**UNIVERSIDADE FEDERAL DE VIÇOSA**

# Effect of high-protein dried distillers grains on meat quality traits of fresh and aged Nellore beef

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*Magister Scientiae*

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Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Mario Luiz Chizzotti

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APPROVED: February 6, 2025.

Assent:

Natália Matos Panosso
Author

Mario Luiz Chizzotti
Adviser

To my Sunshine, Luna Panosso Cruz.
I dedicate.

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To God, for the gift of life.
To my parents and my family, for all the support and affection throughout these years.
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## ABSTRACT

PANOSSO, Natália Matos, M.Sc., Universidade Federal de Viçosa, February, 2025. **Effect of high-protein dried distillers grains on meat quality traits of fresh and aged Nellore beef**. Adviser: Mario Luiz Chizzotti.

The aim of the present study was to evaluate the effects of increasing levels of high-protein dried distillers grains (HP-DDG) on carcass characteristics, meat quality, and lipid oxidation of fresh and aged beef of feedlot Nelore cattle. Forty-four uncastrated Nelore bulls were fed four treatments (n = 11, 100 days): 0, 4, 8, and 12% HP-DDG. Carcass yield, fat thickness, Longissimus muscle area, pH, sarcomere length, and proximate composition were assessed, with no differences observed between diets (P > 0.05). Additionally, meat quality [color, myoglobin redox forms, shear force (WBSF), lipid oxidation (TBARS), and thawing, cooking, and total losses] was evaluated in fresh and aged meat (0, 7, and 14 days). An aging effect was observed for thaw loss, but no differences were detected after Tukey corrections. A reduction in WBSF was observed after 7 and 14 days of aging compared to fresh meat, while aging increased TBARS values (P = 0.001). The inclusion of 12% HP-DDG in the diet reduced (P = 0.001) the L* parameter compared to other diets, and an increase in L* (P = 0.001) was observed after 7 and 14 days of aging compared to unaged beef. The a* (redness) was reduced (P = 0.001) after 14 days of aging, while b* (yellowness) values were greater (P = 0.001) for 7 and 14 days compared to unaged. Greater chroma was observed for meat (P = 0.001) after 7 days compared to unaged and 14 days of aging, while hue angle was decreased (P = 0.001) by aging. The inclusion of 8% HP-DDG in the finishing diets promoted an increase (P = 0.03) in the percentage of OMb compared to 12% inclusion. Greater MMb was observed (P = 0.001) for aged beef, whereas aging reduced (P = 0.001) DMb percentages. Lower OMb percentages were observed (P = 0.001) after 14 days compared to unaged and 7 days of aging. HP-DDG can replace soybean meal (SBM) in finishing diets for Nelore cattle without compromising meat quality. Additionally, aging improves tenderness and induces changes in meat color.



Keywords: Lipid oxidation; Meat color; Rumen undegradable protein; Warner-bratzler shear force.

## RESUMO

PANOSSO, Natália Matos, M.Sc., Universidade Federal de Viçosa, fevereiro de 2025. **Efeito da inclusão de grãos secos de destilaria com alto teor proteico na qualidade da carne de novilhos Nelore**. Orientador: Mario Luiz Chizzotti.

O objetivo deste estudo foi avaliar os efeitos de níveis crescentes de grãos secos de destilaria ricos em proteínas (HP-DDG) sobre as características de carcaça, qualidade da carne e oxidação lipídica de carne fresca e maturada de bovinos Nelore confinados. Quarenta e quatro novilhos Nelore não castrados foram alimentados com quatro tratamentos (n = 11, 100 dias): 0, 4, 8 e 12% de HP-DDG. O rendimento de carcaça, espessura de gordura, área do músculo Longissimus, pH, comprimento do sarcômero e composição proximal foram avaliados, sem diferenças significativas entre as dietas (P > 0,05). A qualidade da carne (cor, formas redox da mioglobina, força de cisalhamento (FC), oxidação lipídica e perdas por descongelamento, cozimento e total) foi avaliada em carne fresca e maturada (0, 7 e 14 dias). A maturação influenciou a perda por descongelamento, mas não houve diferença após correções pelo teste de Tukey. A força de cisalhamento reduziu após 7 e 14 dias de maturação, enquanto a maturação aumentou os valores de TBARS (P = 0,001). A inclusão de 12% de HP-DDG na dieta reduziu (P = 0,001) o parâmetro L* em comparação às outras dietas, e o valor de L* aumentou após 7 e 14 dias de maturação. O a* (vermelhidão) diminuiu (P = 0,001) após 14 dias de maturação, enquanto o b* (amarelo) foi maior para 7 e 14 dias em relação a carne não maturada (P = 0,001). Maior croma foi observado (P = 0,001) após 7 dias em comparação a não maturada e 14 dias, e o ângulo de tonalidade (hue) reduziu com a maturação (P = 0,001). A inclusão de 8% de HP-DDG aumentou (P = 0,03) a porcentagem de oximioglobina em comparação à inclusão de 12%. Maiores percentuais de metamioglobina foram observados em carne maturada, enquanto a maturação reduziu os percentuais de deoximioglobina (P = 0,001). Menores porcentagens de oximioglobina foram observadas após 14 dias de maturação. O HP-DDG pode substituir o farelo de soja nas dietas de confinamento de bovinos Nelore sem comprometer a qualidade da carne. Além disso, a maturação melhora a maciez e provoca alterações na cor da carne.



