

Article

Meat Quality Traits in Beef from Heifers: Effect of including Distiller Grains in Finishing Pasture-Based Diets

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Abstract: Distiller grains (DG), which are the by-product from the bioethanol industry, represent an interesting alternative as animal feedstock. To our knowledge, little information is available on the inclusion of DG on the quality of meat from pasture-fed heifers. Thus, the aim of the present study was to evaluate the effect of DG inclusion in pasture-based systems on the main meat quality attributes of Charolais × Aberdeen Angus heifers. For this purpose, meat from heifers fed with a pasture-based diet without supplementation (P) or with 0.75% of live weight DG supplementation (PDG; DG plus dry-rolled corn, 50:50) or with 0.75% of live weight dry-rolled corn supplementation (PRC) was evaluated. Physical (pH, WHC, color, texture), sensory and nutritional (fat content, fatty acid, and amino acid profile) attributes were evaluated in beef samples. No effect of supplementation was observed on meat pH or color ($p > 0.05$). Meat from PDG heifers showed higher values of WBSF than meat from P heifers ($p = 0.039$). However, the overall tenderness evaluated by trained panelists showed no differences due to supplementation ($p > 0.05$). Our results indicate that the inclusion of DG as a partial corn-replacement supplementation for heifers under grazing represents a strategic tool not only related to meat quality, but also as an alternative to reduce food–feed competition.

Keywords: fatty acid profile; amino acid profile; tenderness; sensorial analysis; meat; distiller grains; meat color



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1. Introduction

The meat industry is an important player within the global agri-food economy. It is projected that meat consumption during the 2020–2029 decade will increase by 12% compared to the 2017–2019 period, with 16% of this increase corresponding to bovine meat. The main drivers of the total demand for meat products are expected to continue being the household income, consumer preferences, and population growth [1]. In the case of the bovine industry, its development in the future will be mainly linked to the reduction of the environmental impact in response to the growing concerns and demands of consumers, along with the improvement of productive yields and the quality of the products [2].

Consumer acceptance of beef is known to depend on two main aspects: its nutritional value and its eating quality (i.e., its juiciness, tenderness, flavor, and color). Meat quality

can be modified by several factors, including the genetics and sex of the animal, and the feeding system. Since the genetics is the main factor that can affect the final quality of beef, its effects are fairly clear and well-established [3].

Regarding the sex of the animals, several reports have stated that heifers present better quality attributes than bulls and steers [4]. This is related to the fact that heifer beef possesses more marbling (i.e., intramuscular fat) because female cattle have genes that efficiently control fat deposition [5]. This increased muscle fat content in heifers has been reported to dilute the connective tissue content in muscle, leading to an increase in tenderness [6]. The fatty acid profile of the beef from heifers is also different from that of bulls and steers, with a higher unsaturated fatty acid content [4].

Regarding the feeding regimes, in recent decades, there has been a resurgence of grass-fed or less intensive beef production, in an attempt to use available resources for resilient production systems, the well-being of the human–animal population, and food security. These interests are linked to several quality benefits, like a potential healthy lipid profile, and the sustainability of future agriculture and food production [7]. In this regard, it has been reported that the beef from heifers finished on a grass silage diet has better overall quality in terms of color, lipid oxidation, and vitamin E levels than that from heifers finished on a corn silage diet [8]. However, grass-fed systems usually need an extra energy input at the finishing stage to avoid low levels of muscle energy at slaughter and to increase the intramuscular fat content. This energy input is generally corn-based, with expensive results for the production system [9]. Thus, one of the main targets of these production systems is the total or partial replacement of this corn supplementation without altering the meat quality attributes. In this context, in the last years, distiller grains (DG) have successfully emerged as a nutrient supplement in animal nutrition. The results of incorporating DG into the finishing diets of steers reared under feedlot systems are encouraging [10–12]. In these steers, previous studies have demonstrated that DG inclusion of up to 30% can lead to a win-win production scheme [13]. This is related to the lower price of DG in comparison to other alternative high protein/energy feedstuffs like soybean/corn grain. Thus, DG incorporation into finishing diets could result not only in an economic opportunity for good-quality beef production, but also in higher resource efficiency under circular bioeconomy practices. However, in heifers, Harris et al. [14] demonstrated that DG inclusion in pasture diets has neither beneficial nor detrimental effects on the physiological functions of heifers, in accordance with other reports [15]. To our knowledge, DG use in extensive heifer beef production systems involving natural pastures remains little studied.

Heifers usually represent the second meat-producing category after steers, reaching up to 30% of total beef production in main meat-producing countries like Argentina and the USA [16,17]. Argentine cattle are traditionally fed on pastures. However, the need to increase the productivity of farms in the central (or Pampas) region has led to the development of beef production systems characterized by a more intensive use of forage resources, particularly the incorporation of energy supplements [18]. Likewise, crossbred heifers constitute a strategy to increase productivity, considering a slaughter destination for all male and female offspring. In this scenario, it is necessary that F1 heifers reach adequate carcass characteristics, slaughter weight, and product quality [19]. In 2019, the central (or Pampas) region of Argentina, which includes the provinces of Buenos Aires, La Pampa, Córdoba, Santa Fe and Entre Ríos, contributed with 83.60% of the bovine production (tons of beef carcass), while the other geographic regions contributed with less than 6.5% each. In addition, 48.99% of this production was due to the slaughter of heifers [20]. Interestingly, 70% of the most important corn-bioethanol processing plants are also located in this region of Argentina. This proximity of bioethanol plants favors the use of DG in animal feeding as a supplement or in feedlot diets [21]. In this way, the reprocessing of by-products from the agri-food industry and the use of their valuable nutrients in animal feeding are also favored [22].

Considering the main aspects previously pointed out, and under a circular economy approach, we hypothesized that the main meat quality traits are not affected by the inclu-

sion of DG as a partial replacement of corn in supplemented diets of grazing systems of heifers. In this context, the objective of the present study was to evaluate the effect of DG inclusion in pasture-based finishing systems on the main meat quality attributes of beef from Charolais × Aberdeen Angus heifers.

