (kg/cm^2)	4.59 ± 0.16	4.07 ± 0.37	4.55 ± 0.24	5.14 ± 0.33	ns	ns

[1]: SE = standard error. [2]: WBSF = Warner–Braztler shear force, L* = lightness, a* = redness, b* = yellowness. [3]: Means with different letters (a, b) are significantly different (SNK $p < 0.05$). In the table ns refers to non-significant results. [4]: ns = non-significant.

No significant correlations were found between meat traits and any variable, except between colorimetric variables measured in the meat.

3.5. Sensory Analysis

The sensory profile analysis of aged meat is presented in Figure 1, where all attributes scored above 5 in all evaluated treatments. Statistical analyses revealed significant differences attributed to slaughter age, except for color and odor intensity, which did not differ between groups, maintaining an average score of 6.14 and 6.12, respectively.

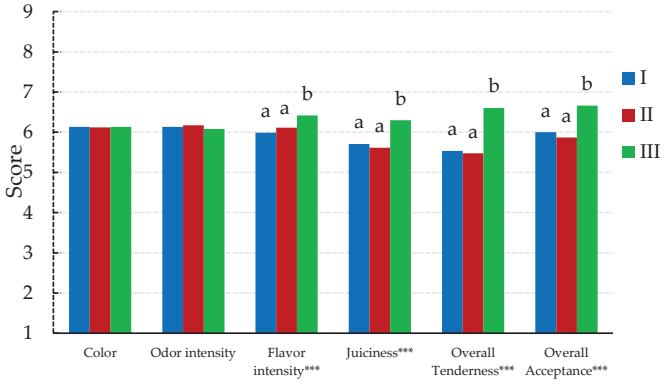

Figure 1. Mean values for the sensory attributes of female Lidia breed meat slaughtered at different ages. Means with different letters (a, b) are significantly different (SNK $p < 0.05$; *** $p < 0.001$). I = Heifer I (24–36 months); II = Heifer II (36–48 months); III = Cull cows (>48 months). A scale of categories from 1 to 9 (low to high intensity, respectively) was used.

The overall acceptance reached an average of 6.34, being significantly higher in cull cows (group III) than in heifers (I and II), with no significant differences between the two latter groups. The most highly rated attribute was flavor intensity, achieving an average of 6.21, while juiciness received the lowest rating at 5.95. Cull cows (group III) obtained significantly higher sensory scores (flavor intensity, overall tenderness and juiciness) than heifers (groups I and II), with no significant differences between these two groups.

4. Discussion

4.1. Carcass Traits

The carcass values observed are somewhat poor compared to what is found in meat breeds and other local breeds reared in the *dehesa*. As the *Lidia* breed has a remarkable temperament, it is difficult to include it in a feedlot system, hindering further conformational and fat development [15]. Regarding body size, the *Lidia* breed, classified as a small to medium-sized rustic breed, showed an age at slaughter (>24 months) higher than that commonly recorded in slaughterhouses (14–16 months) [32]. However, the left half-carcass weight (59.5 kg) was lower than that recorded in specialized and rustic breeds reared in their natural environment [33,34]. This was expected due to the lower weight of *Lidia* females compared to other native Spanish cattle breeds (300–400 kg vs. 400–600 kg), and it was anticipated that the weight of the left half-carcass would be greater in adult animals compared to young animals [35].

The morphometry of carcass *Lidia* females were similar to other rustic bovine breeds [36]. However, this was different from carcasses found in slaughterhouses, especially in the carcass compactness index [33,37–39]. Evaluated carcasses showed conformation and fatness scores lower than those recorded in other native and even in rustic breeds [33,40,41]. This difference in scores could be attributed to their degree of maturity, dietary restrictions, and low-fat content, which may be related to the fact that unimproved breeds have less subcutaneous fat than improved ones [42]. These results are in line with studies emphasizing that fatness scores depend on subcutaneous fat deposition, which can be more easily depleted during periods of low energy input [43].

While it is commonly known that increasing carcass weight improves conformation [44], in this study, a uniform carcass conformation score ($p > 0.05$) was found in all carcasses, in agreement with previous research [39]. The fatness score did not change with age at slaughter, which is consistent with other studies [45]. Based on conformation and fatness scores, the carcasses of *Lidia* females show certain similarities with other native Spanish breeds such as *Avileña*, *Retinta* or *Morucha* [4,39]. The variability and zoometric differentiation among the farms could account for the influence on hind-limb perimeter and carcass conformation [4].

4.2. pH and Carcass Color

The pH_{24} level significantly impacts meat quality, as a lower pH is often linked to enhanced tenderness and results in lighter meat. Ultimate pH values (5.7) in this study fall within the normal beef range (5.4–5.8) [46,47]. Our results are in line with studies on *Pajuna* steers [48] and young bulls from the *Retinta* breed [49], being lower than those for Limousin crossbred heifers fed with agro-industrial by-products [24].

In the CIELab* space, the subcutaneous fat of the carcass of Lidia females showed a lower value of lightness compared to carcass values of Spanish breeds (L* = 59.77 vs. L* = 66–71.2) and higher values of red (a* = 8.22, vs. a* = 2.8 to 3.4) and yellow (b* = 23.48; vs. b* = 7.6 to 18.1) [50–52]. The same was found when compared with adult steers (L* = 61.1–66.4; b* = 10.26 to 13.79). Extensive systems tend to be associated with carcasses with a lower fat lightness and higher yellowness index compared to intensive systems [53,54]. Carcasses from Lidia females showed a similar L* to other breeds, but with higher red (a* = 0.44 to 1.18) and yellow indices (b* = 6.92 to 9.88) [24,55]. The elevated a* value is attributed to increased myoglobin due to the higher physical activity of the *Lidia* breed [56], while the b* values may be the result of nutrition [24].

Concerning age at slaughter, results show higher a* and b* values in adult animals, confirming previous studies [57], partially in agreement with Galli et al. [58], and contrasting with Marenčić et al. [59], who found negligible effects on color parameters. Du Plesis and Hoffman [60] observed lower L* values and higher a* values at 30 months, indicating darker and redder meat.

Differences between farms in b* values from subcutaneous fat can be attributed to variability in pasture quantity and quality in their geographical areas [61], as well as

differences in pH, according to Page et al. [62], who reported a negative correlation between pH and b* values.

The correlations found between the colorimetric variables and the carcass traits are in line with previous studies [63], highlighting that subcutaneous fat darkens as carcass weight and size increase. The positive correlations between carcass size and fat and *m. Rectus abdominis* a*, b* and C* values suggest a relationship between carcass size and composition and the color characteristics of fat and meat [63].

4.3. 6th Rib Cut Dissection

The 6th rib dissection method, proven to offer a more precise prediction of tissue composition, distinguishes our study from earlier research employing different rib cuts [36,55]. Tissue composition in animals from rustic breeds varies from more specialized beef breeds [64], aligning with the leaner meats typical of extensive systems. The dissection of the 6th rib revealed high bone content, a moderate fat percentage and medium to low lean percentage, resembling compositions of rustic and native breeds in extensive systems [33,36,38,65]. In contrast to the work of Vieira et al. [52] on adult steers, *Lidia* females exhibited higher bone values (24.8 vs. 13.5%) and lower fat values (14.3 vs. 27.3%). The percentage of lean and bone content remained consistent with the age at slaughter, contrary to that found by Albertí et al. [66], who reported decreases with increased carcass weight. Allometric indices of bone (~0.6–0.7) and lean (~1), alongside breed maturity, may influence young animals reaching bone and lean development similar to cows, according to [36]. The lean-to-bone relationship similarity ($p > 0.05$) between age groups suggested no significant changes.

The correlations found were as expected, including a negative correlation between intramuscular fat and CS [63,67]. However, the correlation found between FS and tissue composition variables differed from that found by Nogalski et al. [68], although the latter used the last three thoracic ribs, which are considered not as good predictors as the 6th rib [69].

4.4. Meat Traits

After the ageing period, the meat from *Lidia* females (pH = 5.7) was within the optimal range. The meat from group I presented the highest pH values, although there were no significant differences between age groups. Younger animals tend to have lower muscle glycogen contents and pH drop rates, making them more susceptible to stress, which can result in higher pH values [70].

Meat color is a crucial factor for consumers when purchasing [71]. *Lidia* female meat was darker and less red than that found in Spanish rustic [41,48,72] and specialized beef breeds [55], which are classified as very red [73]. Grazing, which leads to higher myoglobin content due to increased physical activity, contributed to the observed darker color [48,53,74]. Meat color did not follow the age-related evolution reported in some studies. The L* values were not affected by age at slaughter, contrary to previous findings [45,74]. However, a* values were lower than those found in the *Morucha* rustic breed [40], and b* values decreased with age [75]. Overall, the color variation was related to body size, fat content and muscle development [72].

The water holding capacity (WHC) is a crucial property for both the industry and consumers [76]. Thawing losses were similar to those in previous studies [49], and the ageing period did not significantly affect the WHC. Thawing losses were within the acceptable range, and meat was classified as juicy [77]. However, some variability in losses was observed, supporting the idea that leaner breeds have a lower WHC [38]. The influence of carcass weight on WHC was inconclusive, with no significant differences between age groups.

The Warner–Bratzler shear force (WBSF) values indicated that *Lidia*-female-cooked meat fell within acceptable limits for tenderness [63]. The values obtained aligned with those considered tender [78] or of intermediate tenderness [79]. No significant differences

in WBSF were observed between age groups, which is possibly attributed to the higher fat content in cull cow carcasses [80]. While the effect of carcass weight on WBSF is not conclusive, some studies have reported significant changes with age at slaughter [45,81]. The specific activity of the calpain enzyme and collagen characteristics have been considered as sources of tenderness variation at different slaughter ages [82].

4.5. Sensory Analysis

The sensory profile analysis revealed significant differences attributed to slaughter age for flavor intensity, juiciness, overall tenderness and overall acceptance. Differences found are usually determined by diet [28,83] and ageing [84,85]. Except for color and odor, sensory attributes showed an increase with the age at slaughter, in contrast to some previous findings [58].

Meat color is influenced by various factors [86], and in this case presented satisfactory results for all evaluated slaughter ages. Odor intensity, often influenced by intramuscular fat content and fatty acid composition, received positive ratings [87,88]. Flavor intensity, a multifactorial trait [89–91], showed improvement with increasing age at slaughter. A higher age at slaughter might have generated a slightly higher amount of intramuscular fat deposits, which could cause the differences found between heifers and cows [92]. Likewise, it would be interesting to explore whether the fatty acid profile in the meat of cows versus heifers varied, which could be a possible justification for the differences found. Although numerically WHC variables were lower in cows, the results were not significant; however, the panel reported significant differences in meat juiciness. Juiciness scores were higher in cull cows, possibly due to a slightly higher fat content associated with age [93]. Overall tenderness improved with age, contrasting with some studies reporting an inverse relationship [94]. Overall acceptance differences correlated closely with flavor, juiciness and overall tenderness, emphasizing their collective importance in consumer perception. The exact hierarchy of importance among these attributes remains a subject of debate in the literature [95,96].

After evaluating the main carcass traits and meat quality of the *Lidia* breed, it would be of interest to subject it to assessment by untrained consumers, aiming for a deeper understanding of how *Lidia* breed meat could better align with the current market preferences.

5. Conclusions

This study reveals that the age at slaughter in *Lidia* females exerts a significant influence on carcass traits and meat quality. The increment of age at slaughter influenced the morphometry and a*, b* and C* color variables of carcass muscle and subcutaneous fat. The tissular composition of cull cows shows higher lean, fat and bone portions, and a higher percentage of fat. In contrast, instrumental variables were not influenced by the age groups studied. Important results were found on sensory variables; cull cows presented higher values in flavor intensity, juiciness, overall tenderness and overall acceptance. Although *Lidia* females are small to medium-sized, with a lower carcass weight compared to other breeds, the instrumental variables were acceptable, and their sensory characteristics suggest a positive potential for consumption.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani14060850/s1, Table S1: Pearson correlations between the analyzed variables.

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Institutional Review Board Statement: The present study complies with the provisions of the Spanish Law on Biomedical Research (Ley 14/2007 del 3 de Julio) and was approved by the Ethical Committee for Research Involving Humans (CEIH) of the University of Córdoba (Ref. CEIH-24-01). Participants were not harmed or affected in any way by being included in this study, nor were any personal or confidential data disclosed.

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Polymorphisms of the *SCD1* Gene and Its Association Analysis with Carcass, Meat Quality, Adipogenic Traits, Fatty Acid Composition, and Milk Production Traits in Cattle

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Simple Summary: This study aimed to reveal the single nucleotide polymorphisms (SNPs) of the bovine Stearoyl-CoA desaturase-1 (*SCD1*) to explore the correlation between genotypes and carcass, meat quality, adipogenic traits, fatty acid composition, and milk production traits in cattle. Four SNPs, g.21272246 A>G, g.21272306 T>C, g.21272422 C>T, and g.21272529 A>G, were found by Sanger sequencing; further statistical analysis showed that these four SNPs of the *SCD1* gene were significantly associated with carcass traits and meat quality, including carcass weight, carcass fat coverage rate, rib eye area, marbling score, adipogenic traits, and fatty acid composition. Additionally, a modest effect on milk production traits, such as average milk yield and milk fat content, was observed in cows. Further haplotype analysis indicated that the combinations of H2H3 and H2H2 of SNPs had a higher value than others. Our results indicate that these four SNPs are potentially effective markers and could be used in marker-assisted breeding to improve meat and milk quality simultaneously in the future.

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Abstract: Stearoyl-CoA desaturase-1 (*SCD1*) is a key enzyme in the biosynthesis of monounsaturated fatty acids and is considered a candidate gene for improving milk and meat quality traits. Sanger sequencing was employed to investigate the genetic polymorphism of the fifth exon and intron of bovine *SCD1*, revealing four SNPs, g.21272246 A>G, g.21272306 T>C, g.21272422 C>T, and g.21272529 A>G. Further variance analysis and multiple comparisons were conducted to examine the relationship between variation sites and economic traits in Chinese Simmental cattle, as well as milk production traits in Holstein cows. The findings revealed these four loci exhibited significant associations with carcass traits (carcass weight, carcass length, backfat thickness, and waist meat thickness), meat quality (pH value, rib eye area, and marbling score), adipogenic traits (fat score and carcass fat coverage rate), and fatty acid composition (linoleic acid and α-linolenic acid). Furthermore, these loci were additionally found to be significantly associated with average milk yield and milk fat content in cows. In addition, a haplotype analysis of combinations of SNPs showed that H2H3 has a significant association with adipogenic traits and H2H2 was associated with higher levels of linoleic acid and α-linolenic acid than the other combinations. These results suggest that the four SNPs are expected to be prospective genetic markers for the above economic traits. In addition, the function of SNPs in exon 5 of *SCD1* on gene expression and protein structure needs to be explored in the future.

Keywords: Stearoyl-CoA desaturase-1 (*SCD1*); single nucleotide polymorphisms (SNPs); cattle; meat quality traits; milk traits

1. Introduction

Early in the 1950s, Simmental cattle were introduced to China and have become the dominant breed in the Chinese beef industry. Over time, through continuous breeding and

adaptation improvements, Chinese Simmental cattle have achieved excellent growth, meat quality, and fat deposition performance, becoming an important breed with significant economic and agricultural value in China. Holstein cows were first introduced into China in the mid-19th century through hybridization with local cattle and long-term breeding, and they formed a better milk production performance, such as milk yield, milk fat content, and milk protein. Bovine milk is an important component of the human diet [1]. As a source of nutrients for human beings, milk is widely popular for its rich content of protein, calcium, zinc, and fatty acids. With the improvement of modern consumption levels and attention to the nutritional value of diet structure, the demand for the quality and quantity of meat and dairy products is also increasing.

Exploring molecular markers that can be used to assist in selecting economically important traits is crucial for advancing genetic improvement in cattle. Marker-assisted selection (MAS), one of the methods in molecular breeding, relies on nucleotide sequence variations among individuals that remain unaffected by environmental and other factors. This method enables direct selection at the DNA level during early stages, significantly reduces breeding time, and enhances efficiency. Single nucleotide polymorphisms (SNPs) are one of the most commonly used forms of genetic markers in genetic polymorphism studies. SNPs can be used as a molecular marker for assisted selection of the economic traits of cattle to analyze the correlation between the SNP locus and beef quality and milk production traits. Therefore, cattle with excellent genetic characteristics can be predicted and selected, providing an important scientific basis and technical means for the genetic improvement of beef cattle and dairy cows. SNP analysis has been widely applied in animal husbandry and agricultural research, providing important tools for molecular genetics in other fields [2].

Stearoyl-Coenzyme A desaturase (*SCD*) belongs to the family of fatty acid desaturases [3]. The *SCD1* gene is a key enzyme regulating fatty acid metabolism [4]. It catalyzes the conversion of saturated fatty acids (SFAs) to monounsaturated fatty acids (MUFAs), which affects the rate of fatty acid synthesis and fat deposition in animals. The fatty acid composition in muscle significantly impacts the flavor development of beef, with the content of unsaturated fatty acids directly influencing the taste of the meat [5]. As a critical enzyme in lipid metabolism, the bovine *SCD1* gene plays an important regulatory role in synthesizing unsaturated fatty acids. In cattle, the *SCD1* gene is primarily expressed in the mammary gland and adipose tissue. It is involved in cellular metabolism and the differentiation of precursor adipocytes, playing a role in regulating the composition of tissue fatty acids [6]. Furthermore, research on the association between the *SCD1* gene and meat quality suggests that *SCD1* may impact meat quality traits by influencing the shear force, marbling, and color [1–3]. As a result, the *SCD1* gene has emerged as an important candidate gene for MAS to improve beef quality. Moreover, studies on the *SCD1* gene in dairy cattle have revealed its crucial role in regulating the composition and content of milk fatty acids. It influences the quality, flavor, and nutritional value of milk and dairy products. The expression level of the *SCD1* gene is associated with the proportion of monounsaturated fatty acids in milk fat [1,7–9]. Studies on the *SCD1* gene encompass various aspects, including fatty acid metabolism, meat quality traits, and milk fatty acid composition. Further research is needed to explore the correlation between *SCD1* gene polymorphisms and important economic traits in cattle.

By detecting variations at single nucleotide sites and establishing SNP markers associated with specific traits, it becomes possible to better predict and select individuals with desirable traits, thereby accelerating the breeding process of livestock breeds. In this study, we used Sanger sequencing to screen potential genetic polymorphic loci in exons and introns of the *SCD1* gene. This study aimed to explore the correlation of different variation loci and genotypes with economic traits of cattle, including carcass traits, meat quality traits, fatty acid composition, and milk production traits and to clarify the potential roles of the *SCD1* gene in improving meat and milk quality traits.

2. Materials and Methods

2.1. Ethics Statements

Animal experiments were performed strictly following the guidance for the care and use of laboratory animals by the Jilin University Animal Care and Use Committee (Permit number: SYXK (Ji) 2008-0010/0011).

2.2. Animals and Sample Collection

A total of 334 Chinese Simmental steers (28 months old) were provided by the Inner Mongolian Baolongshan cattle farm (Tongliao, China) and were randomly selected from the offspring of a Simmental population of approximately 1000 female cows and 21 bulls. All individuals are similar in age, feeding conditions, and physical condition. Blood genomic DNA and tissue samples were extracted in our previous study [10] and stored at −80 °C in the laboratory for further use. After obtaining Dairy Herd Improvement (DHI) data, 73 cows were selected for jugular vein blood collection for subsequent experimental analysis.

2.3. Trait Analysis

Before slaughter, the live weight, the backfat thickness of living, and the area of *longissimus dorsi* muscle (by ultrasound) were measured and recorded; the weight of the carcass, omental fat, mesentery fat, and kidney fat were recorded at the slaughterhouse. After slaughter, the carcasses are stored in a refrigerated room at 0 to 4 °C for 24 h. All the measurements complied with the criterion GB/T 17238-2008 cutting standard of fresh and frozen beef of China (China Standard Publish) [11]. Furthermore, we also measured meat quality traits, including meat color and marbling, and recorded the fat coverage rate, marbling score, fat color score, muscle color score, rib eye area, and backfat thickness after slaughter. The day after slaughter, the 12th and 13th ribs' *longissimus dorsi* muscle samples were collected from ripening carcasses and stored at −20 °C until they were thawed for fatty acid composition analysis and expressed as g/100 g fresh tissue [10,12].

In this study, 36 traits of carcass, meat quality, and adipogenic traits were obtained through measurement, and 14 fatty acid compositions were detected from the longissimus of the back, as described in our previous study [13]. Six milk composition traits, including milk yield, milk fat, milk protein percentage, lactose content, dry matter, and urea nitrogen, were detected and recorded for subsequent analysis.

2.4. Genomic DNA Extraction, PCR Amplification, and Identification

Genomic DNA was extracted from whole-blood samples (10 mL per cattle) using a blood DNA extraction kit (TIANGEN, Beijing, China), according to our previous reports. The purity and concentration of genomic DNA determined by NanoDrop 2000 (Thermo, Scientific, Waltham, MA, USA) showed an absorbance at $OD_{260\,nm}/OD_{280\,nm}$ between 1.8 and 2.0. In addition, the agarose gel electrophoresis detection showed genome DNA without RNA, protein, and ion pollution, which can be used for further experiments. Moreover, 45 randomly selected DNA samples of the detected population were mixed into a genomic DNA pool for a polymerase chain reaction (PCR) analysis to determine potential SNPs in the cattle *SCD1* gene. After obtaining the genetic variation loci in the detected populations, four SNPs located in exon 5 and intron 5 were selected for subsequent PCR, and 334 Chinese Simmental steers and 73 Holstein cattle were used as templates to amplify this polymorphic fragment, respectively.

The cattle *SCD1* gene sequence was obtained from the National Center for Biotechnology Information (NCBI) database query (GenBank: NC_037353.1), and 12 pairs of primers were designed by Primer Premier 6.0 software and synthesized by the Sangon Biotech company (Changchun, China), as shown in Table 1. The PCR reaction was performed in a 20 μL volume containing 1 μL genomic DNA, 0.4 μL primer (forward and reverse primer), 10 μL Green Taq Mix (2×), and 8.2 μL ddH_2O. The PCR amplification procedure was performed as follows: pre-denaturation at 95 °C for 10 min, followed by 35 cycles of

denaturation for 30 s at 95 °C, 55 °C for 30 s during annealing, extension for 30 s at 72 °C, and a final extension of 10 min at 72 °C, then cooling to 4 °C. Then, the PCR products were analyzed on a 1.5% agarose gel. The PCR products were sent to the Sangon Biotech company (Changchun, China) for Sanger sequencing by selecting clear single electrophoresis bands whose fragment sizes matched the expected product size.

Table 1. PCR primer sequences of the *SCD1* gene.

Primer Names	Primer Sequences (5′-3′)	Product Size (bp)	Tm (°C)
SCD1-1	F: TAGTGGGTGACACATTCATAGC R: GGATTGCCTGGGAGGATGA	833	55
SCD1-2	F: AATCATCCTCCCAGGCAATCC R: GCGTAAGAGGTTCAGCCAATG	702	55
SCD1-3	F: CGGGTTTGAGGACACGTCT R: TTTATTCGTTGCCAACAAGGG	599	55
SCD1-4	F: TGTGCAGCATCCAGTTCTTG R: AAGGCGGAAGACAGGGAAG	781	54
SCD1-5	F: ATCTCTAGCTCCTACACAACCA R: AGCCCTCTAAAGTCACTCATCT	704	54
SCD1-6	F: AGGTTAGCAGAAGGTCAGAGG R: AAGACCACAACAGCCAGACT	746	55
SCD1-7	F: GCATTCCACTCACCACATAACC R: TTGTGCCTCTCCTCGCTATG	936	55
SCD1-8	F: TCCTTGCTCCACCACTTCC R: CCACCCAGATGACCCTACTC	832	55
SCD1-9	F: CATTCATTCAACAGCAACAGGT R: CAGGAGAGAAAGGGAGCATACT	1002	54
SCD1-10	F: CTCCCTTTCTCTCCTGACTCTG R: CCATCACTGCCTCTGAATACAC	1002	55
SCD1-11	F: TCACTGAACCACTGTTTCTCTT R: AAGGCATCCAGATAAGTTGTCA	1203	51
SCD1-12	F: ATGCTGACAACTTATCTGGATG R: CAGGGCAATCAGATTCACTTT	981	51

The sequence data were compared with the reference sequences of the *SCD1* gene (NC_037353.1) by using SnapGene 4.1.9 (GSL Biotech LLC, Boston, MA, USA). Based on the sequencing results from PCR, amplifying the genomic DNA pool and individuals' genomic sequences, the SNP loci and genotypes of the *SCD1* gene could be determined.

2.5. Statistical Analysis

Allele and genotype frequency were determined using relevant calculation formulas. The expected heterozygosity (He), effective number of alleles (Ne), and polymorphism information content (PIC) were calculated based on the genotyping results, which could estimate the degree of homozygosity or heterozygosity and the genetic polymorphism and test whether a genetic system is in equilibrium in a cattle population. The HaploView software 4.2 was also used to analyze the linkage disequilibrium of the SNP loci. A general linear model was adopted to analyze whether the genotypes of the different loci have synchronous effects on traits. We employed independent sample *t*-tests and an analysis of variance (ANOVA) in SPSS 25.0 (IBM, New York, NY, USA) to explore the correlations between the genotypes of different SNP loci in the *SCD1* gene and carcass traits, adipogenesis traits, meat quality traits, and fatty acid composition; finally, we used the LSD method for multiple comparisons, with a significance level of $p < 0.05$. We have presented the experimental data as the mean ± standard deviation. The model employed for this analysis was as follows:

$$Y_{ijk} = u + ys_i + m_j + e_{ijk},$$

Y_{ijk} represents the phenotypic observation of the *k*-th individual from the Simmental breed with genotype *j* in the *i*-th year season; u represents the population mean, ys_i represents the year effect in the *i*-th year season, m_j represents the effect of genotype *j*, and e_{ijk} represents the random residual effect that correlates with the observed value [14].

3. Results

3.1. SNP Detection and Genotyping of the SCD1 Gene

A total of 17 SNPs were detected in five PCR productions (*SCD1*-7, *SCD1*-8, *SCD1*-9, *SCD1*-10, *SCD1*-11) by DNA sequencing (Table 2). Previous research found that exon 5 and intron 5 amplified by the primer *SCD1*-8 have potential research significance. Therefore, as key loci for SNP screening, fragments from 334 Chinese Simmental steers and 73 Chinese Holstein cattle were amplified and genotyped.

Table 2. 17 SNPs' information regarding the *SCD1* gene.

Primer Names	SNP	Mutation Region	Number	Variation ID	Gene Position
***SCD1*-7**	A>G	intron3	2	rs41255689	26:21270336
	A>G	intron4		rs41255690	26:21270739
***SCD1*-8**	A>G	exon5	4	rs41255691	26:21272246
	T>C	exon5		rs41255692	26:21272306
	C>T	exon5		rs41255693	26:21272422
	A>G	intron5		rs383175036	26:21272529
***SCD1*-9**	G>T	exon6	3	rs41255694	26:21275659
	G>C	exon6		rs41255695	26:21275732
	C>A	exon6		rs41255696	26:21275851
***SCD1*-10**	C>T	exon6	2	rs41255697	26:21276141
	A>G	exon6		rs41255698	26:21276672
***SCD1*-11**	A>G	exon6	6	rs41255700	26:21277095
	C>T	exon6		rs41255701	26:21277195
	G>A	exon6		rs41255702	26:21277296
	T>G	exon6		rs41255703	26:21277378
	G>A	exon6		rs41255704	26:21277585
	G>A	exon6		rs382676818	26:21277770

The electrophoresis results of the product showed that the bands that appeared on gel electrophoresis were consistent in size with the target fragment (832 bp) and had a good specificity (Figure 1B). Furthermore, based on the position of the bovine *SCD1* gene on the chromosome, these SNPs were marked in the genetic structure of *SCD1* (Figure 1A). The sequencing peak plot illustrated that four SNPs were screened in the fifth exon and intron of the *SCD1* gene, which were named separately as g.21272246 A>G, g.21272306 T>C, g.21272422 C>T, and g.21272529 A>G, and three genotypes were detected in this population (Figure 1C). Among these four SNPs, a missense mutation was detected at the locus g.21272422 C>T, while synonymous mutations were found at the other three loci. Specifically, a CGG to UGG missense mutation was recognized at the 293th codon in exon 5 (C>T) of *SCD1*, resulting in the replacement of alanine by valine (A293V), which is consistent with previous reports [4,5].

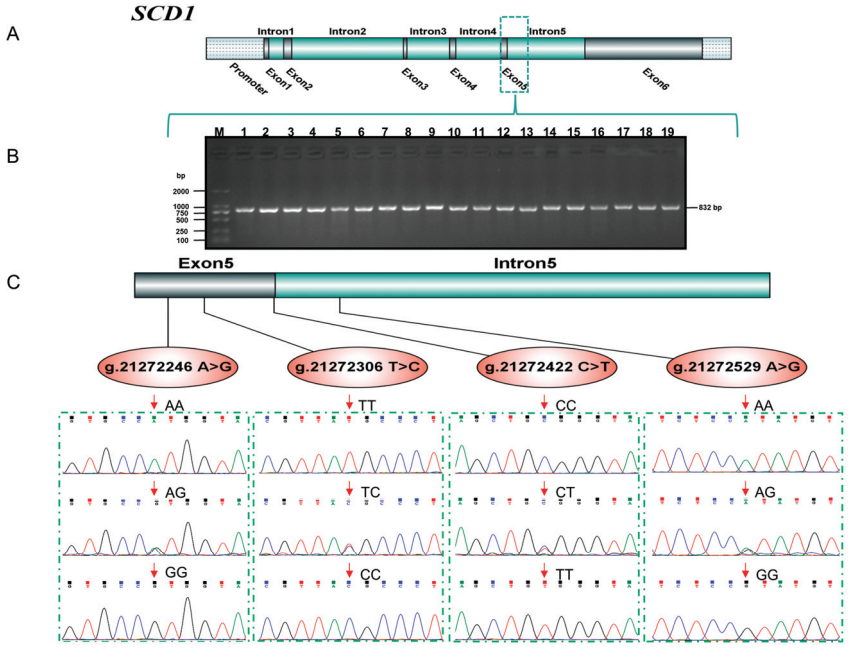

Figure 1. *SCD1* gene structure, agarose gel electrophoresis of PCR products, and SNPs detection. (**A**) Gene structure of *SCD1*; (**B**) Agarose gel electrophoresis of the *SCD1* gene PCR amplification product (partial) M: DL2000 Marker; 1–19: 832 bp PCR product of *SCD1*-8 primers; (**C**) Four SNPs' detection and genotyping of the *SCD1* gene.

3.2. Analysis of Population Genetic Polymorphism, Linkage, and Haplotypes

The genotypic and allelic frequencies and population genetic polymorphism of four SNPs were calculated and are shown in Table 3. At 21272246 A>G and g.21272529 A>G, the heterozygous genotype of AG was the dominant genotype, and allele A (0.575, 0.585) had a higher frequency than allele G (0.425, 0.415), respectively. At the g.21272306 T>C locus, allele T had a frequency of 0.584. Also, the heterozygous genotype TC was the dominant genotype and had a higher frequency (0.407) than the homozygote of TT (0.380) and CC (0.213). In addition, the CC, CT, and TT genotypes were observed for the g.21272422 C>T locus, allele C had a higher frequency (0.588) than allele T (0.412), and CT was the dominant genotype with a high frequency (0.410) (Figure 2A, Table 3). The genotypic frequencies of the four SNPs in the Chinese Holstein population were 0.452 for AA, 0.493 for AG, and 0.055 for GG, respectively. The genotypes of TT, TC/CT, and CC were also the same, the lowest genotype frequency of mutant homozygous was 0.055, and the heterozygous genotype was dominant accordingly. The allele frequency of A, T, and C was 0.699, higher than that of G, C, and T at 0.301 (Figure 2B, Table 3). The results showed that the homozygosity of the four SNPs was higher than the heterozygosity. In Chinese Simmental cattle, the polymorphic information contents of the four SNPs were 0.489, 0.486, 0.484, and 0.485, respectively, and this value was found to be 0.421 in Chinese Holstein cattle, which all belonged to a moderate polymorphic frequency ($0.25 < PIC < 0.5$). Additionally, there was a strong linkage between g.21272422 C>T and g.21272529 A>G ($D' = 1.0$, LOD = 150.45, $r^2 = 0.988$), g.21272306 T>C and g.21272529 A>G ($D' = 1.0$, LOD = 153.03, $r^2 = 0.994$), and g.21272306 T>C and g.21272422 C>T ($D' = 1.0$, LOD = 148.70, $r^2 = 0.982$).

Table 3. Genetic diversities of the *SCD1* gene in Chinese Simmental steer and Chinese Holstein cattle.

SNP	g.21272246 A>G				g.21272306 T>C				g.21272422 C>T				g.21272529 A>G			
Gene position	26:21272246				26:21272306				26:21272422				26:21272529			
Mutation type	synonymous mutation				synonymous mutation				missense mutation				synonymous mutation			
Breed	Chinese Simmental steer $n = 334$		Chinese Holstein cattle $n = 73$		Chinese Simmental steer $n = 334$		Chinese Holstein cattle $n = 73$		Chinese Simmental steer $n = 334$		Chinese Holstein cattle $n = 73$		Chinese Simmental steer $n = 334$		Chinese Holstein cattle $n = 73$	
Genotype frequency	AA (123)	0.368	AA (33)	0.452	TT (127)	0.380	TT (33)	0.452	CC (128)	0.383	TT (33)	0.452	AA (127)	0.380	AA (33)	0.452
	AG (138)	0.413	AG (36)	0.493	TC (136)	0.407	TC (36)	0.493	CT (137)	0.410	TC (36)	0.493	AG (137)	0.410	AG (36)	0.493
	GG (73)	0.219	GG (4)	0.055	CC (71)	0.213	CC (4)	0.055	TT (69)	0.207	CC (4)	0.055	GG (70)	0.210	GG (4)	0.055
Allele frequency	A	0.575	A	0.699	T	0.584	T	0.699	C	0.588	C	0.699	A	0.585	A	0.699
	G	0.425	G	0.301	C	0.416	C	0.301	T	0.412	T	0.301	G	0.415	G	0.301
He	0.413		0.493		0.407		0.493		0.410		0.493		0.410		0.493	
PIC	0.489		0.421		0.486		0.421		0.484		0.421		0.485		0.421	
Ne	1.704		1.972		1.686		1.972		1.695		1.972		1.695		1.972	

Note: He, gene heterozygosity; PIC, polymorphic information content; Ne, effective number of alleles.

