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Effect of non-protein nitrogen supplementation and maturation time in the physicochemical quality of *Longissimus dorsi* muscle of grazing cattle

Efeito da suplementação com nitrogênio não proteico fornecida a pastejo e do tempo de maturação da carne na qualidade físico-química do músculo *Longissimus dorsi* de bovinos

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Highlights

Non-protein nitrogen levels affected tenderness, luminosity, and meat color.
Unsupplemented beef cattle showed a lower cooking loss.
Longissimus dorsi tenderness improved when matured for up to nine days.

Abstract

This study accessed the effect of non-protein nitrogen (NPN) levels in concentrates fed to grazing beef cattle (½ Angus + ½ Nellore) on physicochemical traits of meat subjected to different maturation times. A total of 108 steaks of the *Longissimus dorsi* muscle from 36 entire animals (approximately 20 months old and 400.33 ± 40.87 kg BW) were used in a completely randomized design with a factorial arrangement, with twelve treatments formed by the combination of four pre-slaughter diets and three meat maturation times (one steak per experimental unit totaling nine replications). A diet with no

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supplementation was used as the negative control, and the other three experimental diets contained pasture plus concentrated supplementation (low, medium, and high NPN). No interaction ($p > 0.05$) between diet and maturation times (MT) was observed for luminosity, red to green component, yellow to blue component, pH, and shear force in the meat. Regardless of the MT, unsupplemented animals showed lower ($p < 0.05$) cooking loss (5.92%) and harder meats (3.32 kgf cm^{-3}) than those fed the medium NPN concentrate (2.62 kgf cm^{-3}). Animals fed the diet with low NPN had meats with greater ($p < 0.05$) red pigmentation, as well as lighter post-cooking steaks ($p < 0.05$) than those fed no concentrate and the medium NPN concentrate. Regardless of the NPN level classes, up to nine days of maturation effectively increases the tenderness of cattle steaks. Crossbred cattle (½ Angus + ½ Nellore) fed diets with low or medium non-protein nitrogen supplementation have steaks with better red to green component and tenderness, respectively.
Key words: Meat color. Pasture. Tenderness. Urea.

Resumo

Objetivou-se avaliar o efeito da inclusão de nitrogênio não proteico (NNP) no suplemento e a influência de diferentes tempos de maturação sobre características físico-químicas no músculo de bovinos de corte (½ Angus + ½ Nelore) em pastejo. Foram utilizados 108 bifes do músculo *Longissimus dorsi,* retirados de 36 bovinos não castrados, com aproximadamente 20 meses de idade e peso corporal inicial de 400,33 ± 40,87 kg, em delineamento experimental inteiramente casualizado e esquema fatorial, com doze tratamentos, constituídos pela combinação de quatro dietas pré-abate e três tempos de maturação da carne, sendo um bife por unidade experimental totalizando nove repetições. As dietas pré-abate foram compostas por pasto e suplementação concentrada com baixo, médio e alto teor de NNP e controle (não suplementado). Não houve interação ($p > 0,05$) entre dietas e tempos de maturação (TM) sobre a luminosidade, teor de vermelho e intensidade de amarelo, pH e força de cisalhamento da carne. Independente dos TM, animais não suplementados apresentaram menor ($p < 0,05$) perda de água por cocção (5,92%) e carnes mais duras (3,32 kgf cm^{-3}) que bovinos que receberam dieta com média concentração de NNP (2,62 kgf cm^{-3}). Os animais que receberam dietas com baixa suplementação de NNP apresentaram carnes com maior ($p < 0,05$) pigmentação vermelha, além de bifes mais leves ($p < 0,05$) no pós-cocção do que bovinos não suplementados e aqueles alimentados com dieta contendo média suplementação de NNP. Independente das classes de NNP, a maturação realizada até o 9º dia é eficaz para aumentar a maciez da carne dos bovinos. Bovinos cruzados (½ Angus + ½ Nelore) que consomem dietas com baixa ou média suplementação de nitrogênio não proteico apresentam bifes com melhores condições de intensidade de cor vermelha e de maciez, respectivamente.
Palavras-chave: Coloração de carne. Maciez. Pasto. Ureia.