2. Materials and Methods

The present study assessed the meat quality in beef obtained in a commercial abattoir. Beef samples were from heifers that had been fed in a pasture-based system with grain—corn or DG—supplementation. Details of the feeding regimes of heifers and productive performance have been previously reported [23]. Briefly, animals that had been raised in a pasture diet were randomly allocated into three groups (eight animals per group), each of which received a different finishing diet for 61 days: P: alfalfa-based pasture only; PDG: alfalfa-based pasture plus supplementation with 0.75% of live weight of a mixture of corn dried DG and dry-rolled corn (50:50); and PRC: alfalfa-based pasture plus supplementation with 0.75% of live weight of dry-rolled corn [23]. Animals were weighed every 21 days. Once animals reached an average weight of 358.3 ± 26.1 kg, they were transported to a commercial abattoir. Animals were slaughtered after 18 h of lairage, under standard conditions in compliance with the Argentinian animal welfare regulations of the Servicio Nacional de Sanidad Animal (SENASA). The characterization of DG, in relation to its fatty acid and antioxidant profile, has been previously reported by our group [13,24].

The study was conducted in accordance with the guidelines of the National Council of Scientific and Technological Research (CONICET), RD 20061211#2857. For sensory analysis, all participants of the panel were informed of the purpose of the research and the voluntary nature of participation and read the free and informed consent to participate in the study.

2.1. Meat Samples

Meat samples (*M. longissimus thoracis*, LT) were collected from ribs 11 to 13 of 24 carcasses, at 48 h *postmortem* (1 ± 1 °C) at a commercial abattoir. The carcasses belonged to beef heifers Charolais × Aberdeen Angus raised and fed according to that reported previously [23]. All carcasses were typified as Vq-B1 according to Res. 32/2018 from the Ministry of Agriculture and Livestock of Argentina.

Three steaks (≈ 2.54 cm thick) were obtained from each left rib section, vacuum packaged, and frozen at -20 °C until further analysis.

2.2. Instrumental Color and pH Determination

Steaks were thawed at 4 °C for 24 h and then allowed to bloom 45 min prior to analysis at 20 °C. Objective color measurements were made using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Bergen, NJ, USA) as described by AMSA, 2012 [25]. The instrumental conditions used were artificial illuminant D65, 8 mm port size and a 2° standard angle observer. The colorimeter was calibrated according to the manufacturer's instructions using a white plate ($Y = 93.8$, $x = 0.3155$, $y = 0.3319$). Six scans were performed on each steak, and the average was used for statistical analysis. The parameters are expressed in terms of the CIELab system (L^* , lightness; a^* , redness and b^* , yellowness).

The pH of each steak was recorded in duplicate, using a pHmeter Orion 420Aplus (Thermo Electron Corporation, Waltham, MA, USA).

2.3. Myoglobin (Mb) Content and Browning Index Determination

Muscle homogenates were prepared by homogenizing 3.6 g of ground LT in 18 mL of ice-cold phosphate buffer 0.04 M (pH 6.8), for 40 s at 9000 rpm, using an Ultraturrax T25 homogenizer (Janke & Kunkel GmbH, Labortechnik, Staufen, Germany). During the homogenization process, the muscle tissue and the buffer were kept cold with an ice bath. Then, the sample was held at 0 to 4 °C for 1 h. After that, an aliquot of 20 mL was centrifuged at $14,000 \times g$ for 40 min at 4 °C (Hermle Z 383 K, Hermle Labor Technik, Wehingen, Germany). The supernatant was filtered through Whatman No. 50 filter paper.

The absorbance spectra were recorded using a spectrophotometer (Lambda Bio 20, Perkin-Elmer, Warsaw, Poland). The total Mb concentration was determined by absorbance at 525 nm (A525), which is the isobestic point for all three redox forms of Mb [25], as follows:

$$\text{Mb concentration (mg/g meat)} = ((A525 - A700)/7.6) \times 17 \times 6, \quad (1)$$

where 7.6 is the millimolar extinction coefficient for Mb at 525 nm, and 6 is the factor of dilution. An average of 17 kDa was used as the molecular mass of Mb. Absorbance at 700 nm (A700) was used to compensate for turbidity and was thus subtracted from the absorbance at 525 nm.

Additionally, the browning index, which represents an indirect estimate of metmyoglobin formation, was calculated as the ratio of absorbance at 503 and 580 nm (A503/A580) as described by AMSA, 2012 [25].

2.4. Water Holding Capacity (WHC)

2.4.1. Thawing Loss

The frozen steaks were placed in a plastic container, covered by a plastic mesh with a 1 cm square grid, to prevent the sample from contacting the liquid released, and then thawed at 4 °C for 24 h. Subsequently, steaks were placed at room temperature (ca. 20 °C) for 0.5 h. Thawing loss was expressed as the percentage of weight loss before freezing and after thawing.

2.4.2. Cooking Loss

The thawed steaks were cooked in a pre-heated electric double-side grill at 220 °C (model TB-GRILLV, Turboblender S.A., Mar del Plata, Argentina), until they reached a final internal temperature of 71 °C. K-type thermocouples (Galileo Italy, Beijing, China) coupled to a digital thermometer (model TK, Galileo Italy, Beijing, China) were inserted into the center of each meat sample to monitor the temperature during cooking. Then, the cooked steaks were placed at room temperature (ca. 24 °C) for 30 min. The loss of weight due to cooking was calculated as cooking loss and is expressed as a percentage of the pre-cooked weight.

2.5. Warner-Bratzler Shear Force (WBSF)

WBSF was measured with a TA-XT Plus[®] texture analyzer (Stable Micro Systems Ltd., Surrey, UK) with a Warner-Bratzler shear cell, and a 50 kg load cell (crosshead speed of 2 mm/s, test mode compression). For this measurement, we used the same sample used for the cooking loss test. Measurements were performed in eight cores (2.00 cm height, 1.25 cm diameter) removed parallel to the longitudinal orientation of the muscle fibers of each steak. The results were expressed as the average peak of shear force (Firmness, (N)) and the work of shear (Toughness, (N/mm s)).

2.6. Sensory Analysis

For sensory analysis, meat samples were cooked on a preheated electric clamshell grill (Ingeniería Gastronómica, San Martín, Argentina) at 160 °C until an internal temperature of 70 °C in accordance with the guidelines provided by AMSA, 2016 [26]. The quantitative descriptive analysis (QDA[®]) method was used with eight trained panelists. The experiment was carried out in a sensory evaluation laboratory equipped with panel booths meeting ISO 8589/2007 standards [27]. Each panelist received two cubes of each sample at ≈60 °C, placed in insulated food containers codified with a random three-digit number. All samples were presented simultaneously using a balanced blocked design to avoid presentation bias. The following attributes were assessed: odor intensity, flavor intensity, overall tenderness, fiber tenderness, juiciness, connective tissue amount, and detection of off-odors and off-flavors. In order to obtain the relative intensity measures for each attribute, ballots contained 10 cm line scales with word anchors placed 1 cm from the end. Word anchors, from left to right, were extremely slight to extremely intense for odor and flavor

intensity, tough to tender for tenderness, dry to juicy for juiciness, and none to abundant for connective tissue amount. Off-odors and flavors, if present, were also described. Assessments were carried out in triplicate in different sessions. Distilled water and unsalted soda crackers were provided to purge the palate of residual flavor notes between samples.