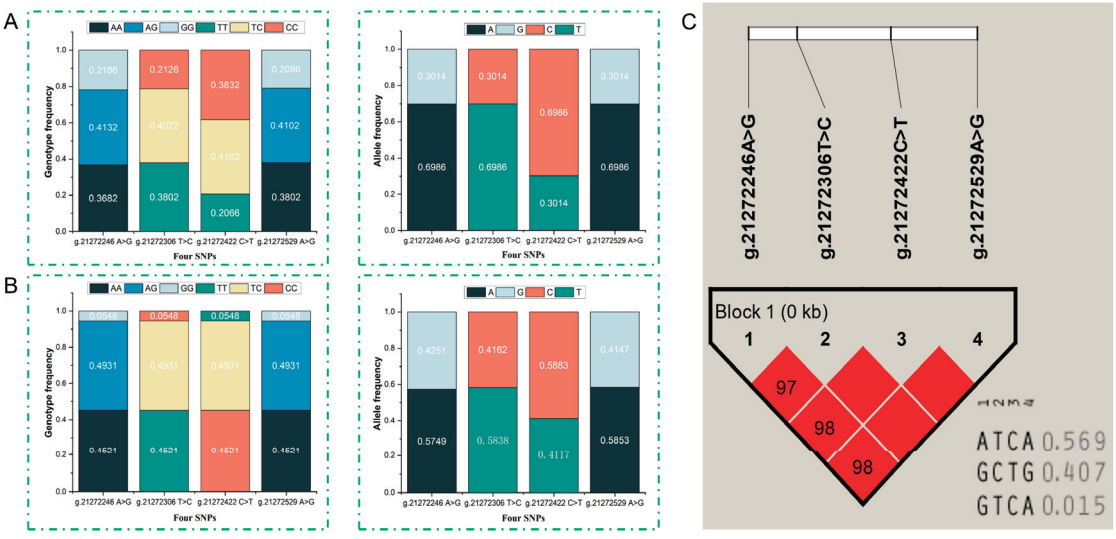

Figure 2. Genotypic and allelic frequencies of SNPs and the haplotypes composed of four SNPs of the *SCD1* gene. (**A**) Genotypic and allelic frequencies of SNPs in the *SCD1* gene in Chinese Simmental Steers; (**B**) Genotype frequency and allele frequency of SNPs in the *SCD1* gene in Chinese Holstein cattle; (**C**) The linkage disequilibrium and haplotypes frequencies in Chinese Simmental steers.

The results of the haplotype analysis of the four SNPs of the *SCD1* gene were Hap1 (ATCA), Hap2 (GCTG), and Hap3 (GTCA). The haplotype frequencies of the three haplotypes were ATCA (0.569), GCTG (0.407), and GTCA (0.015), respectively. ATCA was the dominant haplotype (Figure 2C).

3.3. Association Analyses of SCD1 Gene Polymorphisms with Carcass and Meat Quality Traits in Chinese Simmental Cattle

Associations between the four SNPs and carcass traits, as well as meat quality traits results, are presented in Table 4. Significant associations were observed between the carcass weight, carcass length, backfat thickness, and waist meat thickness with the *SCD1* g.21272246 A>G, g.21272306 T>C, g.21272422 C>T, and g.21272529 A>G SNPs regarding the carcass. Further analysis of the genotypes revealed that individuals with the AG genotype had a higher carcass weight ($p < 0.05$) and backfat thickness ($p < 0.01$) than those individuals with the AA and GG genotype at g.21272246 A>G and g.21272529 A>G; however, the genotypic differences between AA and GG were not significant ($p > 0.05$). Similarly, individuals carrying the TC heterozygous genotype showed a higher carcass weight ($p < 0.05$) and backfat thickness ($p < 0.01$) than TT or CC homozygotes at g.21272306 T>C; at g.21272422 C>T, the individuals with the CT heterozygous genotype displayed an increased carcass weight ($p < 0.05$) and backfat thickness ($p < 0.01$) compared to CC or TT homozygous individuals, but no significant differences were detected between the CC and TT genotypes ($p > 0.05$). Waist meat thickness was significantly higher for the AG heterozygous individuals than those with the AA genotype ($p < 0.05$). In contrast, the carcass length of AA homozygous individuals was higher than that of GG homozygous individuals ($p < 0.05$) at g.21272246 A>G and g.21272529 A>G. In the same way, the waist meat thickness of individuals of the TC genotype was also higher than that of the TT genotype ($p < 0.05$), but the carcass length of TT homozygous individuals was higher than that of CC homozygous individuals ($p < 0.05$); at g.21272306 T>C, CT genotype individuals also had a higher value than that of CC genotype individuals ($p < 0.05$). In contrast, the carcass length of CC homozygous individuals was higher than that of TT homozygous individuals ($p < 0.05$) at g.21272422 C>T. Additionally, individuals with TC

and CC genotypes showed a higher live weight than TT individuals at g.21272306 T>C ($p < 0.05$). A genotype comparison revealed no significant differences between individuals with the TC and CC genotypes ($p > 0.05$). AG genotype individuals had a higher live weight than AA and GG homozygous individuals at g.21272529 A>G ($p < 0.05$). Similarly, no significant associations were observed among individuals with the AA and GG genotypes ($p > 0.05$). No significant associations were observed between the g.21272246 A>G and g.21272422 C>T variants and the live weight measured ($p > 0.05$).

Similar, highly significant associations were also discovered in the meat quality traits at the four locations with the rib eye area, pH, and marbling score. Regarding the rib eye area, which is related to meat quality, it was observed that individuals with the AG genotype exhibited a higher rib eye area compared to those with GG genotype at g.21272246 A>G and g.21272529 A>G ($p < 0.05$). The rib eye area of TC heterozygous individuals was higher than that of CC individuals at g.21272306 T>C; the CT heterozygous individuals had a higher rib eye area than the individuals with the TT homozygous genotype ($p < 0.05$) at g.21272422 C>T, indicating a positive correlation between these genotypes and rib eye area related to meat quality traits. A highly significant correlation was observed with the meat quality trait of pH; individuals of the AA homozygous genotype had a significantly higher pH value at 0 h and 24 h compared with GG homozygous individuals at g.21272246 A>G and g.21272529 A>G ($p < 0.01$), and polymorphisms on g.21272306 T>C and g.21272422 C>T loci were also positively correlated with pH at 0 h and 24 h, respectively ($p < 0.01$). Surprisingly, the GG homozygous individuals displayed a higher marbling score than the AG heterozygous individuals g.21272246 A>G and g.21272529 A>G ($p < 0.05$), individuals with the CC genotype exhibited significantly higher levels compared to those with the TC heterozygous genotype at g.21272306 T>C, and TT homozygous individuals displayed a higher value than CT heterozygous individuals at g.21272422 C>T regarding marbling score, respectively ($p < 0.01$, $p < 0.05$).

Table 4. Association analyses of *SCD1* gene polymorphisms with economic traits in Chinese Simmental cattle.

Traits		g.21272246 A>G			g.21272306 T>C			g.21272422 C>T			g.21272529 A>G		
		AA (n = 123)	AG (n = 123)	GG (n = 73)	TT (n = 123)	TC (n = 123)	CC (n = 71)	CC (n = 128)	CT (n = 123)	TT (n = 67)	AA (n = 127)	AG (n = 137)	GG (n = 70)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Carcass traits	LW[1] (kg)	487.73 ± 58.66	500.31 ± 58.54	487.52 ± 64.2	486.73 ± 57.83 ^{b}	501.28 ± 57.29 ^{a}	488.69 ± 67.38 ^{a}	486.36 ± 57.65	501.78 ± 59.05	487.32 ± 64.67	486.24 ± 57.86 ^{b}	502.41 ± 58.59 ^{a}	486.30 ± 64.76 ^{b}
	CW (kg)	254.11 ± 35.34 ^{b}	262.81 ± 36.77 ^{a}	253.52 ± 41.81 ^{b}	253.7 ± 35.03 ^{b}	263.51 ± 36.22 ^{a}	253.68 ± 43.05 ^{b}	253.50 ± 34.95 ^{b}	263.68 ± 37.05 ^{a}	253.01 ± 41.89 ^{b}	253.39 ± 35.07 ^{b}	264.11 ± 36.75 ^{a}	252.38 ± 41.92 ^{b}
	DP (%)	52.02 ± 2.30	52.42 ± 2.15	51.82 ± 2.86	52.05 ± 2.31	52.46 ± 2.15	51.73 ± 2.85	52.04 ± 2.30	52.44 ± 2.15	51.74 ± 2.88	52.03 ± 2.30	52.47 ± 2.14	51.72 ± 2.86
	CL (cm)	140.52 ± 8.13 ^{a}	139.42 ± 8.36	138.19 ± 7.90 ^{b}	140.34 ± 8.15 ^{a}	139.50 ± 8.23	138.23 ± 8.22 ^{b}	140.44 ± 8.14 ^{a}	139.57 ± 8.33	137.90 ± 7.90 ^{b}	140.36 ± 8.12 ^{a}	139.65 ± 8.38	137.91 ± 7.84 ^{b}
	CD (cm)	64.78 ± 3.14	64.32 ± 3.48	64.14 ± 3.33	64.67 ± 3.09	64.38 ± 3.43	64.17 ± 3.56	64.73 ± 3.11	64.42 ± 3.49	63.99 ± 3.35	64.69 ± 3.07	64.45 ± 3.53	64.01 ± 3.34
	CBD (cm)	65.33 ± 3.95	65.45 ± 3.47	64.91 ± 3.62	65.21 ± 3.89	65.48 ± 3.38	65.03 ± 3.88	65.27 ± 3.90	65.53 ± 3.46	64.84 ± 3.69	65.23 ± 3.88	65.56 ± 3.49	64.87 ± 3.67
	HLC (cm)	48.72 ± 4.08	48.97 ± 3.45	49.06 ± 3.56	48.73 ± 4.03	48.99 ± 3.42	49.07 ± 3.68	48.72 ± 4.02	49.02 ± 3.45	48.97 ± 3.66	48.70 ± 4.03	49.03 ± 3.45	48.99 ± 3.63
	HLW (cm)	44.56 ± 2.92	44.85 ± 2.56	44.56 ± 2.71	44.49 ± 2.92	44.86 ± 2.51	44.73 ± 2.74	44.49 ± 2.94	44.87 ± 2.51	44.66 ± 2.72	44.46 ± 2.92	44.89 ± 2.53	44.66 ± 2.7
	HLL (cm)	80.22 ± 4.40	80.17 ± 4.01	80.01 ± 3.95	80.12 ± 4.47	80.12 ± 3.94	80.24 ± 3.95	80.19 ± 4.48	80.14 ± 3.92	80.11 ± 3.92	80.14 ± 4.46	80.16 ± 3.95	80.16 ± 3.92
	TMT (cm)	17.81 ± 1.81	17.88 ± 1.68	18.20 ± 1.58	17.79 ± 1.82	17.88 ± 1.69	18.29 ± 1.50	17.76 ± 1.81	17.91 ± 1.70	18.26 ± 1.48	17.77 ± 1.82	17.90 ± 1.70	18.25 ± 1.48
	BFT (cm)	0.93 ± 0.61 ^{B}	1.10 ± 0.64 ^{A}	0.93 ± 0.60 ^{B}	0.96 ± 0.63 ^{B}	1.10 ± 0.63 ^{A}	0.87 ± 0.57 ^{B}	0.94 ± 0.63 ^{B}	1.10 ± 0.63 ^{A}	0.89 ± 0.57 ^{B}	0.95 ± 0.63 ^{B}	1.10 ± 0.63 ^{A}	0.88 ± 0.57 ^{B}
	WMT (cm)	6.77 ± 0.92 ^{b}	6.94 ± 0.85 ^{a}	6.91 ± 0.93 ^{ab}	6.78 ± 0.91 ^{b}	6.97 ± 0.87 ^{a}	6.89 ± 0.90 ^{ab}	6.76 ± 0.91 ^{b}	6.96 ± 0.87 ^{a}	6.91 ± 0.90 ^{ab}	6.77 ± 0.91 ^{b}	6.97 ± 0.87 ^{a}	6.89 ± 0.90 ^{ab}
Meat quality traits	pH (0 h)	6.33 ± 0.51 ^{A}	6.19 ± 0.49 ^{B}	6.16 ± 0.51 ^{B}	6.31 ± 0.51 ^{A}	6.18 ± 0.49 ^{B}	6.18 ± 0.52 ^{B}	6.32 ± 0.51 ^{A}	6.19 ± 0.49 ^{B}	6.17 ± 0.52 ^{B}	6.32 ± 0.51 ^{A}	6.19 ± 0.49 ^{B}	6.17 ± 0.51 ^{B}
	pH (24 h)	5.60 ± 0.34 ^{A}	5.55 ± 0.31 ^{AB}	5.49 ± 0.37 ^{B}	5.60 ± 0.34 ^{A}	5.55 ± 0.31 ^{AB}	5.49 ± 0.38 ^{B}	5.60 ± 0.33 ^{A}	5.55 ± 0.31 ^{AB}	5.48 ± 0.38 ^{B}	5.60 ± 0.33 ^{A}	5.55 ± 0.31 ^{AB}	5.49 ± 0.38 ^{B}
	MBS	5.39 ± 0.72 ^{ab}	5.26 ± 0.74 ^{b}	5.48 ± 0.69 ^{a}	5.40 ± 0.72 ^{AB}	5.24 ± 0.75 ^{B}	5.49 ± 0.67 ^{A}	5.40 ± 0.71 ^{ab}	5.26 ± 0.75 ^{b}	5.48 ± 0.68 ^{a}	5.40 ± 0.72 ^{ab}	5.25 ± 0.75 ^{b}	5.49 ± 0.68 ^{a}
	MCS	5.72 ± 1.08	5.57 ± 1.08	5.64 ± 1.12	5.70 ± 1.08	5.62 ± 1.10	5.58 ± 1.10	5.70 ± 1.07	5.61 ± 1.09	5.59 ± 1.12	5.70 ± 1.07	5.61 ± 1.09	5.59 ± 1.11
	REA (cm^2)	79.28 ± 13.44 ^{ab}	81.30 ± 12.56 ^{a}	77.23 ± 11.88 ^{b}	79.21 ± 13.28 ^{ab}	81.12 ± 12.69 ^{a}	77.73 ± 12.11 ^{b}	79.18 ± 13.17 ^{ab}	81.07 ± 12.8 ^{a}	77.78 ± 11.98 ^{b}	79.19 ± 13.23 ^{ab}	81.20 ± 12.68 ^{a}	77.53 ± 12.08 ^{b}

The different letters (a,b) indicate significant differences among the genotypes ($p < 0.05$); The different letters (A,B) indicate highly significant differences among the genotypes ($p < 0.01$); Mean ± SD, mean ± standard deviation. LW[1], live weight; CW, carcass weight; DP, dressing percentage; CL, carcass length; CD, carcass depth; CBD, carcass breast depth; HLC, hind legs' circumference; HLW, hind legs' width; HLL, hind legs' length; TMT, thigh meat thickness; BFT, backfat thickness; WMT, waist meat thickness; pH (0 h), beef pH value after slaughter; pH (24 h), beef pH value 24 h after degassing; MBS, marbling score (the score range of marbling is from No. 1 to 9); MCS, muscle color score (the score range of muscle color is from No. 1 to No. 7); REA, rib eye area.

3.4. Association Analyses of SCD1 Gene Polymorphisms with Adipogenesis Traits and Fatty Acid Composition

A correlation analysis of the SNPs in the *SCD1* gene related to adipogenesis traits and fatty acid composition is presented in Table 5. The individuals with the AA and AG genotypes had a significantly higher score of fat color than those of GG homozygous individuals at g.21272246 A>G and g.21272529 A>G. ($p < 0.05$). Moreover, at g.21272306 T>C, TT homozygous individuals' scores were significantly higher than CC homozygous individuals ($p < 0.05$). Interestingly, the fat color score of CC genotype individuals had a highly significant difference compared to the TT genotype at g.21272422 C>T in the Chinese Simmental cattle population ($p < 0.01$). In carcass fat coverage rate, both heterozygous and mutant individuals at these four loci exhibited significantly higher levels than wild-type individuals ($p < 0.01$). Moreover, regarding the aspects of lung and trachea and kidney weight, the association analysis reflected that these metrics in heterozygous individuals were significantly higher than for homozygotes at the four SNPs of the *SCD1* gene ($p < 0.01$). A highly significant difference was observed in terms of cow penis weight at these four loci, wild-type individuals were higher in this metric than mutant individuals ($p < 0.01$). Heterozygous individuals had a higher spleen weight than that of homozygous individuals at g.21272422 C>T and g.21272529 A>G ($p < 0.05$). Furthermore, the renal adipose weight of heterozygous individuals was significantly higher than that of wild-type individuals at the g.21272306 T>C, g.21272422 C>T, and g.21272529 A>G ($p < 0.01$). In front hoof weight and heart weight, the individuals with the AG genotype had a higher weight of the front hoof and heart than those individuals with AA and GG genotypes at g.21272246 A>G; an identical pattern was observed at the remaining three loci.

As shown in Table 5, the SNPs of the *SCD1* gene had a significant association with linoleic acid and α-linolenic acid. In multiple genotype comparisons at g.21272246 A>G and g.21272529 A>G loci, GG genotype individuals exhibited a higher linoleic acid compared to AG and AA individuals ($p < 0.01$), and similar results were seen for the AA and AG genotypes ($p > 0.05$). Individuals of the CC genotype had a higher linoleic acid content than the TT and TC genotypes, and individuals of the TT genotype had a higher linoleic acid content than the CC and CT genotypes at g.21272306 T>C and g.21272422 C>T, respectively ($p < 0.01$). A higher α-linolenic acid content was significantly elevated in GG genotype individuals compared to that of the AA genotype at g.21272246 A>G and g.21272529 A>G ($p < 0.01$); at g.21272306 T>C locus, α-linolenic acid was significantly higher for CC genotype individuals than for the TT genotype, and TT genotype individuals had a higher α-linolenic acid content than the CC genotype at g.21272422 C>T ($p < 0.01$). It is worth mentioning that a significant association was observed with stearic acid, wherein CC individuals displayed a higher stearic acid content than TT and TC genotype individuals at g.21272306 T>C ($p < 0.05$). No significant associations were observed between the three other loci and stearic acid ($p > 0.05$).

Table 5. Association analyses of *SCD1* gene polymorphisms with adipogenesis traits and fatty acid composition in Chinese Simmental cattle.

Traits		AA (n = 123)	g.21272246 A>G AG (n= 138)	GG (n = 73)	TT (n = 127)	g.21272306 T>C TC (n = 136)	CC (n = 71)	CC (n = 128)	g.21272422 C>T CT (n = 137)	TT (n = 69)	AA (n = 127)	g.21272529 A>G AG (n = 137)	GG (n = 70)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Adipogenesis traits	MBS	5.39 ± 0.72 ^{ab}	5.26 ± 0.74 ^{b}	5.48 ± 0.69 ^{a}	5.40 ± 0.72 ^{AB}	5.24 ± 0.75 ^{B}	5.49 ± 0.67 ^{A}	5.40 ± 0.71 ^{ab}	5.26 ± 0.75 ^{b}	5.48 ± 0.68 ^{a}	5.40 ± 0.72 ^{AB}	5.25 ± 0.75 ^{B}	5.49 ± 0.68 ^{A}
	FCS	2.79 ± 1.03 ^{a}	2.77 ± 0.87 ^{a}	2.53 ± 1.04 ^{b}	2.79 ± 1.02 ^{a}	2.75 ± 0.85	2.55 ± 1.08 ^{b}	2.80 ± 1.02 ^{A}	2.77 ± 0.87 ^{a}	2.49 ± 1.04 ^{B,b}	2.80 ± 1.02 ^{a}	2.76 ± 0.85 ^{a}	2.53 ± 1.07 ^{b}
	BFT (cm)	0.93 ± 0.61 ^{B}	1.10 ± 0.64 ^{A}	0.93 ± 0.60 ^{B}	0.96 ± 0.63 ^{B}	1.10 ± 0.63 ^{A}	0.87 ± 0.57 ^{B}	0.94 ± 0.63 ^{B}	1.10 ± 0.63 ^{A}	0.89 ± 0.57 ^{B}	0.95 ± 0.63 ^{B}	1.10 ± 0.63 ^{A}	0.88 ± 0.57 ^{B}
	FCR%	47.88 ± 21.66 ^{b}	50.44 ± 20.91 ^{a}	51.16 ± 19.93 ^{a}	48.16 ± 21.5 ^{B}	51.21 ± 20.99 ^{A}	49.8 ± 19.78 ^{AB}	47.7 ± 21.65 ^{B}	51.09 ± 20.95 ^{A}	50.43 ± 19.67 ^{AB}	47.91 ± 21.60 ^{b}	50.99 ± 21.07 ^{a}	50.21 ± 19.6 ^{ab}
	RRAW (kg)	7.50 ± 0.98	7.47 ± 0.92	7.55 ± 0.96	7.48 ± 0.98	7.45 ± 0.87	7.62 ± 1.04	7.49 ± 0.98	7.47 ± 0.91	7.57 ± 0.97	7.48 ± 0.98	7.48 ± 0.91	7.57 ± 0.96
	HW[1] (kg)	23.53 ± 2.39	23.84 ± 2.34	23.15 ± 2.58	23.39 ± 2.33	23.86 ± 2.26	23.27 ± 2.76	23.44 ± 2.36	23.89 ± 2.34	23.18 ± 2.64	23.43 ± 2.37	23.92 ± 2.33	23.16 ± 2.62
	FHW (kg)	5.90 ± 0.62 ^{b}	6.04 ± 0.71 ^{a}	5.86 ± 0.71 ^{b}	5.87 ± 0.62 ^{b}	6.06 ± 0.69 ^{a}	5.88 ± 0.74 ^{b}	5.87 ± 0.62 ^{b}	6.07 ± 0.70 ^{a}	5.86 ± 0.71 ^{b}	5.87 ± 0.62 ^{b}	6.07 ± 0.7 ^{a}	5.86 ± 0.71 ^{b}
	HHW (kg)	3.50 ± 1.09	3.40 ± 0.95	3.38 ± 0.98	3.47 ± 1.08	3.39 ± 0.93	3.44 ± 1.03	3.49 ± 1.09	3.04 ± 0.94	3.39 ± 0.99	3.48 ± 1.09	3.41 ± 0.95	3.40 ± 0.99
	OmW (kg)	4.02 ± 0.76	3.93 ± 0.69	3.88 ± 0.66	3.99 ± 0.75	3.92 ± 0.66	3.94 ± 0.74	4.00 ± 0.75	3.94 ± 0.70	3.90 ± 0.67	4.00 ± 0.75	3.94 ± 0.70	3.90 ± 0.67
	HW[2] (kg)	1.78 ± 0.31 ^{B}	1.88 ± 0.35 ^{A}	1.78 ± 0.33 ^{B}	1.77 ± 0.31 ^{B}	1.89 ± 0.35 ^{A}	1.78 ± 0.33 ^{B}	1.77 ± 0.30 ^{B}	1.88 ± 0.35 ^{A}	1.78 ± 0.33 ^{B}	1.77 ± 0.31 ^{B}	1.89 ± 0.35 ^{A}	1.77 ± 0.33 ^{B}
	SW (kg)	0.86 ± 0.17	0.87 ± 0.20	0.83 ± 0.20	0.86 ± 0.17	0.87 ± 0.19	0.83 ± 0.20	0.86 ± 0.17 ^{b}	0.87 ± 0.20 ^{a}	0.82 ± 0.20 ^{b}	0.86 ± 0.170 ^{b}	0.87 ± 0.20 ^{a}	0.82 ± 0.20 ^{b}
	LTW (kg)	3.09 ± 0.42 ^{B}	3.22 ± 0.49 ^{A}	3.14 ± 0.51 ^{AB}	3.09 ± 0.42 ^{B}	3.24 ± 0.49 ^{A}	3.14 ± 0.51 ^{AB}	3.09 ± 0.42 ^{B}	3.23 ± 0.49 ^{A}	3.14 ± 0.52 ^{AB}	3.08 ± 0.42 ^{B}	3.24 ± 0.49 ^{A}	3.14 ± 0.51 ^{AB}
	KW (kg)	1.15 ± 0.18 ^{B}	1.20 ± 0.22 ^{A}	1.16 ± 0.21 ^{AB}	1.15 ± 0.18 ^{B}	1.20 ± 0.22 ^{A}	1.17 ± 0.21 ^{AB}	1.15 ± 0.18 ^{B}	1.20 ± 0.22 ^{A}	1.17 ± 0.21 ^{AB}	1.15 ± 0.18 ^{B}	1.20 ± 0.22 ^{A}	1.17 ± 0.21 ^{AB}
	RAW (kg)	4.58 ± 2.70	4.98 ± 2.80	4.81 ± 2.87	4.57 ± 2.67 ^{B}	5.11 ± 2.83 ^{A}	4.63 ± 2.84 ^{AB}	4.53 ± 2.67 ^{B}	5.09 ± 2.82 ^{A}	4.71 ± 2.84 ^{AB}	4.55 ± 2.67 ^{B}	5.09 ± 2.83 ^{A}	4.67 ± 2.84 ^{AB}
	CPW (kg)	0.45 ± 0.09 ^{A}	0.44 ± 0.08 ^{AB}	0.42 ± 0.08 ^{B}	0.45 ± 0.09 ^{A}	0.44 ± 0.08 ^{AB}	0.42 ± 0.09 ^{B}	0.45 ± 0.09 ^{A}	0.44 ± 0.08 ^{AB}	0.42 ± 0.09 ^{B}	0.45 ± 0.09 ^{A}	0.44 ± 0.08 ^{AB}	0.42 ± 0.09 ^{B}
	TaW (kg)	42.22 ± 6.64	42.47 ± 5.98	41.03 ± 5.90	42.12 ± 6.71	42.31 ± 5.70	41.41 ± 6.32	42.27 ± 6.8	42.34 ± 5.74	41.10 ± 6.01	42.16 ± 6.70	42.43 ± 5.87	41.15 ± 5.98
	TeW (kg)	0.63 ± 0.12	0.61 ± 0.13	0.57 ± 0.11	0.67 ± 0.14	0.68 ± 0.15	0.65 ± 0.15	0.67 ± 0.14	0.68 ± 0.15	0.66 ± 0.15	0.67 ± 0.14	0.68 ± 0.15	0.65 ± 0.15
	GFW (kg)	0.93 ± 0.38	0.89 ± 0.34	0.85 ± 0.34	0.91 ± 0.37	0.89 ± 0.34	0.86 ± 0.35	0.92 ± 0.37	0.89 ± 0.34	0.85 ± 0.34	0.92 ± 0.37	0.89 ± 0.34	0.85 ± 0.34
	OxW (kg)	1.34 ± 0.22	1.38 ± 0.26	1.35 ± 0.27	1.33 ± 0.22 ^{b}	1.39 ± 0.26 ^{a}	1.34 ± 0.26 ^{ab}	1.33 ± 0.22 ^{b}	1.39 ± 0.27 ^{a}	1.34 ± 0.25 ^{ab}	1.33 ± 0.22 ^{b}	1.40 ± 0.26 ^{a}	1.33 ± 0.26 ^{b}
	BW (kg)	20.24 ± 3.03	20.23 ± 3.22	19.77 ± 2.98	20.11 ± 3.04	20.27 ± 3.16	19.92 ± 3.12	20.14 ± 3.04	20.28 ± 3.20	19.84 ± 3.00	20.11 ± 3.03	20.32 ± 3.21	19.81 ± 2.99
Fatty acid composition	Myristic acid	0.020 ± 0.017	0.020 ± 0.017	0.025 ± 0.018	0.020 ± 0.017	0.019 ± 0.017	0.027 ± 0.020	0.02 ± 0.017	0.021 ± 0.018	0.025 ± 0.019	0.020 ± 0.017	0.020 ± 0.018	0.025 ± 0.019
	Myristoleic acid	0.002 ± 0.004	0.002 ± 0.004	0.003 ± 0.004	0.002 ± 0.006	0.002 ± 0.004	0.003 ± 0.004	0.002 ± 0.006	0.002 ± 0.004	0.003 ± 0.004	0.002 ± 0.006	0.002 ± 0.004	0.003 ± 0.004
	Palmitic acid	0.265 ± 0.223	0.246 ± 0.179	0.309 ± 0.183	0.264 ± 0.221	0.237 ± 0.174	0.321 ± 0.188	0.261 ± 0.220	0.250 ± 0.180	0.313 ± 0.186	0.264 ± 0.221	0.247 ± 0.181	0.309 ± 0.183
	Palmitoleic acid	0.031 ± 0.044	0.024 ± 0.020	0.031 ± 0.018	0.031 ± 0.044	0.024 ± 0.020	0.031 ± 0.018	0.03 ± 0.043	0.024 ± 0.020	0.031 ± 0.019	0.031 ± 0.044	0.024 ± 0.020	0.031 ± 0.018
	Margaric acid	0.011 ± 0.007	0.011 ± 0.007	0.013 ± 0.008	0.011 ± 0.007	0.011 ± 0.007	0.014 ± 0.008	0.011 ± 0.007	0.012 ± 0.008	0.014 ± 0.008	0.011 ± 0.007	0.012 ± 0.008	0.013 ± 0.008
	Heptadecenoic acid	0.006 ± 0.009	0.004 ± 0.006	0.005 ± 0.005	0.006 ± 0.009	0.004 ± 0.006	0.006 ± 0.005	0.006 ± 0.009	0.004 ± 0.006	0.006 ± 0.005	0.006 ± 0.009	0.004 ± 0.006	0.005 ± 0.005
	Stearic acid	0.183 ± 0.109	0.186 ± 0.119	0.227 ± 0.126	0.182 ± 0.108 ^{b}	0.178 ± 0.105 ^{b}	0.241 ± 0.140 ^{a}	0.181 ± 0.108	0.189 ± 0.120	0.231 ± 0.128	0.182 ± 0.108	0.188 ± 0.120	0.227 ± 0.126
	Oleic acid	0.401 ± 0.541	0.332 ± 0.233	0.388 ± 0.201	0.400 ± 0.535	0.321 ± 0.230	0.401 ± 0.207	0.395 ± 0.530	0.333 ± 0.234	0.393 ± 0.204	0.400 ± 0.535	0.331 ± 0.235	0.387 ± 0.201
	Linoleic acid	0.096 ± 0.025 ^{B}	0.101 ± 0.026 ^{B}	0.123 ± 0.043 ^{A}	0.097 ± 0.025 ^{B}	0.098 ± 0.023 ^{B}	0.126 ± 0.045 ^{A}	0.097 ± 0.024 ^{B}	0.101 ± 0.027 ^{B}	0.123 ± 0.044 ^{A}	0.097 ± 0.025 ^{B}	0.100 ± 0.027 ^{B}	0.123 ± 0.043 ^{A}
	α-linolenic acid	0.004 ± 0.005 ^{B}	0.006 ± 0.005 ^{AB}	0.009 ± 0.012 ^{A}	0.005 ± 0.005 ^{B}	0.006 ± 0.005 ^{AB}	0.009 ± 0.011 ^{A}	0.004 ± 0.005 ^{B}	0.006 ± 0.005 ^{AB}	0.010 ± 0.012 ^{A}	0.005 ± 0.005 ^{B}	0.006 ± 0.005 ^{AB}	0.009 ± 0.012 ^{A}

The different letters (a,b) indicate significant differences among the genotypes ($p < 0.05$); The different letters (A,B) indicate highly significant differences among the genotypes ($p < 0.01$); Mean ± SD, mean ± standard deviation. MBS, marbling score (the score range of marbling is from No. 1 to 9); FCS, fat color score (the score range of fat color is from No. 1 to 7); BFT, backfat thickness; FCR, carcass fat coverage rate; RRAW, rumen, reticulum, and abomasum weight; HW[1]: head weight; FHW, front hoof weight; HHW, hind hoof weight; OmW, omasum weight; HW[2], heart weight; SW, spleen weight; LTW, lung and trachea weight; KW, kidney weight; RAW, renal adipose weight; CPW, cow penis weight; TaW, tare weight; TeW, testicular weight; GFW, genital fat weight; OxW, oxtail weight; BW, bone weight.

3.5. Association Analysis of the SNPs in the SCD1 Gene with Milk Production Traits in Chinese Holstein Cows

The association analysis is shown in Table 6. These four SNPs had a significant association with the average milk yield and milk fat content. The average milk yield of the GG genotype individuals was significantly higher compared to that of AG individuals at g.21272246 A>G and g.21272529 A>G ($p < 0.05$). Individuals of the CC genotype had a higher average milk yield than the TC genotype at g.21272306 T>C, and the CC genotype individuals had a higher average milk yield than the TC genotype at g.21272422 C>T, respectively ($p < 0.05$). Additionally, individuals with the AA genotype had a higher milk fat content than those with the GG genotype at g.21272246 A>G and g.21272529 A>G ($p < 0.05$). The milk fat content of the TT genotype individuals was higher than that of CC homozygous individuals ($p < 0.05$) at g.21272306 T>C, and CC genotype individuals also had a higher value than that of the TT genotype at g.21272422 C>T in Chinese Holstein cows ($p < 0.05$).

Table 6. Association analyses of *SCD1* gene polymorphisms with milk traits in Chinese Holstein cows.