Introduction

Tropical pastures usually have insufficient protein content to meet the growth potential of beef cattle, making protein supplementation necessary to improve productive performance (Detmann et al., 2014). Non-protein nitrogen (NPN) sources are commonly used to replace true protein in ruminant diets because rumen microorganisms convert NPN into protein of high biological value (Santos & Mendonça, 2011). This nutritional practice can correct forage nutrient deficits, aiming to maximize nitrogen usage by rumen microorganisms and enhance weight gain at early animal ages. However, over the years, there has been concern about dietary supplements and changes in carcass composition and meat quality.

Meat quality encompasses juiciness, tenderness, and color (Lima et al., 2011). Tenderness is considered the most important sensory attribute during beef consumption (Bernardo et al., 2021), while color is the most important when purchasing, as it is the first trait consumers perceive (Pitombo et al., 2013). These attributes can be influenced by the production system in which the animals are raised (Kim et al., 2017), intrinsic (muscle physiology, breed, and age) and extrinsic (feeding, production system, and pre-slaughter and post-mortem conditions) individual factors or their interaction (Farias et al., 2018).

Maturation time can also influence meat quality (Mateus et al., 2018), and in this sense, strategies have been studied to enhance beef physicochemical parameters. Such procedures guarantee improved meat quality if performed properly within the right maturation time to avoid product damage. The vacuum-packed technique is one of the meat preservative proposals (for a few days or weeks) to keep the main physical traits, improve tenderness, and reduce microbiological contamination (Mateus et al., 2018).

Studies addressing the effects of NPN supplementation on the meat quality of beef cattle grazing tropical pastures are scarce in the scientific literature. Thus, this study assessed the effects of increasing dietary levels of NPN on the physicochemical traits of the *Longissimus dorsi* muscle at different maturation times in grazing beef cattle.

Material and Methods

Location of the experiment

All animal procedures were approved by the Ethics Committee on the Use of Animals in Experimentation at the Universidade Estadual do Oeste do Paraná - UNIOESTE (protocol no. 13/2017), Marechal Cândido Rondon, PR, Brazil. The experiment was conducted in the Beef Cattle Center of UNIOESTE, located in Entre Rios do Oeste, PR, Brazil (24°40'34"S and 54°16'39"W) at an average altitude of 261 m. The climate is warm and temperate, classified as Cfa according to the Koppen and Geiger scale (Alvares et al., 2013).

Experimental design and treatments

A total of 108 steaks obtained from the *Longissimus dorsi* muscle from 36 entire beef cattle were used (½ Angus + ½ Nelore, approximately 20 months old, and 400.33

± 40.87 kg of body weight at the beginning of the experiment). Animals were allotted to a completely randomized design with a 4 × 3 factorial arrangement (four pre-slaughter diets and three meat aging times) with twelve treatments, nine replicates, and one steak per experimental unit. All animals were fed a basal diet composed of *Brachiaria brizantha* cv. Xaraés supplemented with concentrates containing different levels of crude protein (CP) in the form of non-protein nitrogen (NPN). Experimental diets were as follows: 1) no concentrate supplementation (negative control; NS); 2) concentrate containing 18% CP (low NPN); 3) concentrate containing 42% CP (medium NPN); and 4) concentrate containing 78% CP (high NPN). The *Longissimus dorsi* muscle steaks obtained from these animals were subjected to three maturation times (MT; 3, 9, and 16 days).

Animals grazed an 8-hectare pasture split into four paddocks. Animals were adapted to experimental diets for 14 days. The concentrate contained corn, soybean meal, urea, and granulated ammonium sulfate and was formulated to contain 30% CP (Table 1). Concentrate was offered daily at 10:00 for 84 days based on BW (0.6%) with adjustments every 28 days. All animals had free access to mineral mixture and water.