Palavras-chave: Cor da carne; Oxidação lipídica; Proteína não degradável no rúmen; Força de cisalhamento Warner-bratzler

## SUMMARY

# Effect of high protein distilled grains on meat quality traits of fresh and aged Nellore beef[1]

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## INTRODUCTION

As worldwide production of bioethanol increases, large amounts of by-products are available to be used in animal nutrition. Most of these by-products are dried and marketed as distillers dried grains with solubles (DDGS). Distilled grains are typically characterized by their high protein content with low ruminal degradation protein (RDP), as readily degradable protein is utilized during fermentation to ethanol production and through Maillard reaction in the drying step (Teodorowicz et al., 2018). In opposite of USA and Europe, most ethanol industries in Brazil produce distillers dried grains without solubles (DDG), as the solubles are destinated to biodiesel production, resulting in a product with higher crude protein and less fiber, fat, and minerals (as phosphorous and sulfur) than DDGS (Schingoethe, 2006).

On average, the DDG and DDGS contain approximately 30 % crude protein (DM basis) and of that 63 % is rumen undegradable protein (RUP) (Castillo-Lopez et al., 2013), while the CP of soybean meal (SBM) is around 46-52 %, with 39 % RUP (Chizzotti et al., 2008). When compared to distiller's co-products, soybean meal has the advantage in consistency of nutrient composition as a result of fractionation technology of ethanol plants continuing to evolve and change (Hoffman and Baker, 2011). However, a recently developed process that aims to improve corn-ethanol yield differs from the traditional dry-milling process because the bran and germ are removed from the corn kernel before fermentation (decreasing the fiber and fat concentrations), while the remaining endosperm and gluten are ground and used as an energy substrate for yeast fermentation, resulting in a high-protein DDG (HP-DDG), with greater CP and RUP than traditional DDGS used in America (Hubbard et al., 2009; Ferreira et al., 2020).

A commercially available high-protein corn DDG (HP-DDG) (50% CP, which 82% is RUP) containing protein derived from *Saccharomyces cerevisiae* and from gluten (BioPass®,

SJC Bioenergia, Quirinópolis, GO, Brazil) may be an alternative ingredient to substitute traditional protein sources such as SBM in feedlot finishing beef diets and increase profitability.

The principal consequence of substituting SBM by DDG into finishing diets is the fact that RUP increases, being the abomasum and the small intestine as its main digestive sites, with the digestible portion of RUP for HP-DDG reaching 97 % RUP (Kelzer et al., 2010). Once the requirements of RDP are met, the provision of RUP could improve the supply of metabolizable protein (MP), increasing the N balance and the availability of nitrogen for anabolic purposes (Dias et al., 2024; Ferreira et al., 2020), as RUP represents an important contribution to the total pool of MP (Hubbard et al., 2009). In addition, when dietary supply of MP is greater than the requirements, deamination of amino acids can occur and the N is recycled to the rumen, which may provide sufficient RDP and can increase energy supply by gluconeogenesis (NRBC, 2016).

While the performance and profitability are the principal factors to consider the HP-DDG as an alternative feed source to SBM for cattle feedlot systems, understanding their effect on meat quality aspects is important, with just a few studies published. Besides that, aging proved to maintain activity of endogenous proteases that breakdown muscle proteins including myofibrillary enhancing meat tenderness (Ribeiro et al., 2019). However, less is known about the tenderizing effect of aging in relation to HP-DDG inclusion in the diet of Nellore cattle. Hart et al. (2019) observed no differences in unaged beef meat, while an increase in lipid oxidation and lightness ($L^*$) and decrease in redness ($a^*$) after 7 d of aging were reported, when they substituted the ground corn by HP-DDG.

Therefore, studies are needed to evaluate the carcass traits and meat quality of animals receiving diets with a high proportion of HP-DDG replacing SBM and submitted to aging process. Considering these aspects, we hypothesized that increasing the dietary inclusion of HP-DDG would improve beef quality traits of Nellore bulls. Therefore, the aim of the present

study was to evaluate the effects of increasing levels of HP-DDG on carcass traits, meat quality and lipid oxidation of fresh and aged beef of feedlot Nellore cattle.

## MATERIALS AND METHODS

### *Animal use and ethics*

Animal care and handling procedures were approved by the Ethics Committee for Animal Use (Protocol CEUAP/DZO/UFV n. 27/2024). The experiment was conducted in the experimental feedlot of the Department of Animal Science of the Universidade Federal de Viçosa, MG, Brazil.

### *Location, design and diets*

A total of forty-four non-castrated Nellore calves of 8 ± 1 months old and 222 ± 29.5 kg of body weight, from the Research, Teaching and Extension Unit in Beef Cattle of the Universidade Federal de Viçosa, were used. The evaluation period lasted 114 days, with 14 days of adaptation to experimental diets, and 100 days of animal performance evaluation. The experiment was conducted using a completely randomized design.

Upon arrival, the animals were immediately identified, weighed, and treated against ecto- and endoparasites. They were then housed in collective pens (48 m²) containing feeders (AF-1000 Master, Intergado LTDA, Contagem, MG, Brazil) and electronic waterers (WD-1000, Master; Intergado, Contagem, MG, Brazil), allowing to record daily intake individually. All animals received ad libitum feeding, with the R:C ratio gradually decreasing (in 20% intervals) every 5 days from the 80:20 ratio until reaching a 20:80 ratio. The animals were randomly divided into four groups, each containing 11 animals, to receive the treatments (diets). On the first and last day of animal performance evaluation period, they were weighed after fasting for 16 h of solids, and the ADG between those days was estimated. The four dietary treatments were defined by the proportion (DM basis) of HP-DDG (BioPass®, SJC Bioenergia,

Quirinópolis, Goiás, Brazil) that replaced the nitrogen concentrate of the diet, resulting in 0, 4, 8, and 12 % HP-DDG dietary inclusion (Table 1), which were formulated according to BR-CORTE recommendations (Valadares Filho et al., 2016), to provide approximately 13.5% crude protein (% total DM of the diet) and to promote a weight gain of approximately 1.2 kg/day.