Traits	g.21272246 A>G			g.21272306 T>C			g.21272422 C>T			g.21272529 A>G		
	AA (n = 33)	AG (n = 36)	GG (n = 4)	TT (n = 33)	TC (n = 36)	CC (n = 4)	CC (n = 33)	CT (n = 36)	TT (n = 4)	AA (n = 33)	AG (n = 36)	GG (n = 4)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Average milk yield (kg/day)	29.38 ± 5.71 ^{ab}	26.36 ± 6.44 ^{b}	31.86 ± 7.54 ^{a}	29.38 ± 5.71 ^{ab}	26.36 ± 6.44 ^{b}	31.86 ± 7.54 ^{a}	29.38 ± 5.71 ^{ab}	26.36 ± 6.44 ^{b}	31.86 ± 7.54 ^{a}	29.38 ± 5.71	26.36 ± 6.44 ^{b}	31.86 ± 7.54 ^{a}
Milk fat content (%)	4.59 ± 0.38 ^{a}	4.53 ± 0.48 ^{ab}	4.15 ± 0.47 ^{b}	4.59 ± 0.38 ^{a}	4.53 ± 0.48 ^{ab}	4.15 ± 0.47 ^{b}	4.59 ± 0.38 ^{a}	4.53 ± 0.48 ^{ab}	4.15 ± 0.47 ^{b}	4.59 ± 0.38 ^{a}	4.53 ± 0.48 ^{ab}	4.15 ± 0.47 ^{b}
Milk protein content (%)	3.31 ± 0.23	3.29 ± 0.34	3.22 ± 0.25	3.31 ± 0.23	3.29 ± 0.34	3.22 ± 0.25	3.31 ± 0.23	3.29 ± 0.34	3.22 ± 0.25	3.31 ± 0.23	3.29 ± 0.34	3.22 ± 0.25
Milk lactose (%)	4.62 ± 0.26	4.52 ± 0.34	4.42 ± 0.36	4.62 ± 0.26	4.52 ± 0.34	4.42 ± 0.36	4.62 ± 0.26	4.52 ± 0.34	4.42 ± 0.36	4.62 ± 0.26	4.52 ± 0.34	4.42 ± 0.36
Dry matter intake (kg)	13.42 ± 0.92	13.29 ± 1.12	12.94 ± 0.87	13.42 ± 0.92	13.29 ± 1.12	12.94 ± 0.87	13.42 ± 0.92	13.29 ± 1.12	12.94 ± 0.87	13.42 ± 0.92	13.29 ± 1.12	12.94 ± 0.87
Milk urea-nitrogen (mg/dl)	18.7 ± 2.10	18.37 ± 1.74	18.07 ± 3.20	18.7 ± 2.10	18.37 ± 1.74	18.07 ± 3.20	18.7 ± 2.10	18.37 ± 1.74	18.07 ± 3.20	18.7 ± 2.10	18.37 ± 1.74	18.07 ± 3.20

The different letters indicate significant differences among the genotypes ($p < 0.05$); Mean ± SD, mean ± standard deviation.

3.6. Association Analysis of Haplotypes and Carcass Traits, Meat Quality, Adipogenesis Traits, and Fatty Acid Composition in Chinese Simmental Cattle

The haplotype analysis results have been described above (Figure 2C). Five haplotype combinations, H1H1, H1H2, H1H3, H2H2, and H2H3, were reconstructed in Chinese Simmental steers as illustrated in Table 7. The correlation analysis indicates that the haplotype combination H2H3 demonstrates significantly higher values for carcass fat coverage rate, renal adipose weight, oxtail weight, and muscle color score compared to the H1H1, H1H2, H1H3, and H2H2 haplotypes ($p < 0.05$), but there is no significant association in the other haplotype combinations. Since the haplotype combinations of H2H3 represented too few individuals to be significant in the statistical analysis, the subsequent correlation analysis between these four haplotype combinations and fatty acid composition is presented in Table 8. The H2H2 haplotype combination exhibits significantly higher levels of linoleic acid and α-linolenic acid than H1H1 and H1H2, while it is similar to the H1H1 and H1H2 haplotype combination individuals ($p > 0.05$).

Table 7. Association analysis of SNPs haplotypes combination and carcass traits, meat quality, adipogenesis traits in Chinese Simmental steers.

Traits	Haplotypes Combination				
	H1H1 (*n* = 121)	H1H2 (*n* = 131)	H1H3 (*n* = 5)	H2H2 (*n* = 68)	H2H3 (*n* = 2)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
LW[1] (kg)	486.81 ± 58.71	499.98 ± 57.88	477.70 ± 42.14	486.54 ± 64.82	543.00 ± 4.36
CW (kg)	253.68 ± 35.46	262.68 ± 36.55	248.84 ± 30.65	252.54 ± 42.01	294.83 ± 10.80
DP (%)	52.03 ± 2.31	52.43 ± 2.15	51.99 ± 2.70	51.72 ± 2.90	54.29 ± 1.64
BW (kg)	20.21 ± 3.03	20.21 ± 3.18	18.60 ± 2.30	19.84 ± 3.02	20.33 ± 0.58
CL (cm)	140.43 ± 8.13	139.17 ± 8.16	139.60 ± 9.32	137.87 ± 7.95	146.00 ± 5.29
CD (cm)	64.72 ± 3.10	64.21 ± 3.35	63.60 ± 2.88	63.98 ± 3.38	66.67 ± 1.53
CBD (cm)	65.28 ± 3.93	65.37 ± 3.36	64.40 ± 2.79	64.84 ± 3.72	66.50 ± 0.87
HLW (cm)	44.52 ± 2.92	44.82 ± 2.51	43.80 ± 2.80	44.64 ± 2.74	44.17 ± 1.26
HLC (cm)	48.71 ± 4.10	48.94 ± 3.47	48.10 ± 2.30	49.01 ± 3.67	49.67 ± 1.44
HLL (cm)	80.17 ± 4.36	80.05 ± 3.88	80.80 ± 6.41	80.14 ± 3.94	78.17 ± 2.02
WMT (cm)	6.77 ± 0.92	6.96 ± 0.86	6.70 ± 0.72	6.91 ± 0.90	7.67 ± 1.31
TMT (cm)	17.8 ± 1.83	17.88 ± 1.67	17.60 ± 1.47	18.26 ± 1.49	18.37 ± 3.01
HW[1] (kg)	23.48 ± 2.38	23.82 ± 2.28	22.61 ± 1.97	23.14 ± 2.63	24.49 ± 1.48
FHW (kg)	5.89 ± 0.62	6.05 ± 0.70	5.46 ± 0.50	5.86 ± 0.72	6.21 ± 0.36
HHW (kg)	3.50 ± 1.09	3.37 ± 0.92	3.33 ± 1.08	3.40 ± 0.99	2.94 ± 0.29
TaW (kg)	42.16 ± 6.66	42.18 ± 5.63	43.74 ± 8.10	41.07 ± 6.05	40.94 ± 1.59
BFT (cm)	0.93 ± 0.61	1.10 ± 0.62	1.22 ± 0.99	0.89 ± 0.57	1.60 ± 0.78
FCR%	48.01 ± 21.66 [b]	51.18 ± 20.67 [b]	43.20 ± 23.50 [b]	50.29 ± 19.78 [b]	73.33 ± 16.07 [a]
GFW (kg)	0.92 ± 0.37	0.88 ± 0.34	0.78 ± 0.33	0.85 ± 0.35	0.75 ± 0.41
RRAW (kg)	7.49 ± 0.98	7.44 ± 0.86	7.42 ± 1.21	7.58 ± 0.98	7.23 ± 0.89
OmW (kg)	4.01 ± 0.76	3.91 ± 0.67	3.75 ± 0.51	3.88 ± 0.67	3.85 ± 0.52
HW[2] (kg)	1.77 ± 0.31	1.88 ± 0.35	1.72 ± 0.19	1.77 ± 0.33	2.10 ± 0.02
LW[2] (kg)	5.87 ± 1.05	6.12 ± 1.10	5.54 ± 0.78	5.84 ± 1.06	6.37 ± 0.59
SW (kg)	0.86 ± 0.18	0.87 ± 0.20	0.80 ± 0.10	0.82 ± 0.20	0.95 ± 0.10
LTW (kg)	3.09 ± 0.42	3.23 ± 0.50	2.93 ± 0.34	3.14 ± 0.51	3.46 ± 0.42
KW (kg)	1.15 ± 0.18	1.21 ± 0.23	1.06 ± 0.19	1.17 ± 0.21	1.08 ± 0.13
RAW (kg)	4.58 ± 2.68 [b]	5.10 ± 2.80 [b]	3.28 ± 1.92 [b]	4.66 ± 2.83 [b]	8.23 ± 0.89 [a]
CPW (kg)	0.45 ± 0.09	0.43 ± 0.08	0.46 ± 0.16	0.42 ± 0.09	0.44 ± 0.06
TeW (kg)	0.67 ± 0.14	0.68 ± 0.15	0.69 ± 0.10	0.65 ± 0.15	0.60 ± 0.30
OxW (kg)	1.34 ± 0.23 [b]	1.39 ± 0.26 [b]	1.25 ± 0.12 [b]	1.34 ± 0.26 [b]	1.75 ± 0.11 [a]
pH (0 h)	6.33 ± 0.51	6.18 ± 0.50	6.14 ± 0.50	6.17 ± 0.52	5.86 ± 0.19
pH (24 h)	5.60 ± 0.34	5.55 ± 0.31	5.52 ± 0.22	5.49 ± 0.37	5.35 ± 0.23
MBS	5.39 ± 0.72	5.24 ± 0.75	5.60 ± 0.55	5.49 ± 0.68	5.00 ± 1.00
MCS	5.71 ± 1.08 [b]	5.60 ± 1.09 [b]	5.20 ± 0.84 [b]	5.57 ± 1.11 [b]	7.00 ± 0.00 [a]
FCS	2.80 ± 1.02	2.75 ± 0.85	2.80 ± 1.10	2.51 ± 1.03	2.33 ± 0.58
REA (cm^2)	79.36 ± 13.48	81.42 ± 12.79	76.6 ± 5.68	77.71 ± 12.05	74.33 ± 5.77

The different letters indicate significant differences among the genotypes ($p < 0.05$); Mean ± SD, mean ± standard deviation; LW[1], live weight; CW, carcass weight; DP, dressing percentage; CL, carcass length; CD, carcass depth; HLW, hind legs' width; CBD, carcass breast depth; HLC, hind legs' circumference; HLL, hind legs' length; WMT, waist meat thickness; TMT, thigh meat thickness; HW[1]: head weight; FHW, front hoof weight; HHW, hind hoof weight; TaW, tare weight; BFT, backfat thickness; FCR, carcass fat coverage rate; GFW, genital fat weight; RRAW, rumen, reticulum, and abomasum weight; OmW, omasum weight; HW[2], heart weight; LW[2], liver weight; SW, spleen weight; LTW, lung and trachea weight; KW, kidney weight; RAW, renal adipose weight; CPW, cow penis weight; TeW, testicular weight; OxW, oxtail weight; BW, bone weight; pH (0 h), beef pH value after slaughter; pH (24 h), beef pH value 24 h after degassing; MBS, marbling score (the score range of marbling is from No. 1 to 9); MCS, muscle color score (the score range of muscle color is from No. 1 to No. 7); FCS, fat color score (the score range of fat color is from No. 1 to 7); REA, rib eye area.

Table 8. Association analysis of SNPs haplotype combination of the *SCD1* gene and fatty acid composition in Chinese Simmental cattle.

Fatty Acid Composition (g/100 g)	Haplotypes			
	H1H1 (n = 43) Mean ± SD	H1H2 (n = 35) Mean ± SD	H1H3 (n = 3) Mean ± SD	H2H2 (n = 21) Mean ± SD
Myristic acid	0.020 ± 0.018	0.019 ± 0.017	0.017 ± 0.009	0.025 ± 0.019
Myristoleic acid	0.002 ± 0.006	0.002 ± 0.004	0.003 ± 0.004	0.003 ± 0.004
Palmitic acid	0.267 ± 0.226	0.241 ± 0.175	0.199 ± 0.097	0.313 ± 0.186
Palmitoleic acid	0.031 ± 0.045	0.024 ± 0.020	0.023 ± 0.018	0.031 ± 0.019
Margaric acid	0.011 ± 0.007	0.011 ± 0.007	0.009 ± 0.001	0.014 ± 0.008
Heptadecenoic acid	0.006 ± 0.009	0.004 ± 0.006	0.003 ± 0.004	0.006 ± 0.005
Stearic acid	0.184 ± 0.110	0.179 ± 0.106	0.136 ± 0.037	0.231 ± 0.128
Oleic acid	0.405 ± 0.547	0.325 ± 0.233	0.297 ± 0.166	0.393 ± 0.204
Linoleic acid	0.096 ± 0.025 ^{B}	0.098 ± 0.023 ^{B}	0.099 ± 0.001 ^{AB}	0.123 ± 0.044 ^{A}
α-linolenic acid	0.004 ± 0.005 ^{B}	0.006 ± 0.005 ^{AB}	0.005 ± 0.007 ^{AB}	0.010 ± 0.012 ^{A}
Arachic acid	0.000 ± 0.001	0.001 ± 0.005	0.000 ± 0.000	0.001 ± 0.002
Eicosanic acid	0.001 ± 0.003	0.001 ± 0.002	0.000 ± 0.000	0.001 ± 0.001
Dihomo-γ-linolenic acid	0.010 ± 0.003	0.010 ± 0.003	0.009 ± 0.001	0.010 ± 0.003
Arachidonic acid	0.049 ± 0.013	0.048 ± 0.011	0.058 ± 0.012	0.054 ± 0.017

The different letters indicate significant differences among the genotypes ($p < 0.05$); The different letters (A,B) indicate highly significant differences among the genotypes ($p < 0.01$); Mean ± SD, mean ± standard deviation.

4. Discussion

In recent years, with the increasing emphasis on healthy eating and the demand for nutrient-rich ingredients, beef and milk as high-quality protein sources have received much attention. Despite the general focus on production and reproductive traits, carcass and meat quality characteristics after slaughter, as well as milk production traits of cows, are more critical factors in determining the success of beef and dairy cattle. As a standard molecular marker, SNPs play an essential role in genetics and molecular breeding and can be used to explore the association between genes and economic traits. SNP association analysis can reveal the genetic influence on economic traits, which can guide the optimization of breed improvement and enhance the effect of economic traits in the selection process [15]. Therefore, it is necessary to select appropriate means to promote the economic traits of improving meat and milk quality.

The *SCD1* gene is abundantly expressed in adipose tissue, liver, and skeletal muscle [16], playing an essential role in the biosynthesis of unsaturated fatty acids in the milk and meat of cattle, and its polymorphisms are frequently considered as an influencing factor [17]. Based on previous reports, the present study identified four known SNPs, g.21272246 A>G, g.21272306 T>C, g.21272422 C>T, and g.21272529 A>G, in the fifth exon and intron of the bovine *SCD1* gene. Three genotypes were found to be present by DNA sequencing, and a missense mutation was found at g.21272422 C>T in exon 5, resulting in a CGG to UGG change at codon 293. This locus has been reported to be significantly associated with marbling and rib thickness in Japanese Black cattle [18], consistent with the association between SNP and marbling in the present study. However, no association was observed for carcass traits measured in northern Australian crossbreed beef cattle [19]. Additionally, the content of monounsaturated fatty acids in milk or meat products was associated with the SCD gene SNP rs41255693 (g.21272422 C>T) in cattle [20,21].

Further research is required to investigate the relationship between SNPs and the carcass traits, meat quality traits, adipogenic traits, and fatty acid composition of Chinese Simmental cattle, as well as their association with the milk production traits of Chinese Holstein cows. The association analyses revealed that four SNPs were significantly associated with carcass traits (carcass weight, carcass length, and backfat thickness, etc.), meat quality traits (marbling score, pH, rib eye area, etc.), adipogenic traits (fat color, fat coverage rate, etc.), and fatty acid composition (linoleic acid, α-linolenic acid) in Chinese Simmental steers. Additionally, these SNPs were correlated with milk production traits for Chinese Holstein cows, for instance, average milk yield and milk fat content. These

findings provide valuable insights into the genetic basis of economically important traits and may have implications for breeding programs to improve livestock productivity.

Haplotypes were also examined to establish relationships with these significant traits. The analysis showed that the H2H3 haplotype had higher values for certain traits (fat coverage rate, muscle color score, etc.) in Chinese Simmental cattle, while the H2H2 haplotype exhibited superior linoleic acid and α-linolenic acid compared to H1H1 and H1H2 in Chinese Holstein cows. Consequently, these four SNPs could be utilized to select individuals with those combinations of haplotypes.

The *SCD1* gene regulates the synthesis of unsaturated fatty acids. Previous studies have shown that fatty acids play an important role in the flavor of ruminant meat [22] and are also key in the fatty acid composition of adipose tissue and animal products such as meat and milk [14]. Smith et al. showed that the flavor produced by beef is related to the composition and type of fatty acids, and the high content of unsaturated fatty acids in fat is more conducive to producing a good flavor [23]. Kim et al. found a significant association between the SNPs of *SCD* and saturated and unsaturated fatty acids in Hanwoo steers [24]. Taniguchi et al. suggested that the *SCD* gene was responsible for the genetic variation in fatty acid composition in steers of the Japanese Black breed [25]. Linoleic acid is an essential fatty acid that can only be obtained from the diet [26]. In this study, we were surprised that four SNPs were statistically significant with linoleic acid and α-linolenic acid content in Chinese Simmental cattle. Our findings imply that these loci in the *SCD1* gene may serve as potential molecular markers to improve fatty acid composition selection in beef cattle.

In addition to its role in carcass, meat quality, and fatty acid composition, the *SCD1* gene also plays a crucial role in determining milk production traits. Studies have shown that the *SCD1* gene affects the fatty acid profile of milk [27,28]. To further explore the impact of SNPs on milk traits, our study was conducted on a population of dairy cattle, and preliminary correlation analysis showed significant associations between the four SNPs and the average milk yield and milk fat content of dairy cows.

Macciotta et al. found that SNPs in exon 5 of the *SCD1* gene were associated with milk production and protein production in Italian Holstein cattle [29]. The influence of the A293V SNP in the *SCD1* gene on milk traits in cattle has been extensively studied [30,31]. Regarding A293V SNP, at locus g.21272422 C>T, resulting in a substitution of alanine by valine at position 293 of the *SCD1* protein, individuals of the TT genotype had a significantly higher average milk yield compared to the CC genotype; our findings support the assertion that the A293V SNP has a positive impact on milk production traits in cattle, and these mutations in exons and introns may be potential genetic markers for improving milk production ability in Chinese Holstein cows. Of course, future studies should confirm these associations in larger cattle populations. Additionally, the effects of SNPs in exon 5 of *SCD1* on gene expression and protein structures need to be further explored.

5. Conclusions

This study identified four SNPs in the fifth exon and intron of the *SCD1* gene. Subsequently, their association with economic traits was further analyzed in Chinese Simmental steers and Holstein cows. The study revealed that the four SNPs of the *SCD1* gene were associated with carcass traits, meat quality, adipogenic traits, and fatty acid composition in beef cattle. Additionally, we observed a modest effect on milk production traits such as average milk yield and milk fat content in cows, especially at locus g.21272422 C>T. Further haplotype analysis indicated that H2H3 and H2H2 had better value than the others. Furthermore, the significance of the well-known *SCD1* gene marker in bovine breed improvement is underscored. These findings could serve as a valuable reference for further exploration into the subsequent functionality and mechanism of the *SCD1* gene.

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Institutional Review Board Statement: Animal experiments were performed strictly following the guidance for the care and use of laboratory animals by the Jilin University Animal Care and Use Committee (Permit number: SYXK (Ji) 2008-0010/0011).

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Article

Effects of Premortem Stress on Protein Expression, Steak Color, Oxidation, and Myofibrillar Fragmentation Index in the *Longissimus Lumborum*

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Simple Summary: This study aimed to determine the effects of premortem stress on beef quality following harvest. Forty castrated Holstein calves underwent an adrenocorticotropic hormone (ACTH) challenge to emulate a stress response. The calves were harvested at different times (2, 12, 24, and 48 h) following the challenge. In addition, cortisol was measured to determine the specific stress response of each animal during the ACTH challenge. Beef quality attributes such as the breakdown of myofibrillar proteins, color, and pH were analyzed in samples at different ages following harvest. The results show that harvest time following the ACTH challenge does impact the quality of beef that is produced. Additionally, steak color, tenderness, and protein expression may be related to stress that occurs prior to harvest.

Abstract: Forty castrated Holstein calves underwent an adrenocorticotropic hormone (ACTH) challenge to assess the effects of premortem stress on the *longissimus lumborum* (LL) following harvest. LL biopsies were collected before the challenge, at different harvest times (2, 12, 24, and 48 h; $n = 10$), and after 14 d aging. The expression of small heat shock proteins (SHSPs), deglycase 1 (DJ-1), and troponin were analyzed. Blood was analyzed throughout the ACTH challenge and at harvest for cortisol, oxidative stress, and complete blood count (CBC). Color and myofibrillar fragmentation index (MFI) were measured in aged samples. Unexpectedly, calves from different harvest times differed ($p = 0.05$) in cortisol response. Calves were divided into two different cortisol response groups (high or low; $n = 20$). Statistical analysis assessed the effects of cortisol response ($n = 20$), harvest time ($n = 10$), and their interaction. Harvest time altered SHSPs ($p = 0.03$), DJ-1 ($p = 0.002$), and troponin ($p = 0.02$) expression. Harvest time and cortisol response impacted steak color ($p < 0.05$), and harvest time altered steak pH ($p < 0.0001$). Additionally, various CBCs were changed ($p < 0.05$) by harvest time. Harvest time changed ($p = 0.02$) MFI. These data demonstrate that the protein expression, color, and MFI of the LL may be influenced by premortem stress.

Keywords: beef; heat shock proteins; oxidation; premortem stress; steak color

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1. Introduction

Tenderness and color are two of several different quality attributes that influence consumer satisfaction and purchasing decisions [1–3]. Despite similar production practices, meat products from beef cattle exhibit undesirable variation in tenderness and stability of

flavor and color [4,5]. These inconsistencies may be a result of the effect that external factors, such as premortem stress, elicit on heat shock proteins (HSPs) and oxidative stress [6,7].

Heat shock proteins are abundant and highly conserved proteins that help mitigate the effects of various stressors [8]. Some HSPs are constitutively expressed, while others are upregulated in response to stressful conditions to protect proteins from damage [9]. Small heat shock proteins (SHSPs) are upregulated in response to stressful conditions and are ATP-independent, so they may still be active after harvest [7,10,11]. Recent research suggests that SHSPs may play a role in the development of beef tenderness and color; however, their exact role is unknown [6,7,12].

Oxidative stress following a stressful event may also play a role in the development of tenderness, color stability, and the flavor of meat from beef cattle. Like tenderness, color is also an important characteristic that consumers consider while purchasing beef [13]. Oxidation causes color deterioration, undesirable flavors, and rancidity development in beef [14,15]. There are many different methods through which oxidative stress can be assessed. Lipid peroxidation forms byproducts known as thiobarbituric acid reactive substances (TBARSs), one of which is malondialdehyde (MDA) [16]. Malondialdehyde is a commonly known marker of oxidative stress [17]. Additionally, there are several different proteins that are known to be involved in oxidative stress.

The protein deglycase 1 (DJ1) is involved in cellular protection from apoptosis, and several studies have demonstrated that its abundance is related to beef tenderness [18–21]. Premortem stress may cause a cascading effect of certain pathways to decrease the quality of meat due to decreased tenderness and color stability; however, the effects that premortem stress has on SHSPs and oxidative stress are poorly understood. As such, the goal of this research was to understand how the time of harvest following premortem stress impacts biological pathways involved in stress, proteolysis, and color development in the *longissimus lumborum* (LL). It was hypothesized that as more time elapsed following a stressful event premortem, there would be decreased expression of SHSPs and less oxidative stress as the muscle returned to a homeostatic state, resulting in improved beef quality.

2. Materials and Methods

All experiments with animals were conducted following procedures approved by the USDA-ARS Livestock Issues Research Unit (LIRU) Care and Use Committee under protocol #1808S. A total of forty castrated Holstein calves (103.5 kg $\pm$ 1.6), approximately three months old, were obtained from a single commercial source for this study. Although steers at industry harvest weight and of beef breeds would have been ideal for analyzing beef quality parameters, castrated Holstein calves were used based on ease of handling, lower amounts of handling-induced stress, and availability. After arriving at the USDA-ARS LIRU research complex, castrated calves were given one week to acclimate to individual pens, while having ad libitum access to a general grower ration and water. All castrated calves were housed in an indoor, thermoneutral, climate-controlled facility throughout the duration of the study. In addition, animals were not restricted feed regardless of harvest time, and all animals were harvested in the facility where the study took place.

2.1. Initial Skeletal Muscle Samples

After the one-week acclimation period, skeletal muscle biopsies were collected from the right side of the LL at the last rib, following previously described methods [22]. This sample served as an initial control prior to the ACTH injection. In brief, castrated calves were led calmly to a cattle chute and were immobilized. The loin area of each animals was clipped, washed, and disinfected. Lidocaine was given to anesthetize the area before performing a 1 cm incision using a sterile scalpel to expose the muscle. An approximately 2 g sample was collected and was immediately snap-frozen in liquid nitrogen and stored at -80 °C for subsequent analyses.

2.2. ACTH Injection

Prior to the ACTH injection, castrated calves were fitted with a rectal temperature monitoring device, as previously described [23], and an indwelling jugular vein catheter, as previously described [24], for serial blood collection. The following day, all calves were injected intravenously with porcine ACTH at a dose of 0.1 IU/kg of body weight (Bachem Chemicals, Torrance, CA, USA) to initiate a stress response. Following the ACTH challenge, castrated calves were serially harvested in groups of 10 animals at the following time points after 6 h exposure to the ACTH-induced stress response: 2 h, 12 h, 24 h, and 48 h (Figure 1).

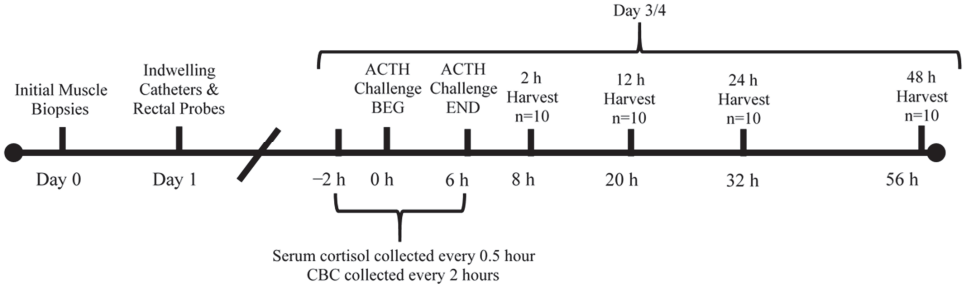

Figure 1. Timeline of sample collection during the adrenocorticotropic hormone (ACTH) challenge. CBC = complete blood count, BEG = beginning of ACTH challenge, END = end of ACTH challenge.

2.3. Blood Analyses

A ProCyte DX hematology analyzer (IDEXX Laboratories, Westbrook, ME, USA) was used to measure complete blood count (CBC) every 2 h from −2 to 6 h relative to the administration of the ACTH injection at 0 h. In addition, serum was collected for analysis of cortisol every 0.5 h from −2 to 6 h relative to the administration of the ACTH challenge at 0 h; this was stored at −80 °C until analyzed. Serum cortisol concentrations were determined using an enzyme immunoassay (Arbor Assays, Ann Arbor, MI, USA) according to the manufacturer's instructions. Intra- and inter-assay coefficients of variation were less than 8% and 12%, respectively. Following the collection of blood, all catheters were flushed with 5 mL of saline (0.9% *w*/*v* NaCl) followed by 5 mL of heparinized saline (1 mL of heparin 10,000 IU/mL in 500 mL of saline) to replace fluid volume and maintain catheter patency.

2.4. Sample Collection

At harvest, serum was collected from blood samples. Blood collected at harvest will be subsequently referred to as the "final" sample. Within 30 m of exsanguination, an approximately 5 g skeletal muscle sample from the right side of the LL was collected, approximately 5 cm anterior from the initial biopsy site. All muscle samples collected for protein and oxidative measures were immediately snap-frozen in liquid nitrogen and were stored at −80 °C for subsequent analysis. The whole LL from the left side was excised and vacuum packaged, and was then held at refrigeration (2–4 °C) for 14 d until a final LL muscle sample was collected from the posterior portion of the muscle.

2.5. Western Blotting

Skeletal muscle samples were ground under liquid nitrogen, and total protein was extracted following previously described procedures [25]. In brief, ground tissue was added to total protein extraction buffer (50 mM Tris-HCl (pH 7.52), 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Tergitol, 0.1% SDS, and 0.5% sodium deoxycholate) containing phosphatase and protease inhibitor tablets (Roche, Indianapolis, IN, USA). Samples were homogenized and rocked for 10 min at 4 °C. Samples were centrifuged at 10,000× *g* for 10 min at 4 °C. The supernatant was removed and stored at −80 °C. Total protein was quantified using a Pierce™ BCA Protein Assay Kit (Thermo Scientific,

Waltham, MA, USA). Proteins (0.3 μg total protein added per lane) were separated using SDS—10% polyacrylamide gels for 90 min at 140 V at 4 °C. Proteins were transferred to an immobilidon-polyvinylidene fluoride (PVDF) membrane at 4 °C for 90 min at 100 V. Western blot analyses were completed on samples collected prior to the ACTH challenge, immediately following harvest, and after 14 d of aging to measure the abundance of HSPβ1, phosphorylated-HSPβ1 (P-HSPβ1), HSPβ5, DJ1, and troponin I using specific primary and secondary antibodies (Table 1).

Table 1. Description of primary bovine-specific antibodies and concentration used for Western blots.

Protein Name [a]	Antibody Company	Host	Product Number	Primary Concentration	Secondary Concentration
HSPβ-1	Invitrogen [b]	rabbit	PA1-25494	1:500	1:1000
PHSPβ-1	Invitrogen [b]	rabbit	PA5-23340	1:2000	1:1000
HSPβ-5	Invitrogen [b]	mouse	MAS-27708	1:4000	1:7500
DJ1	Abcam [c]	rabbit	ab18257	1:1000	1:1000
Troponin	Invitrogen [b]	rabbit	PA5-42108	1:500	1:1000

[a] Heat shock protein β-1 (HSPβ-1), phosphorylated-HSPβ-1 (PHSPβ1), heat shock protein β-5 (HSPβ-5), protein deglycase (DJ1), troponin I (Troponin); [b] Invitrogen, Rockford, IL, USA; [c] Abcam, Cambridge, MA, USA.

All membranes were blocked in 5% non-fat milk plus 1X tris-buffered saline (1X TBS) for 1 h at room temperature, and were then incubated in primary antibody overnight at 4° C. Membranes were washed in 1X TBS and were incubated with secondary anti-rabbit antibody (HSPβ1, P-HSPβ1, DJ1, and troponin I) (7074S, Cell Signaling Technology, Beverly, MA, USA) or anti-mouse antibody (HSPβ5) (31430, Invitrogen, Rockford, IL, USA) conjugated to horseradish peroxidase (HRP) for 2 h at room temperature. Bands were visualized using a C-DiGit® Blot Scanner (LI-COR, Lincoln, NE, USA) and were quantified with Image Studio™ (LI-COR, Lincoln, NE, USA). One skeletal muscle sample not associated with this study was run on each blot; this was considered as the internal standard to account for differences between different blots. Protein abundance values were adjusted to the internal standard. See Figure 2 for the representative blots and target proteins analyzed. Blots were randomized by treatment and harvest time. As such, samples in different treatment groups were not compared visually across the same blot.

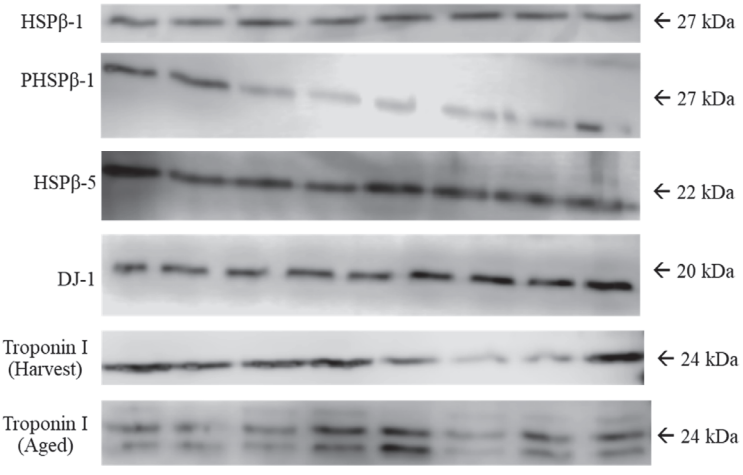

Figure 2. Representative Western blot images from the castrated Holstein calves. The representative proteins are heat shock protein β-1 (HSPβ-1), phosphorylated heat shock protein β-1 (PHSP-β1), protein deglycase (DJ-1), and troponin at harvest and after 14 d of aging. Samples were randomized by treatment groups and harvest time across each blot. Each blot is a representation of each target protein that was analyzed.

2.6. Myofibrillar Fragmentation Index

Skeletal muscle from samples aged for 14 d postmortem were ground under liquid nitrogen, and myofibril fragments were extracted as previously described [26]. In brief, ground tissue was added to myofibrillar fragmentation index (MFI) buffer (100 mM KCl, 20 mM KPO4-pH 7, mM EGTA, 1 mM MgCl2, and 1 mM NaN3) and was homogenized for 30 s. Samples were centrifuged twice at 1000× *g* for 15 min at 4 °C; supernatant was decanted, and fresh MFI buffer was added each time. Myofibrillar fragments were then quantified using the Pierce™ BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). After quantification, samples were diluted, plated, and read at 540 nm on a BioTek Synergy H1 plate reader (Agilent, Santa Clara, CA, USA) to determine absorbance. All assays were run in triplicate and the average value was reported. The measurement of MFI = 200 × absorbance.

2.7. Color and pH

Steaks aged for 14 d were used for retail display color analysis. Steak color was measured on d 0, 1, 2, 3, and 4. Steaks were placed onto foam trays with absorbent pads and were over-wrapped with a PVC film (oxygen-permeable polyvinyl chloride fresh meat film; 15,500 to 16,275 cm^3 O_2 m^{-2} 24 h^{-1} at 23 °C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film, Koch Supplies, Kansas City, MO, USA). After packaging, steaks were placed in a coffin-style open display case maintained at 2 °C ± 1 under continuous lighting (1612 to 2152 lx, Philips Deluxe Warm White Fluorescent lamps; Andover, MA, USA; color rendering index = 86; color temperature = 3000 K). All packages were rotated daily to minimize variances in light intensity and/or temperature caused by specific case locations. The surface color was measured using a HunterLab MiniScan XE Plus spectrophotometer (Model 45/0 large area view, 2.5 cm diameter aperture, Illuminant A, 10° Observer; HunterLab, Reston, VA, USA) on respective display days. Both reflectance spectra from 400 to 700 nm (10 nm increments) and CIE L*, a*, and b* values were measured on each steak at three random locations, and subsamples were averaged for statistical analyses. The pH of each steak was recorded using an Accumet 50 pH meter (Fisher Scientific, Fairlawn, NJ, USA) in duplicate, and the values were averaged.