Table 1
Chemical composition of pasture and concentrates

		Concentrate[1]		
Ingredients (%)	Pasture	Low NPN	Medium NPN	High NPN
Ground corn	-	45.67	66.24	91.50
Soybean meal	-	54.33	30.10	0.00
Urea and Ammonium Sulphate (9:1)	-	0.00	3.66	8.50
Crude protein (% MS)	9.00	30.70	30.40	30.80
NPN (% N total)	24.30	17.70	42.10	78.00

[1]Low, medium, and high NPN: concentrate containing 18%, 42%, and 78% non-protein nitrogen (NPN; total N%), respectively.

Pasture samples were collected every 28 days via hand plunking (grazing simulation technique). The concentrate ingredients and pasture samples were dried at 55 ºC for 72 h, ground, and analyzed for dry matter, CP, and NPN content as previously described by Detmann et al. (2021).

Animal slaughter and steak sampling

Animals were weighed at 16:00 (no fasting) and then transported in a truck (1.53 m^2 per animal) to a commercial slaughterhouse where they were kept in pens (2.5 m^2 per animal) and subjected to a 14 h fasting (solids and liquids).

The slaughter was carried out on days 85, 124, 131, and 158 of the experiment. A similar number of animals per treatment was slaughtered on each day. Animals were subjected to experimental treatments until the slaughter, which was performed via stunning using a pneumatic penetrating captive bolt gun followed by exsanguination (jugular and carotid cuts).

Carcasses were cooled at 4.0 ºC for 24 hours. Then, the *Longissimus dorsi* muscle was sampled between the 12th and 13th ribs from each carcass (left half). Samples were identified, packed in plastic bags, placed on ice in a thermal box, and transported (5 km) to the food laboratory of Unioeste.

Three replicate steaks (2.54 cm thick) were extracted from each muscle in the shape of the original piece. Cuts followed the parallel direction of muscle fibers (Wheeler et al., 1997). The replicates were individually vacuum-packed in plastic bags, identified with the respective maturation time, and subjected to air removal with the aid of a sealing machine.

pH of steaks

After 3, 9, and 16 days of maturation, pH was measured at the package opening using a portable digital pH meter with an insertion electrode (Asko, model AK103, São Leopoldo, RS, Brazil) in *Longissimus dorsi* muscle obtained from the left half carcass between the 12th and 13th ribs.

Steak samples were withdrawn from the refrigerator and remained at room temperature for 20 minutes to be oxygenated and recover normal color. Then, the electrode was inserted, and two pH readings were performed in steaks at each maturation time. Mean values were used in data analysis.

Meat color

The *post-mortem* color of *Longissimus dorsi* steaks was assessed according to the CIELAB color system using a portable colorimeter (Konica Minolta, model CR-400). Color parameters were measured as L* (luminosity), which expresses the brightness or reflectance rate, in which the values ranged from 0 (black) to 100 (white); a* (red-green component); and b* (yellow-blue component). Three readings were taken per sample to obtain a mean value.

Cooking loss

After opening the vacuum packages, the uncooked matured weight of steaks (UMW; g) was recorded using a semi-analytical scale (SHIMADZU, model UX-4200H). Then, steaks were placed in oven trays (covered with aluminum foil) containing small holes to drain liquid exudates. The oven was preheated to 100 °C, and this temperature was kept throughout cooking. The internal temperature was measured using a digital stick thermometer inserted in the center of the steaks. After reaching 40 °C, steak samples were turned over and kept in the oven until 71 °C was reached (Bridi, 2006). Subsequently, steaks were removed from the oven, cooled at room temperature, wrapped in plastic films, placed in styrofoam trays, and kept at 4.0 ºC for 24 hours. Immediately after cooling, the post-cooking weight of steaks (PCW; g) was recorded using the same semi-analytical scale mentioned before.

The PCW was corrected for a 190 g steak using the following equation: PCCW = (PCW × 190) ÷ UMW , where PCCW is the weight of matured steaks after cooking

corrected for a 190 g steak. The correction for a 190 g steak was based on the estimated mean value of the UMW obtained in the experimental sample.

Cooking loss (CL; %) was obtained from the difference between refrigerated and cooked weight (g) as follows: CL = ((UMW - PCW) ÷ UMW) × 100.