2.7. Fat Content and Fatty Acid Profile

The content of intramuscular fat (IMF) was determined by the Soxhlet method (SOX-TEC SYSTEM HT 1043 Extraction Unit, FOSS Tecator, Höganäs, Sweden) on two 5 g meat samples, as described by Garcia et al. [28]. The fatty acid profile was determined according to Park and Goins [29] with modifications. Briefly, fatty acids were converted to fatty acid methyl esters (FAMES) in situ by incubation of 0.1 g of lyophilized meat samples with dichloromethane—NaOH/MeOH at 90 °C for 10 min. This step was repeated using boron trifluoride. FAMES were extracted with NaCl 0.9%/hexane. Fatty acids in the hexane fraction were determined using a GC-FID equipment (Nexis GC-2030, Shimadzu, Kyoto, Japan) and a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm i.d., 0.20 µm film thickness, Varian CP7489) as described in Garcia et al. [28]. The results are expressed as % of total fatty acids detected. The atherogenic (AI) and thrombogenic (TI) indexes were calculated as proposed by Ulbricht and Southgate [30], as follows:

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / (\Sigma MUFA + \Sigma PUFA) \quad (2)$$

$$TI = (C14:0 + C16:0 + C18:0) / (0.5 \times (\Sigma MUFA + \Sigma n - 6 PUFA) + 3 \times \Sigma n - 3 PUFA + \Sigma n - 3 PUFA / \Sigma n - 6 PUFA) \quad (3)$$

where PUFA stands for polyunsaturated fatty acids and MUFA stands for monounsaturated fatty acids.

2.8. Amino Acid Profile

The total amino acid profile was determined by the method of Spackman et al. [31]. Briefly, samples of meat containing 2 mg of nitrogen (Kjeldahl method) were digested with 1 mL of hydrochloric acid 6 N at 110 °C for 24 h under a vacuum atmosphere. Then, the hydrolysates were allowed to cool at room temperature, buffered to pH 2.2 with NaOH and centrifuged at 10,000 rpm for 10 min (Hermle Z 383 K, Hermle Labor Technik, Wehingen, Germany). The separation of amino acids was performed by cation exchange chromatography and post-column derivatization with ninhydrin using a Biochrom-30 Amino Acid Analyzer (Biochrom Ltd., Cambridge, UK) with pH and temperature gradients (2.2 to 13.6 and 52 to 85 °C, respectively). Detection was made at two wavelengths: 440 and 570 nm. For amino acid identification and quantification, internal (L-norleucine) and external (Sigma AA-18 amino acid standards and L-Cysteic acid monohydrate, L-methionine sulphoxide, L-methionine sulfone, tryptophan, L-ornithine monohydrochloride) were used. The results are expressed as % of total amino acids detected.

2.9. Statistical Analysis

Statistical analysis was conducted using Infostat Software 2020 e-version [32]. Data normality was checked using Shapiro–Wilk test and homogeneity of variances using Levene’s Test. Data that did not show a normal distribution or homogeneity of variances ($p < 0.05$) were analyzed using Kruskal–Wallis test. A general linear model was used to assess the effect of the supplementation source on the meat quality characteristics, and an individual animal was considered an experimental unit [33]. The least significant differences were set at a 5% level and means were compared by Tukey’s test. Trend towards significance was set at $0.05 < p < 0.10$.

Data from sensory analysis were analyzed using a two-way analysis of variance, with assessors as random effect and samples as fixed effect (Equation (4)). The assessor by sample interaction was also tested. The least significant differences were set at a 5% level

and means were compared by Fisher's test. SenPAQ V6.3 and XLSTAT 2019.2.1 software were used.

$$y_{ij} = \mu + \text{Assessor}_i + \text{Sample}_j + \text{Assesor} \times \text{Sample}_{ij} + \text{Error}_{ij} \quad (4)$$

3. Results

3.1. Meat pH, WHC, Color and Texture (WBSF)

The results of meat pH, WHC, texture, and meat and fat color are presented in Table 1.

Table 1. Meat quality attributes of beef from heifers finished in three dietary three dietary treatments: P (pasture only), PDG (pasture plus distiller grains and dry-rolled corn) and PRC (pasture plus dry-rolled corn).

Parameter	Diets ¹			SEM ²	p-Value
	P	PDG	PRC		
pH	5.56	5.63	5.58	0.05	0.55
Thawing loss, %	4.48	3.15	3.38	0.29	0.10
Cooking loss, %	24.90 ^b	26.25 ^a	23.89 ^b	0.42	0.005
Meat color					
Lightness L*	31.10	30.67	30.34	0.74	0.77
Redness a*	22.57	22.46	22.75	0.92	0.97
Yellowness b*	9.71	8.85	9.74	0.69	0.60
Fat color					
Lightness L*	68.32	67.36	70.22	1.34	0.33
Yellowness b*	18.72 ^a	14.55 ^b	14.82 ^b	0.93	0.01
Mb, mg/g meat	3.36 ^a	2.37 ^b	3.24 ^{ab}	0.24	0.02
Browning index (A503/A581)	0.51	0.56	0.61	0.07	0.23
WBSF, N	40.65 ^b	46.86 ^a	47.70 ^a	1.92	0.039
Toughness, N/mm s	201.55	212.48	225.57	12.89	0.35

¹ Finishing diets: P, pasture only; PDG, pasture plus supplementation with 0.75% of live weight of a mixture of corn dried distiller grains and dry-rolled corn (50:50); PRC, pasture plus supplementation with 0.75% of live weight of dry-rolled corn. ² SEM, standard error of the mean. Means in the same row having different letters are significant at the $p \leq 0.05$ level.

Meat WHC was determined through thawing losses and cooking losses. LT samples from heifers fed the P diet tended to have higher values of thawing losses than LT samples from those fed the PRC and PDG diets ($p = 0.10$). LT samples from heifers fed the PDG diet showed the highest values ($p = 0.005$) of cooking loss, while LT samples from those fed the P and PRC diets showed similar values (average value of $24.39 \pm 0.71\%$).

The pH values determined in the present study (mean value 5.59 ± 0.04) were within the normal expected range for ultimate pH (5.4 to 5.8), and no effect of the feeding diets was observed ($p > 0.05$). It is widely recognized that *post mortem* pH decline and ultimate pH highly influence meat WHC and color [34]. Therefore, this result suggests that differences in meat WHC or meat color would not be attributable to pH values.