The diets were provided twice a day, at 8:00 AM and 4:00 PM, ensuring that leftovers remained at approximately 5% of the offered amount. The quantities offered and leftovers were weighed daily, and water was provided ad libitum. Every 25 days, the animals were weighed to monitor weight gain, and at the end of the 182 days, they were slaughtered.

***Slaughter of animals and sample collection***

The animals were transported to an Experimental Slaughterhouse at Universidade Federal de Viçosa. The truck stocking density was 0.8 ± 0.2 bulls/$m^2$ and transport distance was < 3 km. To mitigate potential influences of the slaughter day on the evaluated parameters, staggered slaughters were conducted over five days, with animals from all assessed treatments slaughtered each day, chosen randomly.

Basically, the animals were stunned using a captive-bolt pistol, and they were bled by exsanguination by cutting the neck vessels. The head, hide, viscera, tail, legs, diaphragm, and excess internal fat were removed. Afterwards, the carcasses were divided medially from the sternum and spine, resulting in two similar halves, which were weighed to calculate the hot carcass weight (HCW) and estimate hot carcass dressing percentage (HCD). Then, the half-carcasses were washed, identified, and stored in a chilling chamber at 4 °C, where they remained for a 24 h period. After that period, they were weighed again to calculate the cold carcass weight (CCW) and estimate cold carcass dressing percentage (CCD).

***Carcass measurements and meat sampling***

The pH and temperature were recorded at 0.5 ($pH_{0h}$ and $Temperature_{0h}$) and 24 h ($pH_{24h}$ and $Temperature_{24h}$) postmortem using a portable pH meter, Pro2Go (Mettler Toledo, Columbus, Ohio, USA) and a digital food thermometer (-50 to + 300 °C), respectively. The pH meter was calibrated and inserted in the *Longissimus thoracis* muscle at 13$^{th}$ rib of the left carcass side about 2.5 cm depth. Following the 24-hour chilling of the carcasses, a sample of the *Longissimus* muscle was obtained from the region between the 6th and 13th ribs of the left half-carcass for analysis of qualitative characteristics, and the subcutaneous fat thickness (SFT) was measured at the level of the 12$^{th}$ rib after a cross-section in the *L. thoracis* with a manual caliper ruler (Starret®, Athol, Massachusetts, USA) and the rib eye area (REA) was delimited on a transparency film and REA was measure using ImageJ software (ImageJ 1.48g, Bethesda, Maryland, USA). The animal performance and carcass traits data with standard deviation (SD) are displayed in Table 2.

Samples were divided into seven portions of three one-inch (2.54 cm) thick steaks using a butcher band saw, properly weighted, labeled, and vacuum-packed individually. Three portions were immediately frozen at – 20 °C (unaged beef [0 d]), and four others were kept at 4 ± 1 °C until 7- and 14-days of aging (2 thick steaks for each), simulating typical Brazilian market conditions. After reaching the desired maturation time, the samples were kept frozen at – 20 °C until subsequent analysis (< 1 month of storage).

***Meat quality analyses***

All analyses were performed at the Meat Science Laboratory of the Department of Animal Science at the Universidade Federal de Viçosa. One steak from each unaged sample (1-day postmortem) was utilized to determine the proximate composition analysis. The steak edges were trimmed avoiding any subcutaneous and intermuscular fat, minced and freeze-dried. Later, dried samples were ground using a stainless ball mill. Total ash content was determined

using a muffle furnace set at 550 °C for 3h. Total fat content was determined in duplicate using Ankom XT4 filter bags and Ankom XT10 fat extractor machine (ANKOM Technologies, Macedon, NY) with petroleum ether as extraction solvent, and following the manufacturer recommendations. Dry matter was estimated (method 934.01; AOAC International, 2012), and moisture was obtained by difference (100 – dry matter content, %). The Kjeldahl method was utilized to determine crude protein (CP) (method 990.03 AOAC International, 2012).

Meat color parameter was obtained after steaks were thawed for 16 hours at 4 ± 1 °C and removed from vacuum packaging to sit in the air at room temperature for 30 min to enable the reoxygenation of muscle myoglobin. Meat color parameters were obtained from an average of five readings across the surface using a Hunter MiniScan EZ colorimeter (4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA). Readings were taken for the *L** (lightness), *a** (redness), and *b** (yellowness) ranges according to the CIELab scale. The estimated chroma and hue values were calculated using the equations provided in the American Meat Science Association (AMSA) Meat Color Measurement Guidelines (King et al., 2023): $Chroma = [(a^{*2} + b^{*2})^{0.5}]$ and *Hue = [(arctangent (b*/a*)].*

The wavelengths were used to determine the percentage of metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb) following AMSA equations (King et al., 2023): $\%MMb = \{1.395 - [(A572 - A730) \div (A525 - A730)]\} \times 100$; $\%DMb = \{2.375 \times [1 - (A474 - A730) \div (A525 - A730)]\} \times 100$; and *%OMb = 100 – (%MMb + %DMb).*

Thawed steak weight was estimated by the weight difference between frozen and thawed steaks. The same steaks previously thawed for meat color measurements were weighted, vacuum packed and cooked in a preheated water bath (Model NT 268, Novatecnica, Piracicaba, SP, Brazil) at 70 °C for 40 min. Then, the steaks were placed in an ice bath for 10 min to stop cooking and kept in refrigerator for 24 h. Lastly, they were removed from the package and weighed again to obtain water cooking loss. The estimation of total loss of water of each steak

was then performed using the following equation: Total water loss (%) = [(frozen steak weight – cooked steak weight) / frozen steak weight] x 100.