Shear force

The shear force (kgf cm^{-3}) was measured using a texture meter (Brookfield Engineering, model CT3) coupled with a 3/100 size TA probe and a TA-SBA fixture device (TA Single Blade Assembly) with a 1.0 mm thick V blade. Shear force analysis was performed using six cores (1.27 cm) removed from each cooked sample (muscle fibers longitudinally orientated) with the system calibrated for force (5.0 kg), deformation (20 mm), and speed (200 mm/min). Each core was cut only once, and the result was based on the average number of replicates.

Statistical procedures

The significance level (α) was initially declared at 0.05 for all hypothesis tests. Then, the non-influence ($p > \alpha$) of the treatments on the difference in days to slaughter (1, 40, 47, and 74 days) of animals (DIF_S) was verified (Banzatto & Kronka, 2008) via analysis of variance (ANOVA).

Then, an ANOVA and an analysis of covariance (ANCOVA) with homogeneous betas were carried out to verify the effect of DIF_S on the traits. An ANCOVA, including the interaction between the covariate DIF_S and diets (DIF_S*D), was performed to confirm the homogeneity of the 1st-degree linear regression coefficients (betas) (Statistical Analysis System [SAS], 2024). The joint nullity hypothesis for the equivalence of the slopes of the regression lines of the four diets (D) was expressed as $\beta_{D1} = \beta_{D2} = \beta_{D3} = \beta_{D4}$.

The statistical model of homogeneous ANCOVA was described as $Y_{ijk} = m + D_i + MT_j + D*MT_{ij} + \beta(X_{ijk} - \bar{X}...) + \varepsilon_{ijk}$, where: Y_{ijk} = the dependent variable in each plot, measured in the i-th class of diet, the j-th level of maturation time, and the k-th repetition; m = effect of the overall mean; D_i = effect of diet classes (i = 1, 2, 3 and 4); MT_j = effect of maturation time levels (j = 1, 2 and 3); $D*MT_{ij}$ = effect of interaction between diets and maturation times; β = regression coefficient of Y over X; X_{ijk} = observation of the covariate (DIF_S) in each plot, measured in the i-th class of diet and the k-th replication; $\bar{X}...$ = overall mean for covariate X; ε_{ijk} = random errors of the plots associated with class i, level j, and repeat k, independent, homoscedastic, and normal distributed.

After adjusting the model, the normality and homogeneity of variances of the experimental errors among treatments for the various traits were assessed using the Shapiro-Wilk and Levene tests, respectively.

The interaction effects D*MT, D, and MT were verified in the initial ANOVA or ANCOVA via F test. If $p \leq \alpha$ for D*MT, the hierarchical effects of the D classes given MT (D/MT) and the levels of MT given D (MT/D) were verified using the F test in ANOVA or intermediate ANCOVA (SAS, 2024).

The means of D/MT and D independent of MT were compared using the t-test for the difference among least squares means (*lsmeans*).

If $p \leq \alpha$ for MT/D and MT, the means among levels of MT hierarchized in each

class of D and the means of MT independent of D were compared using the F test after unfolding the degrees of freedom and the sums of squares of the sources of variation MT/SS, MT/low NPN, MT/mean NPN, MT/high NPN and MT in three orthogonal groups of orthogonal contrasts (Banzatto & Kronka, 2008), in which the simple contrasts among lsmeans associated with the effects described were the contrasts of interest.

Results and Discussion

No interaction ($p > 0.05$) between diet and maturation time (D*MT) was observed for luminosity index (L*), red to green component (a*), yellow to blue component (b*), pH, and shear force in the *Longissimus dorsi* muscle steaks (Table 2).