Regarding meat color parameters, no effect of the diets was observed. Regarding fat color parameters, yellowness b* showed higher values in LT samples from heifers fed the P diet than in those from heifers fed the PDG and PRC diets ($p = 0.01$).

Regarding Mb content, LT samples from heifers fed the P diet showed greater values than those from heifers fed the PDG diet, while LT samples from heifers fed the PRC diet showed intermediate values ($p = 0.02$). Regarding the browning index (A503/A581), which is a ratio developed to measure the relative proportions of brown color (metmyoglobin) and red color (carboxymyoglobin and/or OxyMb) in Mb solutions [35], no effect of the feeding diets was observed ($p > 0.05$).

Finally, regarding texture, when analyzing WBSF, IMF% was included as a covariate term, but was then removed from the model due to non-significance ($p > 0.05$). A significant

effect of the feeding diets was observed for WBSF ($p = 0.04$), while no effect was observed for the work of shear, measured as toughness ($p > 0.05$). LT samples from heifers fed the P diet showed lower WBSF values than those from heifers fed the PDG and PRC diets.

3.2. Sensory Analysis

The results from the sensory analysis are depicted in Figure 1. No effect of the dietary treatment was observed on the overall tenderness, juiciness, and flavor intensity ($p > 0.05$). For these attributes, LT samples were rated as “moderately tough” to “slightly tender”, “slightly dry” to “slightly juicy”, and “slight” to “moderately slight”, respectively. For fiber tenderness, samples from heifers fed the P and PDG diets were rated as less tender than samples from heifers fed the PRC diet ($p = 0.02$). The amount of connective tissue was also affected by the dietary treatment, and LT samples from heifers fed the P and PDG diets exhibited greater values (“slight” to “moderate”) than those from heifers fed the PRC diet (“traces” to “slight”) ($p = 0.002$).

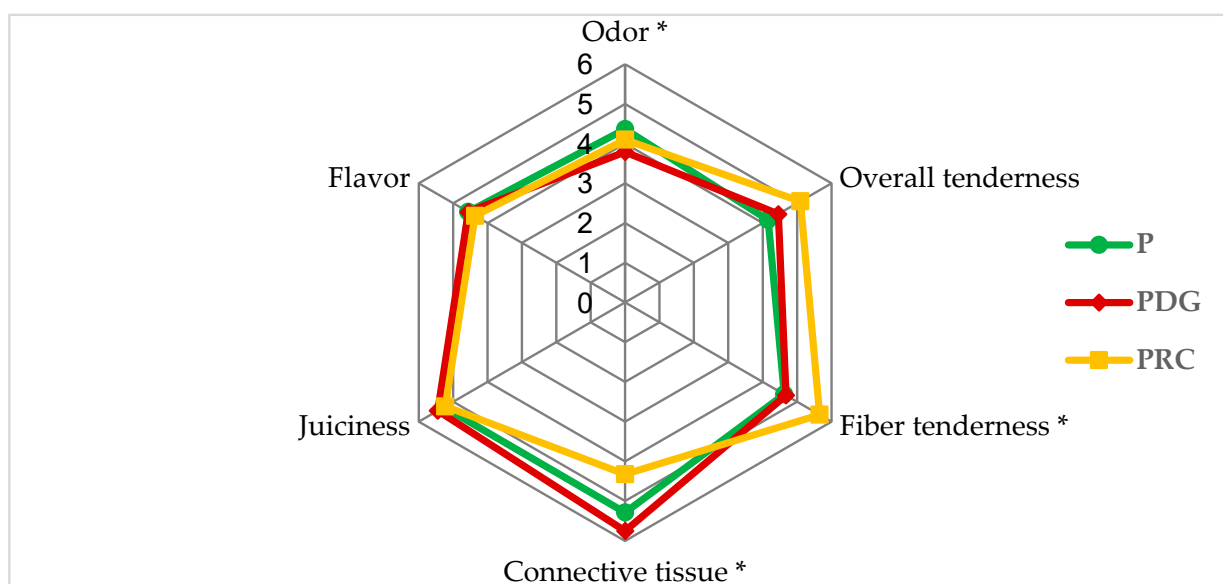


Figure 1. Spider web chart with mean sensory scores from QDA[®] of meat samples from three dietary treatments: P (pasture only), PDG (pasture plus distiller grains and dry-rolled corn) and PRC (pasture plus dry-rolled corn) evaluated by an eight-member trained panel. The data shown are means of the scores given by an eight-member panel over a 10 cm scale. For clarity, data are plotted up to 6 cm. Notations: * indicates significance at a p -value < 0.05 .

Meat samples from heifers fed the PDG diet exhibited “very slight” to “slight” odor intensity, while those from heifers fed the P diet exhibited “slight” to “moderately slight” odor intensity ($p = 0.03$). Meat samples from heifers fed the PRC diet exhibited intermediate values. Besides, none of the samples presented off-flavors or off-odors.

3.3. Nutritional Quality

3.3.1. IMF and Fatty Acid Profile

The results from the total IMF content and fatty acid profile are shown in Table 2. As can be seen, the supplementation did not modify the total fat content of beef. In general terms, the supplemented diets did not change the contents of saturated (SFA) and monounsaturated fatty acids (MUFA) ($p > 0.05$), but did lead to increased ($p = 0.041$) PUFA content in beef, mainly due to n-6 PUFA ($p = 0.05$), leading to an increased PUFA/SFA ratio ($p = 0.047$).

Table 2. Total intramuscular content (IMF) and fatty acid profile of beef from heifers finished in three dietary treatments: P (pasture only), PDG (pasture plus distiller grains and dry-rolled corn) and PRC (pasture plus dry-rolled corn).