Warner-Bratzler shear force (WBSF) was determined using the cooked steaks after cooled for 24 h at 4 °C (AMSA, 1995). Six cylindrical samples (1.27 cm diameter) were removed parallel to the longitudinal orientation of the muscle fibers from each cooked steak, using a stainless-steel device for the extraction of samples (AMSA, 1995). A perpendicular incision of the muscle fibers of each cylinder of meat were performed to determine the shear force, by Warner-Bratzler shear device (G-R Electrical Manufacturing Company, Manhattan, KS, USA) equipped with a 1.016 mm thick V-notched (60° angle) cutting blade at a constant speed of 2 mm/s.

Sarcomere length was estimated following the helium-neon laser diffraction technique described by Cross et al. (1981). Six individual muscle fibers were teased from the muscle bundle using tweezers and were placed on a microscope slide with a drop of 0.2 M sucrose solution (0.2 M glucose and 0.1 M $NaHPO_4$ with pH 7). The slides prepared with the filaments were placed on a suspended holder where the laser diffraction (632.8 nm) was incident on the filaments, using a 05-LHR-021 laser (Melles Griot, Carlsbad, CA, USA). The diffraction bands were drawn on white paper beneath the holder, obtaining six diffraction bands for each sample, with the average value used to obtain the sarcomere length according to the following equation: Sarcomere length (μm) = [0.6328 x D x $\sqrt{}$(T/D)2 + 1]/T; in which: D = distance in mm from the specimen-holding device to the screen (throughout this experiment, a value of 120 mm was used) and T = the separation (mm) between the zero and the first maximum band.

The thiobarbituric acid-reactive substance (TBARS) content was determined using the method of Buege and Aust (1978). This involved homogenizing 5 g of strip loin sample with 15 mL of distilled water, followed by the addition of 50 μL of 7.2% butylated hydroxyanisole to 1 mL of the homogenate to inhibit oxidation. Then, 2 mL of this mixture was combined with

184 TCA/TBA reagent, heated at 90 °C for 15 min, and centrifuged at 2000 × *g* for 10 min. The
185 absorbance of the supernatant was measured at 531 nm using a UV/VIS spectrophotometer
186 (Molecular Devices, M2e, Sunnyvale, CA, USA). A blank sample was prepared similarly with
187 distilled water only.

188 ***Statistical analysis***

189 All statistical analyses were performed using SAS University Edition (SAS/STAT®, SAS
190 Institute Inc., NC, USA). Dietary treatment effects were evaluated through assessed using the
191 Tukey Test. The effect of aging (0 [unaged beef], 7 and 14 days) on instrumental meat color;
192 WBSF; thawing, cooking and total water losses and lipid oxidation was analyzed, and
193 differences between the means for different aging times were also assessed using the Tukey
194 Test. Diet (and aging, when applicable) was considered as fixed effect and the animals as
195 random effect. The diets and diet × aging interaction means were computed using the
196 LSMEANS option. For both analyses the effects were considered as significant when $P \leq 0.05$.

197

198 **RESULTS**

199 The HP-DDG inclusion did not affected ($P > 0.05$) pH, before (6.68, on average) and
200 after (5.82, on average) the *rigor mortis* (Table 3). In addition, no changes ($P > 0.05$) in SFT
201 (0.99 cm, on average), and final sarcomere length (1.55 µm, on average) were observed by the
202 diet effect. For the chemical composition (moisture, crude protein, ether extract and ash), no
203 differences were observed ($P > 0.05$) by the HP-DDG diet inclusion (Table 4).

204 The diet inclusion of 12% HP-DDG (39.6) reduced $L^*$ parameter (lightness; Table 5;
205 $P = 0.001$) compared to the other diets (42.0, on average). On the other hand, an increase was
206 observed ($P = 0.001$) in the $L^*$ values after the aging period of 7 (41.7) and 14 days (42.6),
207 compared to the unaged beef (0 days; 39.8). The $a^*$ (redness) was reduced ($P = 0.001$) after 14
208 days (12.3) compared to unaged (13.7) and 7 days of aging (13.7), while the $b^*$ (yellowness)
209 values were greater ($P = 0.001$) for the 7 (11.8) and 14 days (11.9) compared to unaged beef

(11.0). Greater *chroma* was observed ($P = 0.001$) after 7 days (18.1) compared to unaged (17.6) and 14 days of aging (17.2), while greater *hue* angle was observed ($P = 0.001$) for unaged beef (51.5°), intermediate for 7 days (49.3°), and lower values for 14 days of aging (46.1°).

Regarding the color of the backfat of *L. dorsi*, lower $L^*$ values ($P = 0.01$; Table 5) were observed for the aging periods of 7 days (66.5) and 14 days (66.6) compared to unaged beef (67.8). In addition, greater $a^*$ ($P = 0.01$) were observed for the aging of 7 days (6.84) and 14 days (6.99) compared to unaged beef (6.27). For the $b^*$ parameter, lower values were observed ($P = 0.001$) after 14 days (15.2) compared to unaged (16.7) and 7 days of aging (16.2). Similarly, a reduction ($P = 0.001$) in *chroma* values occurred after 14 days (16.8) compared to unaged (17.9) and 7 days of aging (17.7). Greater *hue* angles were observed ($P = 0.001$) after 14 days (24.6°), intermediate for 7 days (22.8°), and lower for unaged beef (20.4°).

The inclusion of 8% HP-DDG (53.1 %) in the finishing diets promoted an increase ($P = 0.03$; Table 6) in the percentage of OMb compared to 12% inclusion (46.9 %), while 0% and 4% of inclusion (51.1 %, on average) were similar to all treatments. However, HP-DDG inclusion did not alter ($P > 0.05$) the percentages of MMb (26.4%, on average) and DMb (23.1%, on average). The 14-day aging period had the greatest ($P = 0.001$) percentages of MMb (30.8 %), with 7 days of aging promoting intermediate percentages (25.9 %), and unaged beef had the lowest (22.4 %). Greater percentages of DMb were observed ($P = 0.001$) for unaged (25.8 %), intermediate percentages for 7 days (20.3 %), and lower for 14 days of aging (22.9 %). Lower percentages of OMb were observed ($P = 0.001$) after 14 days (46.2 %) compared to unaged (51.8 %) and 7 days of aging (53.8 %).