Table 2
Physical and chemical traits of beef cattle steaks (½ Angus + ½ Nellore), according to diets and maturation times (MT)

Item[1]	MT[2]	Diets[3]				P-value
		Pasture	Low NPN	Medium NPN	High NPN	MT*Diets
Luminosity (L*)	3	34.16±1.22	38.66±1.37	36.87±1.12	40.23±1.19	0.947
	9	36.37±1.22	38.84±1.50	36.90±1.12	41.79±1.19	
	16	36.45±1.22	40.19±1.37	39.20±1.12	42.61±1.19	
Red to green component (a*)	3	15.10±0.64	17.16±0.78	14.35±0.62	15.47±0.66	0.749
	9	16.42±0.64	17.79±0.78	15.47±0.62	16.07±0.66	
	16	15.58±0.64	18.25±0.78	16.60±0.62	16.51±0.66	
Yellow to blue component (b*)	3	4.89±0.52	6.80±0.62	5.76±0.51	5.15±0.53	0.265
	9	5.97±0.52	6.28±0.62	6.29±0.51	6.16±0.53	
	16	4.81±0.52	6.48±0.62	6.93±0.51	6.86±0.53	
pH	3	5.95±0.10	5.56±0.13	5.77±0.18	5.98±0.12	0.731
	9	6.00±0.10	5.76±0.13	6.05±0.18	5.84±0.12	
	16	5.99±0.10	5.56±0.13	5.79±0.18	5.81±0.12	
PCCW (g)	3	184.4±3.26^{a}A	149.8±3.95^{b}	159.6±3.46^{b}	156.4±3.56^{b}A	0.033
	9	178.6±3.26^{a}AB	142.7±3.95^{c}	157.7±3.26^{b}	144.4±3.56^{c}B	
	16	173.1±3.26^{a}B	140.2±3.95^{b}	167.6±3.26^{a}	142.8±3.56^{b}B	
CL (%)	3	2.93±1.72^{b}B	21.14±2.08^{a}	15.98±1.82^{a}	17.70±1.88^{a}B	0.033
	9	5.94±1.72^{c}AB	24.92±2.08^{a}	17.02±1.72^{b}	24.00±1.88^{a}A	
	16	8.88±1.72^{b}A	26.21±2.08^{a}	11.79±1.72^{b}	24.84±1.88^{a}A	
SF (kgf cm^{-3})	3	3.99±0.29	3.39±0.35	3.09±0.29	3.98±0.31	0.705
	9	3.02±0.29	2.84±0.35	2.56±0.29	2.80±0.31	
	16	2.95 ±0.29	2.60±0.35	2.20±0.29	2.19±0.31	

[1]PCCW: post-cooking corrected steak weight (relative to a 190 g steak); CL: cooking loss; SF: shear force; [2]MT: maturation times (days); [3]Low, medium, and high NPN: diets containing 18%, 42%, and 78% non-protein nitrogen (NPN) in the concentrate%, respectively.
Means followed by lowercase letters in the row and uppercase letters in the column differ by the F test associated with orthogonal groups of orthogonal contrasts at 5% probability.

pH values were similar among treatments ($p > 0.05$) and remained within the appropriate range of 5.7 to 6.0 (Nelis et al., 2022). The pH after carcass cooling is directly related to the luminosity and color of the meat (Favaro et al., 2021). Several traits are altered when pH is not reduced sufficiently during post-mortem glycolysis, generating darker and undesirable meats (Ijaz et al., 2020).

An interaction ($p < 0.05$) between diet and maturation time for post-cooking corrected weight (PCCW) and cooking loss (CL) (Table 2).

An effect ($p < 0.05$) of D/MT3, D/MT9, D/MT16, MT/SS, and MT/high NPN on the PCCW was observed. However, no effects of MT were observed by fixing the "low and medium NPN" diet classes (Table 2). The estimated average PCCW of non-supplemented animals on day 3 (184.43 g) and 9 of maturation (178.63 g) were higher ($p < 0.05$) than the respective average PCCW of those fed the other diets with different concentrations of NPN (Table 2). This effect was possibly due to the smaller size of the muscle fibers of animals fed exclusively pasture, reflecting less water loss when their steaks were cooked. Beline et al. (2021) observed that animals with a higher growth rate had a larger muscle fiber diameter and greater cooking loss.

The differences in PCCW may be related to the extra dietary protein sources (NPN and true protein from corn and soybean meal). According to Ramos and Gomide (2017), supplementation improves growth performance structures for grazing beef cattle. However, the water loss capacity will define the potential for reducing meat weight after slaughter, as any processing promotes natural moisture loss due to the free water in tissues.