Parameter ¹	Diets ²			SEM ³	p-Value
	P	PDG	PRC		
IMF (g/100 g muscle)	2.21	2.48	2.00	0.42	0.73
Fatty acid profile					
C 14:0, %	2.76	2.83	2.71	0.25	0.94
C 15:0, %	0.71	0.73	0.72	0.08	0.99
C 16:0, %	27.12	27.14	26.53	0.67	0.77
C 16:1 c-9, %	3.28	3.37	3.50	0.23	0.81
C 17:0, %	0.75 ^a	0.62 ^b	0.64 ^{a,b}	0.03	0.024
C 18:0, %	13.51	12.50	12.42	0.46	0.21
C 18:1 t, %	1.32 ^b	2.14 ^a	1.61 ^{a,b}	0.21	0.038
C 18:1 n-9 cis, %	37.93	36.53	34.99	0.81	0.07
C 18:1 c-11, %	1.46	1.46	1.60	0.06	0.16
C 18:1 c-12, %	0.26	0.14	0.16	0.04	0.17
C 18:2 n-6, %	3.26	3.79	4.71	0.42	0.08
C 18:3 n-3, %	0.89 ^b	0.88 ^b	1.30 ^a	0.09	0.007
CLA, %	0.28	0.31	0.35	0.02	0.10
C 20:4 n-6, %	0.73	1.22	1.66	0.26	0.07 [†]
C 20:5 n-3, %	0.26	0.39	0.54	0.08	0.07
C 22:4 n-6, %	0.10 ^b	0.08 ^b	0.17 ^a	0.02	0.012
C 22:6 n-3, %	0.48	0.68	0.86	0.12	0.11
SFA, %	43.40	42.46	41.67	0.89	0.41
MUFA, %	41.21	39.91	38.49	0.95	0.16
PUFA, %	5.74 ^b	7.64 ^{a,b}	9.22 ^a	0.87	0.041
n-3 PUFA, %	1.81	1.94	2.69	0.27	0.07
n-6 PUFA, %	3.28 ^b	3.88 ^{a,b}	4.87 ^a	0.42	0.05
n-6 PUFA/n-3 PUFA	2.09	2.10	1.83	0.12	0.22
MUFA/SFA	0.95	0.94	0.93	0.03	0.89
PUFA/SFA	0.14 ^b	0.19 ^{a,b}	0.23 ^a	0.02	0.047
TI	1.58	1.52	1.39	0.07	0.18
AI	0.79	0.82	0.79	0.05	0.84

¹ IMF, intramuscular fat; CLA, total conjugated linoleic acid; SFA, saturated fatty acids (C14:0 + C16:0 + C18:0); MUFA, monounsaturated fatty acids (C16:1 + C18:1); PUFA, polyunsaturated fatty acids (n-6 + n-3); n-3 PUFA, (C18:3 + C20:5 + C22:5 + C22:6); n-6 PUFA, (C18:2 + C18:3 + C20:4 + C22:4), TI, thrombogenic index; AI, atherogenic index. ² Finishing diets: P, pasture only; PDG, pasture plus supplementation with 0.75% of live weight of a mixture of corn dried distiller grains and dry-rolled corn (50:50); PRC, pasture plus supplementation with 0.75% of live weight of dry-rolled corn. ³ SEM, standard error of the mean. [†], statistical trend $p > 0.05$ to $p \leq 0.10$. Means in the same row having different letters are significant at the $p \leq 0.05$ level.

With respect to the individual fatty acid contents, the C 14:0, C 15:0, C 16:0, C 16:1 c-9, C 18:0, C 18:1 c-11, C 18:1 c-12, C 18:2 n-6, C 20:4 n-6, C 20:5 n-3, and C 22:6 n-3 acids were similar between treatments ($p > 0.05$). The content of C 17:0 in the beef from heifers fed the PRC and PDG diets was lower than that in the samples from heifers fed the P diet ($p = 0.024$). On the other hand, the content of C 18:1 t in the beef from heifers fed the PDG diet was higher than the beef from heifers fed the P diet ($p < 0.05$), while the contents of C 18:3 n-3 and C 22:4 n-6 were higher ($p < 0.05$) in beef from heifers fed the PRC diet.

For all the diets, the highest content was that of C 18:1 n-9 cis, reaching an average value of 36.48 ± 1.20 . This content was not modified by the supplementation and is comparable to values previously reported in beef from steers finished in pasture-based diets [28].

Regarding n-6 PUFA, linoleic acid (C 18:2 n6) was the most abundant in the samples analyzed, while for n-3 PUFA, alpha-linolenic acid (C 18:3 n3) was the most abundant and was significantly higher ($p = 0.007$) in samples from heifers fed the PRC diet than in those from heifers fed the P and PDG. The n-6 PUFA/n-3 PUFA ratio was not modified ($p > 0.05$) by the dietary treatments.

3.3.2. Amino Acid Profile

The results of the amino acid profile are shown in Table 3. As can be seen, the supplementation partially modified the content of both essential (EAA) and non-essential amino acids (NEAA) in beef. Hydroxyproline could not be quantified, whereas ornithine was not detected in most samples, and was thus not included in Table 3.

Table 3. Total amino acid profile of beef from heifers finished in three dietary treatments: P (pasture only), PDG (pasture plus distiller grains and dry-rolled corn) and PRC (pasture plus dry-rolled corn).

Parameter	Diets ¹			SEM ²	p-Value
	P	PDG	PRC		
Alanine (Ala), %	6.37 ^a	5.94 ^b	6.07 ^{a,b}	0.10	0.048
Arginine (Arg), %	0.35 ^b	7.26 ^a	7.30 ^a	0.06	0.0001
Aspartate (Asp), %	10.49	8.68	10.24	0.44	0.05
Cysteine (Cys), %	0.94	0.98	0.88	0.06	0.598
Glutamate (Glu), %	14.73 ^a	13.31 ^b	13.46 ^{a,b}	0.29	0.027
Glycine (Gly), %	4.94 ^a	4.65 ^b	4.70 ^b	0.05	0.009
Histidine (His), %	6.60	6.99	6.60	0.15	0.186
Isoleucine (Ile), %	4.11	4.36	4.21	0.12	0.423
Leucine (Leu), %	8.12 ^a	7.69 ^b	7.60 ^b	0.06	0.001
Lysine (Lys), %	9.10 ^a	8.68 ^b	8.75 ^{a,b}	0.08	0.021
Methionine (Met), %	3.69	3.13	3.18	0.15	0.075 [†]
Phenylalanine (Phe), %	4.34 ^c	4.59 ^a	4.48 ^b	0.02	0.001
Proline (Pro), %	6.72 ^a	4.69 ^{a,b}	2.17 ^b	0.75	0.015
Serine (Ser), %	5.34	5.43	5.62	0.15	0.446
Threonine (Thr), %	4.70	4.67	4.85	0.10	0.457
Tryptophan (Trp), %	0.40 ^b	0.22 ^c	0.63 ^a	0.02	0.001
Tyrosine (Tyr), %	4.05	4.25	4.22	0.12	0.518
Valine (Val), %	4.12	4.42	2.96	0.16	0.051 [†]
Ratio Met/Trp	9.35 ^b	14.61 ^a	5.06 ^b	1.17	0.003

¹ Finishing diets: P, pasture only; PDG, pasture plus supplementation with 0.75% of live weight of a mixture of corn dried distiller grains and dry-rolled corn (50:50); PRC, pasture plus supplementation with 0.75% of live weight of dry-rolled corn. ² SEM, standard error of the mean. [†], statistical trend $p > 0.05$ to $p \leq 0.10$. Means in the same row having different letters are significant at the $p \leq 0.05$ level.