2.8. TBARSs

TBARSs were measured in serum at 2 h increments throughout the study and at exsanguination utilizing a modification of previously described methods [27,28]. A standard curve was prepared using a trichloroacetic acid (TCA)/thiobarbituric acid (TBA) reagent (15% TCA (*w*/*v*) and 20 mM TBA in DI water) and a diluted 1, 1, 3, 3-tetra-ethoxypropanone (TEP) standard. Samples were ground under liquid nitrogen, and 2.5 g was added to 7.5 mL of cold DI water. Samples were then vortexed for 90 s and were then centrifuged for 15 min at 4500× *g*. Following centrifugation, 2 mL of supernatant was added to 4 mL of TCA/TBA reagent; then, 100 µL of butylated hydroxyanisole (BHA) was added. Samples were then vortexed for approximately 1 min. For the serum, 250 µL of sample was added to 500 µL of TCA/TBA reagent and 10 µL of BHA and was vortexed for approximately 1 min. All prepared samples and standards were heated for 1 h at 90 °C, and were then submerged in an ice water bath for 20 min. For standards, tubes were then brought to ambient temperature before each standard was plated for evaluation. Heated and cooled serum and tissue samples were centrifuged for 15 min at 4500× *g*, then 200 µL of supernatant was plated onto a 96-well plate for evaluation at 531 nm. Standard curves were used to determine the concentrations of MDA in all samples.

2.9. Statistical Analysis

Statistical analysis for all measurements was performed using the MIXED Procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The main effect of time of harvest (n = 10) was analyzed with each individual calf as a random variable. The differences in cortisol response after the initiation of the ACTH challenge was not anticipated by the authors. It was hypothesized that cortisol response would be similar amongst groups, as

they were randomly placed into harvest groupings and had similar backgrounds. However, since the cortisol response was found to differ ($p < 0.05$) among harvest groups 0.5 h after administration of ACTH, the effect of the ACTH-induced cortisol response was included as a fixed effect in the model. A total of two cortisol response groups (high or low), with 20 calves per group ($n = 20$), were assigned based on the delta area under the curve (AUC) for cortisol. This was calculated by measuring the difference in the AUC for serum cortisol concentration collected every 0.5 h from −2 to 0 h and 0 to 2 h relative to ACTH challenge (Figure 3). The effect of the delta AUC of cortisol will be referred to as cortisol response throughout the paper. The final statistical model assessed the main effects of harvest time, cortisol response, and their interaction. Protein abundance was found to be different in samples collected before the ACTH challenge, as such relative abundance was calculated and used in statistics to account for individual animal variation. Relative protein abundance was calculated by dividing the initial protein abundance of the skeletal muscle biopsy by the abundance in the samples collected at harvest and after 14 d of aging. Steak color, TBARSs, and CBC were analyzed using repeated measures and PROC MIXED, where harvest time, cortisol response, and their interaction were used as fixed effects and individual calves served as random effects in the model. In all analyses, when treatment differences were found to be significant ($p < 0.05$), least square means were separated using Tukey–Kramer adjustments. A $p < 0.05$ was considered statistically significant, whereas a $p < 0.10$ was considered a trend. All data are presented as the least square mean ± SEM.

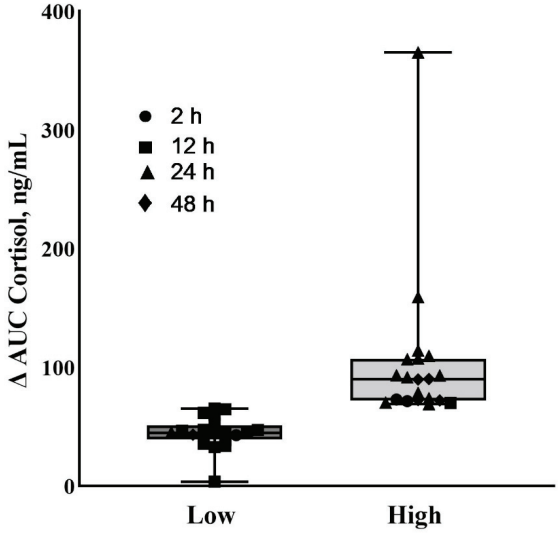

Figure 3. Different quartile groupings of castrated Holstein calves (103.5 kg ± 1.6) based on the delta area under the curve (AUC) cortisol. This was measured by finding the difference between the AUC of cortisol −2 to 0 h before initiation of the adrenocorticotropic hormone (ACTH) challenge (0.1 IU/kg of body weight) to initiate a stress response and the AUC of cortisol 0 to 2 h after ACTH was given. Castrated calves were grouped as having a low or high delta AUC cortisol ($n = 20$). Differently shaped data points represent different harvest times following ACTH challenge. Individual data points represent the delta AUC cortisol response of each individual castrated calf within each group. Shaded boxes represent the delta AUC cortisol. Error bars represent the minimum and maximum data point in each cortisol response group.

3. Results

3.1. Cortisol

Unexpectedly, the concentration of cortisol in the serum collected every 0.5 h relative to the ACTH challenge was shown to be impacted by both time of harvest ($p < 0.001$) and

time ($p < 0.001$) relative to the ACTH challenge (Figure 4). Differences among groups that were to be harvested at different times were present 0.5 h after the initiation of the ACTH challenge, such that calves that were to be harvested 48 h after the ACTH challenge had an increased ($p < 0.001$) serum cortisol concentration compared to calves that were to be harvested 12 h after the ACTH challenge (Figure 4A). However, no differences ($p > 0.05$) among harvest time groups were observed through the remainder of the ACTH challenge. In addition, the cortisol response measured as ΔAUC was different ($p < 0.05$) between the different harvest times, such that animals harvested at 12 h had a decreased ($p < 0.05$) ΔAUC compared to calves harvested at 2 h, but were no different ($p > 0.05$) than those harvested at 24 or 48 h (Figure 4B).

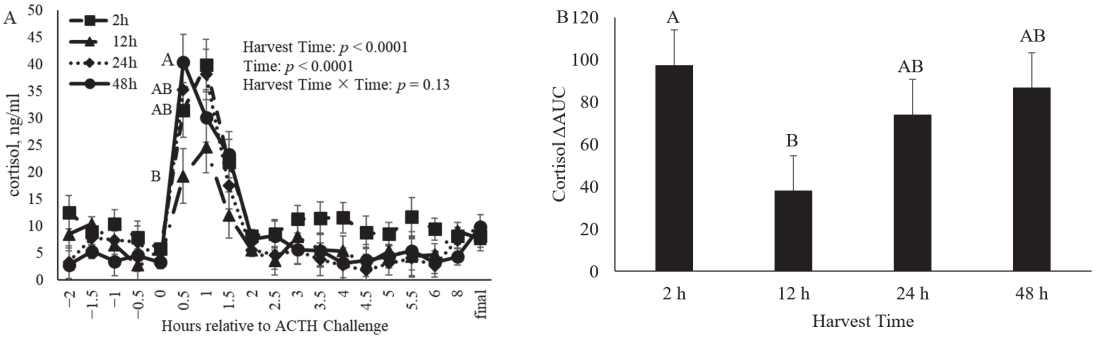

Figure 4. (**A**): Cortisol concentration in serum of castrated Holstein calves (103.5 ± 1.6 kg) relative to the beginning of the adrenocorticotropic hormone (ACTH) challenge given at a dose of 0.1 IU/kg of body weight. Treatment groups consisted of four groups of calves that were harvested at different time points (2 h, 12 h, 24 h, and 48 h; $n = 10$) following the ACTH challenge. Values represent the least squares means ± SEM of cortisol concentration in serum collected relative to the ACTH challenge for harvest times. Points with different letters differ ($p < 0.05$) among harvest times at that specific time point. (**B**): Differences in the delta area under the curve (ΔAUC) of cortisol calculated as the AUC from 0 to 2 h of the challenge–AUC from −2 to 0 h of the challenge between animals harvested at different time points. Bars with different letters indicate a difference ($p < 0.05$) among the different harvest times.

3.2. Rectal Temperature

The time of harvest altered ($p < 0.0001$) rectal temperature over time relative to the ACTH challenge (Figure 5A). Castrated calves that were to be harvested 12 h after the ACTH challenge had a decreased ($p < 0.0001$) rectal temperatures compared to calves that were to be harvested 2 h and 48 h after the ACTH challenge (Figure 5A). There was also a relationship ($p < 0.0001$) between cortisol response and rectal temperature, such that calves that had a low cortisol response had an increased rectal temperature compared to calves that had a high cortisol response (Figure 5B).

3.3. Protein Abundance

Quantifications of five proteins using antibody-specific Western blotting (Figure 2) demonstrated that both harvest time and cortisol response affected protein expression. There was no effect ($p = 0.22$) of time of harvest on relative HSPβ1 abundance in samples collected at harvest (Table 2). However, samples aged for 14 d had altered ($p = 0.03$) HSPβ1 abundance in the LL between harvest times (Table 2). In addition, cortisol response did not alter the expression of HSPβ1 at harvest ($p = 0.29$) or after 14 d of aging ($p = 0.19$) (Table 3).

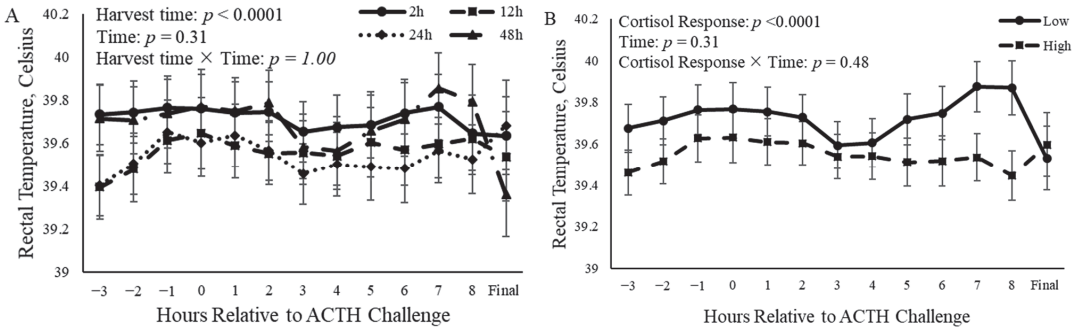

Figure 5. Rectal temperature of castrated Holstein calves (103.5 ± 1.6 kg) relative to the beginning of the ACTH challenge given at a dose of 0.1 IU/kg of body weight. (**A**): Treatment groups consisted of four groups of calves that were harvested at different time points (2 h, 12 h, 24 h, and 48 h; $n = 10$) following the ACTH challenge. (**B**): Calves were grouped based on cortisol response relative to ACTH challenge and were split into two different groups (low or high; $n = 20$). Repeated measures analyses were completed to determine the effects of harvest time, cortisol response, time, harvest time × time, and harvest time × cortisol response. Values represent the least squares mean ± SEM for (**A**) harvest times or (**B**) cortisol responses.

Table 2. Relative abundance of proteins in the longissimus lumborum at either harvest or after 14 d of aging relative to the initial muscle biopsy collected prior to the adrenocorticotropic hormone (ACTH) challenge.

	Harvest Time Relative to End of ACTH Challenge [a]					
	2 h [b]	**12 h [b]**	**24 h [b]**	**48 h [b]**	**SEM [c]**	***p*-Value [x]**
Harvest						
HSPβ1 [d]	2.26	6.10	4.15	1.13	1.54	0.22
P-HSPβ1 [e]	122.83 [xy]	−86.06 [x]	45.73 [xy]	380.38 [y]	107.03	**0.03**
HSPβ5 [f]	−0.20	2.27	7.66	−0.41	3.75	0.67
DJ1 [g]	6.44 [xy]	−17.76 [x]	73.25 [y]	16.10 [xy]	20.10	**0.002**
Troponin [h]	−24.52	127.26	995.10	162.52	320.74	0.22
14 d Aged						
HSPβ1 [d]	−0.02	5.48	2.05	1.94	1.10	**0.03**
P-HSPβ1 [e]	0.26 [x]	0.97 [y]	0.54 [xy]	0.16 [x]	0.15	**0.001**
HSPβ5 [f]	−0.73	11.05	25.66	−2.31	9.82	0.43
DJ-1 [g]	2.55 [x]	−5.18 [x]	54.57 [y]	12.32 [x]	7.72	**<0.0001**
Troponin—Entire Band [i]	1.07 [xy]	0.19 [x]	2.98 [xy]	3.37 [y]	0.73	**0.01**
Troponin—Lower Band [j]	0.31 [x]	0.21 [x]	0.48 [xy]	1.34 [y]	0.24	**0.02**

[a] Treatment groups consisted of four groups of castrated Holstein calves (103.5 ± 1.6 kg) that were harvested at different time points (2 h, 12 h, 24 h, and 48 h; $n = 10$) following the ACTH challenge given at a dose of 0.1 IU/kg of body weight. [b] Columns are least square means for the relative abundance of each protein. Protein abundance was calculated by dividing the initial protein abundance of the skeletal muscle biopsy by the abundance in the initial skeletal muscle biopsy. This calculation was carried out for samples collected both at harvest and after 14 days of aging. [c] SEM = standard error of the mean. [d] Heat shock protein β1. [e] Phosphorylated heat shock protein β1. [f] Heat shock protein β5. [g] Protein deglycase. [h] Troponin at harvest is one large band that is intact. [i] Entire band of troponin is measured by analyzing protein abundance of all troponin bands that appear after degradation following harvest. [j] Lower band of troponin indicates protein abundance of the lower of two bands of protein, which indicates degradation. [x,y] Values within a row with different letters (i.e. x, y, etc.) are different ($p < 0.05$) from one another.

Time of harvest affected ($p = 0.03$) relative P-HSPβ1 abundance in the LL at harvest, such that castrated calves that were harvested 48 h after the ACTH challenge had an increased ($p = 0.03$) abundance of P-HSPβ1 compared to calves that were harvested 12 h after the ACTH challenge (Table 2). The relative P-HSPβ1 abundance of samples aged for 14 d differed ($p = 0.001$) between harvest times, where calves that were harvested 2 h and 48 h after the ACTH challenge had a decreased ($p = 0.001$) abundance of P-HSPβ1 compared to calves that were harvested 12 h after the ACTH challenge (Table 2). Cortisol

response did not impact the expression of P-HSPβ1 at harvest ($p = 0.15$) nor after 14 d of aging ($p = 0.81$) (Table 3).

Table 3. Relative abundance of proteins in the longissimus lumborum at either harvest or after 14 d of aging relative to the initial muscle biopsy collected prior to the adrenocorticotropic hormone (ACTH) challenge between steers differing in cortisol response.

	Cortisol Response [a]			
	Low [b]	**High [b]**	**SEM [c]**	***p*-Value [x]**
Harvest				
HSPβ1 [d]	2.29	4.14	1.15	0.29
P-HSPβ1 [e]	210.32	21.12	80.04	0.15
HSPβ5 [f]	0.52	4.14	2.80	0.40
DJ1 [g]	38.77	0.24	15.03	0.07
Troponin [h]	188.44	441.74	239.86	0.49
14 d Aged				
HSPβ1 [d]	1.52	3.20	1.67	0.19
P-HSPβ1 [e]	0.45	0.51	0.11	0.81
HSPβ5 [f]	2.14	14.69	7.34	0.25
DJ-1 [g]	23.49	8.64	5.99	0.08
Troponin—Entire Band [i]	2.14	1.66	0.54	0.52
Troponin—Lower Band [j]	0.56	0.61	0.18	0.88

[a] Treatment groups consisted of four groups of castrated Holstein calves (103.5 ± 1.6 kg) that were harvested at different time points (2 h, 12 h, 24 h, and 48 h; $n = 10$) following the ACTH challenge given at a dose of 0.1 IU/kg of body weight. [b] Columns are least square means for the relative abundance of each protein. Protein abundance was calculated by dividing the initial protein abundance of the skeletal muscle biopsy by the abundance in the initial skeletal muscle biopsy. This calculation was carried out for samples collected both at harvest and after 14 days of aging. [c] SEM = standard error of the mean. [d] Heat shock protein β1. [e] Phosphorylated heat shock protein β1. [f] Heat shock protein β5. [g] Protein deglycase. [h] Troponin at harvest is one large band that is intact. [i] Entire band of troponin is measured by analyzing protein abundance of all troponin bands that appear after degradation following harvest. [j] Lower band of troponin indicates protein abundance of the lower of two bands of protein, which indicates degradation. [x] Values within a row with different letters are different ($p < 0.05$) from one another.

Analysis of the expression of HSPβ5 from samples collected at harvest and after 14 d of aging was not different ($p = 0.67$ and $p = 0.43$, respectively) relative to the time of harvest (Table 2). Similarly, analysis of the relative HSPβ5 expression was not altered by cortisol response at harvest ($p = 0.40$) or after 14 d of aging ($p = 0.25$) (Table 3).

The relative expression of DJ1 was different at both harvest ($p = 0.002$) and after 14 d of aging ($p < 0.0001$) among castrated calves harvested at different times after the ACTH challenge (Table 2). At harvest, calves that were harvested 12 h after the ACTH challenge had a decreased ($p = 0.002$) DJ-1 expression compared to calves that were harvested 24 h after the ACTH challenge. After 14 d of aging, samples from calves that were harvested 24 h after the ACTH challenge had an increased ($p < 0.0001$) expression of DJ1 compared to the other three harvest time groups (Table 2). Cortisol response tended to affect the relative DJ1 expression at harvest and after 14 d of aging in the LL ($p = 0.07$ and $p = 0.08$, respectively) (Table 3).

Time of harvest did not have an effect ($p = 0.22$) on the relative troponin expression in the LL at harvest (Table 2). Further, cortisol response had no effect ($p = 0.49$) on troponin expression at harvest (Table 3). The expression of troponin analyzed after 14 d aging was quantified as the entire band, and the upper and lower bands were quantified as degradation of this protein had occurred during aging. The time of harvest impacted ($p = 0.01$) the expression of the entire band of troponin. Calves that were harvested 48 h after the ACTH challenge had an increased ($p = 0.01$) troponin expression compared to calves that were harvested 12 h after the ACTH challenge (Table 2). The relative expression of the entire band of troponin after 14 d of aging was not impacted ($p = 0.52$) by cortisol response (Table 3).

The time of harvest affected ($p = 0.02$) the expression of the lower band of troponin I after 14 d of aging, such that samples from calves that were harvested 48 h after the

ACTH challenge had an increased ($p = 0.02$) expression compared to samples from calves harvested at 2 h and 12 h after the ACTH challenge (Table 2). Cortisol response did not impact ($p = 0.88$) the lower band of troponin I after 14 d of aging (Table 3).

3.4. Complete Blood Counts

The time of harvest affected ($p < 0.0001$) the red blood cell (RBC) concentration (Figure 6A). Castrated calves that were to be harvested 24 h after the ACTH challenge had a decreased ($p < 0.05$) concentration compared to calves that were to be harvested at the other three time points (Figure 6A). Additionally, the time of harvest altered ($p < 0.0001$) the hemoglobin concentration, where calves that were to be harvested 2 h after the ACTH challenge had an increased ($p < 0.05$) hemoglobin concentration compared to calves that were to be harvested 24 and 48 h after the ACTH challenge (Figure 6B). Additionally, calves that were to be harvested 12 h after the ACTH challenge had increased ($p < 0.05$) hemoglobin concentrations compared to those that were harvested 24 h after the ACTH challenge (Figure 6B). Furthermore, hematocrit was impacted ($p = 0.0003$) by the time of harvest, such that calves harvested 48 h after the ACTH challenge had a decreased ($p < 0.05$) hematocrit compared to those harvested 2 and 12 h after the challenge (Figure 6C). The platelet concentration was also altered ($p = 0.01$) by the time of harvest, although no differences ($p > 0.05$) among groups that were to be harvested at different times were observed (Figure 6D). The final concentrations of hemoglobin, hematocrit, and platelets collected at harvest did not differ ($p = 0.10$, $p = 0.18$, and $p = 0.59$, respectively) among harvest times; however, RBC concentrations were different ($p = 0.01$).

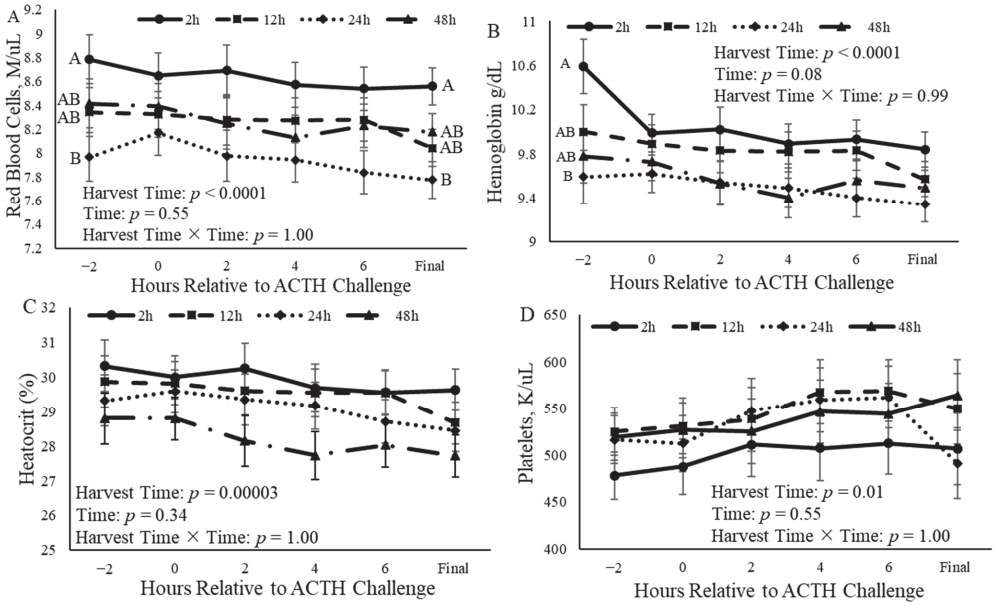

Figure 6. Concentration of blood components of castrated Holstein calves (103.5 ± 1.6 kg) ((**A**): red blood cells; (**B**): hemoglobin; (**C**): hematocrit; (**D**): platelets) measured every 2 h relative to adrenocorticotropic hormone (ACTH) challenge given at a dose of 0.1 IU/kg of body weight between calves grouped into harvest time (2 h, 12 h, 24 h, and 48 h; $n = 10$). Repeated measures analyses were completed to determine the effects of harvest time, time, and harvest time × time. Values represent the least squares mean ± SEM for the respected blood component. Points with different letters differ ($p < 0.05$) among harvest times at each time point.

Concentrations of white blood cells (WBCs), neutrophils, monocytes, eosinophils, and lymphocytes each varied ($p = 0.01$, $p = 0.002$, $p < 0.0001$, $p = 0.01$, and $p = 0.05$, respectively)

with the time of harvest (Figure 7). White blood cells were increased ($p = 0.01$) in castrated calves harvested 2 h after the ACTH challenge when compared to calves harvested 12 h after the ACTH challenge. Calves that were harvested at 24 h after the ACTH challenge had increased ($p = 0.01$) neutrophil concentrations compared to calves harvested 12 h after the challenge (Figure 7A). Calves that were harvested 2 h and 48 h after receiving ACTH had increased ($p = 0.001$) monocytes compared to those calves that were harvested 12 h and 24 h after the challenge (Figure 7C). Eosinophils were increased ($p < 0.0001$) in calves harvested 12 h after the ACTH challenge when compared to calves harvested at the three other time points (Figure 7D). Additionally, the concentrations of WBCs, neutrophils, monocytes, and eosinophils changed ($p < 0.05$) over time (Figure 7). In each of these, the concentration increased ($p < 0.05$) 2 h after the ACTH challenge and then decreased after that time point. There was no relationship ($p = 0.31$) between the time of harvest and the concentration of basophils (Figure 7F). The final concentration of WBCs, neutrophils, monocytes, eosinophils, and lymphocytes collected at harvest did not differ ($p = 0.22$, $p = 0.13$, $p = 0.21$, $p = 0.22$, and $p = 0.93$, respectively) among harvest times (Figure 7). However, the final concentration of basophils differed ($p = 0.006$) among harvest times (Figure 7F).

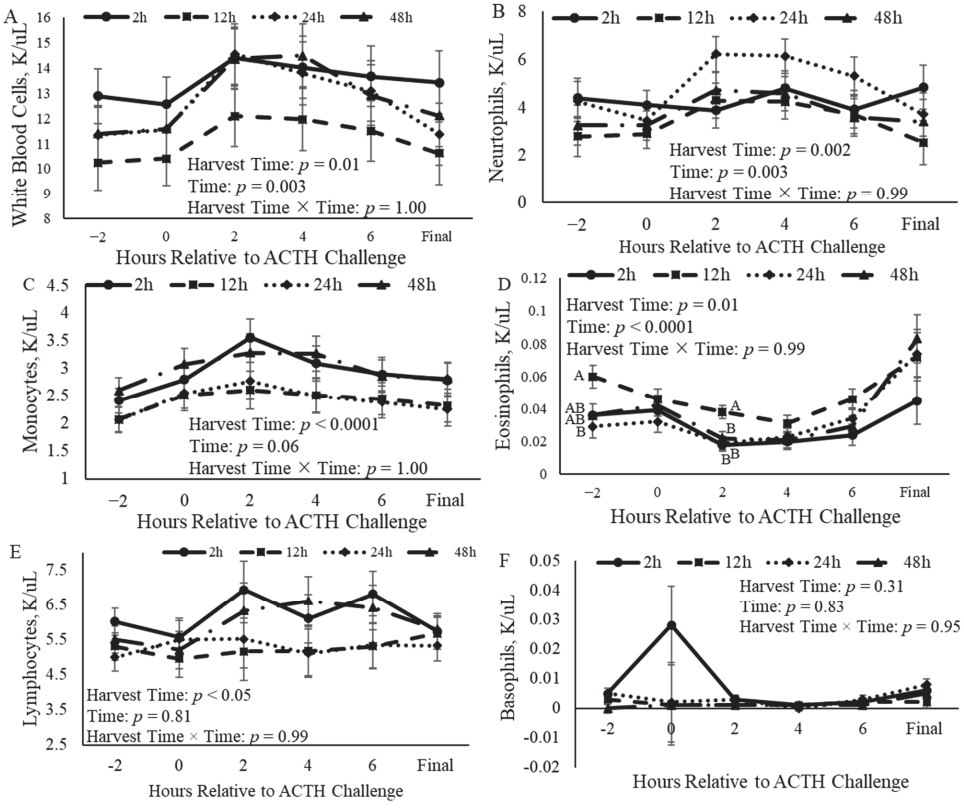

Figure 7. Concentration of blood components of castrated Holstein calves (103.5 ± 1.6 kg) ((**A**): white blood cells, (**B**): neutrophils, (**C**): monocytes, (**D**): eosinophils, (**E**): lymphocytes, (**F**): basophils) measured every 2 h relative to adrenocorticotropic hormone (ACTH) challenge given at a dose of 0.1 IU/kg of body weight between calves grouped into harvest time (2 h, 12 h, 24 h, and 48 h; $n = 10$). Repeated measures analyses were completed to determine the effects of harvest time (treatment), time, and treatment × time. Values represent the least squares mean ± SEM for the respected blood component. Points with different letters differ ($p < 0.05$) among harvest times at each time point.

The time of harvest tended to affect ($p = 0.07$) the neutrophil–lymphocyte ratio (N:L) (Figure 8A). However, cortisol response did not affect ($p = 0.72$) the N:L ratio (Figure 8B).

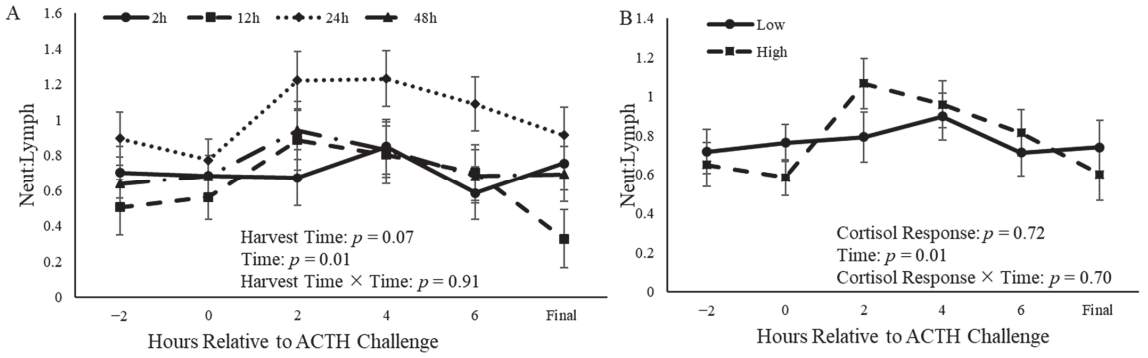

Figure 8. Neutrophil and lymphocyte ratio (neut:lymph) of castrated Holstein calves (103.5 ± 1.6 kg) relative to the beginning of the adrenocorticotropic hormone (ACTH) challenge. (**A**): Calves were grouped based on time of harvest following ACTH challenge given at a dose of 0.1 IU/kg of body weight (2 h, 12 h, 24 h, and 48 h; $n = 10$). (**B**): Calves were grouped based on cortisol response relative to the ACTH challenge and were split into two different groups (low and high; $n = 20$). Repeated measures analyses were completed to determine the effects of harvest time, cortisol response, time, harvest time x time, cortisol response x time, and harvest time × cortisol response. Values represent the least squares mean ± SEM of neut:lymph.

3.5. Steak Color

A harvest time × cortisol response ($p = 0.02$) was observed for a* values (Figure 9). Additionally, the a* value was affected by the time of harvest ($p < 0.0001$) and retail day ($p < 0.0001$) (Figure 9A). On d 3 and d 4 of retail display, steaks from castrated calves that were harvested 24 h and 48 h after the ACTH challenge had an increased ($p = 0.03$) a* value compared to steaks from calves harvested 12 h after the ACTH challenge (Figure 9A). Cortisol response did not affect ($p = 0.57$) a* values (Figure 9B).

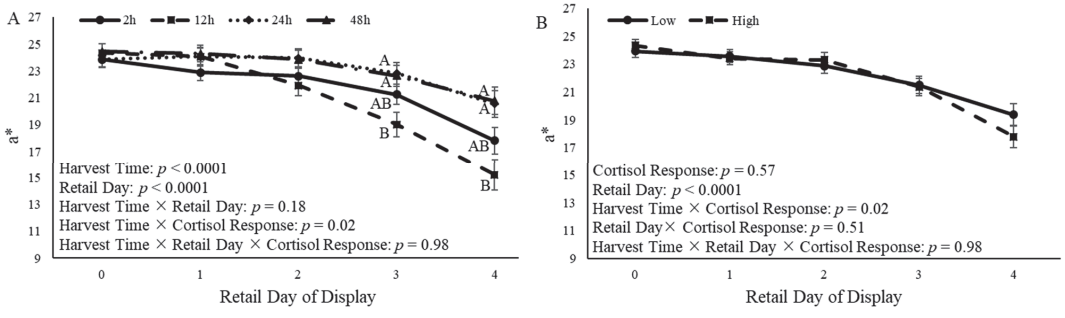

Figure 9. a* (redness) color measurement from the *longissimus lumborum* of castrated Holstein calves (103.5 ± 1.6 kg) after 14 d of aging. (**A**): Calves were grouped based on time of harvest following adrenocorticotropic hormone (ACTH) challenge given at a dose of 0.1 IU/kg of body weight (2 h, 12 h, 24 h, and 48 h; $n = 10$). (**B**): Calves were grouped based on cortisol response relative to the ACTH challenge and were split into two different groups (low and high; $n = 20$). Repeated measures analyses were completed to determine the effects of harvest time, cortisol response, retail day, harvest time × retail day, harvest time × cortisol response, and harvest time × retail day × cortisol response. Values represent the least squares mean ± SEM of a* measurements. Points with different letters differ ($p < 0.05$) among harvest times or cortisol responses at each time point.

A harvest time × cortisol response ($p = 0.002$) was observed for b* values (Figure 10). The time of harvest ($p = 0.006$) and retail day of display ($p < 0.0001$) each also affected b* values (Figure 10A). After 4 d of retail display, steaks from castrated calves that were harvested 24 h and 48 h after the ACTH challenge had increased ($p = 0.006$) b* values compared to steaks from calves harvested 12 h after the ACTH challenge (Figure 10A). Cortisol response did not affect ($p = 0.22$) b* values (Figure 10B).

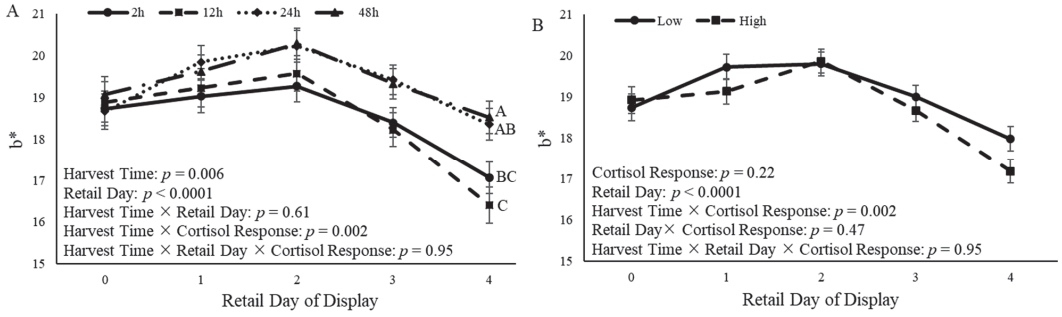

Figure 10. b* (yellowness) color measurement from the *longissimus lumborum* of castrated Holstein calves (103.5 ± 1.6 kg) after 14 d of aging. (**A**): Calves were grouped based on time of harvest following adrenocorticotropic hormone (ACTH) challenge given at a dose of 0.1 IU/kg of body weight (2 h, 12 h, 24 h, and 48 h; $n = 10$). (**B**): Calves were grouped based on cortisol response relative to the ACTH challenge and were split into two different groups (low and high; $n = 20$). Repeated measures analyses were completed to determine the effects of harvest time, cortisol response, retail day, harvest time × retail day, harvest time × cortisol response, and harvest × retail day × cortisol response. Values represent the least squares mean ± SEM of b* measurements. Points with different letters differ ($p < 0.05$) among harvest times or cortisol responses at each time point.