Unsupplemented grazing animals showed lower PCCW ($p < 0.05$) on day 16 of maturation (MT16; 173.13 g) compared to day 3 (MT3; 184.43 g). PCCW averages were similar ($p > 0.05$) among the maturation times when cattle were fed concentrates with low and medium NPN. As animals were fed diets with low NPN, the mean PCCW values were 149.83 (MT3), 142.66 (MT9), and 140.20 g (MT16) (Table 2). However, when animals were fed the concentrate with high NPN, the mean PCCW on day 3 (156.36 g) was greater ($p < 0.05$) than on days 9 (144.40 g) and 16 of maturation (142.80 g). These results suggest MT greater than three days had an effect ($p < 0.05$) on the PCCW (Table 2).

For cooking water loss (CL), an effect ($p < 0.05$) of D/MT3, D/MT9, D/MT16, MT/pasture, and MT/high NPN was observed. However, no effects ($p > 0.05$) of the levels of MT were observed by fixing the diet classes with low and medium concentrations of NPN. The estimated means for CL (%) of steaks from unsupplemented animals on days 3, 9, and 16 were 2.93, 5.94, and 8.88%, respectively. The highest CL was observed on day 16 of maturation (8.88%), which differed ($p < 0.05$) from the estimated mean on day 3 (Table 2).

The longer steaks matured, the greater the CL when animals were fed the concentrate with high NPN on day 9 (24.00%) and 16 (24.84%) of maturation. These means differed ($p < 0.05$) from the average CL of steaks matured for three days (17.70%). Similar results were observed by Mateus et al. (2018), who reported greater CL with nine days of maturation, which was believed to be related to protein degradation promoted by the enzyme complex involved in the maturation period, in addition to the folding capacity of collagen when subjected to cooking above 60 °C.

In general, the mean CL values we observed in unsupplemented animals were lower ($p < 0.05$) than in those fed concentrates with different levels of NPN, considering each MT (Table 2).

Regarding the shear force (FC), the values ranged from 2.19 to 3.98 kgf cm^{-3} (Table 2) and could be considered an adequate range for consumers and slaughterhouses. Indeed, tenderness in beef is considered acceptable when CF is less than 4.5 kgf cm^{-3} (Oliveira et al., 2012). A possible explanation for obtaining this degree of tenderness was the young animals subjected to the present study, as increasing the cattle slaughter age reduces meat tenderness (Li et al., 2022).

Rodrigues and Silva (2016) reported that intrinsic individual factors, such as breed and age, and extrinsic factors, such as *post-mortem,* affect meat tenderness. As animals age, the number of cross-links within and among the tropocollagen molecules of collagen increases. These bonds increase both molecule stability and collagen insolubility. Consequently, the meat becomes less tender (Kopuzlu et al., 2018). Taurine animals have high calpain activity and reduced calpastatins activity compared to zebu. The lower action of calpastatin promotes greater calpain activity and, hence, greater meat tenderness (Malheiros et al., 2018).

Regardless of the maturation time, diets did not influence ($p > 0.05$) b* in the steaks, but there was an effect ($p < 0.05$) of diets on luminosity (L*), pH, PCCW, CL, and SF (Table 3). L* in steaks from unsupplemented grazing animals (35.66) was lower ($p < 0.0001$) than in those fed the concentrates containing low (39.23) or high NPN (41.54). This result suggests a darker meat color in steaks from unsupplemented animals.

Table 3
Physical and chemical characteristics of beef cattle steaks (½ Angus + ½ Nellore), according to diets and maturation times (MT)