The supplementation of pasture-based diets led to a modification in the total amino acid profile of heifer meat. Regarding EAA, the PDG diet increased phenylalanine ($p = 0.001$) and tryptophan ($p = 0.001$) and decreased lysine ($p = 0.021$) and leucine ($p = 0.001$) contents in beef. Regarding the NEAA profile, both the PRC and PDG diets led to increased levels of arginine ($p = 0.0001$) and decreased levels of proline ($p = 0.015$), when compared to the P diet. The PDG diet also decreased the beef contents of glutamate ($p = 0.027$), glycine ($p = 0.009$) and alanine ($p = 0.048$).

The cumulative quantity of EAA in the beef was at the level of 38.56% for the P diet, whereas the supplementation led to a slight decrease ($p > 0.05$), leading to 36.70% and 37.76% for the PRC and PDG diets, respectively. Also, a significant difference ($p = 0.01$) was observed for NEAA. The P diet showed a value of 60.53%, versus values of 61.25% and 62.18% for the PRC and PDG diets, respectively. No significant difference ($p > 0.05$) was observed in the EEA/NEAA ratio among treatments, being 0.62 ± 0.02 on average.

4. Discussion

Feeding ruminants under grazing conditions poses several advantages including meat quality characteristics, and ethical and environmental issues related to production systems, both of which are consumers' concerns [36,37]. However, pasture-based systems are highly dependent on climate conditions and producers occasionally need to turn to supplements to provide the necessary nutrients to animals, a fact associated with high costs [38]. In this sense, the use of by-products obtained after the processing of crops as feeding supplements

results in a potential strategy, since these by-products have lower costs than crops and reduce food-feed competition [39]. However, to include them in a feeding scheme, a full evaluation of their use and impact is necessary [40].

DG are the main by-product obtained from the fuel bioethanol industry and have been extensively used in animal feeding due to their high content of fat, protein, fiber, vitamins and minerals [10]. Most studies carried out on meat quality from ruminants, mainly on steers, have analyzed their inclusion as supplements and compared them with other grains in concentrate rations [10,12]. One of the effects observed in meat quality is related to oxidative stability, as a consequence of greater PUFA content, and antioxidant vitamin concentration in DG, in comparison with grains [11,13,24]. A recent study has shown that rearing management in heifers (from birth to slaughter) and diet characteristics during growth and fattening periods could influence certain meat characteristics [41]. However, to our knowledge, there is limited information on the quality of meat from heifers finished on a pasture-based diet with DG supplementation.

Meat quality can be defined through different approaches since it should be considered along all the stages from the farm to the table. Thus, meat quality could be defined as the combination between the quality attributes of fresh beef and the end user's expectations [42]. As it is known, the physical, sensorial and nutritional properties of meat depend on animal genetics, livestock practices and post-slaughter conditions [34,42,43].

Among physical properties, WHC describes the ability of fresh meat to retain its own water during slicing, pressing, thawing, and cooking, among other processing operations [34,44]. In the present study, the values obtained for thawing losses were lower than those reported by Lage et al. [45] for meat from heifers of different genetic groups (Nellore, $1/2$ Angus \times $1/2$ Nellore, and $1/2$ Simmental \times $1/2$ Nellore) and fed with two levels of concentrate in the finishing diets (average of 4.86%).

WHC in *post-mortem* muscles depends on complex biochemical processes [46]. Water loss can be delayed by factors that prevent the oxidation of lipids and thus help to stabilize cell membranes [46–48]. Also, water could be entrapped in the network developed between sarcoplasmic and myofibrillar proteins [49]. During the meat cooking process, water is lost as a consequence of the denaturation of muscle proteins due to the effect of heat, which affects the structural characteristics of meat, including the destruction of cell membranes, transverse and longitudinal contraction of muscle fibers, aggregation and formation of gel-like structures by sarcoplasmic proteins, and the contraction and solubility of connective tissue [34,44,50]. Feeding DG could increase the PUFA content in the sarcoplasmic membrane, which may enhance its instability and increase the susceptibility of muscle tissues to rapid oxidation [51]. Then, meat WHC could be reduced and favor cooking loss. Chao et al. [51] analyzed the meat from Continental \times British steers fed for 147 days on either a corn-based diet with 0% wet DG or 50% wet DG. The authors hypothesized that feeding wet DG may result in increased total PUFA and decreased total phospholipids in the sarcoplasmic reticulum membrane, contributing to its instability. In the present study, these findings could help to explain the difference observed in cooking loss in meat from heifers fed the PDG diet compared to the other diets. Bearing in mind that the present study was performed in a pasture-based system, it would be necessary to deepen the antioxidant status achieved in the meat from each diet to better understand the underlying mechanisms.

Among meat quality attributes, beef color is one of the most relevant that influence meat purchasing decision [52]. The overall color of the meat perceived by consumers is generated by chromatic and achromatic attributes. The former is dominated by the contribution of myoglobin (associated with a^* and b^* , in the CIE- $L^*a^*b^*$ color space) and the latter is determined by the physical and structural properties of the muscle (associated with L^* , in the CIE- $L^*a^*b^*$ color space) [53]. Both attributes are affected by animal management, nutritional background, slaughtering conditions, and processing conditions [52]. In this study, the redness (a^*) of LT samples was not affected by the diet and the values observed could be considered acceptable for consumers' satisfaction since they were above the threshold

value (14.5) established by Holman et al. [54]. These results agree with those obtained by Coria et al. [55], who reported no effect of different diets with a forage/concentrate ratio during finishing on the muscle color of steers. Regarding DG inclusion in the finishing diets, previous studies have shown no clear trend on meat color due to the variability in DG sources or level used in the feeding diets [10]. This is explained by the fact that DG supply anti- and pro-oxidants, and mixed with the other feeding ingredients, affect the quantity of pigments and their stability on fresh meat [10,56,57]. Previous studies performed on meat from animals fed DG-supplemented diets on concentrate rations have reported no effect of the DG level on meat redness [11,58], whereas a recent study has reported greater redness values on fresh patties from animals fed corn DG-supplemented diets compared to rolled barley-supplemented diets [59]. Some authors have postulated that the greater values of meat redness could be explained by the presence of xanthophylls, which are carotenoid compounds, in corn DG [56]. In this sense, it is known that, after fermentation, DGs retain all nutrients from corn, except sugar, and that the content of xanthophylls could be two-fold higher than that in corn grain [57]. However, results recently published by our group have shown that although DG-supplemented diets have greater content of carotenoid compounds, they led to no differences in meat redness [24]. Interestingly, in the present study, the yellowness of fat differed due to the supplementation. The subcutaneous fat of the meat from the P diet showed greater values of yellowness than that of the subcutaneous fat of the meat from the supplemented diets due to the dilution of carotenoid supply [60].