Additionally, L* values were impacted by harvest time × cortisol ($p = 0.02$). However, the values for L* were not affected ($p = 0.14$) by the retail display time relative to harvest time (Figure 11A). Additionally, the cortisol response altered ($p = 0.003$) L* values over time. Castrated calves with a low cortisol response had increased ($p = 0.05$) L* values compared to calves with a high cortisol response on d 3 of retail display (Figure 11B).

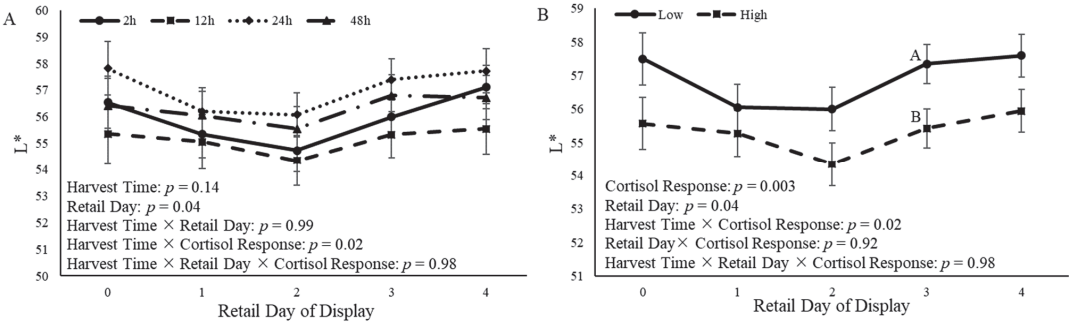

Figure 11. L* (lightness) color measurement from the *longissimus lumborum* of castrated Holstein calves (103.5 ± 1.6 kg) after 14 d of aging. (**A**): Calves were grouped based on time of harvest following adrenocorticotropic hormone (ACTH) challenge given at a dose of 0.1 IU/kg of body weight (2 h, 12 h, 24 h, and 48 h; $n = 10$). (**B**): Calves were grouped based on cortisol response relative to the ACTH challenge and were split into two different groups (low and high; $n = 20$). Repeated measures analyses

were completed to determine the effects of harvest time, cortisol response, retail day, harvest time × retail day, harvest time × cortisol response, and harvest × retail day × cortisol response. Values represent the least squares mean ± SEM of L* measurements. Points with different letters differ ($p < 0.05$) among harvest times or cortisol responses at each time point.

3.6. Steak pH

The steak pH was affected by both harvest time and retail day of display ($p < 0.0001$ and $p = 0.007$, respectively) (Figure 12A). Steaks from castrated calves that were harvested 48 h after the ACTH challenge had greater ($p < 0.0001$) pH values than steaks from calves that were harvested 2 h and 12 h after the ACTH challenge at 1 d and 2 d of retail display (Figure 12A). At 3 d and 4 d of retail display, steaks from calves that were harvested 48 h after the ACTH challenge had greater ($p < 0.0001$) pH values compared to steaks from calves that were harvested 2 h, 12 h, and 24 h after the ACTH challenge (Figure 12A). However, cortisol response did not ($p = 0.85$) impact steak pH (Figure 12B).

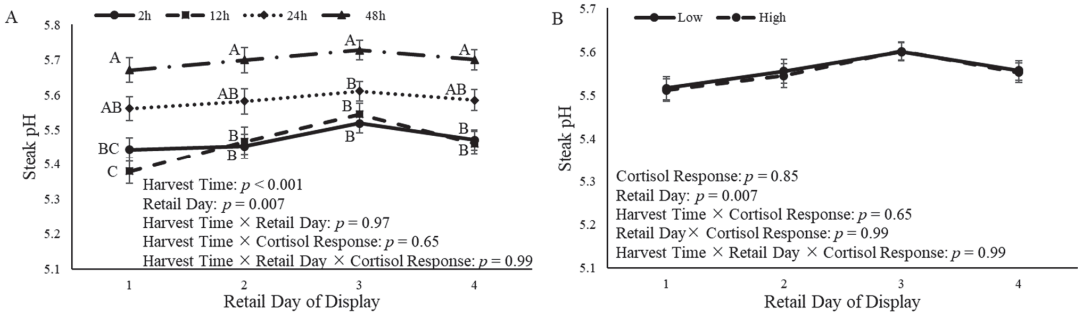

Figure 12. Steak pH measurement from the *longissimus lumborum* of castrated Holstein calves (103.5 ± 1.6 kg) after 14 d of aging. (**A**): Calves were grouped based on time of harvest following adrenocorticotropic hormone (ACTH) challenge given at a dose of 0.1 IU/kg of body weight (2 h, 12 h, 24 h, and 48 h; $n = 10$). (**B**): Calves were grouped based on cortisol response relative to the ACTH challenge and were split into two different groups (low and high; $n = 20$). Repeated measures analyses were completed to determine the effects of harvest time, cortisol response, retail day, harvest time × retail day, harvest time × cortisol response, and harvest × retail day × cortisol response. Values represent the least squares mean ± SEM of steak pH measurements. Points with different letters differ ($p < 0.05$) among harvest times or cortisol responses at each time point.

3.7. Oxidation

The harvest time × cortisol response altered ($p < 0.0001$) serum TBARSs (Figure 13). Additionally, both harvest time ($p < 0.0001$) and cortisol response ($p < 0.0001$) affected serum concentrations of TBARSs (Figure 13). At 8 h relative to the ACTH challenge, concentrations of TBARSs were increased ($p = 0.001$) in the serum of castrated calves harvested 24 h after the ACTH challenge when compared to those harvested 2 and 12 h after (Figure 13A). Final serum TBARSs increased ($p = 0.0009$) in calves harvested 24 h after the ACTH challenge compared to the other three harvest times (Figure 13A). Additionally, cortisol response had an effect ($p = 0.0001$) on TBARSs concentration, such that calves that had a low concentration of cortisol had increased concentrations of TBARSs compared to calves that had a high cortisol response (Figure 13B).

3.8. Myofibrillar Fragmentation Index

The time of harvest changed ($p = 0.016$) MFI, such that castrated calves that were harvested 24 h after the ACTH challenge had an increased ($p < 0.05$) MFI value compared to calves that were harvested 12 h after the ACTH challenge (Figure 14A). However, cortisol response did not affect ($p = 0.25$) MFI (Figure 14B).

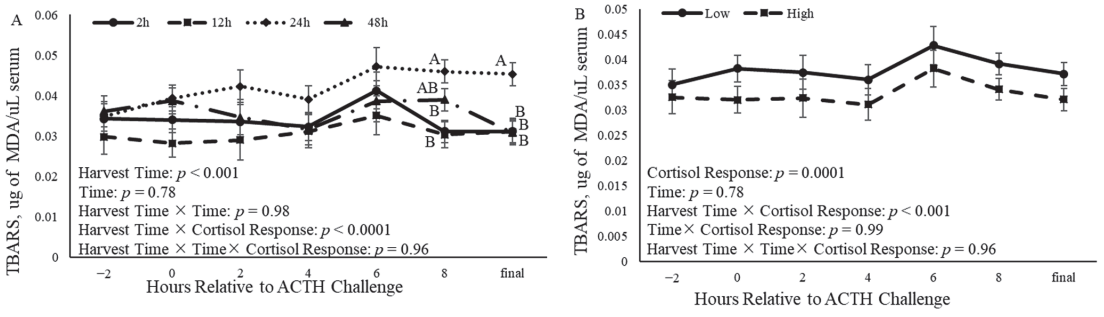

Figure 13. Concentration of TBARSs in serum of castrated Holstein calves (103.5 ± 1.6 kg) collected every 2 h relative to adrenocorticotropic hormone (ACTH) challenge given at a dose of 0.1 IU/kg of body weight. Panel (**A**) describes TBARSs concentration in serum relative to the ACTH challenge in calves that were harvested at different time points (2 h,12 h, 24 h, and 48 h; $n = 10$) following the ACTH challenge. Panel (**B**) describes TBARSs concentration in serum relative to the ACTH challenge in calves that had different cortisol responses (low and high; $n = 20$) relative to the ACTH challenge. Repeated measures analyses were completed to determine the effects of harvest time, cortisol response, time, harvest × time, time x cortisol response, harvest time × cortisol response, and harvest time × time × cortisol response. Values represent the least squares mean ± SEM of TBARSs concentration in the serum. Points with different letters differ ($p < 0.05$) within harvest time or cortisol response at each time point.

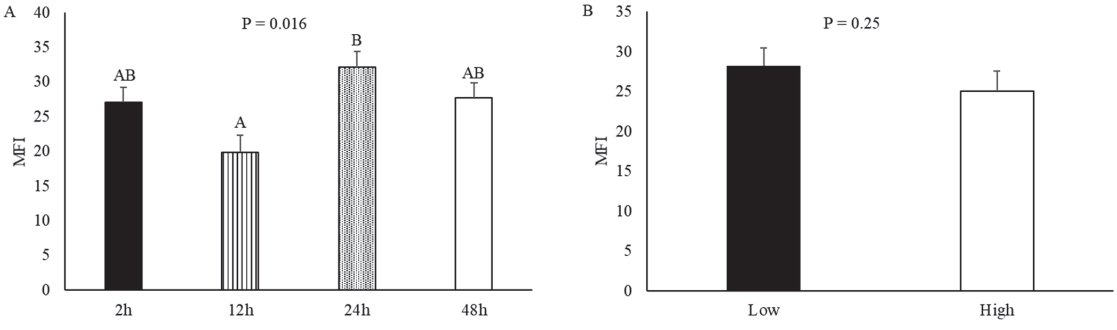

Figure 14. Myofibrillar fragmentation index (MFI) of 14 d aged muscle tissue collected from the *longissimus lumborum* of castrated Holstein calves (103.5 ± 1.6 kg). Panel (**A**) describes the MFI of samples collected from calves that were harvested at different time points (2 h, 12 h, 24 h, and 48 h; $n = 10$) following adrenocorticotropic hormone (ACTH) challenge given at a dose of 0.1 IU/kg of body weight. Panel (**B**) describes the MFI of samples collected from calves that had different cortisol responses (low and high; $n = 20$) after the initiation of the ACTH challenge. Values represent the least squares mean ± SEM of MFI for 14 d aged muscle tissue. The *p*-value above each time point represents the effect of harvest time (**A**) or cortisol response (**B**). Bars with different letters are different ($p < 0.05$) from one another.

4. Discussion

The rate and extent of the postmortem breakdown of myofibrillar proteins are major determinants of tenderness [20,29]. Although the development of end-product tenderness is a well-researched area, inconsistencies in tenderness still exist [30]. Premortem stress may be involved in decreased meat quality by modulating HSP abundance. Heat shock proteins are highly conserved proteins that can be constitutively expressed or upregulated during stressful conditions [8,9], and act as molecular chaperones to maintain cellular homeostasis [8]. Small heat shock proteins play a similar role, acting as molecular chaperones, but are ATP-independent, indicating that they can still be active postmortem [31,32].

Proteomic studies of muscle have identified that some SHSPs are upregulated in post-mortem muscle [33]. Following a stressful event, oxidative stress may affect the tenderness, color stability, and flavor of meat by causing color deterioration, undesirable flavors, and rancidity in meat [15]. As such, the goal of this research was to understand how biological pathways, proteolysis, and color are changed in beef following a stressful event prior to harvest. To study the effects of acute stress, such as transport, castrated calves were challenged with ACTH prior to being serially harvested. Differences in cortisol concentrations 0.5 h after initiation of the ACTH challenge were observed between harvest groups; therefore, cortisol response was also included as a main effect in the model. It should be mentioned that the difference in serum cortisol concentration was not anticipated by the authors.

The calves used in the present study were much smaller and younger than industry-standard finished feedlot cattle. In addition, the present study used a dairy breed rather than a beef breed. To the authors' knowledge, no previous research has analyzed beef quality traits on castrated calves of this size or age in relation to stress. As such, many comparisons are made with beef breeds and/or finished feedlot cattle throughout the discussion.

In the present study, most blood components in the serum differed over time based on the time of harvest relative to the ACTH challenge. Calves allowed 2 h of rest following the ACTH challenge had an increased concentration of WBCs compared to calves harvested 12 h after the challenge. However, the concentration of WBCs from calves that were harvested 48 h after the challenge did not differ from other harvest times. However, a study involving Limousine feedlot beef cattle that were transported for 5 h observed an increased WBCs concentration 48 h following transportation [34]. In the present study, the platelet count was not affected by harvest time; however, hematocrit was increased in calves that were harvested 2 h and 12 h after the ACTH challenge compared to calves that were harvested 48 h after the ACTH challenge. This could be a result of minor dehydration due to stress during the ACTH challenge, although calves had ad libitum access to water until harvest. The current study showed a slight increase in eosinophil concentration at harvest compared to eosinophil concentration throughout the ACTH challenge in all animals, while no apparent changes were observed in monocytes, neutrophils, or lymphocytes. However, a study that observed the effects of cold stress and ACTH administration in Japanese Black steers saw that monocytes, eosinophils, and neutrophils increased, while lymphocytes decreased after ACTH was administered in a cold environment [35]. The differences between this study and the present study may be due to the additional cold stress that the Japanese Black steers were experiencing, or the age and breed differences in the animals utilized in the respective trials. Taken together, the results of this study demonstrate that some blood cell populations are affected slightly during a stressful period. However, intense stress or an increased cortisol response compared to the current study may affect blood cell populations more intensely. The neutrophil–lymphocyte ratio can be used as an indicator of stress in steers [36]. However, in the current study, stress did not impact N:L. Conversely, other studies have found that steers that experience stress have an increased N:L ratio [36–38]. The difference in results between the present study and other studies could be a result of actual cortisol concentration at harvest. In the present study, cortisol concentration at harvest was not different among harvest times.

The cortisol concentration relative to the ACTH challenge showed that the ACTH challenge did indeed induce a cortisol response, with peak concentrations observed between 0.5 and 1 h after ACTH was administered. Although the calves were the same breed, uniform in size, the same age, housed in the same environment, and sourced from one farm, differences in cortisol response were observed among harvest time groups 0.5 h after initiation of the ACTH challenge. However, cortisol concentration did not differ at harvest. This may be the result of differing behavior traits due to individual animal variation among the harvest time groups, as a difference in cortisol response was observed 0.5 h after the initiation of the ACTH challenge. The baseline cortisol concentration of the calves in the present study is similar to that in other studies [39–41]; however, the peak cortisol concentrations in the present study are lower compared to previous studies that

performed an ACTH challenge on Swedish Red and White heifers and mature dairy cows, respectively [39,40]. Although the authors are unsure why lower peak cortisol concentrations were observed, it could be attributed to the environment the calves were housed in, the breed of the calves, or previous exposure to stressful events. Further, the dose of ACTH could have also been a factor.

Time of harvest and cortisol response were both shown to impact rectal temperature. Calves that had a low cortisol response had increased rectal temperatures. However, other studies have observed increased rectal temperatures during a stressful event. Stressful events like transportation and arrival at a new facility have been shown to increase rectal temperature [42]. Another study observed decreased rectal temperatures in calm Brahman bulls compared to temperamental- and intermediate-tempered bulls [43]. In addition, temperamental bulls had increased cortisol levels compared to calm bulls [43]. In Holstein heifers, rectal temperatures and cortisol concentrations were increased in heifers that were relocated via transportation compared to heifers that were not relocated [42]. It is important to note that although calves within the different harvest time groups displayed different rectal temperatures, the differences were not extreme and may not be of biological significance.

As molecular chaperones, SHSPs bind to and stabilize unstable proteins [7]. Various stressors result in the synthesis of HSPs [8]. The increase in cortisol prompts cellular adaptation, which is accomplished through the synthesis of HSPs [44]. The postmortem environment creates a "stressful" environment and in an attempt to maintain homeostasis, cells will increase the expression of SHSPs [33]. It has been shown that SHSPs are upregulated in different time frames based on the type of stress that the cell is undergoing, the intensity of the stress, and the specific SHSP [45]. Furthermore, during periods of stress, HSPβ1 can undergo post-translational modifications, which results in phosphorylation [7]. In response to oxidative stress, HSPβ1 can be phosphorylated, and in this form, it interacts with actin and protects it from fragmentation [46]. The SHSP HSPβ5 rapidly binds to and accumulates on myofibrils during stress to protect myofibrillar filament organization [47]. In the present study, we had initially hypothesized that calves that were given more time to recover following a stressful event would have a decreased expression of SHSPs. Relative HSPβ1 levels did not differ at harvest; however, they were increased after 14 d of aging in steaks from calves harvested 12 h after the ACTH challenge compared to other harvest times. Additionally, it is also important to note that no differences in HSPβ1 or P-HSPβ1 were found relative to cortisol response. Further, the expression of HSPβ5 did not differ based on the time of harvest nor cortisol response. Other studies have shown increased HSPβ1 abundance caused by various stressors. Heat stress caused by increased ambient temperatures in Brazil was measured using rectal temperature in Nelore and Caracu [48]. Increased heat stress resulted in increased relative HSPβ1 gene expression in the serum [48]. The expression of HSPβ1 mRNA in peripheral blood lymphocytes increased after 9 h of transportation, and gradually declined in Xia Nan cows [49]. To date, the authors are unaware of any research that has been completed on P-HSPβ1 or HSPβ5 in cattle. Additional research would be beneficial to determine how stress specifically impacts the abundance of HSPβ1 in skeletal muscle.

In the present study, MFI and troponin expression were used as an indication of tenderness development. The time of harvest affected MFI; however, cortisol levels 0.5 h after the challenge did not. The tenderness measurement, Warner–Bratzler shear force (WBSF), was not used in this study due to the inferior size of loins collected from the calves. A strong negative correlation between MFI and WBSF has been well documented [26,48]. In the present study, MFI was increased in calves that were harvested 24 h after the ACTH challenge, indicating that steaks from this group of calves may have an increased tenderness due to increased myofibrillar fragmentation. Other studies have observed that beef tenderness was affected following an acute stressor. Following 4 h of transportation, Hereford and Bradford steers were allowed to rest in lairage for various lengths of time, and steers that were in the long lairage group showed decreased WBSF values than steers from the short lairage group [49]. Another study showed that Nellore steers with increased

chute scores or flightier temperaments had increased WBSF values [50]. Although MFI is shown to be correlated to WBSF and is a common measurement of tenderness, MFI is as a result of rather than a cause of postmortem aging [51]. As such, future studies need to include additional measurements, such as WBSF, to determine how the time of harvest following a stressful event affects end-product tenderness.

As mentioned above, troponin degradation was used in the present study to assess tenderness. The troponin complex is a principle regulatory component of the thin filament in skeletal muscle, and contains three subunits (troponin I, troponin C, and troponin T) [52]. The increased degradation of troponin is related to the increased tenderness of meat [53]. The present study used troponin I in the troponin degradation analysis. Troponin I is part of the tropomyosin–troponin complex and is an important component of myofibrils. In the present study, calves that were harvested 48 h after a stress exposure had an increased relative expression of troponin I in 14 d aged samples. In addition, calves that were harvested 24 h after stress exposure had a relatively large expression of troponin at harvest; however, this measurement was not significantly different from the other harvest groups. A study that characterized pre-harvest stress by dark, firm, and dry (DFD) meat in Rubi Gallega cattle observed that troponin C was increased in normal meat compared to the DFD meat in the *longissimus thoracis* (LT) [54]. In addition, researchers observed that DFD meat was more tender than normal meat [54]. Another study showed that Angus-influenced heifers designated for kosher slaughter are calmer than non-kosher steers; however, kosher heifers had increased WBSF values and an increased troponin T expression in the LL than non-kosher steers [55]. The effects of a stressful event prior to harvest on troponin expression and MFI have not been studied at length. More research in this area would be beneficial to fully elucidate this interaction.

The highly conserved protein, DJ1, is present in the cytoplasm as well as in the intracellular organelles and protects against oxidative stress [18]. In the present study, 0 calves that were allowed to rest for 24 h following a stressful event had the greatest expression of DJ1 at harvest and after 14 d of aging compared to calves in the other three harvest times. Little to no research has been conducted on the effects of premortem stress on DJ1 abundance in cattle. However, several studies have analyzed the relationship of DJ1 and the tenderness of meat. Some researchers have reported that tenderness was associated with the abundance of DJ1 [19,21]. Interestingly, the calves that were allowed to rest for 24 h after the ACTH challenge had the highest MFI compared to the other three treatment groups, which agrees with other studies that state that the protein abundance of DJ1 is linked to increased tenderness. In addition to DJ1 expression, serum TBARSs were used as a measurement of lipid peroxidation. Both the time of harvest and the cortisol response has a relationship with serum TBARSs concentration in the present study. Calves that were to be harvested 24 h after the ACTH challenge had an increased concentration of TBARSs in the serum 8 h after initiation of the challenge and at harvest. Researchers observed a three-fold increase in MDA in crossbred steers after transportation compared to before transportation [56]. In addition, another study observed a strong positive correlation between concentration of cortisol and TBARSs in calves after 2 h of transportation [57]. In cows infected with *Theileria annulata,* researchers observed increased lipid peroxidation measured in hemolysate compared to that of healthy cows [58]. It is apparent that certain stressors can increase the oxidation of tissues, which may affect meat quality [8,59]. However, more research would be beneficial to understand the effects of stress on the expression of DJ1 in skeletal muscle and the concentration of TBARSs in serum.

Steak color is an important quality that consumers consider when purchasing beef [13]. In the present study, time of harvest and cortisol response had an effect on a*, and time of harvest and cortisol response tended to affect b*. Cortisol response affected L*, such that calves that had a low cortisol response had increased L* values compared to calves with a high cortisol response. Calves that were harvested 24 h and 48 h after the ACTH challenge had steaks that were more red and yellow in color than steaks from calves that were harvested 12 h after the ACTH challenge. This is similar to what has been observed in previous studies. One study found that Hereford and Braford steers that were transported

for 4 h had increased a* and b* values in the long lairage group; however, L* values were not significantly different between the short and long lairage times [49]. In another study, *Bos indicus* x *Bos taurus* cross cattle were allowed to rest in lairage for 3 h or 18 h after transportation, and steaks collected from the LL were aged for either 1 d or 14 d [60]. Researchers observed a lairage x aging interaction for a* and b* values, and cattle that were held in lairage for longer had increased a* and b* values in 14 d aged samples [60]. However, other researchers observed different results than the present study. A study utilizing Hungarian Simmental bulls observed increased L* values 24 h after harvest in bulls that were held in lairage for 48 h and 72 h compared to bulls that were held in lairage for 24 h; however, no differences were reported in a* values based on lairage time, and no differences in steak color 7 d after harvest were reported [61]. Friesian steers that were transported for 3 h or 16 h and kept in lairage for 3, 6, 12, or 24 h found that steers that were transported for 3 h and harvested 12 h after transportation had increased L* values compared to the other treatment groups [62]. These differences may be a result of differing lairage times and aging periods from the current study. The measurement of color closer to harvest would have been beneficial to better understand the observed color differences.

The measurement of pH is a good indicator of meat quality as it relates to the depletion of muscle glycogen [63–65]. Stressful events before harvest decrease glycogen reserves in the muscle, which, in turn, affects meat pH [63]. A normal pH for beef is approximately 5.5; however, dark-cutting beef may have a pH of 5.8 to 6.2 [66]. The pH of the LL was < 5.8 in the present study. In addition, the harvest time affected the pH of the LL. Calves that were harvested 48 h after the ACTH challenge produced meat that had greater pH values compared to the other harvest times. Interestingly, calves that were harvested 48 h after the ACTH challenge also had greater cortisol concentrations 0.5 h after the administration of ACTH, and greater a* values. Previous research has observed increased pH values in the *longissimus thoracis* of Friesian steers that were transported for 16 h compared to those transported for 3 h [62]. Additionally, another study observed the effects of using an electric prod before harvest on feedlot cattle and did not observe differences in the pH values of the meat produced from control animals compared to those that were handled with an electric prod [67]. Further, another study observed the effects of the temperament of steers on the pH and did not find any differences in pH values among the temperament groups [68]. The results of the present study may suggest that stressful events before harvest affect muscle pH; however, harvest time following a stressful event may have a greater impact on muscle pH.

5. Conclusions

The current study portrayed the complicated relationship between harvest time and cortisol response following a stressful event and how these factors may impact beef quality. From these data, we can observe that harvest time and the level of stress during a stressful event does impact the quality of beef in different ways. It can be generalized that animals that were harvested 12 h after the ACTH challenge resulted in an increased quality of beef due to the differences related to improved steak color, lower steak pH, decreased oxidation, and decreased MFI. The findings of this study are significant because they demonstrate that the time of harvest and cortisol response following a stressful event affects beef quality. Additional research, especially on industry-finished beef cattle, is needed to determine how premortem stress and cortisol response is involved in beef quality to provide consumers with beef of a consistent quality.

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Carcass and Meat Characteristics of Cull Heifers from Different Genetic Groups Fed Diets with Different Sources of Nonprotein Nitrogen in Confinement

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Simple Summary: The slaughter of females represents a significant proportion of all cattle slaughtered in Brazil. Despite this, the number of studies on the quality of the carcasses and meat of these animals is still low, which leads to less knowledge about the quality and commercial value of the meat. The aim of this study was to evaluate the carcass and meat quality of heifers from different genetic groups fed diets containing different sources of nonprotein nitrogen. The main differences found were in relation to the genetic group, with effects on carcass weight, fat content in the carcass and meat, and also on the fatty acid profile of the meat.

Abstract: The aim of this study was to evaluate the effect of genetic groups and diets with different sources of nonprotein nitrogen (NPN) on the carcass and meat characteristics of beef heifers. The meat from 40 heifers (20 $\frac{1}{2}$ Angus $\frac{1}{2}$ Nellore (A × N) and 20 $\frac{1}{2}$ Charolais $\frac{1}{2}$ Nellore (L × N)), finished in feedlots, was used. The heifers were fed diets containing different sources of NPN—(1) a diet with livestock urea and protected urea (LPU) and (2) a diet with extruded urea (EU)—in a completely randomized design with a 2 × 2 factorial arrangement. Carcass, composition and meat quality evaluations were carried out. There were no significant interactions between diet and genetic group for most of the variables evaluated ($p > 0.05$). The A × N heifers had higher hot carcass weights (305.73 vs. 279.80 kg), loin eye areas (80.87 vs. 75.45 cm^2), subcutaneous fat thicknesses (8.69 vs. 6.35 mm) and lower shear forces (6.98 vs. 7.7 kg) compared to the C × N heifers ($p < 0.05$). The meat from the A × N heifers had higher proportions of saturated fatty acids (49.41 vs. 47.95%), with no effects on the proportions of monounsaturated (47.57%) and polyunsaturated (4.01%) fatty acids. The A × N heifers had better carcass and meat characteristics, while the C × N heifers had meat and fat with better fatty acid profiles.

Keywords: collagen; extruded urea; fatty acid profile

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1. Introduction

There are many studies in the literature on the carcass and meat characteristics of beef cattle. However, most of these studies were conducted on male cattle. Even if a reasonable proportion of females were included in the total number of cattle slaughtered in Brazil [1–3],

this would lead to an undervaluation by slaughterhouses. Historically, the meat from cull heifers/cows has been considered be of lower quality than that of male animals.

One of the reasons for the increase in female culling in recent years has been the interest of livestock farmers in inseminating young females, on average 14 months old, in order to shorten the production cycle. Some of the heifers that do undergo insemination fail to become pregnant and are discarded. To make better use of these heifers, they are finished in confinement and can be sold to premium meat markets [4]. They have desirable characteristics, such as good finishing [5], softness and marbling [1].

With the increasing use of crosses between breeds for the production of more efficient animals with better meat quality [4] and the diversified nature of the Brazilian production system, it is essential to evaluate the production performance and carcass and meat characteristics of cull heifers. Such characteristics are determined by aspects intrinsic to the animal [6], food [7] and environment [8].

As with the use of more specialized animals, diet formulation is a critical factor in production. It directly contributes to the growth rate, carcass and meat quality and profitability of the production system [9]. Protein is an essential component of the diet, although it is the most expensive nutrient. Substitutes for this nutrient, such as nonprotein nitrogen (NPN) sources, in finishing diets can reduce feed costs without compromising performance [10,11]. The NNP sources used in ruminant diets have different solubility rates, determining the source or combination of NNP sources that has better synchronization with dietary carbohydrates may increase the microbial protein synthesis and, consequently, improve weight gain [12].

We hypothesized that the carcass and meat characteristics of crossbred cull heifers finished indoors would be unaffected by the genetic group and nonprotein nitrogen sources in the diet. The objective was to evaluate the effect of genetic group and nonprotein nitrogen sources added to the total diet on carcass and meat characteristics of cull heifers ($\frac{1}{2}$ Angus $\frac{1}{2}$ Nellore and $\frac{1}{2}$ Charolais $\frac{1}{2}$ Nellore) finished in confinement.

2. Materials and Methods

2.1. Animals, Treatments and Experimental Design

The experiment was carried out at the Vertente da Pedra Feedlot, located in Água Clara, MS, and at the College of Veterinary Medicine and Animal Science of the Federal University of Mato Grosso do Sul/UFMS. All procedures used in this study were approved by the Ethics Committee for the Use of Animals (ECUA)—UFMS, according to protocol no. 1216/2022. Meat from 40 crossbred cull heifers was used, 20 $\frac{1}{2}$ Angus $\frac{1}{2}$ Nellore (A × N) and 20 $\frac{1}{2}$ Charolais $\frac{1}{2}$ Nellore (C × N), with initial average body weights (BWs) of 374.23 ± 55 kg and ages of 24.0 ± 2 months, kept in the confinement for 102 days. These cull heifers come from the farm's production system, which discards the heifer after a diagnosis of nonpregnancy in two consecutive insemination protocols.

The foods used in the diets and their compositions are presented in Table 1. Mixed silage (millet silage and Piatã grass planted in intercropped fields), ground corn grain, DDGS (distillery-dried grains with solubles), protected fat (Nutri Gordura®, Nutricorp—Araras, SP, Brazil), livestock urea (Reforce-N, Petrobras), protected urea (Prote-N®- VitallTech do Brasil-Nutrição Animal, Sarandi, RS, Brazil), extruded urea (Amireia-200 S®; Pajoara Ind & Com Ltd.a., Campo Grande, MS, Brazil) and mineral mix (Guabinucleo confinement SPM- Guabi Nutrição e Saúde Animal S. A., Indaiatuba, SP, Brazil).

Table 1. Chemical composition of the foods used in the experimental diets (g/kg of dry matter).

Item	DM [1]	OM [2]	CP [3]	EE [4]	NDF [5]	ADF [6]	Ash [7]
Silage [8]	293.70	964.70	67.18	13.74	755.25	657.76	35.30
Ground corn	872.76	988.83	72.72	22.23	137.48	35.16	11.17
DDGS [9]	913.25	965.01	415.87	65.47	622.56	314.06	34.99

Table 1. *Cont.*

Item	DM [1]	OM [2]	CP [3]	EE [4]	NDF [5]	ADF [6]	Ash [7]
Protected urea	994.96	999.80	2560.00	58.45	-	-	0.20
Livestock urea	975.09	999.59	2809.10	-	-	-	0.40
Protected fat	963.86	783.77	-	810.85	-	-	216.23
Extruded urea	950.39	995.81	2281.28	26.11	24.73	7.13	4.19
Mineral mix	984.95	118.63	-	-	-	-	881.37

[1] Dry matter. [2] Organic matter. [3] Crude protein. [4] Ethereal extract. [5] Neutral detergent fiber. [6] Acid detergent fiber. [7] Mineral matter. [8] Millet silage and Piatã grass planted and intercropped. [9] Distillery dried grains with solubles.

Amireia is obtained from the extrusion of livestock urea, corn grain and sulfur. The products are ground and subjected to a process involving temperature, pressure and humidity. Protected urea is also produced from livestock urea, but in this case, the urea grain is coated with a polymer. Both products are available on the market as slow-release urea sources.

The diets studied had the same protein and energy contents but different sources of NPN. The first diet (LPU) contained a combination of livestock urea and protected urea, while the second diet (EU) contained only extruded urea as the source of NPN (Table 2). The diets were formulated to meet maintenance requirements and an average daily gain of 1.5 kg, based on the nutritional requirements described in [13]. Metabolizable energy (ME) levels were estimated according to the equations proposed in [13]. The heifers were weighed at the beginning and end of the confinement period after a 16 h fast on solids to determine the average daily gain (ADG), which was calculated by dividing the difference in weight by the confinement period. Dry matter intake (DMI) was determined daily by weighing the amount fed and the leftovers. Feed conversion was estimated by dividing DMI by ADG.

Table 2. Formulations and compositions of the experimental diets (g/kg DM).

Item	Diet	
	LPU [1]	EU [2]
Silage [3]	326.2	348.8
DDGS [4]	71.2	95.5
Ground corn	557.9	519.2
Protected fat	10.0	-
Livestock urea	4.9	-
Protected urea	8.8	-
Extruded urea	-	15.6
Mineral mix [5]	21.1	20.9
Chemical composition (g/kg DM)		
Dry matter	691.91	678.41
Organic matter	954.63	956.04
Crude protein	151.55	162.20
Ethereal extract	26.64	20.77
Neutral detergent fiber	251.88	275.70
Acid detergent fiber	145.34	163.36
Ash	659.90	596.10
Nonfibrous carbohydrates	691.91	678.41
In vitro dry matter digestibility	954.63	956.04
Metabolizable energy (Mcal/kg DM)	2.72	2.69

[1] Diet with livestock urea and protected urea. [2] Diet with extruded urea. [3] Millet silage and Piatã grass planted and intercropped. [4] Distillery dried grains with solubles. [5] P 27 g/kg; F 27 g/kg; Na 80 g/kg; Mg 25 g/kg; S 32 g/kg; Co 30 mg/kg; Cu 680 mg/kg; I 51 mg/kg; Mn 1.100 mg/kg; Se 9 mg/kg; Zn 2.750 mg/kg; vitamin A 100.000 U.I.; 25-hidroxivitamin D3 20.000 U.I.; vitamin E 600 mg; monensin 1.100 mg/kg; Virginiamycin 730 mg/kg.