Aging and diet did not affect ($P > 0.05$; Table 7) cooking (17.6%, on average) and total water losses (26.8 %, on average). An aging effect was observed ($P = 0.05$) for thawing loss, but no differences between days were observed after Tukey corrections (9.21 %, on average). There was a reduction ($P = 0.001$; Table 8) in WBSF values after 7 days (3.48 kgf)

and 14 days (3.20 kgf) compared to unaged beef (4.92 kgf). An aging effect ($P$ = 0.001) was also observed for TBARS, with the greatest concentration at 14 days of aging (1.36 mg MDA/kg), intermediate at 7 days of aging (0.895 mg MDA/kg), and the lowest value for unaged beef (0.568 mg MDA/kg). No diet effect was observed for WBSF (3.87 kgf) and TBARS (0.941 mg MDA/kg) concentrations.

## DISCUSSION

While the majority of the literature has concentrated on evaluating diets with varying proportions of dent corn and either DDGS or WDGS—predominantly under conditions in the United States—there has been a marked increase in recent studies exploring the inclusion of corn distillers' grains in diets for Bos indicus cattle. (Silva et al., 2021; Hoffman et al., 2021; Sousa et al., 2024; Dias et al., 2024). However, limited data exists comparing the use of HP-DDG to replace SBM, in diets with flint corn under tropical conditions. This study represents a pioneering effort to explore the effects to explore the effects of HP-DDG on carcass traits and meat quality of Nellore beef cattle fed high-concentrate diets within Brazilian feedlot systems.

From a nutritional perspective, the diets were formulated using prediction equations who suggest that the requirements for CP, RDP and RUP would amount to 947, 730 and 344 g/day, respectively, for Zebu beef cattle with an average weight of 367 kg and an estimated daily gain of 1.2 kg/day (Valadares Filho et al., 2016). In addition, our study primarily aims to analyze the effects of these substitutions on meat quality, and thus, the diets were formulated to be isoenergetic and isonitrogenous.

Inclusion of HP-DDG is expected to enhance metabolizable protein balance due to the elevated supply of amino acids to the small intestine. These amino acids can serve as an energy source and facilitate the recycling of urea to fulfill RDP needs in the rumen (Kelzer et al., 2010; Ferreira et al., 2020; Pouzo et al., 2023). Apparently, this mechanism might have compensated

for the reduction in RDP and starch (less ground corn) in the diets (Table 1) as DDG was incorporated. Additionally, most feedlots in Brazil use flint corn in their finishing diets with coarse grinding (Pinto and Millen, 2019), and probably RDP requirements may be reduced because of the lower energy supply (Ferreira et al., 2020).

Nonetheless, despite the lower energy content of HP-DDG (without the addition of solubles), our findings align with previous research indicating similar HCW, dressing percentage, REA, and BFT when SBM (Pittaluga et al., 2021; Dahmer et al., 2022) and cottonseed meal (Hoffman et al., 2021) were replaced by DDGS. Ross et al. (2024) observed similar DMI, ADG, HCW and BFT, when replaced SBM by DDGS, however, the dressing percentage and REA were higher and lower, respectively, for DDGS diet. In addition, the lack of dietary effects on meat proximal composition aligns with other report (Hart et al., 2019). These results underscore the considerable potential of HP-DDG as a substitute for soybean meal in finishing diets.

The WBSF serves as an indirect measure of meat tenderness, influenced by factors such as sarcomere length, which exhibits a positive correlation with tenderness (Cross et al., 1981; Rhee et al., 2004). A sarcomere may range in length from about 1.5μm–2.7μm, being fully compressed and fully extended sarcomere, respectively (Braden, 2013), while sarcomere length of at least 2μm is accompanied by increased tenderness (Wheeler et al., 2000). In our study, sarcomere length was not affected by the treatments, and the average was 1.54 μm, probably influenced by the lower BFT observed in Nellore carcasses. Concomitantly, WBSF values indicated tough meat tenderness as classified by Sullivan and Calkins (2011), who categorize values above 4.6 kgf as tough, with no differences between diets, in consistency with findings from other studies involving distilled grains (Koger et al., 2010; Mello et al., 2018; Ribeiro et al., 2019).

However, postmortem muscle proteolysis, influenced by diet, also impacts tenderness (Lonergan et al., 2010). Aging is known to enhance myofibrillar proteolysis in younger cattle,

285 as evidenced in previous research (Ribeiro et al., 2019; Silva et al., 2019a), and the decrease of
286 WBSF with aging indicated that aging decreased the toughness of the steaks, which could then
287 be classified as tender after 7 days of aging (< 3.9 kgf; Sullivan and Calkins, 2011). The ten-
288 derization could be related to weakening of myofibrillar proteins (as troponin) by either endog-
289 enous enzymes (Koohmaraie and Geesink, 2006; Silva et al., 2019b) or ionic solubilization
290 (Takahashi, 1996). Recently, Senaratne (2012) observed an increase in sarcoplasmic calcium
291 concentration with prolonged aging, with calcium playing a crucial role in meat tenderization,
292 as the calpain system, primarily responsible for myofibrillar protein proteolysis, requires cal-
293 cium for activation (Koohmaraie and Geesink, 2006; Lonergan et al., 2010).