On the other hand, meat color depends on the Mb content, its redox stability and the form that is prevalent [53]. In turn, the content of Mb is affected by the energy metabolism of muscles. The proposed hypothesis is that muscle responds to different levels of dietary nutrients by altering general energy metabolism and, subsequently, impacting on the muscle-to-meat conversion process [33]. These changes then affect the color of the meat. Apaoblaza et al. [33] observed a darker color in meat from steers from a grass-fed system and a relative abundance of Mb approximately two-fold higher than in that from their concentrate-system counterparts. The data reported herein showed that while meat from the P diet showed higher Mb content than that from the PDG diet, no significant differences were observed in the color parameters. However, the differences in Mb content were not so large. Also, no significant differences were observed in the browning index, which represents an indirect estimate of metmyoglobin formation. Regarding this issue, Franco et al. [61] found no effect of the finishing time (30 and 60 days) on the Mb content in meat from Holstein-Friesian cull cows. In that study, animals from the control group were fed in pasture, while animals from the other groups were finished in an area without pasture, with a diet based on a commercial concentrate and corn silage, following a pasture period of 90 days. The authors reported values of Mb content (6.59 ± 0.57 mg/g fresh meat) greater from those reported in the present study, a difference that could be associated with the older age of the animals evaluated [62].

One of the most important attributes in consumer's eating satisfaction is tenderness, a trait positively related to an increase in consumers' purchase frequency and willingness to pay higher prices [4,63,64]. This attribute is influenced by several factors, e.g., animal genetics, feeding systems, composition of diets, slaughtering and post-harvest conditions, and numerous molecular pathways are involved in its development, which makes it very complex to predict [34,65,66]. Some studies performed on meat quality have shown that DG inclusion in feedlot diets has no detrimental effect on WBSF values [11,52,67]. However, our results are not in accordance with this, since beef from heifers fed the P diet showed lower WBSF values than that from heifers fed the supplemented diets. This result could be explained by a greater calpain/calpastatin ratio in meat from a pasture diet, which could be related to the proteolytic capacity of the muscle [55]. Coria et al. [55] reported that the composition of the diet affected the expression of the calpain system at the mRNA level.

Different values of WBSF have been proposed as a threshold value for consumer unacceptability. A value of 4.4 kg (43.12 N) has been proposed by Platter et al. [68], whereas a value of 55.9 N has been proposed by Miller et al. [63]. In the present study, all the

samples were below the 55.9 N threshold, but 55% of the samples were between 43.12 N and 55.9 N. Then, it will be necessary to analyze the response of consumers in the intent market to define product acceptability.

In our study, tenderness was also assessed by sensory analysis with trained panelists. The panel rated LT samples as “moderately tough” to “slightly tender”, independently of the dietary treatment, and samples from the P and PDG diets were perceived as less tender than samples from the PRC diet. Previous reports on heifer meat quality in feedlot systems have reported higher overall and myofibrillar tenderness with increasing levels of DG in the diets, which was explained by a decrease in the amount of connective tissue perception [58]. In agreement, in our study, the samples that were perceived as less tender (P and PDG) also showed greater scores for connective tissue amount. Other authors have also reported no differences in overall tenderness assessed by a trained panel and an effect of DG-supplemented diets on WBSF values [69]. In this sense, it has been stated that correlations between WBSF and sensory assessment of beef tenderness are highly variable, depending on the muscle type, sample preparation, fiber orientation, shear apparatus, cooking method, measurement procedure and panel type [64,70].

Finally, regarding beef odor, in our study, beef from heifers fed the P diet exhibited greater scores of odor intensity than that from heifers fed the PRC and PDG diets. In agreement, Resconi et al. [71] reported slight differences in beef odor, which were negatively associated with the level of concentrate in the diets. Other authors have reported no effect of DG inclusion in pasture diets on the odor of beef patties after 7 days of refrigerated storage [59].

Meat nutritional value is an important contributor to the overall quality of a product, and consumers are increasingly demanding healthy meat products, with special focus on their fat content and composition [42,43]. In general terms, the results obtained in the present study regarding the IMF content and fatty acid profile are in accordance with that observed in beef from animals fed on a pasture-based diet [19,72]. Regarding this issue, the beef obtained in the present study could be classified as extra-lean meat (<5 g of fat in 100 g of beef) according to the USDA beef grading. Concerning the fatty acid profile, the supplementation with both DG and rolled corn increased the content of PUFA in the heifer beef. This increase was mainly due to n6-PUFA. This finding is in accordance with previously published results and could be explained by the increased availability of n6-PUFA present in the corn-derived food matrixes used to supplement the pasture diets [28,43].

The supplementation did not modify SFA in meat, especially C14:0 or C16:0, saturated fatty acids mainly involved in increased cholesterol levels and cardiovascular diseases in humans. Consequently, no differences in the TI or AI were found between treatments. In this sense, other authors also found no effect of DG supplementation on SFA, TI, or AI when finishing steers were fed with a silage-based diet [59].

In the present study, supplementation of pasture diets increased the C18:1 trans fatty acid. This finding agrees with the increased values of PUFA also observed in meat, leading to believe that the diets provided an increased substrate availability for ruminal biohydrogenation, a main source of this fatty acid [73]. It has been previously reported that the levels of C18:1 trans fatty acids increase when the content of PUFA in diets is up to 80 g/kg dry matter [74]. Indeed, previous works have observed a linear correlation between the levels of C18:1 trans fatty acids in meat and the PUFA available in the diet [13,75]. Despite the increase found in the C-18:1 trans fatty acid level due to DG or rolled corn supplementation—the main substrate for CLA synthesis through the enzyme delta-9-desaturase—no significant difference was found in the CLA content in the heifer meat. However, a numerical increase in CLA content was found in the meat of both supplemented diets. It could be assumed that the main reason for this finding is the low level of supplementation. In heifers finished in pasture-based diets, Pordomingo et al. [76] also found CLA levels below 0.5% and related them to the feedlot background of animals. These results indicate that it is necessary to deepen the study of the effects of the different feeding

strategies on heifers in order to fully understand their impact on the resulting profile of fatty acids in meat.