Item[1]	Diets[2]				P-value
	Control	Low NPN	Medium NPN	High NPN	
Luminosity (L*)	35.66±0.82^{c}	39.23±0.83^{b}	37.66±0.67^{bc}	41.54±0.71^{a}	<0,001
Red to green component (a*)	15.70±0.39^{b}	17.73±0.47^{a}	15.48±0.36^{b}	16.02±0.38^{b}	0,002
Yellow to blue component (b*)	5.22±0.33	6.52±0.37	6.33±0.31	6.06±0.31	0,077
pH	5.98±0.06^{a}	5.63±0.08^{b}	5.87±0.10^{ab}	5.88±0.07^{a}	0,014
PCCW (g)	178.7±1.94^{a}	144.2±2.30^{c}	161.6±1.98^{b}	147.9±2.22^{c}	<0,001
CL (%)	5.92±1.02^{c}	24.09±1.21^{a}	14.93±1.04^{b}	22.18±1.17^{a}	<0,001
SF (kgf cm^{-3})	3.32±0.17^{a}	2.94±0.20^{ab}	2.62±0.17^{b}	2.99±0.18^{ab}	0,036

[1]PCCW: post-cooking corrected steak weight (relative to a 190 g steak); CL: cooking loss; SF: shear force; [2]MT: maturation times (days); [3]Low, medium, and high NPN: diets containing 18%, 42%, and 78% non-protein nitrogen (NPN) in the concentrate%, respectively.
Means followed by lowercase letters in the row and uppercase letters in the column differ by the t-test for the difference between least squares means at 5% probability.

The highest value ($p < 0.05$) for the red to green component (a*) was 17.73, measured in the steaks of animals fed diets with low NPN (Table 3). Nassu et al. (2016) observed similar values of L* (37.40) and a* (14.71%) in Canchim cattle raised in an integrated crop-livestock system. Meat is a heterogeneous product, and the differences observed in its color involve numerous factors, among which pH is paramount (Lima et al., 2011).

pH may be responsible for the redder meat because the pH in the steaks from animals supplemented with low NPN was 5.63, a value lower ($p < 0.05$) than the mean pH in steaks from unsupplemented (5.98) and supplemented with high NPN (5.88). The reduction in pH must occur gradually so that the meat has an acceptable color to consumers. The glycogen stored in muscles before slaughter increases the concentration of lactic acid, reducing pH. Meat industries have recommended that pH be measured in chilled meat after 24 hours to disqualify carcasses with a pH greater than 5.8 (Nelis et al., 2022).

The PCCW in unsupplemented animals was greater ($p < 0.05$) than in those fed diets with NPN supplementation, possibly due to the lower ($p < 0.05$) CL in steaks from grazing animals (5.92%) compared to those fed concentrates (Table 3). This result suggests a lower liquid loss after cooking in steaks from unsupplemented animals. Pitombo et al. (2013) reported that lower CL contributes to softer and juicier meat.

The steaks from animals fed concentrates with medium NPN were more tender ($p < 0.05$) (2.62 kgf cm^{-3}) than those that were unsupplemented (3.32 kgf cm^{-3}). Still, no differences ($p > 0.05$) were observed for the shear force (SF) in the steaks from these animals compared to those from animals fed low and high NPN (Table 3). The mean SF observed in the meat from unsupplemented cattle was similar to the one observed in meat from steers fed forage cactus and sorghum silage supplemented with urea and cottonseed meal (3.35 kgf cm^{-3}) (Silva et al., 2019). Likewise, it was similar to the average SF value (3.52 kgf cm^{-3}) observed in the Longissimus dorsi muscle from Nellore steers (Ribeiro et al., 2016).

That feeding affects meat tenderness, specifically with the lower SF in cattle supplemented with a medium concentration of NPN compared to those unsupplemented, may be associated with an increased starch digestibility provided by a greater dietary CP, especially in diets with whole grains. Hence, blood glucose and insulin tend to increase intramuscular fat deposition, which ensures meat with more tenderness, aroma, flavor, and juiciness due to the spaces formed among the fibers when the meat is subjected to cooking (Bridi et al., 2011).

The estimated average luminosity (L*) in steaks (½ Angus + ½ Nellore) that matured for 16 days (39.61) was higher ($p < 0.05$) than in steaks that matured for three days (37.48) regardless of the diet. However, the mean L* did not differ ($p > 0.05$) among the steaks on day 3 and day 9 of maturation (38.48) (Table 4).