To determine the chemical composition, samples of the ingredients, complete diets and leftovers were analyzed. The samples were dried in a forced-air oven at 55 °C for 72 h and then ground in a knife mill with a 1 mm sieve. Dry matter (DM) was determined in an oven at 105 °C for 24 h (method 930.15 [14]), and the total nitrogen (method 976.05 [14]), ethereal extract (method 920.39 [14]), minerals (method 942.05 [14]) and organic matter were calculated on the basis of the mass loss by combustion. Neutral detergent fiber (NDF) and acid detergent fiber (FDA) were determined by the method described in [15]. The in vitro dry matter digestibility (ivDMD) of the diets was determined using the method described in [16]. Nonfiber carbohydrates (NFCs) were estimated using the equation described in [17], as follows: NFCs = 100 − (%CP + %NDF + %EE + %MM).

The extraction of lipids from the diets was carried out according to the method proposed in [18], using methanol, chloroform and distilled water as reagents. The analysis was carried out on a gas chromatograph with a flame ionization detector (GC/FID) (Thermo, model: Trace GC Ultra). The column used had the following characteristics: 10% cyanopropylphenyl—90% biscyanopropyl polysiloxane stationary phase, 105 m length, 0.25 mm internal diameter, and 0.2 μm film thickness (105 m, the initial temperature was 120 °C for 5 min, with an increase of 5 °C/min until a temperature of 230 °C was reached, which was maintained for 21 min [120 °C (5 min)–230 °C at 5 °C/min–230 °C (21 min)]). Hydrogen (H) was used as the carrier gas at 40 cm 3/s. The injector temperature was 250 °C, with the injection in the split mode with a split ratio of 1:20 and an injection volume of 1.0 μL. Helium gas was used as the carrier gas at a constant flow rate of 1.5 mL/min with a detector temperature of 270 °C. Fatty acids (FAs) were identified by comparing the retention times of the methyl esters in the samples using helium gas as an internal standard. Methyl nonadecanoate (Sigma-Aldrich, São Paulo, SP, Brazil) was used at a concentration of 1 mg/mL in each sample. Fatty acids were quantified by normalizing the area under the methyl ester curve and then estimated using the equation described in [19].

The proportions of the saturated fatty acids (SFAs%), monounsaturated fatty acids (MUFAs%), and polyunsaturated fatty acids (PUFAs%) were calculated in relation to the total amounts of the acids. The sum of the n6 (∑n6) and n3 (∑n3) fatty acids was calculated, and, finally, the ratios of PUFAs/SFAs and n6/n3 were calculated by dividing the amounts of PUFAs by SFAs and n6 by n3 (Table 3)s.

Table 3. Fatty acid profiles of the experimental diets (mg/100 g lipids in the diet).

Item	Diet	
	LPU [1]	EU [2]
C6:0	0.44	0.38
C12:0	0.31	0.29
C13:0	9.20	10.58
C14:0	1.17	-
C16:0	72.22	73.94
C17:0	0.54	0.56
C18:0	22.43	13.88
C18:1n9c	126.92	149.37
C18:2n6c	141.41	175.69
C18:3n6	2.29	2.53
C18:3n3	9.80	12.15
C20:1n9	0.96	1.04
SFAs [3] (%)	27.42	22.62
MUFAs [4] (%)	32.98	34.15
PUFAs [5] (%)	39.59	43.23
∑ n-6	143.70	178.23
∑ n-3	9.80	12.15
PUFAs/SFAs	1.44	1.91
n-6/n-3	14.66	14.67

[1] Diet containing the combination of livestock urea and protected urea. [2] Diet containing extruded urea. [3] Saturated fatty acids. [4] Monounsaturated fatty acids. [5] Polyunsaturated fatty acids.

2.2. Slaughter and Carcass Evaluations

At the end of the confinement period, the heifers were slaughtered at a commercial abattoir in accordance with the rules established by the Regulations for the Industrial and Sanitary Inspection of Products of Animal Origin—RIISPOA [20]. After slaughter, carcasses were individually identified, sectioned longitudinally, weighed to obtain the hot carcass weight (HCW), and stored at 4 °C for 24 h. The carcass yield (CY) was calculated from the HCW and BW, as follows: CY (%) = (HCW/FBW) × 100.

On the day after slaughter, the left halves of the carcasses were visually evaluated to assess the degree of finishing, fat distribution in the hindquarters, conformation and maturity, according to the methodology described in [21]. Subcutaneous fat distribution was assessed at the heights of the 6th, 9th and 12th ribs, and the following classification scale was used: absent (1.0 ± 0.3), scant (2.0 ± 0.3), median (3.0 ± 0.3), uniform (4.0 ± 0.3) and excessive (5.0 ± 0.3). The conformation of the carcass was assessed visually, and the classification was according to the musculature with variation (+ 0 -); the data were converted into numerical values, as follows: convex—15 to 13; subconvex—12 to 10; rectilinear—9 to 7; subrectilinear—6 to 4; concave—3 to 1.

The carcass pH was then measured between the 12th and 13th ribs, using a portable digital potentiometer with a penetration probe (model HI 99163, Hanna Instruments, Woonsocket, RI, USA), calibrated before analysis with pH 4 and pH 7 standard solutions.

A sample of the longissimus muscle was taken between the 9th and 12th ribs to measure the subcutaneous fat thickness (SFT) and rib eye area (REA). The STF was measured with a digital caliper and classified as follows: <1.0 mm, absent; 1–3 mm, sparse; 3–6 mm, intermediate; 6–10 mm, uniform; and >10.0 mm, excessive. The REA was drawn on tracing paper, and the area (cm^2) was then estimated using an LI-3100C leaf area meter (Li-Cor Inc., Lincoln, NE, USA). The longissimus muscle sample collected was identified, packed in Styrofoam on ice and then stored at −20 °C until the meat quality analysis.

2.3. Meat Composition

The moisture (MO), dry matter (DM), crude protein (CP) and mineral matter (ash) were determined using methods 930.15, 976.05 and 942.05, respectively, as described in [14]. Ethereal extract (EE) was determined using an Ankom XT10 fat extractor (Ankom Technology, NY, USA) in XT4® bags according to method 920.39 [14].

2.4. Meat Quality

2.4.1. pH and Color of Meat

The pH of the meat was measured on the thawed steak, and the calibration procedures were the same as those described for assessing the pH of the carcass. The steaks were then kept at room temperature (25 °C) for 30 min, after which the meat color (L*, a* and b*) was measured using a portable spectrophotometer (Meter CR400, Konica® Minolta, Osaka, Japan).

2.4.2. Cooking Losses and Shear Force

Cooking losses and shear force were calculated according to the methodology described by the American Meat Science Association [22]. The steaks were baked in an electric oven (Layr, Crystal model, with upper and lower heating elements, São Paulo, Brazil) until they reached an internal temperature of 71 °C. To monitor the internal temperature of the steaks, rods with temperature sensors (Taylor, model 1478-21, Cincinnati, OH, USA) were inserted into the geometric center of each steak. After reaching the temperature, they were removed from the oven and weighed. Cooking losses were estimated based on the difference in the weights of the steak before and after roasting. After roasting, the steaks were stored for 24 h at 2 °C; then, 7 subsamples of 1.27 cm in diameter were taken from each steak to determine the shear force (SF). The subsamples were taken in the direction of the muscle fiber, using a bench drill. The SF was determined using a texture analyzer (CT3 Warner Bratzler, Brookfield Engineering, Middleborough, MA, USA).

2.4.3. Lipid Oxidation and Myofibrillar Fragmentation Index (MFI)

The lipid oxidation of the meat was assessed by the concentration of malonaldehyde (MDA), mg/kg of meat, according to the methodology described in [23]. The myofibrillar fragmentation index (MFI) was determined according to the method described in [24].

2.4.4. Cholesterol and Collagen Concentration

The total cholesterol concentration was determined by the enzymatic method, according to the methodology described in [25]. The soluble and insoluble collagens were determined from the concentration of hydroxyproline [26]. The collagen concentration in the fresh meat sample (g/100 g) was calculated according to the equations described in [27].

2.4.5. Fatty Acid Profile of Fat and Meat

Lipids were extracted from the meat and fat samples using the technique described in [28]. Fresh samples of 5 g of meat and 1.5 g of fat were used. For the methylation of the fatty acids, the methodology adapted from [29] was applied, and the FA concentrations were calculated using the equations described in [19]. The determination of FAs was carried out following the same procedures used to analyze the diets.

2.5. Statistical Analysis

Data were analyzed using a 2 × 2 factorial design with two genetic groups and two diets. All variables were analyzed by analysis of variance using the Proc Glimix procedure in the SAS Studio software, version 9.2 (SAS Institute Inc., Cary, NC, USA) [30], and the means per treatment were compared using the Tukey test at a significance level of 0.05.

3. Results

3.1. Carcass Evaluation

There was no significant interaction between GG and diet for final weight and carcass characteristics ($p > 0.05$) (Table 4). The A × N heifers had higher final weights (501.42 vs. 483.26 kg), ADGs (1.29 vs. 0.15 kg/day) and DMIs (10.33 vs. 9.92 kg/day) compared to the C × N heifers ($p < 0.01$). A significant interaction was observed for FC, where lower averages were observed in the A × N heifers fed the LPU and EU diets, followed by the C × N heifers fed the LPU diet and, finally, the C × N heifers fed the EU diet ($p < 0.01$).

Table 4. Carcass characteristics of beef heifers from different genetic groups finished in confinement.

Item	A × N [1]		C × N [2]		SEM [3]	P > F		
	LPU [4]	EU [5]	LPU	EU		GG [6]	Diet	GG × Diet
Body weight initial (kg)	373.54	372.99	374.23	372.66	2.83	0.95	0.72	0.86
Body weight final (kg)	508.02	494.81	487.52	478.99	3.96	<0.01	<0.01	0.54
Average daily gain (kg/day)	1.30	1.27	1.16	1.14	0.35	<0.01	0.49	0.91
Dry matter intake (kg/day)	10.26	10.39	9.99	9.85	0.10	<0.01	0.94	0.16
Feed conversion [7]	7.32 d	8.04 c	8.30 b	8.73 a	0.07	<0.01	<0.01	0.04
Hot carcass weight (kg)	302.0	309.45	275.50	284.10	5.91	<0.01	0.18	0.92
Carcass yield (%)	55.65	55.17	55.40	55.30	0.44	0.89	0.52	0.67
pH carcass	5.60	5.65	5.62	5.59	0.04	0.59	0.87	0.40
Rib eye area (cm^2)	84.05	77.69	76.39	74.50	2.32	0.02	0.08	0.33
Subcutaneous fat thickness (mm)	8.77	8.61	6.64	6.06	0.67	<0.01	0.56	0.74
Fat distribution (score) [8]	2.05	2.50	1.65	1.80	0.19	<0.01	0.11	0.42
Conformation (score) [9]	11.30	7.10	8.00	8.10	1.29	0.17	0.11	0.09
Physiological maturity (months)	26.00	25.60	25.60	25.80	1.28	0.94	0.94	0.87

Means followed by different letters differ according to the Tukey test ($p < 0.05$). [1] $\frac{1}{2}$ Angus $\frac{1}{2}$ Nellore heifers. [2] $\frac{1}{2}$ Charolais $\frac{1}{2}$ Nellore heifers. [3] Standard error of the mean. [4] Diet containing the combination of livestock urea and protected urea. [5] Diet containing extruded urea. [6] Genetic group. [7] Dry matter intake (kg/day)/average daily gain (kg/day). [8] Evaluation scores: absent (1.0 ± 0.3), scant (2.0 ± 0.3), median (3.0 ± 0.3), uniform (4.0 ± 0.3) and excessive (5.0 ± 0.3). [9] Evaluation scores: convex—15 to 13; subconvex—12 to 10; rectilinear—9 to 7; subrectilinear—6 to 4; concave—3 to 1.

The HCWs were higher in the A × N heifers, at 305.73 kg, compared to the C × N heifers, at 279.8 kg ($p < 0.01$). The CY and carcass pH were not influenced by the genetic group or diets studied ($p > 0.05$). Measurements of the REA, SFT, and fat distribution were higher in the A × N heifers, at 80.87 cm^2 and 8.69 mm, compared to the C × N heifers, 75.44 cm^2 and 6.35 mm ($p < 0.05$), with no effect of diets. There was no significant difference in the physiological maturities ($p > 0.05$).

3.2. Meat Composition

There was no effect of GG and diet on the moisture content of the meat (Table 5). A significant interaction was found in the ether extract contents ($p < 0.05$); while the A × N heifers had higher concentrations of EE when they were fed the LPU diet, in the C × N heifers, a greater amount of EE was observed with the EU diet. The concentrations of minerals in the meat was not significant ($p > 0.05$).

Table 5. Composition and quality of meat from beef heifers from different genetic groups finished in confinement.

Item	A × N [1]		C × N [2]		SEM [3]	P > F		
	LPU [4]	EU [5]	LPU	EU		GG [6]	Diet	GG × Diet
Moisture (%)	72.19	71.96	72.66	72.38	0.26	0.09	0.34	0.93
Crude protein (%)	25.65	26.28	26.48	25.84	0.33	0.58	0.99	0.07
Ethereal extract (%)	3.51 a	2.90 ba	2.21 b	2.73 ba	0.30	<0.01	0.86	0.04
Ash (%)	1.05	1.07	1.07	1.09	1.09	0.44	0.32	0.87
Cholesterol (mg/100 g meat)	57.04	63.30	61.37	62.24	2.67	0.56	0.20	0.33
Myofibrillar fragmentation index	92.30	96.41	97.31	97.95	2.57	0.21	0.36	0.50
Lipid oxidation	2.44	2.95	2.77	2.69	0.43	0.93	0.63	0.51
pH meat	5.62	5.68	5.65	5.60	0.03	0.36	0.99	0.08
Total collagen (g/100 g meat)	0.16	0.16	0.15	0.15	<0.01	0.21	0.98	0.79
Soluble collagen (%)	93.81	94.06	94.65	94.42	0.47	0.19	0.99	0.61
Insoluble collagen (%)	6.18	5.94	5.35	5.58	0.47	0.19	0.99	0.60
Meat color								
*L**	37.17	35.62	35.02	36.45	0.78	0.40	0.94	0.06
*a**	20.42	18.90	20.30	19.63	0.68	0.66	0.12	0.54
*b**	11.15	9.89	10.89	10.62	0.42	0.58	0.08	0.25
Shear force (kg)	7.03	6.92	7.82	7.58	0.22	<0.01	0.44	0.76
Cooking losses (%)	19.78	18.79	18.57	19.81	0.99	0.92	0.89	0.24

Means followed by different letters differ according to the Tukey test ($p < 0.05$). [1] ½ Angus ½ Nellore heifers. [2] ½ Charolais ½ Nellore heifers. [3] Standard error of the mean. [4] Diet containing the combination of livestock urea and protected urea. [5] Diet containing extruded urea. [6] Genetic group.

3.3. Meat Quality

No significant interactions were observed between the genetic group and diet for cholesterol, MFI and lipid oxidation in the meat ($p > 0.05$). Similar trends were found for the levels of total collagen, soluble collagen and insoluble collagen (Table 5). The shear force was 10.5% lower in meat from the A × N heifers (6.97 kg) compared to meat from the C × N heifers (7.70 kg). There was no interactions or significant effects of genetic group and diet on cooking losses ($p > 0.05$).

Significant interaction between the genetic group and diet was found for palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), stearic (C18:0), oleic (C18:1n9c), linoleic (C18:2n6c), arachidic (C20:0), y-linolenic (C18:3n6) and behenic (C22:0), with higher concentrations of these acids in the meat from the A × N heifers fed the EU diet (Table 6).

The concentration of capric acid (C10:0) was influenced only by the genetic group, being higher in the C × N heifers, at 0.41 vs. 0.37 ($p < 0.05$). Lauric (C12:0), myristic (C14:0), myristoleic (C14:1), pentadecanoic (C15:0) and α-linolenic (C18:3n3) acids were not influenced by genetic group or diet ($p > 0.05$).

There was a significant interaction between the concentration of arachidonic acid (C20:4n6) and the amount of SFAs in relation to the total amount of acids ($p < 0.05$). The proportion of MUFAs and PUFAs and the amount of n-3 were not influenced by genetic

group and diet ($p > 0.05$). There was a significant interaction between genetic group and diet for the n-6 and the n-6/n-3 ratio averages (Table 6).

Table 6. Composition of fatty acids (mg/100 g of meat) in meat from beef heifers from different genetic groups fed and finished in confinement.

Item	A × N [1]		C × N [2]		SEM [3]	P > F		
	LPU [4]	EU [5]	LPU	EU		GG [6]	Diet	GG × Diet
C10:0	0.36	0.38	0.40	0.43	0.03	0.03	0.43	0.67
C12:0	0.47	0.46	0.50	0.53	0.04	0.17	0.76	0.61
C14:0	22.28 b	25.12 a	20.87 b	23.74 a	1.3	0.31	0.04	0.99
C14:1	4.14	4.94	4.16	4.61	0.49	0.76	0.21	0.72
C15:0	1.81	2.13	2.16	1.90	0.19	0.73	0.88	0.13
C16:0	187.18 b	220.75 a	183.22 b	183.9 2b	7.50	<0.01	0.03	0.03
C16:1	18.12 b	26.25 a	22.81 ba	24.12 ba	1.69	0.46	<0.01	0.04
C17:0	5.01 b	6.07 a	5.32 ba	5.22 b	0.23	0.24	0.04	0.01
C18:0	101.71 b	120.95 a	101.73 b	100.03 b	4.00	0.01	0.03	0.01
C18:1n9c	277.18 b	342.63 a	293.48 b	290.57 b	13.11	0.18	0.02	0.01
C18:2n6c	15.72 b	21.70 a	20.19 ba	17.60 ba	1.27	0.89	0.19	<0.01
C20:0	0.18 b	0.25 a	0.23 ba	0.21 ba	0.02	0.89	0.11	<0.01
C18:3n6	0.58 b	0.67 a	0.61 ba	0.58 b	0.02	0.07	0.07	<0.01
C18:3n3	1.31	1.22	1.38	1.20	0.12	0.83	0.28	0.77
C22:0	1.62 b	2.49 a	2.23 ba	1.82 b	0.12	0.86	0.21	<0.01
C20:4n6	5.17 b	6.98 a	7.19 a	6.26 b	0.58	0.26	0.44	0.02
SFAs [7] (%)	50.18 a	48.63 ba	47.56 b	48.34 ba	0.53	<0.01	0.47	0.03
MUFAs [8] (%)	46.42	47.80	48.24	47.80	0.57	0.12	0.41	0.12
PUFAs [9] (%)	3.74	3.95	4.35	3.99	0.28	0.21	0.78	0.29
∑ n-6	22.61 b	29.29 a	27.87 a	25.02 ba	1.71	0.77	0.25	<0.01
∑ n-3	1.32	1.22	1.38	1.21	0.12	0.83	0.28	0.77
PUFAs/SFAs	0.07	0.08	0.09	0.08	<0.01	0.08	0.51	0.10
n-6/n-3	19.07 b	25.61 a	20.87 b	20.40 b	1.34	0.19	0.02	<0.01

Means followed by different letters differ according to the Tukey test ($p < 0.05$). [1] $\frac{1}{2}$ Angus $\frac{1}{2}$ Nellore heifers. [2] $\frac{1}{2}$ Charolais $\frac{1}{2}$ Nellore heifers. [3] Standard error of the mean. [4] Diet containing the combination of livestock urea and protected urea. [5] Diet containing extruded urea. [6] Genetic group. [7] Saturated fatty acids. [8] Monounsaturated fatty acids. [9] Polyunsaturated fatty acids.

There was a significant interaction between genetic group and diet for the concentrations of FAs C10:0, C12:0 C14:0, C16:0, C17:0, C18:1n9c, C18:3n6 and C18:3n3, of which greater amounts were observed in the fat of the C × N heifers that received the EU diet ($p < 0.05$) (Table 7).

Table 7. Composition of fatty acids (mg/100 g of fat) of fat from beef heifers from different genetic groups fed and finished in confinement.

Item	A × N [1]		C × N [2]		SEM [3]	P > F		
	LPU [4]	EU [5]	LPU	EU		GG [6]	Diet	GG × Diet
C10:0	0.34 b	0.28 b	0.36 ba	0.40 a	0.03	<0.01	0.67	0.04
C12:0	0.49 b	0.39 b	0.51 ba	0.57 a	0.04	<0.01	0.56	0.05
C14:0	24.80 b	21.85 b	22.76 b	28.48 a	1.34	0.09	0.30	<0.01
C14:1	5.38	5.15	5.29	8.24	0.84	0.02	0.30	0.19
C15:0	2.67	2.45	2.48	2.79	0.20	0.72	0.81	0.20
C16:0	180.79 a	172.46 ba	158.25 b	190.82 a	5.85	0.72	0.04	<0.01
C16:1	23.18	22.35	24.34	30.23	2.03	0.03	0.22	0.10
C17:0	5.69b a	5.63 ba	5.15b	6.23 a	0.24	0.92	0.04	0.02
C18:0	97.37	93.12	82.22	96.22	4.31	0.36	0.51	0.10
C18:1n9c	288.06 b	283.44 b	272.28 b	333.69 a	12.05	0.12	0.02	<0.01
C18:2n6c	5.69	6.55	5.78	7.23	0.43	0.38	<0.01	0.50
C18:3n6	0.74 a	0.67 b	0.66 b	0.77 a	0.04	0.96	0.47	0.03
C18:3n3	0.71a	0.58 b	0.62 b	0.73 a	0.05	0.53	0.82	0.02
SFAs [7] (%)	49.62	48.64	47.06	46.50	0.64	<0.01	0.22	0.75
MUFAs [8] (%)	49.92	51.16	52.47	52.28	0.88	<0.01	0.82	0.64
PUFAs [9] (%)	1.14	1.28	1.22	1.24	0.06	0.76	0.20	0.34

Table 7. *Cont.*

Item	A × N [1]		C × N [2]		SEM [3]	P > F		
	LPU [4]	EU [5]	LPU	EU		GG [6]	Diet	GG × Diet
∑ n-6	6.44	7.24	6.44	8.01	0.46	0.40	0.01	0.41
∑ n-3	0.72 ba	0.58 b	0.62b a	0.74 a	0.05	0.53	0.82	0.02
PUFAs/SFAs	0.02	0.02	0.02	0.02	<0.01	0.38	0.20	0.30
n-6/n-3	10.2 6	13.62	11.41	11.40	0.99	0.59	0.09	0.09

Means followed by different letters differ according to the Tukey test ($p < 0.05$). [1] $\frac{1}{2}$ Angus $\frac{1}{2}$ Nellore heifers. [2] $\frac{1}{2}$ Charolais $\frac{1}{2}$ Nellore heifers. [3] Standard error of the mean. [4] Diet containing the combination of livestock urea and protected urea. [5] Diet containing extruded urea. [6] Genetic group. [7] Saturated fatty acids. [8] Monounsaturated fatty acids. [9] Polyunsaturated fatty acids.

There was an effect of genetic group on the concentrations of C16:0, C18:3n6, C18:3n3, C14:1 and C16:1, with a higher concentration in the fat of the A × N heifers ($p < 0.05$). There was no effect of genetic group or diet on the concentrations of C15:0 and C18:0 ($p > 0.05$). The amounts of SFAs and MUFAs varied according to genetic group, where fat from the A × N heifers had a higher concentration of SFAs (49.13% vs. 46.78%) and the fat from the C × N heifers had more MUFAs (52.38% vs. 49.28%), with no effect on the PUFA concentration ($p > 0.05$). The sum of n6 was only significant for the NPN content, with a higher average with the EU diet (7.23 vs. 6.84 mg/100 g fat). There was a significant interaction in the sum of n3 ($p < 0.05$), while the concentration of MUFAs and the ratios of PUFAs/SFAs and n6/n3 were not influenced by genetic group or diet ($p > 0.05$).

4. Discussion

4.1. Carcass Evaluation

The higher final weights of the A × N heifers can be explained by the high genetic potential of the Angus breed, which provides better heterosis when crossing with Nellore [6]. Similar results were also found in [31], which reported higher HCWs, REAs, and SFTs in A × N heifers compared to Nellore or Simmental × Nellore heifers slaughtered at 18 months. Similarly, Ref. [3] recorded higher final weights for A × N cull cows compared to Caracu × Nellore or Nellore cows. On the other hand, Ref. [32] reported a significant difference only in CY and REA for Nellore × Angus cows compared to Angus, Nellore and Hereford cows.

The different solubility rates of the NPN sources may have favored the development of the rumen microbiota and, consequently, better use of nutrients and production of microbial protein, which led to greater cold carcass weights in the A × N heifers. Ref. [12] evaluated the performance of beef steers fed with different sources and combinations of NPN sources, noting that the highest performance was observed with the combination of livestock urea + extruded urea + protected urea, with fast, medium, and slow solubilities, respectively. Recent studies have demonstrated that extruded urea can partially replace true protein sources in ruminant diets [10,11]. According to these works, the advantages of this source of NPN range from a lower risk of intoxication to better synchronization of NPN with other nutrients for microbial protein synthesis and lower dietary cost.

According to [3], slaughter weight has positive correlations with CY, REA and SFT, which may justify the higher REA and SFT values in the A × N heifers. These are characteristics of interest to a slaughterhouse due to their relationship with the yield of commercial cuts and the protection of the carcass during the cooling process [33]. The higher fat content in the A × N heifers also allowed for a better distribution of fat in the carcass. In all treatments, the SFTs were between 6 and 10 mm and classified as uniform finishing, which is much higher than 3 mm minimum required by the slaughterhouse.

In a study that compared the carcass and meat characteristics of A × N cattle of different genders (castrated males, noncastrated males, and females) finished in confinement, with slaughter at 20 months, Ref. [4] reported higher cold carcass weights and REAs in

uncastrated males. However, heifers had higher SFTs and marbling, which are factors that influence the sensory attributes of meat [34].

4.2. Meat Composition

The lack of differences in the protein, moisture, and ash contents of the meat can be explained by the similarity in ages and finishings, in addition to the animals being of the same sex. As demonstrated in [35] while observing the compositions of meat from cattle of different crosses, the percentages of moisture, crude protein, and ash were little influenced by the genetic group, and only the lipid content showed greater variations. Our results are in line with those described in [35], in which a difference was observed only in the ether extract content. Although the lipid content may vary according to the genetic group, Ref. [2] found no significant difference in the meat compositions of A × N heifers and steers finished in confinement. According to these authors, the lack of significant effect is related to the finishing standard, which was the criterion used for slaughter.

In a study to evaluate the effects of different genetic groups on carcass and meat quality, Ref. [31] found no significant differences in the meat compositions of Nellore, Angus × Nellore and Simmental × Nellore heifers finished in confinement. The average moisture content (72.37%), ether extract (3.12%) and mineral matter (1.31%) are close to those found in this work. There was a difference observed only in the concentration of crude protein, with a higher average in our study (26.06% vs. 20.64%).

4.3. Meat Quality

The lower SF observed for the meat from the A × N heifers may be related to better performance. As reported in [36], animals with greater potential for weight gain tend to have lower calpastatin activity, which is directly related to the tenderness of the meat, since calpastatin inhibits the action of calpain, which acts to soften the meat. The SF of meat from the A × N heifers found in this study (6.97 kg) was lower than the 7.91 kg described in [37] while evaluating meat from 148 13-month-old A × N heifers finished in confinement.

The MFI is also related to meat tenderness, with values above 60 indicating high meat tenderness [24]. However, although the MFI results in this study are above this value, the SF data do not confirm the good tenderness of the meat. Lower values for SF could be obtained with maturation for 7 or 14 days, as demonstrated in [3,37]. Another variable linked to meat tenderness is collagen concentration, which is related to genetics, age, animal growth rate, and days of maturation [38]. The absence of significant differences in this variable can be explained by the similarity in the ages and finishes of the heifers at the time of slaughter. Ref. [39] demonstrated through a meta-analysis that there are no differences in the collagen contents of the longissimus muscle among beef breeds, with the same weight and age at slaughter. According to the authors, differences can be found in certain muscles and days of maturation.

Meat color is affected by nutrition, growth rate [40], preslaughter management and, mainly, the animal's age [41]. As the animal ages, the myoglobin concentration in the meat increases, making it darker [42]. The heifers evaluated in this study were the same age, had the same management during the experiment and preslaughter, and had adequate fat coverage and carcass pH. Although the diets had different sources of NPN, this is not a nutrient that can cause changes in the characteristics of the meat [11].

According to [33], cholesterol and EE levels are associated variables, since EE includes marbling and intracellular fat, where the highest cholesterol levels are concentrated. However, despite the greater amount of EE in the meat of A × N heifers, there was no difference in cholesterol concentration between GG and diet. The cholesterol content found in this study (60.99 mg/100 of meat) was higher than that found in [43] (49.71 mg/100 g meat) in the Longissimus muscle of Nellore × Charolais heifers finished in confinement.

The fatty acid profile of meat changes according to the genetic group and nutrition. Ref. [35] reported that crosses between the Nellore breed and continental breeds produce less C14:0 and C16:0, which was confirmed in this work. The greater amounts of C16:0 and

SFAs in the meat of the A × N heifers may also be related to the greater proportion of SFAs in the diet and the greater amount of fat in the carcass. These fatty acids are undesirable because they are related to heart disease and cancer [44].

According to [45], the C14:0 and C16:0 contents arms health when the sum of these two FAs is greater than 35% of the total acids. In the treatments evaluated, the FAs mentioned were below this value, with an average of 31.56 ± 0.81%. Although there was an effect on the amount of SFAs, the amounts of MUFAs and PUFAs were similar among treatments. As described in [35], C18:0 is one of the most abundant acids in beef, but this FA is not related to increased serum cholesterol levels. On the other hand, C18:1n9c and PUFAs are related to the reduction in cholesterol and increase in high-density proteins (HDLs) [46].

The n6 acids were more abundant in the meat of the C × N heifers, which did not differ from the amount found in the meat of the A × N heifers that received the EU diet. Of these acids, the one that had the greatest proportion was C18:2n6. According to [35], this FA is responsible for causing an imbalance in the proportions of n6/n3, where the ideal is a proportion of up to 4.0. The values observed for the proportions of n6/n3 were higher, which may lead to concern, as they are related to heart problems and cancer for the meat consumer. One factor that may have contributed to the high n6/n3 ratio is the small amount of n3 detected in the analysis.

The PUFAs/SFAs ratio was higher in the C × N heifers; however, it was lower than that described in [40] for crosses between Charolais × Caracu (0.21), Angus × Nellore (0.18) and crossbred heifers (0.13). It was also lower than the 0.17 found in [46], which determined the fatty acid profiles of meat from Nellore heifers finished in confinement. The lower PUFAs/SFAs ratio found in this study, compared to the works cited is mainly due to the lower proportion of PUFAs in the total amount of FAs. The amount and proportion of MUFAs in meat can be altered by including seeds or oilseed oil, such as linseed [44], cottonseed, soybean [47], or sunflower [48]. The main FAs impacted by the inclusion of these foods in the diet are n-6 and n-3. Different sources of NPN are not mentioned in the literature as factors capable of altering the fatty acid profile of meat.

The fatty acid profile of the subcutaneous fat was similar to the meat profile for most FAs, with a greater participation of C18:1n9c in both cases. However, differences were observed in the proportion of FAs, with a reduction in the amount of PUFAs and an increase in the proportions of MUFAs. Similar results were found in [49] when analyzing subcutaneous fat from the longissimus muscle of heifers, where the proportions of SFAs and MUFAs were 48.26% and 48.23%, respectively. The proportions of MUFAs (3.42%) and n-3 (0.91%) were higher, while n-6 was higher in our study. The low amount of PUFAs resulted in a low PUFAs/SFAs ratio and, as described for the meat fat, the C18:2n6c acid was responsible for causing an imbalance in the n6/n3 ratio. As reported in [49], fatty acid profiles can vary by type of fat (intramuscular, intermuscular, external and internal) and the amount of fat in the carcass. In the latter case, the increase in fat in the carcass causes an increase in SFAs.

5. Conclusions

The A × N heifers had better carcass characteristics and softer meat than the C × N heifers. The meat compositions for heifers from different genetic groups differed only in the fat contents, with higher amounts in the meat from A × N heifers, with no difference among sources of NPN. The C × N heifers had lower concentrations of SFAs in the meat and fat, which provided a better fatty acid profile for this crossbreed. Regardless of the genetic group, the proportion of PUFAs and the PUFAs/SFAs ratios were lower in the subcutaneous fat compared to fat extracted from the meat.

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Muscle-Specific Effects of Genotype, Animal Age, and Wet Aging Duration on Beef Color, Tenderness, and Sensory Characteristics

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Simple Summary: The meat quality, especially color and tenderness, varies significantly among different muscles within a single carcass. Therefore, this study investigated how genotype, animal age, and aging durations affected the beef color, tenderness and sensory characteristics of different muscles. We found that tenderloin and rib-eye muscles improved in color, tenderness, and sensory traits with up to seven days of aging. The sirloin and rump muscles continued to improve for up to 14 days. The tenderloin was the most tender and acceptable, while the sirloin had the best color and oxidative stability. These results highlighted the importance of applying muscle-specific aging strategies to enhance meat quality in both humped and humpless bulls.

Abstract: This study investigated the effects of genotype, animal age, muscle type, and aging duration on meat quality characteristics of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus Medius* (GM) muscles. The PM, LT, LL, and GM muscles were sourced from a total of 32 bulls, consisting of 16 humped (*Bos indicus*) and 16 humpless (*Bos indicus* × *Bos taurus*) bulls aged 21 ± 2 and 30 ± 3 months. The muscles underwent aging durations of 0, 7, and 14 days. Meat pH, color, drip loss, cooking loss, instrumental shear force, lipid oxidation (thiobarbituric acid reactive substances/TBARS), and sensory analysis were performed. Our results indicated that humped bulls had superior color, while humpless bulls exhibited better sensory characteristics. The 30 ± 3 months of age bulls showed improved color and sensory characteristics with higher TBARs values than the 21 ± 2 months of age bulls. The color, tenderness, and sensory characteristics improved in PM and LT at 7 days, whereas in LL and GM they improved at 14 days. PM showed better tenderness and overall acceptability among different muscles, while LL showed better color and oxidative stability. This study suggested the necessity of muscle-specific aging strategies to enhance the meat quality characteristics of humped and humpless bulls. Further research could explore additional aging durations and other muscle types to better understand their impact on meat quality characteristics.