The PUFA/SFA ratio was increased in meat from supplemented heifers. This finding is related to the increased content of PUFA and the lack of modifications in the SFA content. In agreement with these findings, other authors have reported a similar behavior when finishing heifers with increasing levels of DG in feedlot system [58] and supplementing silage-based diets with DG [59].

Interestingly, most of the studies carried out on DG-supplemented diets have reported an increase in n-6/n-3 PUFA, due to the higher content of n-6 PUFA, while, in the present study, DG supplementation did not affect this ratio. This could be explained by the fact that n-3 PUFA content in beef from heifers fed the PDG diet tended to be higher than in that from heifers fed the P diet. The mean n6-PUFA/n3-PUFA ratio found was also similar to that recorded in steer beef finished in pasture systems, in coincidence with that reported by Silva et al. [77].

It is known that the nutritional value of beef is determined by the nutrient content necessary for the human body. This issue is especially relevant for a balanced composition of amino acids. In this regard, it has been stated that meat proteins provide all EAA, i.e., lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, and valine, with no limiting amino acid [78]. EAA are needed to ensure the harmonious functioning of the complex biology of the human body and should be supplied with food in sufficient quantities. Among them, methionine, tryptophan and lysine remain as the most important in human nutrition.

In general terms, the ratio of the content of EAA to the total amount of amino acids found in the present study is similar to that published in recent reports that have demonstrated levels at 39–41% in different muscles of beef carcasses [79]. In all the dietary treatments here evaluated, the EAA/NEAA ratios were higher than the recommendations by WHO/FAO/UNU [80] for human intake, since a value of 0.37 can be calculated from this ratio considering these recommendations. Regarding the content of the main EAA for human requirements, i.e., methionine, tryptophan and lysine, the beef from the different dietary treatments showed similar cumulative amounts (13.23%, 12.56% and 12.03% for the P, PRC and PDG diets, respectively). Among indicators of the quality of the amino acid profile in food matrixes, it has been proposed that the methionine/tryptophan ratio provides a means to assess the significance of a matrix in the diet [81]. A higher value of this indicator would imply greater benefits in the incorporation of the food matrix into the human diet to enhance the balance of amino acid composition. In the present study, the methionine/tryptophan ratio for beef from the P treatment was 9.35. Supplementation led to changes in this ratio: the PRC diet significantly decreased this ratio while the incorporation of DG significantly increased it, when compared to the P diet (5.06 and 14.61 vs. 9.35, respectively). These ratios are higher than those previously reported by Alekseeva and Kolchina [81], who calculated methionine/tryptophan ratio values of 1.22 and 1.23 in bulls of Aberdeen Angus and Hereford breed, respectively, raised in pasture-based production system. Differences with the results found in the present study could be related to the methodology used for amino acid analysis and the effect of sex, breed and diet from each production system [82]. The latter can be especially involved since the rumen-produced microbial proteins are the most important source of amino acids for many domestic and wild ruminants. A possible mechanism that has been stated is that the ruminal conditions generated by the feed composition can be modified, affecting in this way the dynamics of intestinal/pancreatic digestive proteases such as trypsin and chymotrypsin and the absorption of amino acids [22,83]. The differential activity of these enzymes may lead to different amino acid availability and further assimilation.

In the last years, it has been stated that forage- and pasture-based production models for both milk and beef, and inclusion of by-products in the diet as well, may result in the upgrading of the quantity and quality of human-edible protein compared with using plant materials directly as human foods [84]. Results obtained in the present study show

that pastured-based systems supplemented with DGs may modify the amino acid profile of heifer beef, leading to increased content of several amino acids of nutritional interest. Pecka-Kiełb et al. [22] stated that using DGs in the animal diet increases the content of most amino acids in the meat, when compared to the use of corn grains in the diet. In the present study, this effect was only seen in the amino acids phenylalanine and proline. Moreover, the partial replacement of rolled corn by DG may lead to an increased methionine/tryptophan ratio in the beef, which would suggest an increased profitability of the amino acid pool provided. Undoubtedly, further studies in the animal nutrition field should be focused on the mechanisms involved in the effect of DG inclusion in pasture-based diets and on the amino acid profile and amino acid profitability in heifer beef.

In certain physiological conditions, like growing children and pregnant women, arginine and histidine are considered EAA [85]. Results found in the present study show that both PRC and PDG increased the content of arginine in heifer beef, suggesting that supplementation led to a better source of this amino acid in the beef obtained. Considering the importance of the incorporation of these amino acids through the diet in specific population sectors, future studies should deepen this aspect.

5. Conclusions

The originality of this study relies on the assessment of meat quality from heifers fed with DG supplements as a partial replacement to dry-rolled corn supplements, in an alfalfa-based pasture finishing system. Our results indicate that the inclusion of DGs in supplement diets of grazing heifers had no negative impact on meat pH, meat color, intramuscular fat content, or amino acid profile. Furthermore, the meat from DG supplementation exhibited an intermediate PUFA/SFA ratio, without an increase in the n6-PUFA/n3-PUFA ratio or the atherogenic and thrombogenic indexes with respect to the P diet. The profitability of the amino acid profile also seems to be increased by DG supplementation. Beef flavor was not affected by DG supplementation; although a detrimental effect was observed on beef WBSF, this effect was not perceived by the panelists.

In summary, the findings of this study indicate that the inclusion of DG as a partial corn-replacement supplement for grazing heifers can be considered a strategic approach, since it has no detrimental effect on the meat quality and represents a feedstock alternative that reduces food–feed competition. However, there is still a knowledge gap regarding the influence of DG and pasture on the amino acid profile and oxidative stability of meat under commercial conditions. Further research is needed to address this issue.

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Institutional Review Board Statement: For this study, meat samples were collected in a commercial abattoir. Animal handling was in accordance with the Handbook of Procedures for Animal Welfare of the National Service of Animal Health (Servicio Nacional de Sanidad Animal, SENASA) of Argentina. Details regarding the production assay have been previously described in Kloster et al. [23]. The donor animals were managed following the guidelines and recommendations of the Ag Guide of the Federation of Animal Science Societies [86]. The study was conducted in accordance with the guidelines of the National Council of Scientific and Technological Research (CONICET): Lineamientos para el comportamiento ético en las Ciencias Sociales y Humanidades Resolución RD 20061211#2857. All participants of the sensory panel were informed of the purpose of the research, and the voluntary nature of participation, and read the free and informed consent to participate in the study. All information gathered during this study was treated with full confidentiality.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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