Table 4
Physical and chemical characteristics of beef cattle steaks (½ Angus + ½ Nellore), according to diets and maturation times (MT)

Item[1]	Maturation times (days)			P-value
	3	9	16	
Luminosity (L*)	37.48±0.59b	38.48±0.61ab	39.61±0.59a	0.044
Red to green component (a*)	15.52±0.34b	16.44±0.34ab	16.74±0.34a	0.033
Yellow to blue component (b*)	5.65±0.27	6.18±0.27	6.27±0.27	0.217
pH	5.82±0.07	5.92±0.07	5.79±0.07	0.358
PCCW (g)	162.6±1.75a	155.8±1.73b	155.9±1.73b	0.010
CL (%)	14.44±0.92b	17.97±0.91a	17.93±0.91a	0.010
SF (kgf cm^{-3})	3.61±0.16a	2.80±0.16b	2.48±0.16b	<0.001

[1]PCCW: post-cooking corrected steak weight (relative to a 190 g steak); CL: cooking loss; SF: shear force; [2]MT: maturation times (days); [3]Low, medium, and high NPN: diets containing 18%, 42%, and 78% non-protein nitrogen (NPN) in the concentrate%, respectively.
Means followed by lowercase letters in the row and uppercase letters in the column differ by the F test associated with orthogonal groups of orthogonal contrasts at 5% probability.

The darker color of the meat resulted from the lower luminosity, with more intensity on day 3 of maturation. L* may be associated with a higher concentration of muscle myoglobin provided by physical activity and age, which may decrease meat L* and change the red to green component (a*) (Maggioni et al., 2012). Our results corroborate this previous report, as the average value of a* in the meat on day 3 of maturation (15.52) was lower ($p < 0.05$) than the one observed on day 16 (16.74). However, MT did not affect b* and pH (Table 4).

As the temperature (TEMP) of steaks was stabilized, the PCCW (g) tended to be steadfast. That is, the stabilization of the TEMP may have contributed to the non-occurrence of changes ($p > 0.05$) in the PCCW from day 9 onwards because the average PCCW on day 3 was 162.56 g, a higher value ($p < 0.05$) than the others we observed. However, no differences ($p > 0.05$) were observed between the means of PCCW on days 9 and 16.

A lower ($p < 0.05$) CL was observed in meat samples that matured for three days (14.44%) than in those that matured for nine (17.97%) and 16 days (17.93%), which showed similar means ($p > 0.05$) (Table 4). The greater the CL, the greater the liquid extracted and the lower the tenderness. Thus, drier meat is obtained, especially due to the water-soluble micronutrients that migrate to the exudate (Roldán et al., 2013).

Steaks with the lowest shear force (SF) matured for nine 2.80 and 16 days (2.80 and 2.48 kgf cm^{-3}, respectively), which did not differ from each other ($p > 0.05$). Between three and nine days of maturation, meat tenderization ($p < 0.05$) occurred,

representing a difference of 0.81 kgf cm^{-3}, equivalent to an average SF reduction of 0.135 kgf cm^{-3} per day. In the last seven days of maturation (day 9 to 16), the decrease was not significant ($p > 0.05$), averaging 0.32 kgf cm^{-3}, which is equivalent to a SF reduction of 0.046 kgf cm^{-3} per day (Table 4).

Greater daily SF reductions were obtained by Farias et al. (2018), who reported decreases of 0.11 kgf cm^{-3} per day between the seven and 14 days of maturation in steaks (cutting thickness of 7.5 cm) of *Longissimus thoracis lumborum* muscle from Nellore cattle with six or eight permanent teeth. The authors reported that a proper MT for meat from adult cattle should be at least 14 days.

In general, our results were similar to those reported by Mateus et al. (2018), who also observed that meat maturation advances during the evaluation period, allowsing us to detect changes in the physical and chemical aspects of the meat. This information can guide the industry in developing appropriate packaging to keep the meat quality to consumers.

Conclusion

Dietary supplementation of beef cattle with 42% crude protein in non-protein nitrogen and maturation up to nine days provides meat with high tenderness and juiciness.

Dietary supplementation of beef cattle with low non-protein nitrogen content and maturation for more than nine days is recommended to provide meat with an intense red color.

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