Keywords: aging; bull age; breed; meat quality; muscle-specific

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1. Introduction

Beef color and tenderness are the most dominant quality characteristics affecting the consumers' acceptance of beef, eating fulfillment, and repeat buying decisions [1]. The bright reddish-purple color indicates the beef's freshness, and consumers are ready to spend an additional cost for fresh-colored and guaranteed tender beef [2]. However, beef color, tenderness, drip loss, cooking loss, and sensory characteristics are highly variable;

muscle-specific characteristics and various muscles from the same carcass exhibit significant differences in meat quality [3].

It is reported that the round and chuck muscles are mainly involved in locomotion. Therefore, these muscles are tougher and have less consumer acceptability than rib and loin muscles associated with providing support [3]. Different muscles in a bull carcass show distinct variations in biochemical characteristics such as muscle composition, lipid oxidation [4], and the extent of proteolysis of structural proteins [5]. These variations contribute to intermuscular differences in color, oxidative stability, and beef tenderness [6].

Cattle breeds also affect different beef muscles' color stability and tenderness [7,8]. Bressan et al. [9] compared the physiochemical characteristics of beef from *Bos taurus* and *Bos indicus* breeds, reporting greater tenderness in *Bos taurus* compared to *Bos indicus* bulls. Miguel et al. [10] reported higher redness in the *Longissimus thoracis* (LT) muscle from Nellore than crossbred (Nellore × Aberdeen Angus) bulls. Animal age at slaughter is another crucial meat quality determinant that affects the color and tenderness of various beef muscles [11]. The color of different beef muscles becomes darker with age, mainly due to increased myoglobin content [12]. Various studies reported that the tenderness of different beef muscles decreases with age, which is largely attributed to an increase in connective tissue along with an increase in their mechanical and thermal stability [11–13].

Postmortem wet aging of different meat cuts under the vacuumed packaging condition is a widely adopted practice used to improve the color and tenderness of beef in different regions of the world [3]. The biochemical changes, including the proteolysis of specific structural proteins by endogenous enzymes, are responsible for improving beef quality characteristics during postmortem aging [14]. Traditionally, wet aging is performed on different sub-primal beef cuts; therefore, various muscles in a beef cut undergo a similar aging procedure. However, the beef industry has emphasized marketing single-muscle cuts to provide better beef quality. The muscles differ in physiochemical and quality characteristics; therefore, they might respond differently to postmortem aging.

In South Asia, particularly in Pakistan, where cattle serve as a primary source of red meat, there is no specific beef breed. Instead, beef producers and processors typically classify cattle into humped and humpless categories [15]. These cattle types differ in color, tenderness, oxidative stability, and sensory characteristics [16]. Additionally, early-age slaughtering of calves is a common practice in the region, which adversely affects carcass yield and overall meat quality. Extending the rearing period of these calves by a few months could result in heavier carcasses with better overall meat quality while minimally compromising tenderness. Despite these observations, the impact of genotype, animal age, and aging duration on the meat quality characteristics of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus Medius* (GM) muscles from humped and humpless cattle remains unexplored. These muscles represent the premium beef cuts, a range of anatomical positions, and functions within a carcass.

This study is innovative as it uniquely evaluated the combined effect of cattle type, animal age, and aging duration on muscle-specific quality characteristics. Furthermore, it explores the potential for these muscles to be marketed individually, which will enhance the quality of these muscles and facilitate the export of these muscles to the higher-end markets of major beef-importing countries. By addressing the research gap in these cattle types and leveraging extended rearing and aging practices, this work would facilitate meeting the growing demand for quality beef. Therefore, the current study aimed to determine the muscle-specific effect of genotype, animal age, and aging duration on the quality characteristics of PM, LT, LL, and GM muscles from humped and humpless cattle bulls.

2. Materials and Methods

2.1. Animals and Sample Preparation

The meat samples used in the current study were collected from a randomly selected subset of thirty-two animals from our previous research [15]. In summary, a total of $n = 32$ cattle bulls were obtained from a research and development farm, Big Feed Pvt. Ltd.,

Lahore, Pakistan, comprising 16 humped (*Bos indicus*, hump height $\geq$ 120 mm) and 16 humpless (*Bos indicus* $\times$ *Bos taurus*, hump height $\leq$ 45 mm) bulls. Each group was further divided by age, with eight bulls from each breed aged 21 $\pm$ 2 months and eight bulls from each breed aged 30 $\pm$ 3 months. The bulls were housed intensively and raised under similar feeding and management conditions. Ethical approval (Ethic number Dr/451) was obtained from the Institutional Ethical Review Committee, Office of Research Innovation and Commercialization (ORIC), University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. The bulls were transported from the farm to a lairage facility at UVAS, Lahore, located 30 km away, with a travel time of approximately 1 h on smooth asphalt roads. Upon arrival, the bulls were rested for 24 h to alleviate transportation stress. During this period, they had unrestricted access to feed and fresh water for the initial 12 h, followed by a 12 h period without feed to minimize cross-contamination from gastrointestinal contents during slaughter. The mean live weight, carcass weight, rib-eye area, and backfat thickness are shown in Supplementary Table S1. Following slaughter, the hump height was measured from the carcass by positioning a ruler parallel to the surface of the sawn chine and aligning it perpendicularly to the first thoracic vertebrae. The ruler was moved to the position of the greatest hump width. After 24 h postmortem, four muscles (PM, LT, LL, and GM) were detached from both sides of the beef carcass. Left- and right-sided muscles were divided into one and two sections, alternatively. Each section within each muscle was randomly allocated for either 0, 7, or 14 days of aging at 2 °C. The muscle sections designated for 7 and 14 days of aging were individually placed in nylon–polyethylene bags (90 µm thickness, oxygen transmission rate of 50 $cm^3/m^2/24$ h) and vacuum-sealed using a C100 vacuum packer machine (Multivac® Ltd., Geprüfte Scherhert, AGW, Wolfertschwenden, Germany). Subsequently, the muscle sections underwent wet aging for periods of 7 and 14 days. Following aging, the muscle sections were unwrapped from the vacuum packaging, and each section was divided into four steaks, each 2.50 cm thick: one for color stability; one for thiobarbituric acid reactive substances (TBARS) and drip loss; one for pH, cooking loss, and Warner–Bratzler shear force (WBSF); and one for sensory analysis. Color stability and TBARS were measured for up to seven days of retail display following the completion of the designated aging duration.

2.2. pH

The pH of each muscle was assessed using a portable pH meter by directly inserting its probe into the steak. Measurements were taken after 0, 7, and 14 days of aging using a pH meter (WTW, pH 3210-SET 2, Weilheim, Germany). Before use, the pH probe underwent calibration with pH 4.00 and 7.00 buffers at room temperature. Three readings were taken from different locations within each muscle, and results were averaged to perform statistical analysis.

2.3. Instrumental Color

After 0, 7, and 14 days of wet aging, the steaks chosen for color measurements were packaged in modified atmosphere trays (HiO_2 MAP containing 80% O_2 and 20% CO_2) using the Multivac® T200 gas packer machine (Multivac® Ltd., Geprufte Scherhert, AGW, Wolfertschwenden, Germany) equipped with a gas mixer (MAP MIX 9001 ME, Dansensor, Ringsted, Denmark). The MAP trays were made from polypropylene, and the MAP film used was PET-PVDC-PE, featuring an oxygen permeability of 5 $cm^3/24$ h/m^2/atm, a carbon dioxide permeability of 20 $cm^3/24$ h/m^2/atm, and a water vapor permeability of 4 g/24 h/m^2. The steaks were placed under a simulated display for seven days. The color parameters were taken on days 1, 3, 5, and 7 of the simulated retail display using a Minolta chromameter (Konica Minolta® CR-410, Tokyo, Japan), equipped with a 2° standard observer, C illuminant, and 50 mm aperture. The chromameter was calibrated using a standard white tile according to the manufacturer's instructions. The CIE color parameters, including lightness (L*), redness (a*), yellowness (b*), and chroma (C*, saturation index), were measured by positioning the Minolta head over the MAP trays. Three separate

readings were recorded for each steak, and the average of these readings was used for statistical analysis.

2.4. Drip Loss (%)

The standard bag method was used to determine the drip loss of the steaks from PM, LT, LL, and GM muscles [17]. The weight of each steak was measured individually with a digital compact weighing balance (SF-400, 7000 g × 1 g, Yongkang, China) and then suspended in a sealed container over 48 h at 4 °C. The drip loss was expressed as a percentage relative to the initial weight of the steak.

2.5. Cooking Loss (%) and Instrumental Shear Force

The steaks were weighed using a digital scale (SF-400, 7000 g × 1 g, Yongkang, China) after 0, 7, and 14 days of wet aging, placed in polyethylene bags, and cooked in a water bath maintained at 80 °C. The steaks were cooked in the water bath (Memmert WNB45, Schwabach, Germany) until they reached an internal temperature of 72 °C, monitored with a digital thermometer (TP-101, temperature range of −50 °C to 300 °C, Shanghai, China). After cooking, the steaks were allowed to cool to room temperature (20 °C) for approximately 45 min, and any excess moisture was removed using a hand towel. The steaks were then weighed again. The percentage change in steak weight before and after cooking was used to calculate cooking loss.

To measure shear force, strips of meat with dimensions 5 cm × 1 cm × 1 cm were excised parallel to the muscle fibers using a scalpel blade. These strips were then subjected to shear force perpendicular to the muscle fibers using the 'V-Slot' blade on a texture analyzer (TA.XT plus® texture analyzer, Stable Micro System, Godalming, UK). The shear force was recorded in Newtons (N), with five individual readings taken from each steak, and the results were averaged to perform statistical analysis.

2.6. Thiobarbituric Acid Reactive Substances (TBARS) Analysis

Lipid oxidation in the meat samples after completing 0, 7, and 14 days of aging was evaluated on days 1, 3, 5, and 7 of the simulated retail displays using the thiobarbituric acid reactive substances (TBARS) method. A mixture was prepared by combining 5 g of ground meat from each steak with 20 mL of distilled water and 25 mL of a 20% aqueous solution of trichloroacetic acid (TCA). Homogenization of the mixture was performed using a Polytron homogenizer (PT 10/35, Brinkman Instruments Inc., Riverview, FL, USA) and was subsequently allowed to incubate at 25 °C for 1 h. The mixture was then centrifuged at 2000 rpm for 15 min, after which the filtrate was diluted to 50 mL with distilled water. Subsequently, 5 mL of the diluted filtrate was combined with 5 mL of a 0.02 M aqueous solution of 2-thiobarbituric acid (TBA) in a test tube. The mixture was incubated in a hot water bath for 15 min to develop color and then cooled in ice water for approximately 10 min. The absorbance of the resulting supernatant was measured at 532 nm using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) against a distilled water blank. The TBARS value was expressed as mg of malondialdehyde (MDA) per kg of meat (mg MDA/kg), following the method described by Xiong et al. [18], using the following formula:

$$\text{TBARS value (mg MDA/kg of meat)} = 7.8 \times \text{absorbance at 532 nm} \quad (1)$$

2.7. Sensory Analysis

The trained panelists evaluated the tenderness, juiciness, and flavor of the 0, 7, and 14 days aged steaks from all four (PM, LT, LL, and GM) muscles. A total of 384 beef steaks were randomly evaluated (2 bull types × 2 age groups × 4 muscles × 3 aging times × 8 replicates) during 32 sessions (12 samples in a session and two sessions/day). Thirty-two cooking batches and 12 steaks were randomly selected from a replicate (1 replicate = 2 bull types × 2 age groups × 4 muscles × 3 aging durations). The panelists, consisting of assistant professors, lecturers, and postgraduate students from the Department of Meat Science and Technology,

underwent a four-month training program. This program included one-hour sessions held twice a week, totaling 32 h of training. Following this training, a triangle test (ISO 4120:2021) [19] was conducted to select nine panelists capable of discerning variations in tenderness, juiciness levels, and off-flavors in cooked beef steaks. The selected nine panelists evaluated all steaks from all treatments, and the same panelists were used for all sessions. The steaks were cooked on a hot plate until they reached an internal temperature of 72 °C. The steaks were sliced into 1 cm^2 cubes and randomly served to the panelists at 60 ± 1 °C. The panelists evaluated the steaks using the 9-point hedonic scale for juiciness (9 = extremely juicy and 1 = extremely dry), flavor (9 = full beef flavor and 1 = no beef flavor), tenderness (9 = extremely tender and 1 = not at all tender), and overall acceptability (9 = extremely acceptable and 1 = extremely unacceptable).

2.8. Statistical Analysis

The data were analyzed through a linear mixed model using Minitab software (Version 17.3.1). The effects of bull type, age, aging duration, and muscle type were taken as fixed effects, with aging duration as repeated measures and animal as random effects to measure the correlation within samples from the same animals. Mean values were considered significantly different at a significance level of $p \leq 0.05$. The statistical model used for the analysis is as follows:

$$Y_{ijkl} = \mu + F_{1i} + F_{2j} + F_{3k} + F_{4l} + animal_{ij} + \varepsilon_{ijkl} \quad (2)$$

where Y_{ijkl} is the response variable, μ is the overall population mean, F_{1i} is the fixed effect of bull type, F_{2j} is the fixed effect of bull age, F_{3k} is the fixed effect of aging duration, F_{4l} is the fixed effect of muscle type, $animal_{ij}$ is the random effect of the individual animal nested within bull type and age, and ε_{ijkl} is the residual error term.

No statistically significant interactions were observed between the fixed effects. Consequently, these interactions were excluded from the tables. Sensory evaluation data for juiciness, flavor, tenderness, and overall acceptability were analyzed using a linear mixed model (PROC GIMMIX), with the fixed effects of bull type, animal age, aging duration, and muscle type along with the random effects of panelist sessions, cooking batches, and individual bulls. The normal distribution of variables was confirmed using histograms and QQ plots. Statistical analysis indicated non-significant differences in random effects, leading to their exclusion from the final model. Tukey–Kramer tests were used for the comparison of mean values, with significance established at $p \leq 0.05$. Moreover, Pearson correlation analysis was conducted to evaluate the relationships among the various analyzed meat quality characteristics.

3. Results and Discussion

3.1. pH

The pH of PM, LT, LL, and GM muscles was significantly affected by the bull age and aging duration ($p < 0.05$, Figure 1). All muscles showed a lower ($p < 0.05$) pH in the 30 ± 3 months of age bulls than in the 21 ± 2 months of age bulls. The muscle pH serves as an indicator of glycolysis extent and is also correlated with other meat quality attributes [20]. Older animals generally have higher glycogen reserves [21] and more developed muscles with increased proportions of connective tissue. These factors can influence overall muscle composition and biochemical properties, potentially affecting the ultimate pH. That could be the possible reason for a lower pH in the 30 ± 3 months of age bulls than in the 21 ± 2 months of age bulls. Contrarily, ref. [22] reported an increase in pH values with an increase in age, which might be due to a decrease in glycogen reserves with the age of the bull. In the current study, the pH within all muscles remained higher at 14 days, followed by 7 and 0 days of aging. The increased pH during the aging process could be due to gradual alkalinization induced as a consequence of nitrogenous compound formation during proteolysis [6,23]. Sadowska et al. [24] documented a similar rise in beef pH as the aging duration progressed.

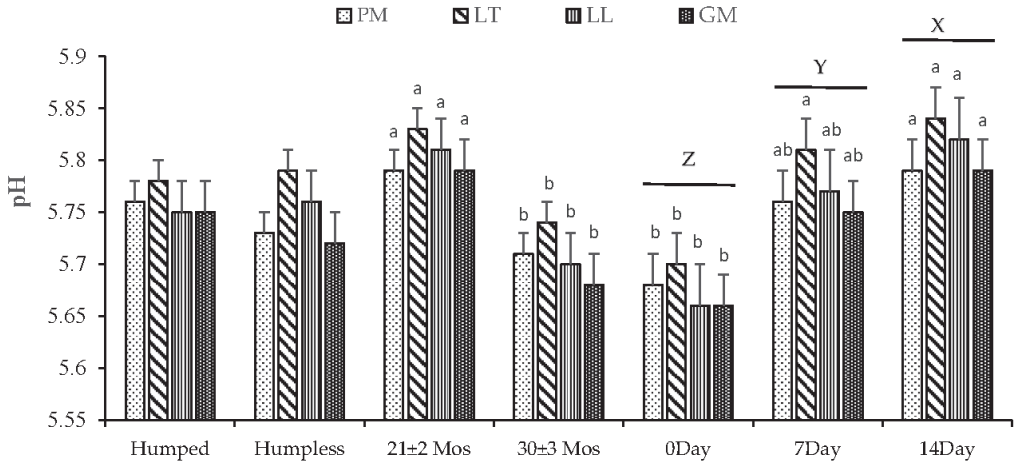

Figure 1. Effects of bull type, age, and wet aging on pH values of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus Medius* (GM) steaks. a, b: Different alphabets among the same muscle indicated a significant difference ($p < 0.05$) within a treatment. X–Z: Different alphabets among different aging times showed a significant difference ($p < 0.05$). The data were expressed as means ± standard errors.

3.2. Instrumental Color

The L*, a*, b*, and C* of PM, LT, LL, and GM muscles showed significant variations caused by bull type, age, and aging duration ($p < 0.05$, Tables 1–4). In the current study, all muscles presented higher ($p < 0.05$) L*, a*, b*, and C* intensities and stabilities in humped bulls than humpless bulls. The higher values of the color intensity and stability of different muscles in humped bulls could be due to lower TBARS values, as reported in the present study. Increased lipid oxidation is known to diminish the intensity and stability of L*, a*, and C* values, while leading to a rise in b* values as a result of heme pigment oxidation. [25]. This is further supported by the correlation analysis, which revealed a strong negative correlation between TBARS and L*, a*, and C* values, while indicating a moderate positive correlation between TBARS and the b* value. (Supplementary Table S2). Similarly, Miguel et al. [10] also reported higher L* and a* values in *M. longissimus thoracis* from Nellore cattle (humped) than from the crossbred (Nellore × Aberdeen Angus, humpless) cattle.

In the current study, all muscles showed higher ($p < 0.05$) L*, a*, b*, and C* values in the 30 ± 3 months of age bulls than in the 21 ± 2 months of age bulls. Similarly, Pastsart et al. [20] detected an increase in the color L* and a* values with the increase in the age of bulls. As reported in the current study, the increase in L* values in the bulls aged 30 ± 3 months may be due to their lower pH compared to the bulls aged 21 ± 2 months. This finding is supported by the correlation analysis, which showed a strong negative correlation between pH and L* values (Supplementary Table S2). The lower pH causes more protein denaturation and higher L* values in beef [26]. The a* values depend on the myoglobin's concentration, chemical state, and muscle surface structure and composition. An increase in the a* value in the meat of older bulls was found, which is probably due to an increased amount of myoglobin since its content in the meat increases with age [27].

Table 1. Effects of bull type, age, and wet aging on lightness (L*) values of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus medius* (GM) steaks during 1, 3, 5, and 7 days of retail display.

Lightness (L*)	Days	Bull Type		Bull Age (Months)			Aging Duration				*p*-Value		
		Humped	Humpless	21 ± 2	30 ± 3	SE	0 Days	7 Days	14 Days	SE	Bull Type	Bull Age	Aging Duration
PM	1	49.21 ^{bcde}	48.05 ^{bcde}	48.02 ^{bcde}	49.24 ^{bcde}	0.32	47.71 ^{B,bcdef}	48.98 ^{AB,abcd}	49.20 ^{A,bcdef}	0.39	0.014	0.010	0.022
	3	48.61 ^{cdef}	47.25 ^{cdef}	47.48 ^{cde}	48.41 ^{cdef}	0.33	46.87 ^{B,cdefg}	48.42 ^{A,abcde}	48.54 ^{A,cdef}	0.40	0.005	0.047	0.007
	5	47.87 ^{def}	46.42 ^{efg}	46.68 ^{def}	47.62 ^{efg}	0.32	46.10 ^{B,efgh}	47.69 ^{A,bcdef}	47.64 ^{A,efgh}	0.39	0.002	0.042	0.008
	7	47.44 ^{efg}	46.00 ^{fgh}	46.52 ^{efg}	46.91 ^{fgh}	0.29	45.66 ^{B,fgh}	47.31 ^{A,cdef}	47.19 ^{A,fgh}	0.36	0.001	0.355	0.003
LT	1	51.00 ^{ab}	49.71 ^{ab}	49.53 ^{ab}	51.18 ^{ab}	0.39	49.84 ^{B,ab}	49.84 ^{B,ab}	51.39 ^{A,ab}	0.48	0.024	0.005	0.038
	3	50.28 ^{abc}	49.01 ^{abc}	48.93 ^{abc}	50.36 ^{abc}	0.38	48.91 ^{B,abc}	49.20 ^{B,abcd}	50.83 ^{A,ab}	0.46	0.022	0.011	0.011
	5	49.62 ^{abcd}	48.32 ^{abcd}	48.26 ^{bcd}	49.67 ^{abcde}	0.38	48.37 ^{B,abcd}	48.56 ^{AB,abcd}	49.97 ^{A,abcd}	0.54	0.020	0.011	0.036
	7	49.41 ^{bcd}	47.89 ^{bcde}	48.05 ^{bcde}	49.26 ^{bcde}	0.39	48.00 ^{B,bcde}	48.29 ^{AB,abcde}	49.66 ^{A,abcde}	0.48	0.009	0.034	0.042
LL	1	51.40 ^{a}	50.18 ^{a}	49.95 ^{a}	51.64 ^{a}	0.39	50.22 ^{B,a}	50.30 ^{AB,a}	51.85 ^{A,a}	0.47	0.033	0.004	0.032
	3	50.68 ^{ab}	49.48 ^{ab}	49.35 ^{ab}	50.82 ^{ab}	0.37	49.29 ^{B,ab}	49.66 ^{B,abc}	51.30 ^{A,ab}	0.46	0.030	0.008	0.008
	5	50.01 ^{abc}	48.79 ^{abc}	48.68 ^{abc}	50.13 ^{abc}	0.38	48.76 ^{B,abc}	49.02 ^{AB,abcd}	50.44 ^{A,abc}	0.46	0.027	0.009	0.029
	7	49.81 ^{abc}	48.37 ^{abcd}	48.46 ^{abc}	49.72 ^{abcd}	0.39	48.38 ^{B,abcd}	48.75 ^{AB,abcd}	50.13 ^{A,abcd}	0.49	0.013	0.028	0.034
GM	1	47.76 ^{defg}	46.73 ^{defg}	46.71 ^{def}	47.79 ^{defg}	0.25	46.38 ^{B,defgh}	47.22 ^{AB,def}	48.14 ^{A,defg}	0.31	0.006	0.004	0.001
	3	46.84 ^{fgh}	45.67 ^{fgh}	45.64 ^{fgh}	46.87 ^{fgh}	0.29	45.61 ^{B,fgh}	46.15 ^{AB,efg}	47.00 ^{A,fgh}	0.36	0.007	0.005	0.030
	5	45.96 ^{gh}	44.98 ^{gh}	44.95 ^{gh}	45.99 ^{gh}	0.30	44.73 ^{B,gh}	45.66 ^{AB,fg}	46.02 ^{A,gh}	0.36	0.023	0.016	0.043
	7	45.43 ^{h}	44.18 ^{h}	44.36 ^{h}	45.25 ^{h}	0.28	44.26 ^{B,h}	44.47 ^{B,g}	45.69 ^{A,h}	0.34	0.002	0.026	0.009

PM; *Psoas major*, LT; *Longissimus thoracis*, LL; *Longissimus lumborum*, GM; *Gluteus Medius*. ^{A,B} Means with different superscripts in a row are statistically different ($p < 0.05$). $^{a–h}$ Means with different superscripts in a column are statistically different ($p < 0.05$).

Table 2. Effects of bull type, age, and wet aging on redness (a*) values of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus medius* (GM) steaks during 1, 3, 5, and 7 days of retail display.

Redness (a*)	Days	Bull Type		Bull Age (Months)			Aging Duration				*p*-Value		
		Humped	Humpless	21 ± 2	30 ± 3	SE	0 Days	7 Days	14 Days	SE	Bull Type	Bull Age	Aging Duration
PM	1	21.03 ^{bcd}	19.83 ^{cd}	19.86 ^{cd}	21.00 ^{bcde}	0.30	19.18 ^{B,cd}	20.96 ^{A,cde}	21.16 ^{A,cde}	0.37	0.007	0.011	0.001
	3	19.54 ^{def}	18.09 ^{ef}	18.26 ^{ef}	19.36 ^{efg}	0.34	17.51 ^{B,defg}	19.25 ^{A,efg}	19.68 ^{A,efg}	0.41	0.004	0.025	0.001
	5	18.55 ^{efg}	17.15 ^{fg}	17.35 ^{fg}	18.35 ^{gh}	0.35	16.63 ^{B,fgh}	18.11 ^{A,gh}	18.18 ^{A,fg}	0.43	0.007	0.049	0.003
	7	17.81 ^{fg}	16.22 ^{g}	16.40 ^{g}	17.63 ^{gh}	0.35	15.81 ^{B,h}	17.28 ^{AB,h}	17.95 ^{A,g}	0.42	0.002	0.015	0.003
LT	1	23.50 ^{a}	22.69 ^{a}	22.21 ^{a}	23.98 ^{a}	0.21	22.48 ^{B,a}	23.33 ^{AB,a}	23.47 ^{A,ab}	0.25	0.008	0.000	0.017
	3	22.40 ^{ab}	21.49 ^{ab}	21.17 ^{abc}	22.71 ^{ab}	0.25	21.00 ^{B,ab}	22.04 ^{AB,abc}	22.78 ^{A,abc}	0.31	0.013	0.000	0.001
	5	21.50 ^{bc}	20.68 ^{bc}	20.41 ^{bcd}	21.76 ^{bcd}	0.27	19.93 ^{B,bc}	21.39 ^{A,abcd}	21.93 ^{A,abcd}	0.33	0.038	0.001	<0.001
	7	20.49 ^{cd}	19.65 ^{cd}	19.37 ^{de}	20.78 ^{cdef}	0.28	18.68 ^{B,cde}	20.55 ^{A,cdef}	20.99 ^{A,cde}	0.35	0.040	0.001	<0.001

Table 2. *Cont.*

Redness (a*)	Days	Bull Type		Bull Age (Months)			Aging Duration				*p*-Value		
		Humped	Humpless	21 ± 2	30 ± 3	SE	0 Days	7 Days	14 Days	SE	Bull Type	Bull Age	Aging Duration
LL	1	23.60 ^{a}	22.44 ^{a}	22.20 ^{a}	23.85 ^{a}	0.23	22.38 ^{B,a}	22.95 ^{AB,ab}	23.74 ^{A,a}	0.28	0.001	<0.001	0.006
	3	22.46 ^{ab}	21.35 ^{ab}	21.38 ^{ab}	22.43 ^{abc}	0.25	21.22 ^{B,ab}	22.04 ^{AB,abc}	22.46 ^{A,abcd}	0.30	0.003	0.004	0.018
	5	21.54 ^{bc}	20.37 ^{bcd}	20.47 ^{bcd}	21.43 ^{bcd}	0.26	20.09 ^{B,bc}	21.21 ^{A,bcd}	21.55 ^{A,bcde}	0.32	0.003	0.015	0.008
	7	20.46 ^{cd}	19.61 ^{cd}	19.59 ^{de}	20.49 ^{def}	0.25	19.10 ^{B,cd}	20.37 ^{A,cdef}	20.64 ^{A,def}	0.31	0.021	0.014	0.002
GM	1	20.27 ^{cde}	18.97 ^{de}	19.09 ^{de}	20.14 ^{def}	0.23	18.82 ^{B,cd}	19.46 ^{B,defg}	20.56 ^{A,def}	0.27	<0.001	0.002	0.000
	3	19.46 ^{def}	17.98 ^{ef}	18.25 ^{ef}	19.19 ^{fgh}	0.27	17.90 ^{B,def}	18.69 ^{AB,fgh}	19.57 ^{A,efg}	0.33	<0.001	0.016	0.003
	5	18.41 ^{fg}	17.30 ^{fg}	17.34 f ^{g}	18.37 ^{gh}	0.28	17.01 ^{B,efgh}	17.78 ^{AB,gh}	18.76 ^{A,fg}	0.34	0.007	0.012	0.003
	7	17.50 ^{g}	16.40 ^{g}	16.39 ^{g}	17.53 ^{h}	0.27	15.95 ^{B,gh}	17.18 ^{A,h}	17.73 ^{A,g}	0.33	0.005	0.004	0.001

PM; *Psoas major*, LT; *Longissimus thoracis*, LL; *Longissimus lumborum*, GM; *Gluteus Medius*. ^{A,B} Means with different superscripts in a row are statistically different ($p < 0.05$). $^{a–h}$ Means with different superscripts in a column are statistically different ($p < 0.05$).

Table 3. Effects of bull type, age, and wet aging on yellowness (b*) values of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus medius* (GM) steaks during 1, 3, 5, and 7 days of retail display.

Yellowness (b*)	Days	Bull Type		Bull Age (Months)			Aging Duration				*p*-Value		
		Humped	Humpless	21 ± 2	30 ± 3	SE	0 Days	7 Days	14 Days	SE	Bull Type	Bull Age	Aging Duration
PM	1	10.08 ^{h}	8.85 ^{i}	8.64 ^{g}	10.29 ^{g}	0.36	8.49 ^{B,h}	9.53 ^{AB,h}	10.37 ^{A,f}	0.44	0.020	0.002	0.016
	3	10.47 ^{gh}	9.37 ^{hi}	9.08 ^{fg}	10.76 ^{fg}	0.35	8.99 ^{B,gh}	9.98 ^{AB,gh}	10.78 ^{A,ef}	0.43	0.032	0.002	0.019
	5	10.82 ^{fgh}	9.80 ^{ghi}	9.46 ^{fg}	11.15 ^{efg}	0.36	9.38 ^{B,fgh}	10.41 ^{AB,fgh}	11.13 ^{A,def}	0.43	0.046	0.001	0.021
	7	11.39 ^{efgh}	10.37 ^{efghi}	9.98 ^{efg}	11.78 ^{defg}	0.35	9.79 ^{B,efgh}	10.99 ^{AB,efgh}	11.86 ^{A,bcdef}	0.42	0.044	0.001	0.005
LT	1	12.45 ^{bcde}	11.70 ^{bcdef}	11.46 ^{bcd}	12.69 ^{bcd}	0.17	11.78 ^{B,abcd}	11.87 ^{B,bcdef}	12.58 ^{A,abcde}	0.21	0.002	0.000	0.016
	3	12.83 ^{abcd}	12.13 ^{abcd}	11.89 ^{bc}	13.07 ^{abcd}	0.16	12.08 ^{B,abcd}	12.32 ^{B,abcde}	13.06 ^{A,abcd}	0.20	0.003	<0.001	0.003
	5	13.21 ^{abc}	12.53 ^{abc}	12.35 ^{abc}	13.44 ^{abc}	0.15	12.58 ^{B,abc}	12.73 ^{AB,abcd}	13.38 ^{A,abc}	0.33	0.002	<0.001	0.011
	7	13.67 ^{ab}	12.82 ^{ab}	12.75 ^{ab}	13.74 ^{ab}	0.15	12.93 ^{B,ab}	13.17 ^{AB,ab}	13.63 ^{A,ab}	0.35	<0.001	0.001	0.038
LL	1	11.94 ^{cdef}	11.24 ^{cdefg}	11.00 ^{cde}	12.18 ^{cdef}	0.16	11.19 ^{B,bcde}	11.43 ^{B,cdefg}	12.15 ^{A,bcdef}	0.20	0.003	<0.001	0.003
	3	12.57 ^{bcde}	11.89 ^{bcde}	11.72 ^{bcd}	12.74 ^{bcd}	0.15	11.76 ^{B,abcd}	12.12 ^{B,bcde}	12.81 ^{A,abcd}	0.18	0.002	0.001	0.001
	5	13.33 ^{abc}	12.67 ^{abc}	12.57 ^{ab}	13.42 ^{abc}	0.14	12.44 ^{B,abc}	13.05 ^{A,abc}	13.49 ^{A,ab}	0.17	0.001	<0.001	<0.001
	7	14.17 ^{a}	13.60 ^{a}	13.50 ^{a}	14.27 ^{a}	0.14	13.40 ^{B,a}	13.94 ^{AB,a}	14.29 ^{A,a}	0.18	0.007	0.001	0.005

Table 3. *Cont.*

Yellowness (b*)	Days	Bull Type		Bull Age (Months)			Aging Duration				*p*-Value		
		Humped	Humpless	21 ± 2	30 ± 3	SE	0 Days	7 Days	14 Days	SE	Bull Type	Bull Age	Aging Duration
GM	1	10.84 ^{fgh}	9.80 ^{ghi}	9.48 ^{fg}	11.16 ^{efg}	0.35	9.38 ^{B,fgh}	10.41 ^{AB,fgh}	11.17 ^{A,def}	0.43	0.042	0.001	0.018
	3	11.26 ^{efgh}	10.23 ^{fghi}	9.89 ^{efg}	11.59 ^{defg}	0.34	9.86 ^{B,efgh}	10.87 ^{AB,efgh}	11.50 ^{A,cdef}	0.42	0.038	0.001	0.027
	5	11.91 ^{defg}	10.69 ^{defgh}	10.47 ^{def}	12.13 ^{cdef}	0.33	10.46 ^{B,defg}	11.30 ^{AB,defg}	12.15 ^{A,bcdef}	0.34	0.012	0.001	0.018
	7	12.41 ^{bcde}	11.23 ^{cdefg}	11.07 ^{cde}	12.56 ^{bcde}	0.33	11.05 ^{B,cdef}	11.75 ^{AB,bcdef}	12.65 ^{A,abcde}	0.33	0.014	0.002	0.024

PM; *Psoas major*, LT; *Longissimus thoracis*, LL; *Longissimus lumborum*, GM; *Gluteus Medius*. ^{A,B} Means with different superscripts in a row are statistically different ($p < 0.05$). $^{a–i}$ Means with different superscripts in a column are statistically different ($p < 0.05$).