294 Cooking loss decreased linearly at 7 days of aging with the inclusion of HP-DDG, likely
295 indicating an increase in juiciness. Increased water-holding capacity and decreased thawing loss
296 is expected after aging since postmortem proteolysis can enhance water binding and entrapment
297 by muscle cells (Aroeira et al., 2016; Kristensen and Purslow, 2001). However, no changes
298 between aging in thawing loss, cooking loss, and total loss were observed in the present study.

299 The $pH_{24h}$ of the carcasses from animals that received DDG in their diet was slightly
300 higher than 5.8, indicating a moderate risk for dark-cutting (Holdstock et al., 2014). In addition,
301 diet effects were observed for *L** and *b**, with the 12% HP-DDG inclusion reducing both pa-
302 rameters. The inclusion of HP-DDG reduces the starch content of the diets linearly (Table 1)
303 due to a lower percentage of ground corn and the removal of starch during ethanol production.
304 This reduction in starch content decreases the glycolytic potential and fat deposition, resulting
305 in those differences, as postmortem muscle color is directly associated with muscle glycogen
306 content and ultimate muscle pH (Koger et al., 2010). In addition, oxygen can bind less effi-
307 ciently to myoglobin, diminishing oxymyoglobin percentages (King et al., 2012). Several au-
308 thors agree that including DDGS during finishing phase reduces *a** and *L** color values (De-
309 penbusch et al., 2009; Segers et al., 2011). However, this does not guarantee that meat quality

was critically impacted during the conversion of muscle into meat during the early postmortem stage and does not guarantee that these differences are visually perceptible. This is especially true considering that the observed fresh meat color indicates normal meat attributes that are acceptable to consumers ($L^* \geq 34$, $a^* \geq 9.5$, Khliji et al., 2010; Ponnampalam et al., 2017), in addition to the absence of WBSF differences between dietary effects.

Aging increased the *L** and *b** and decreased *a** and *hue* meat values, making the meat appear less vibrant and appealing, with increased percentages of metmyoglobin. Those results are expected, as prolonged aging time combined with exposure to both oxygen and light (pro-oxidants) can result in oxidation of oxymyoglobin (bright cherry red) into the brownish color associated with metmyoglobin, resulting in discoloration of meat (Seideman et al., 1984), regardless of the dietary treatments, as observed in other studies (Mello et al., 2018; Koger et al., 2010). Chroma represents the color intensity, describing how vivid or dull the color is, and is a good indicator of the oxygenation of meat recently exposed to air (Aroeira et al., 2017). An increase in *chroma* and a reduction in deoxymyoglobin was observed after 7 days of exposure compared to unaged and 14 days of aging, but without any apparent biological explanation. Chroma and *b** of backfat were decreased due lipid and carotenoids oxidation, which contributed to paler fat appearance while allowing red pigments to become more visible, while some oxidation products absorb more light, reducing *L** (Domínguez et al., 2019). The hue angle is based on the arctangent of the *b**/*a** ratio, and their increase in backfat due aging represents a shift in the balance of color toward a reddish or pinkish hue, with less yellow influence (King et al., 2023).

Meat color is commonly used by consumers as a parameter of freshness and quality, especially at the time of purchase, when other sensory attributes have not yet been evaluated. After purchasing meat, consumers can detect lipid oxidation through the presence of off-flavors, which involves the oxidation of unsaturated fatty acids in phospholipids (Gray et al.,

335 1996). Previous studies have shown that feeding DDGS can increase polyunsaturated fatty acid
336 concentrations in beef, leading to faster lipid oxidation and discoloration during retail shelf life
337 (Depenbusch et al., 2009; Koger et al., 2010), which may deter consumer purchases. The inclu-
338 sions of HP-DDG were lower than 15 % DM, and as previously reported, HP-DDG contains
339 less corn oil than DDGS (3-4% and 10-11% EE, respectively; Diaz et al., 2012). Because of
340 that, TBARS was only affected by aging. However, the values of TBARS were all lower than
341 the threshold value of 2 mg malondialdehyde per kg of meat that would be necessary for con-
342 sumers to reject the meat (Campo et al., 2006). Unfortunately, the fatty acid composition was
343 not analyzed in the present study, making it difficult to provide specific explanations for lipid
344 oxidation.
345
346 **CONCLUSIONS**
347 The HP-DDG can replace SBM in Nellore bulls finishing feedlot diets without
348 compromising meat quality. In addition, aging increases tenderness and promotes changes in
349 meat color and lipid oxidation.
350

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534

535 **TABLES**

536

Table 1. Proportions of ingredients and chemical composition of nutrients in experimental diets

| Item | Concentrate | | | | Diets | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 4% DDG | 8% DGG | 12% DDG | Control | 4% DDG | 8% DGG | 12% DDG |
| | Proportion of ingredients (g/kg DM) | | | | | | | |
| Corn silage | - | - | - | - | 200 | 200 | 200 | 200 |
| Ground corn | 874 | 854 | 833 | 812 | 699 | 683 | 666 | 650 |
| HP-DGG | - | 50.0 | 100 | 150 | 0.0 | 40.0 | 80.0 | 120 |
| Soybean meal | 74.9 | 49.9 | 25.0 | 0.0 | 59.9 | 40.0 | 20.0 | 0.0 |
| Ureia + AS[2] | 13.2 | 8.8 | 4.4 | 0.0 | 10.6 | 7.1 | 3.5 | 0.0 |
| Mineral premix[3;4] | 37.5 | 37.5 | 37.5 | 37.5 | 30.0 | 30.0 | 30.0 | 30.0 |
| | Chemical composition (g/kg DM)[1] | | | | | | | |
| DM | 890 | 892 | 895 | 897 | 623 | 623 | 624 | 625 |
| ASH | 52.7 | 51.8 | 50.7 | 49.6 | 54.2 | 53.5 | 52.6 | 51.8 |
| OM | 947 | 948 | 949 | 951 | 946 | 947 | 947 | 948 |
| CP | 143 | 143 | 142 | 142 | 130 | 130 | 129 | 129 |
| EE | 29.2 | 32.1 | 35.0 | 37.9 | 29.3 | 31.6 | 33.9 | 36.2 |
| NDFap | 136 | 145 | 154 | 164 | 222 | 229 | 237 | 244 |
| NFC | 655 | 648 | 641 | 605 | 581 | 575 | 570 | 541 |
| N | 22.8 | 22.4 | 22.2 | 21.9 | 20.7 | 20.4 | 20.2 | 20.0 |