Table 4. Effects of bull type, age, and wet aging on chroma (C*) values of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus medius* (GM) steaks during 1, 3, 5, and 7 days of retail display.

Chroma (C*)	Days	Bull Type		Bull Age (Months)			Aging Duration				*p*-Value		
		Humped	Humpless	21 ± 2	30 ± 3	SE	0 Days	7 Days	14 Days	SE	Bull Type	Bull Age	Aging Duration
PM	1	22.37 ^{cde}	21.09 ^{def}	20.73 ^{cde}	22.74 ^{bcde}	0.36	20.78 ^{B,cd}	21.87 ^{AB,cdef}	22.50 ^{A,cdef}	0.45	0.017	<0.001	0.029
	3	20.56 ^{efg}	19.25 ^{gh}	18.99 ^{fg}	20.83 ^{efgh}	0.39	18.83 ^{B,ef}	19.87 ^{AB,fghi}	21.02 ^{A,efgh}	0.47	0.021	0.002	0.008
	5	19.76 ^{fg}	18.19 ^{hi}	18.36 ^{gh}	19.59 ^{ghi}	0.42	17.77 ^{B,fg}	18.93 ^{AB,hi}	20.22 ^{A,fgh}	0.52	0.011	0.044	0.006
	7	18.96 ^{g}	17.13 ^{i}	17.35 ^{h}	18.74 ^{i}	0.44	16.84 ^{B,g}	18.33 ^{AB,i}	18.96 ^{A,h}	0.53	0.005	0.028	0.021
LT	1	24.86 ^{ab}	23.78 ^{ab}	23.13 ^{ab}	25.51 ^{a}	0.26	23.44 ^{B,a}	24.45 ^{AB,ab}	25.07 ^{A,ab}	0.32	0.006	<0.001	0.003
	3	23.19 ^{bcd}	22.16 ^{cde}	21.81 ^{bcd}	23.54 ^{bc}	0.30	21.26 ^{B,bc}	23.08 ^{A,abcd}	23.68 ^{A,abcd}	0.36	0.019	<0.001	<0.001
	5	22.51 ^{cde}	21.61 ^{def}	21.27 ^{cde}	22.84 ^{bcd}	0.30	20.63 ^{B,cde}	22.45 ^{A,bcde}	23.09 ^{A,bcde}	0.37	0.038	0.001	<0.001
	7	21.59 ^{def}	20.65 ^{efg}	20.46 d^{ef}	21.79 ^{cdef}	0.31	19.68 ^{B,cde}	21.41 ^{A,defg}	22.29 ^{A,cdef}	0.38	0.037	0.004	<0.001
LL	1	25.52 ^{a}	24.24 ^{a}	23.94 ^{a}	25.83 ^{a}	0.25	24.30 ^{B,a}	24.79 ^{AB,a}	25.55 ^{A,a}	0.32	0.001	<0.001	0.026
	3	24.31 ^{abc}	23.30 ^{abc}	23.32 ^{ab}	24.29 ^{ab}	0.28	23.15 ^{B,ab}	23.67 ^{AB,abc}	24.60 ^{A,abc}	0.34	0.013	0.018	0.014
	5	23.16 ^{bcd}	22.28 ^{bcd}	22.25 ^{bc}	23.19 ^{bc}	0.28	21.37 ^{C,bc}	22.69 ^{B,abcd}	24.09 ^{A,abcd}	0.35	0.034	0.024	<0.001
	7	22.23 ^{de}	21.42 ^{def}	21.38 ^{cde}	22.27 ^{cde}	0.27	20.28 ^{C,cde}	21.92 ^{B,cdef}	23.26 ^{A,abcde}	0.31	0.029	0.017	<0.001
GM	1	21.56 ^{def}	20.95 ^{def}	20.70 ^{cde}	21.81 ^{cdef}	0.20	20.66 ^{B,cde}	21.03 ^{B,defg}	22.07 ^{A,def}	0.25	0.038	<0.001	0.001
	3	21.09 ^{ef}	20.26 ^{fg}	20.11 ^{ef}	21.24 ^{defg}	0.24	20.19 ^{B,cde}	20.50 ^{AB,efgh}	21.33 ^{A,efg}	0.30	0.019	0.002	0.027
	5	20.17 ^{fg}	19.11 ^{gh}	19.03 ^{fg}	20.24 ^{fghi}	0.27	18.94 ^{B,def}	19.71 ^{AB,ghi}	20.26 ^{A,fgh}	0.33	0.007	0.002	0.022
	7	18.91 ^{g}	18.14 ^{hi}	17.99 ^{gh}	19.06 ^{hi}	0.25	17.67 ^{B,fg}	18.52 ^{AB,hi}	19.38 ^{A,gh}	0.31	0.033	0.004	0.001

PM; *Psoas major*, LT; *Longissimus thoracis*, LL; *Longissimus lumborum*, GM; *Gluteus Medius*, $^{A–C}$ Means with different superscripts in a row are statistically different ($p < 0.05$). $^{a–i}$ Means with different superscripts in a column are statistically different ($p < 0.05$).

The postmortem aging duration significantly ($p < 0.05$) affected the L*, a*, b*, and C* values of the PM, LT, LL, and GM muscles during 1, 3, 5, and 7 days of simulated retail display. All the muscles presented higher ($p < 0.05$) L*, a*, b*, and C* values at 14 days, followed by 7 and 0 days of aging during 1, 3, 5, and 7 days of simulated retail display. Various studies reported a rise in the CIE L*, a*, b*, and C* values with the aging duration [28–30]. The increase in L* values observed throughout the aging process is likely due to protein denaturation caused by endogenous enzymes, which destabilize the protein matrix and result in increased light scattering [6]. The non-significant difference in the L* values of the PM muscle between 7 and 14 days of aging might be due to a decrease in the protein denaturation (also indicated by WBSF values) that resulted in less light scattering. The changes in the a* values could be attributed to the ability of beef muscles to develop bloom during the aging process. Jacob [31] noted that this effect is linked to a reduction in the activity attributed to the action of oxygen-consuming enzymes and enhanced oxygen penetration, resulting in a thicker oxymyoglobin layer as the aging duration increases.

The color intensity and stability could be affected by the muscle types that have varied rates of oxygen consumption and activities of metmyoglobin reduction [23]. In the present study, LL steaks showed higher while GM and PM steaks showed lower ($p < 0.05$) L*, a*, b*, and C* values measured during simulated retail display ($p < 0.05$, Tables 1–4). All muscles exhibited a decrease in L*, a*, and C* values, along with an increase in b* values throughout the simulated retail display. However, these changes in meat color values remained within the acceptable range. The differences in the L*, a*, b*, and C* values among PM, LT, LL, and GM can be credited to the muscle fiber differences. The LT and LL muscles composed of type IIB fibers demonstrated greater lightness and redness than the PM and GM muscles composed of type I fibers [32]. The fiber type of LT and LL muscles could favor the glycolytic potential of these muscles and cause rapid postmortem pH decline leading to more protein denaturation and higher L* values due to more light reflectance. The chroma (C*) value indicates the color intensity. Similarly to the current study, Rooyen et al. [33] also found a muscle-specific effect on the C* values of different beef muscles. The PM and GM muscles are considered color labile muscles as these have enhanced mitochondria and a higher oxygen consumption rate. Therefore, these muscles showed lower b* and C* values than the LL and LT muscles.

3.3. Drip Loss (%)

The bull age and aging duration significantly affected the drip loss of PM, LT, LL, and GM muscles ($p < 0.05$, Figure 2). All muscles showed higher ($p < 0.05$) drip loss in the 30 ± 3 months of age bulls than in the 21 ± 2 months of age bulls. Similarly, Schönfeldt and Strydom [34] reported a higher drip loss in two or more cattle with permanent incisors than in cattle with no permanent incisors. However, Du Plessis and Hoffman [35] observed no differences in the drip loss of 18 and 30 months of age steers. In the current study, bulls aged 30 ± 3 months exhibited lower pH levels, leading to greater protein denaturation and higher drip losses compared to bulls aged 21 ± 2 months. This observation is further supported by the correlation analysis, which revealed a strong negative correlation between pH and drip loss values (Supplementary Table S2). Protein denaturation reduces the water-holding capacity of the meat, causing more water to expel and contributing to higher drip losses.

In the present study, the drip loss of all muscles was higher at 14 days, followed by 7 and 0 days of aging. Similarly, Sadowska et al. [24] also reported higher drip losses up to 21 days of aging in different beef muscles. Moreover, among different muscles, PM and LT showed higher drip loss than LL and GM, but only at 14 days of aging. Likewise, Sadowska et al. [24] also reported higher drip losses in different beef muscles after 14 days of aging. Different muscles have different metabolic and proteolytic potentials [36] that result in differences in meat quality characteristics. PM and LT muscles typically have a different fiber type from LL and GM muscles. PM and LT muscles, which have a higher proportion of

fast-twitch fibers [37], are more susceptible to proteolytic activity. This increased proteolysis could be the reason for elevated drip loss.

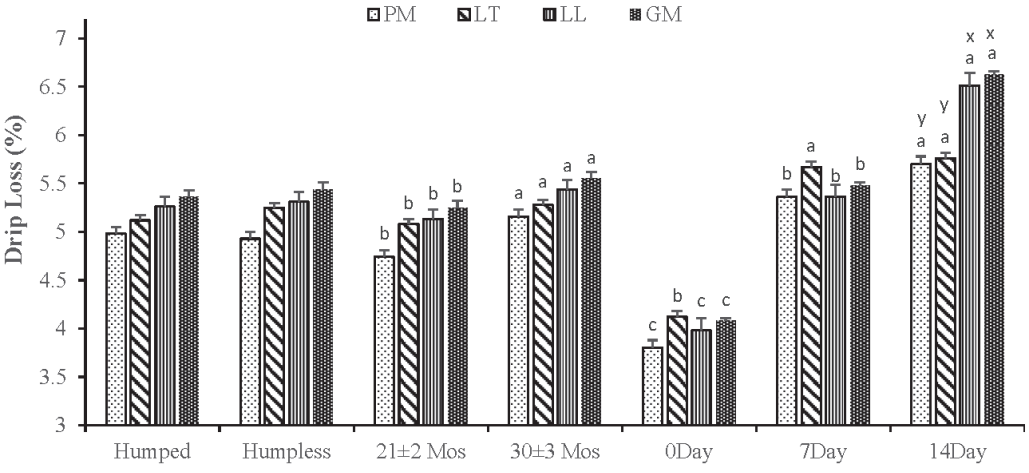

Figure 2. Effects of bull type, age, and wet aging on drip loss (%) of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus Medius* (GM) steaks. a, b: Different alphabets among the same muscle indicate a significant difference ($p < 0.05$) within a group. x, y: Different alphabets among different muscles indicate a significant difference ($p < 0.05$) within a treatment. The data were expressed as means ± standard errors.

3.4. Cooking Loss (%) and Instrumental Shear Force

The cooking loss displays the water loss from the cooked meat that occurs due to the denaturation of proteins during cooking. The cooking loss of PM, LL, LT, and GM was significantly ($p < 0.05$) affected by the bull type, animal age, and aging duration (Table 5). Only the GM muscle from the humpless bulls showed higher cooking loss than the humped bulls. The increased cooking loss of the GM muscle from humpless bulls could be due to increased proteolysis, which is also evident by the decreased WBSF values in humpless bulls in comparison to humped bulls. Furthermore, the GM muscle in humpless bulls may exhibit unique structural characteristics compared to humped bulls, such as variations in muscle fiber type, connective tissue density, and fat content. These differences in muscle composition contributed to the observed variations in cooking loss [38]. The LL, LT, and GM muscles showed higher ($p < 0.05$) cooking loss in the 30 ± 3 months of age bulls than in the 21 ± 2 months of age bulls. Similarly, Schönfeldt and Strydom [34] observed a rise in cooking loss with increased animal age. A rise in cooking loss with the progression of age might be due to the upsurge of collagen cross-linkages with age, resulting in more cooking loss channels [39].

In the present study, the cooked loss of PM, LL, LT, and GM muscles was significantly increased ($p < 0.05$) with the aging duration. All the muscles showed a higher ($p < 0.05$) cooking loss at 14 days of aging. However, the cooking loss of PM and LT muscles was non-significant between 7 and 14 days of aging. The aging induces an increase in protein denaturation that reduces the proteins' water-retention ability, consequently increasing cooking losses [30]. However, Hughes et al. [40] found non-significant differences in the cooking loss of beef during aging. During aging, meat undergoes protein denaturation, which can affect its water-holding capacity and cooking loss. In our study, the lack of a significant difference in cooking loss between the PM and LT muscles after 7 and 14 days of aging may be attributed to a stabilization in protein denaturation and an increase in pH following the initial aging period. This stabilization and increase in pH could result in consistent cooking loss values over the subsequent aging days [41]. The GM muscle

showed the highest ($p < 0.05$) cooking loss among different muscles in comparison to PM, LT, and LL muscles. Similarly, Rhee et al. [42] reported a higher cooking loss in GM than in the LL muscle. The increased drip loss of GM might be due to the difference in the fat contents [38], as higher marbling is associated with decreased cooking losses.

Table 5. Effects of bull type, age, and wet aging on cooking loss (%) and Warner–Bratzler shear force (WBSF) values of *Psoas major* (PM), *Longissimus thora*cis (LT), *Longissimus lumborum* (LL), and *Gluteus medius* (GM) steaks.

Parameter	Muscle	Bull Type		Bull Age (Months)			Aging Duration				*p*-Value		
		Humped	Humpless	21 ± 2	30 ± 3	SE	0 Days	7 Days	14 Days	SE	Bull Type	Bull Age	Aging Duration
Cooking Loss (%)	PM	31.76 ^{b}	32.00 ^{b}	31.58 ^{b}	32.18 ^{b}	0.28	30.18 ^{B,b}	32.53 ^{A,b}	32.93 ^{A,b}	0.34	0.541	0.129	<0.001
	LT	31.63 ^{b}	31.87 ^{b}	31.22 ^{b}	32.28 ^{b}	0.30	29.85 ^{B,b}	32.10 ^{A,b}	33.30 ^{A,b}	0.37	0.562	0.017	<0.001
	LL	31.70 ^{b}	32.29 ^{b}	31.42 ^{b}	32.58 ^{b}	0.10	29.94 ^{C,b}	32.32 ^{B,b}	33.73 ^{A,ab}	0.27	0.066	0.001	<0.001
	GM	33.22 ^{a}	34.01 ^{a}	33.19 ^{a}	34.05 ^{a}	0.24	31.38 ^{C,a}	34.23 ^{B,a}	35.24 ^{A,a}	0.30	0.027	0.015	<0.001
WBSF (N/cm^2)	PM	33.43 ^{b}	31.03 ^{b}	30.83 ^{c}	33.63 ^{b}	0.78	37.13 ^{A,c}	30.66 ^{B,c}	28.91 ^{B,c}	0.96	0.036	0.016	<0.001
	LT	41.78 ^{a}	39.00 ^{a}	38.22 ^{b}	42.55 ^{a}	0.53	48.57 ^{A,b}	37.12 ^{B,b}	35.47 ^{B,b}	0.65	0.001	<0.001	<0.001
	LL	44.63 ^{a}	42.64 ^{a}	42.13 ^{ab}	45.14 ^{a}	0.48	51.21 ^{A,ab}	42.87 ^{B,a}	36.82 ^{C,a}	0.58	0.005	<0.001	<0.001
	GM	45.12 ^{a}	43.05 ^{a}	42.74 ^{a}	45.42 ^{a}	0.24	52.29 ^{A,a}	44.09 ^{B,a}	35.86 ^{C,a}	0.30	0.003	<0.001	<0.001

PM; *Psoas major*, LT; *Longissimus thoracis*, LL; *Longissimus lumborum*, GM; *Gluteus Medius*, WBSF; Warner–Bratzler shear force. $^{A–C}$ Means with different superscripts in a row are statistically different ($p < 0.05$). $^{a–c}$ Means with different superscripts in a column are statistically different ($p < 0.05$).

The shear force of the PM, LT, LL, and GM muscles was significantly ($p < 0.05$) affected by bull type, animal age, and aging duration (Table 5). All the muscles exhibited higher ($p < 0.05$) shear force values in humped bulls than in humpless bulls. The humped bulls have an advanced level of calpastatin activity as compared to the humpless bulls [40]. This increase in calpastatin activity in humped bulls decreases protein proteolysis during aging and negatively impacts meat tenderness [43,44].

The bull age has a significant impact on meat tenderness. The PM, LT, LL, and GM muscles from the 30 ± 3 months of age bulls presented higher ($p < 0.05$) WBSF values than the 21 ± 2 months of age group. The rise in the shear force readings in the 30 ± 3 months of age bulls could be attributed to an increase in connective tissue with the advancement of age. Furthermore, meat becomes tougher due to a rise in collagen's mechanical and thermal stability with age. As the age of the animal increases, it might result in mature cross-linkages that are more stable and resistant to heat, ultimately leading to less solubilization during the cooking and tougher meat [45].

The postmortem aging time ($p < 0.05$) reduced the WBSF values of the PM, LT, LL, and GM muscles from one to fourteen days of aging. All the muscles showed the lowest ($p < 0.05$) WBSF values at 14 days of aging. However, the PM and LT muscles showed a limited response to aging duration compared to LL and GM, with non-significant changes in WBSF values between 7 and 14 days of aging. This indicated the muscle-specific response of aging duration on the tenderness of different beef muscles. Some muscles, such as LL and *Bicep femoris*, benefit from postmortem aging in terms of tenderness due to their higher proteolytic activity compared to muscles such as PM and *Infraspinatus*, which have limited proteolytic activity [46].

Similarly, Nair et al. [3] also reported a limited response of PM muscle compared to LL muscle to the aging process. Among different beef muscles, PM showed the lowest while GM presented the highest ($p < 0.05$) shear force values. Differences among muscles in the structure, composition, sarcomere length, intermuscular fat, proteolysis rate and extent, and solubility of intramuscular connective tissues contribute to distinct variations in meat tenderness. The present study reported that no single biochemical change could describe the variations in the tenderness of all muscles; therefore, further studies are needed to determine the activity of different proteases and proteomes or any other muscle-specific biochemical change to better evaluate the tenderness of all muscles.

3.5. Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS values, indicating lipid oxidation of the PM, LT, LL, and GM muscles were significantly affected by bull type, animal age, and aging duration ($p < 0.05$, Table 6). In the present study, all the muscles presented higher ($p < 0.05$) TBARS values in humpless bulls than in humped bulls. The increased TBARS values in humpless bulls might be due to an increase in unsaturated fatty acid levels in humpless bulls compared to humped bulls [15]. Bull age also affected the ($p < 0.05$) TBARS values. In the current study, all the muscles showed higher ($p < 0.05$) TBARS values in the 30 ± 3 months of age bulls than in the 21 ± 2 months of age bulls. The higher lipid oxidation in elder bulls might be due to increased myoglobin and unsaturated fatty acids compared to younger bulls, as age changes the unsaturation levels in beef lipids [47]. Moreover, lipid oxidation in older bulls is also associated with mitochondrial dysfunction and is due to an increase in the levels of non-heme iron with the age of the bull [48].

Table 6. Effects of bull type, age, and wet aging on thiobarbituric acid reactive substances (TBARS) values of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus medius* (GM) steaks during 1, 3, 5, and 7 days of retail display.

TBARS (mg MDA/kg)	Days	Bull Type		Bull Age (Months)			Aging Duration				*p*-Value		
		Humped	Humpless	21 ± 2	30 ± 3	SE	0 Days	7 Days	14 Days	SE	Bull Type	Bull Age	Aging Duration
PM	1	0.89 ^{cde}	1.04 ^{bcdef}	0.90 ^{cdefg}	1.03 ^{bcde}	0.04	0.73 ^{C,bcd}	0.95 ^{B,cdef}	1.21 ^{A,bcdefg}	0.05	0.008	0.011	<0.001
	3	0.94 ^{bcde}	1.10 ^{abcde}	0.95 ^{bcdef}	1.08 ^{abcde}	0.04	0.79 ^{C,abcd}	0.99 ^{B,bcdef}	1.27 ^{A,abcdef}	0.04	0.004	0.016	<0.001
	5	0.99 ^{abcd}	1.14 ^{abcde}	1.00 ^{abcde}	1.12 ^{abcd}	0.03	0.83 ^{C,abc}	1.05 ^{B,abcde}	1.31 ^{A,abcd}	0.04	0.006	0.020	<0.001
	7	1.04 ^{abc}	1.19 ^{abc}	1.06 ^{abcd}	1.18 ^{abc}	0.04	0.88 ^{C,abc}	1.11 ^{B,abc}	1.36 ^{A,abc}	0.04	0.006	0.022	<0.001
LT	1	0.72 ^{e}	0.94 ^{def}	0.75 ^{fg}	0.93 ^{de}	0.05	0.71 ^{B,bcd}	0.83 ^{AB,ef}	0.98 ^{A,h}	0.07	0.002	0.023	0.019
	3	0.86 ^{cde}	1.08 ^{abcdef}	0.84 ^{defg}	1.10 ^{abcde}	0.05	0.84 ^{B,abc}	0.95 ^{AB,cdef}	1.11 ^{A,efgh}	0.06	0.004	0.001	0.017
	5	0.95 ^{abcd}	1.14 ^{abcde}	0.91 ^{bcdefg}	1.19 ^{abcd}	0.05	0.92 ^{B,ab}	1.04 ^{AB,bcdef}	1.18 ^{A,cdefgh}	0.07	0.016	0.000	0.024
	7	1.04 ^{abc}	1.22 ^{abcd}	1.01 ^{abcde}	1.25 ^{abc}	0.04	1.02 ^{B,a}	1.07 ^{B,abcde}	1.30 ^{A,abcdef}	0.06	0.010	0.001	0.003
LL	1	0.71 ^{e}	0.85 ^{f}	0.71 ^{g}	0.84 ^{e}	0.03	0.57 ^{C,d}	0.78 ^{B,f}	0.97 ^{A,gh}	0.04	0.002	0.003	<0.001
	3	0.79 ^{de}	0.91 ^{ef}	0.78 ^{efg}	0.92 ^{de}	0.03	0.67 ^{C,cd}	0.85 ^{B,def}	1.03 ^{A,fgh}	0.03	0.004	0.001	<0.001
	5	0.85 ^{cde}	0.97 ^{cdef}	0.84 ^{defg}	0.98 ^{cde}	0.03	0.71 ^{C,bcd}	0.92 ^{B,cdef}	1.10 ^{A,defgh}	0.03	0.003	0.001	<0.001
	7	0.94 ^{bcde}	1.04 ^{abcdef}	0.92 ^{bcdefg}	1.06 ^{abcde}	0.02	0.82 ^{C,abc}	0.99 ^{B,bcdef}	1.16 ^{A,bcdefgh}	0.03	0.004	0.000	<0.001
GM	1	1.02 ^{abcd}	1.12 ^{abcde}	1.00 ^{abcd}	1.13 ^{abcd}	0.03	0.81 ^{C,abc}	1.10 ^{B,abcd}	1.30 ^{A,abcde}	0.04	0.020	0.010	<0.001
	3	1.07 ^{abc}	1.18 ^{abcd}	1.08 ^{abc}	1.18 ^{abc}	0.03	0.87 ^{C,abc}	1.15 ^{B,abc}	1.36 ^{A,abc}	0.04	0.016	0.019	<0.001
	5	1.13 ^{ab}	1.22 ^{ab}	1.12 ^{ab}	1.23 ^{ab}	0.03	0.91 ^{C,ab}	1.21 ^{B,ab}	1.40 ^{A,ab}	0.03	0.020	0.013	<0.001
	7	1.17 ^{a}	1.28 ^{a}	1.18 ^{a}	1.29 ^{a}	0.03	0.97 ^{C,a}	1.27 ^{B,a}	1.45 ^{A,a}	0.04	0.015	0.015	<0.001

PM; *Psoas major*, LT; *Longissimus thoracis*, LL; *Longissimus lumborum*, GM; *Gluteus Medius*, TBARS; thiobarbituric acid reactive substances. $^{A–C}$ Means with different superscripts in a row are statistically different ($p < 0.05$). $^{a–h}$ Means with different superscripts in a column are statistically different ($p < 0.05$).

The postmortem aging duration significantly ($p < 0.05$) affected the lipid oxidation in PM, LT, LL, and GM muscles. All the muscles showed higher ($p < 0.05$) lipid oxidation at 14 days of aging. However, all the muscles maintained the TBARS values within the maximum acceptable limits of 2.0 mg MDA/kg throughout the aging duration. The increase in lipid oxidation over time could potentially be attributed to the depletion of endogenous antioxidants [49]. Furthermore, iron is released from molecules such as myoglobin and ferritin during the aging process. The amino acids use this iron (produced because of proteolysis during aging) to form the chelates that are the active catalysts of lipid oxidation [50].

Oxidative intensity and stability could be affected by the muscle type and retail display time [51]. The postmortem aging duration significantly ($p < 0.05$) affected the TBARS values of the PM, LT, LL, and GM muscles during 1, 3, 5, and 7 days of simulated display. The LT and LL muscles on day 1 showed the lowest TBARS values while the GM muscle on day 7 showed the highest TBARS values. The difference in the oxidative stability of different muscles could be due to the difference in the muscle fiber type. Muscles such as PM and GM are composed of type I fiber and showed higher mitochondria and oxidative metabolism levels than muscles composed of type IIB such as LT and LL [32].

Moreover, the muscles comprising type I muscle fibers contain higher lipid and myoglobin concentrations than the muscles consisting of type IIB fibers [52]. These differences in muscle fiber types could be the reason for the higher lipid oxidation in PM and GM

muscles than in LT and LL muscles. Furthermore, Joseph et al. [53] reported a lower level of the antioxidant protein in the PM muscle than in the LL muscles, which might be another possible reason for the lower oxidative stability in the PM and GM than in the LT and LL muscles in the current study.

3.6. Sensory Analysis

The juiciness, flavor, tenderness, and overall acceptability of PM, LL, LT, and GM were significantly ($p < 0.05$) affected by the bull type, age, and aging duration (Table 7). Breed difference is one of the significant features affecting sensory characteristics [43]. In the current study, all the muscles showed better ($p < 0.05$) sensory characteristics in humpless bulls than in humped bulls. The superior juiciness and flavor of humpless bulls may be attributed to differences in marbling levels compared to humped bulls [54]. Furthermore, a decrease in the sensory tenderness in humped bulls could be due to increased calpastatin activity in comparison to the humpless bulls [55]. Bull age at slaughter also affected ($p < 0.05$) the sensory characteristics of the PM, LL, LT, and GM muscles. Juiciness, flavor, and overall acceptability were increased while the sensory tenderness score was decreased in 30 ± 3 months of age bulls in comparison to the 21 ± 2 months of age bulls. An increase in the juiciness, flavor, and overall acceptability score could be due to the increase in the marbling levels with the age of the bull. Furthermore, the quantity and strength of the connective tissue are increased with the advancement of the age of the bull [56], which might result in a decrease in sensory tenderness in the present study. Similarly, Du Plessis et al. [35] observed increased juiciness and a decreased sensory tenderness score in the 30 months of age cattle in comparison to the 18 months of age cattle. Additionally, sensory tenderness also showed a strong negative correlation with WBSF values (Supplementary Table S2).

Table 7. Effects of bull type, age, and wet aging on sensory evaluations of *Psoas major* (PM), *Longissimus thoracis* (LT), *Longissimus lumborum* (LL), and *Gluteus medius* (GM) steaks.

Sensory Evaluation	Muscle	Bull Type		Bull Age (Months)			Aging Duration				*p*-Value		
		Humped	Humpless	21 ± 2	30 ± 3	SE	0 Days	7 Days	14 Days	SE	Bull Type	Bull Age	Aging Duration
Juiciness	PM	5.90 ^{b}	6.67 ^{b}	5.98 ^{b}	6.60 ^{b}	0.13	5.20 ^{B,b}	6.65 ^{A,b}	7.00 ^{A,bc}	0.16	<0.001	0.001	<0.001
	LT	7.00 ^{a}	7.83 ^{a}	7.13 ^{a}	7.70 ^{a}	0.14	6.65 ^{B,a}	7.65 ^{A,a}	7.95 ^{A,a}	0.17	<0.001	0.005	<0.001
	LL	6.80 ^{a}	7.70 ^{a}	6.93 ^{a}	7.57 ^{a}	0.13	6.60 ^{B,a}	7.40 ^{A,a}	7.75 ^{A,ab}	0.16	<0.001	0.001	<0.001
	GM	5.67 ^{b}	6.43 ^{b}	5.73 ^{b}	6.37 ^{b}	0.13	5.10 ^{C,b}	6.20 ^{B,b}	6.85 ^{A,c}	0.15	<0.001	0.001	<0.001
Flavor	PM	7.23 ^{a}	7.73 ^{a}	7.27 ^{a}	7.70 ^{a}	0.12	6.65 ^{B,a}	7.75 ^{A,a}	8.05 ^{A,a}	0.15	0.005	0.013	<0.001
	LT	6.33 ^{b}	7.03 ^{b}	6.50 ^{a}	6.87 ^{b}	0.12	5.65 ^{B,b}	7.05 ^{A,b}	7.35 ^{A,b}	0.15	<0.001	0.039	<0.001
	LL	6.53 ^{ab}	7.27 ^{ab}	6.57 ^{a}	7.23 ^{ab}	0.13	5.95 ^{B,ab}	7.25 ^{A,ab}	7.50 ^{A,ab}	0.16	<0.001	0.001	<0.001
	GM	7.16 ^{a}	7.73 ^{a}	7.13 ^{a}	7.77 ^{a}	0.16	6.55 ^{B,a}	7.85 ^{A,a}	7.95 ^{A,ab}	0.20	0.018	0.009	<0.001
Tenderness	PM	7.17 ^{a}	7.83 ^{a}	7.77 ^{a}	7.23 ^{a}	0.13	6.65 ^{B,a}	7.80 ^{A,a}	8.05 ^{A,a}	0.16	0.001	0.005	<0.001
	LT	6.80 ^{a}	7.63 ^{a}	7.53 ^{a}	6.90 ^{a}	0.13	6.60 ^{B,a}	7.40 ^{A,a}	7.65 ^{A,a}	0.16	<0.001	0.001	<0.001
	LL	7.00 ^{a}	7.50 ^{a}	7.53 ^{a}	6.97 ^{a}	0.17	6.40 ^{B,a}	7.40 ^{A,a}	7.95 ^{A,a}	0.21	0.043	0.023	<0.001
	GM	5.53 ^{b}	6.23 ^{b}	6.17 ^{b}	5.60 ^{b}	0.14	5.00 ^{B,b}	6.20 ^{A,b}	6.45 ^{A,b}	0.17	0.001	0.004	<0.001
Overall Acceptability	PM	7.00 ^{a}	7.63 ^{a}	6.87 ^{a}	7.77 ^{a}	0.20	6.65 ^{B,a}	7.45 ^{AB,a}	7.85 ^{A,a}	0.28	0.027	0.002	0.003
	LT	5.83 ^{b}	6.57 ^{b}	5.90 ^{b}	6.50 ^{b}	0.18	5.65 ^{B,ab}	6.30 ^{AB,b}	6.65 ^{A,b}	0.22	0.005	0.020	0.007
	LL	5.87 ^{b}	6.80 ^{b}	6.03 ^{b}	6.63 ^{b}	0.17	5.45 ^{C,b}	6.35 ^{B,b}	7.20 ^{A,ab}	0.20	<0.001	0.013	<0.001
	GM	5.67 ^{b}	6.37 ^{b}	5.77 ^{b}	6.27 ^{b}	0.17	5.20 ^{C,b}	6.05 ^{B,b}	6.80 ^{A,b}	0.20	0.004	0.037	<0.001

PM; *Psoas major*, LT; *Longissimus thoracis*, LL; *Longissimus lumborum*, GM; *Gluteus Medius*. $^{A–C}$ Means with different superscripts in a row are statistically different ($p < 0.05$). $^{a–c}$ Means with different superscripts in a column are statistically different ($p < 0.05$).

Postmortem aging is commonly used to improve sensory quality characteristics, especially tenderness [57,58]. The 14 days of postmortem aging ($p < 0.05$) improved the juiciness, flavor, tenderness, and overall acceptability of the PM, LL, LT, and GM muscles. The structural and biochemical changes during the aging process improve the juiciness, flavor, tenderness, and overall acceptability [59]. In the present study, the non-significant differences in sensory characteristics between 7 and 14 days of aging duration could be due to a decrease in the structuring and biochemical changes during the mentioned period. Different muscles from the same carcass show considerable variation in sensory characteristics. In the current study, the LT and LL muscles exhibited the highest ($p < 0.05$) juiciness score,

while the PM showed the highest flavor, tenderness, and overall acceptability score. The highest juiciness score of the LT and LL muscles might be due to the higher marbling levels in these muscles [38]. Like instrumental tenderness, the PM muscle also showed the highest sensory tenderness score in comparison to the remaining muscles. The highest tenderness of the PM muscle could be the primary reason for the highest overall acceptability score in comparison to the remaining muscles.

4. Conclusions

The current study's findings indicated the muscle-specific effects of animal age and aging duration on the meat quality characteristics of humped and humpless bulls. Humped bulls showed better color and oxidative stability, whereas humpless bulls showed better instrumental tenderness and sensory characteristics. The 30 $\pm$ 3 months of age bulls showed better pH color, sensory juiciness, flavor, and an overall acceptability score than the 21 $\pm$ 2 months of age bulls. Interestingly, the PM and LT muscles respond differently to the postmortem aging duration compared to the LL and GM muscles. The LL and GM muscles showed improvement in meat tenderness at 14 days of aging, while 7 days of aging improved the tenderness of the PM and LT muscles. The LT and LL muscles showed the highest color and oxidative stability among muscles in comparison to PM and GM. Overall, muscle-specific aging strategies should be used to improve the color, oxidative stability, instrumental tenderness, and sensory characteristics of humped and humpless bulls. Further research could explore additional aging durations and other muscle types to better understand their impact on meat quality characteristics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani14243593/s1, Table S1. Effects of bull type, and age on live weight, hot carcass weight, backfat thickness, and ribeye area of humped and humpless bulls. Table S2: Pearson correlation analysis among various meat quality characteristics.

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