[1]DM = Dry matter; ASH = Ash; OM = Organic Matter; CP = Crude Protein; EE = Ethereal Extract; NDFap = Neutral detergent fiber corrected for ash and crude protein; NFC = Non-fibrous carbohydrates; N = Nitrogen.

[2]Urea/ammonium sulfate (AS) ratio of 9:1.

[3]Basic composition of the product: Calcitic limestone; Sodium chloride (Common salt); Ventilated sulfur; Monodicalcium phosphate; Magnesium oxide; Probiotic additive; Raspberry aroma; Mixed herbal aroma; Sodium bicarbonate; Silicon dioxide; Onion extract; Grape seed extract; Calcium iodate; Monensin sodium; Manganese oxide; Zinc oxide; Sodium Selenite; Cobalt sulfate; Copper sulfate; Iron sulfate; Vehicle; Vitamin A; Vitamin D3; Vitamin E.

[4]Guarantee levels per kg of product: Mixed Herbal Flavor (min) 5.00MG; Onion extract (min) 9.00MG; Grape seed extract (min) 1.60MG; Monensin 1038.00MG; Saccharomyces cerevisiae (min) 100X 10E9 UFC, Calcium (min) 198.00G; Calcium (max) 290.00G; Sulfur (min) 24.50G; Fluoride (max)

222.75MG; Phosphorus (min) 11.00G; Magnesium (min) 19.00G; Sodium (min) 61.50G; Cobalt (min) 11.10MG; Copper (min) 335.00MG; Iron (min) 370.00MG; Iodine (min) 27.70MG; Manganese (min) 1668.00MG; Selenium (min) 7.40MG; Vitamin A (min) 112000.00UI; Vitamin D3 (min) 14900.00UI; Vitamin E (min) 136.00UI; Zinc (min) 2223.00MG.

537
538

539 Table 2. Animal performance and carcass characteristics of bulls fed diets containing either 0,
540 4, 8, or 12% high-protein dried distiller's grains (HP-DDG).

| Item | HP-DDG diet inclusion (DM basis) | | | | SD |
|---|---|---|---|---|---|
| | 0% | 4% | 8% | 12% | |
| IBW, kg | 303 | 303 | 302 | 297 | 29.6 |
| FBW, kg | 447 | 446 | 446 | 427 | 34.7 |
| HCW, kg | 265 | 264 | 264 | 251 | 22.0 |
| HCD, % | 59.1 | 59.0 | 59.3 | 58.7 | 1.31 |
| CCW, kg | 259 | 257 | 257 | 244 | 22.3 |
| CCD, % | 57.9 | 57.6 | 57.7 | 57.2 | 1.48 |
| REA, $cm^2$ | 81.0 | 77.5 | 80.5 | 74.7 | 8.37 |

541 IBW = initial body weight; FBW = final body weight; HCW = hot carcass weight; HCD = hot
542 carcass dressing; CCW = cold carcass weight; CCD = cold carcass dressing; REA = rib eye
543 area; SD: standard deviation.

544 Table 3. Proximal composition, pH and sarcomere length of *Longissimus thoracis* muscle
545 from bulls-fed diets containing either 0, 4, 8, or 12% high-protein dried distiller's grains (HP-
546 DDG).

| Item | HP-DDG diet inclusion (DM basis) | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|
| | 0% | 4% | 8% | 12% | | |
| $pH_{0.5h}$ | 6.62 | 6.74 | 6.72 | 6.64 | 0.0642 | 0.40 |
| $pH_{24h}$ | 5.70 | 5.86 | 5.86 | 5.86 | 0.0886 | 0.50 |
| SFT, cm | 1.06 | 0.982 | 0.982 | 0.973 | 0.0654 | 0.72 |
| Sarcomere length (μm) | 1.570 | 1.570 | 1.541 | 1.502 | 0.0373 | 0.53 |

547 SFT = subcutaneous fat thickness; SEM = standard error of the mean.
548 Means within a row with different online letters (a-b) differ ($P \leq 0.05$).
549

550 Table 4. Proximal composition, pH and sarcomere length of *Longissimus thoracis* muscle
551 from bulls-fed diets containing either 0, 4, 8, or 12% high-protein dried distiller's grains (HP-
552 DDG).

| Item | HP-DDG diet inclusion (DM basis) | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|
| | 0% | 4% | 8% | 12% | | |
| Moisture (%) | 76.4 | 76.8 | 76.7 | 77.0 | 0.259 | 0.52 |
| CP (%) | 19.5 | 19.0 | 19.4 | 19.4 | 0.192 | 0.30 |
| EE (%) | 4.74 | 4.85 | 4.48 | 4.44 | 0.231 | 0.52 |
| Ash (%) | 5.48 | 5.64 | 5.36 | 5.12 | 0.228 | 0.38 |

553 SEM = standard error of the mean.

554 Means within a row with different online letters (a-b) differ ($P \leq 0.05$).

555

556 Table 5. Meat color of *Longissimus thoracis* muscle from bulls-fed diets containing either 0,
557 4, 8, or 12% high-protein dried distiller's grains (HP-DDG) and submitted to 0, 7 or 14 d of
558 aging.

| Item | HP-DDG inclusion (DM basis) | | | | Aging (days) | | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 4% | 8% | 12% | 0 | 7 | 14 | | Diet | Aging | D x A |
| | | | | | Meat | | | | | | |
| *L** | 42.1a | 41.7a | 42.2a | 39.6b | 39.8b | 41.7a | 42.6a | 0.894 | 0.001 | 0.001 | 0.99 |
| *a** | 13.3 | 13.3 | 13.5 | 12.9 | 13.7a | 13.7a | 12.3b | 0.351 | 0.20 | 0.001 | 0.52 |
| *b** | 11.9a | 11.7a | 12.0a | 10.7b | 11.0b | 11.8a | 11.9a | 0.478 | 0.002 | 0.008 | 0.97 |
| *Chroma* | 17.9 | 17.7 | 18.1 | 16.8 | 17.6b | 18.1a | 17.2b | 0.498 | 0.16 | 0.001 | 0.28 |
| *Hue* | 48.0 | 48.8 | 48.6 | 50.7 | 51.5a | 49.3b | 46.1c | 1.113 | 0.28 | 0.001 | 0.98 |
| | | | | | Backfat | | | | | | |
| *L** | 67.4 | 67.4 | 66.8 | 66.1 | 67.8a | 66.5b | 66.6b | 0.709 | 0.08 | 0.01 | 0.98 |
| *a** | 6.54 | 6.93 | 6.55 | 6.78 | 6.27b | 6.84a | 6.99a | 0.345 | 0.43 | 0.01 | 0.70 |
| *b** | 15.8 | 16.1 | 16.4 | 16.0 | 16.7a | 16.2a | 15.2b | 0.399 | 0.37 | 0.001 | 0.67 |
| *Chroma* | 17.2 | 17.6 | 17.7 | 17.4 | 17.9a | 17.7a | 16.8b | 0.435 | 0.74 | 0.001 | 0.30 |
| *Hue* | 22.4 | 23.3 | 21.7 | 23.0 | 20.4c | 22.8b | 24.6a | 0.999 | 0.35 | 0.001 | 0.86 |

559 SEM = standard error of the mean.

560 Means within a row with different online letters (a-b) differ ($P \leq 0.05$).

561

562 Table 6. Myoglobin oxidation of *Longissimus thoracis* muscle from bulls-fed diets containing
563 either 0, 4, 8, or 12% high-protein dried distiller's grains (HP-DDG) and submitted to 0, 7 or
564 14 d of aging.

| Item | HP-DDG inclusion (DM basis) | | | | Aging (days) | | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 4% | 8% | 12% | 0 | 7 | 14 | | Diet | Aging | D x A |
| | | | | | Meat | | | | | | |
| MMb (%) | 27.8 | 26.3 | 25.2 | 26.2 | 22.4c | 25.9b | 30.8a | 1.896 | 0.70 | 0.001 | 0.49 |
| DMb (%) | 20.3 | 23.7 | 21.7 | 26.8 | 25.8a | 20.3c | 22.9b | 2.867 | 0.32 | 0.001 | 0.92 |
| OMb (%) | 51.9ab | 50.4ab | 53.1a | 46.9b | 51.8a | 53.8a | 46.2b | 1.935 | 0.03 | 0.001 | 0.16 |

565 MMb = metmyoglobin; DMb = deoxymyoglobin; OMb = oxymyoglobin; SEM = standard
566 error of the mean.
567 Means within a row with different online letters (a-b) differ ($P \leq 0.05$).
568

569 Table 7. Water losses of *Longissimus thoracis* muscle from bulls-fed diets containing either 0,
570 4, 8, or 12% high-protein dried distiller's grains (HP-DDG) and submitted to 0, 7 or 14 d of
571 aging.

| Item | HP-DDG inclusion (DM basis) | | | | Aging (days) | | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 4% | 8% | 12% | 0 | 7 | 14 | | Diet | Aging | D x A |
| Thaw loss, % | 9.04 | 9.25 | 9.44 | 9.10 | 9.53 | 8.62 | 9.48 | 0.560 | 0.81 | 0.05 | 0.43 |
| Cooking loss, % | 17.5 | 18.3 | 17.7 | 16.8 | 17.4 | 17.9 | 17.5 | 0.820 | 0.14 | 0.61 | 0.78 |
| Total loss, % | 26.6 | 27.5 | 27.1 | 25.9 | 26.9 | 26.5 | 26.9 | 1.110 | 0.26 | 0.80 | 0.71 |

572 SEM = standard error of the mean.

573 Means within a row with different online letters (a-b) differ ($P \leq 0.05$).

574

575 Table 8. Warner Bratzler shear force (WBSF) and thiobarbituric acid reactive substances
576 (TBARS) of *Longissimus thoracis* muscle from bulls-fed diets containing either 0, 4, 8, or
577 12% high-protein dried distiller's grains (HP-DDG) and submitted to 0, 7 or 14 d of aging.

| Item | HP-DDG inclusion (DM basis) | | | | Aging (days) | | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | 4% | 8% | 12% | 0 | 7 | 14 | | Diet | Aging | D x A |
| WBSF, kgf | 4.14 | 3.91 | 3.76 | 3.66 | 4.92a | 3.48b | 3.20b | 0.269 | 0.13 | 0.001 | 0.75 |
| TBARS, mg MDA/kg | 0.982 | 1.04 | 0.878 | 0.864 | 0.568c | 0.895b | 1.36a | 0.113 | 0.15 | 0.001 | 0.79 |

578 SEM = standard error of the mean.
579 Means within a row with different online letters (a-b) differ ($P \leq 0.05$).
580