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Effect of rib fat thickness on the quality of aged meat from Nellore young bulls

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ABSTRACT. This trial aimed to evaluate the quality of aged beef from Nellore young bulls under two yield grade (YG). Fourteen animals with approximately 450 ± 30 kg body weight were evaluated for backfat thickness (BFT) at the beginning of the experimental period. Seven animals had BFT of 0-3 mm and seven animals, 3.1-6 mm. Two groups were formed at the end of the experiment: animals finished with 3-6 mm BFT (seven animals) and animals finished with 6.1 to 10 mm BFT (seven animals). Every 28 days, we evaluated by ultrasound the BFT between the 12nd 13rd ribs. There was no interaction between YG and aging for beef color, pH, cooking losses and shear force ($p > 0.05$). There was no effect of YG on sarcomere length ($p = 0.11$). However, there was interaction between YG and aging on water holding capacity ($p < 0.01$). The yield grades evaluated did not interfere with meat quality, but carcasses with 6.1 - 10 mm of backfat thickness showed highest water holding capacity. The aging of the longissimus muscle for up to 14 days improves beef tenderness.

Keyword: aging, longissimus, subcutaneous fat.

Efeito da espessura de gordura subcutânea sobre a qualidade da carne maturada de tourinhos da raça Nelore

RESUMO. Objetivou-se neste trabalho avaliar a qualidade da carne maturada de tourinhos Nelore, sob dois graus de acabamento (GA) de carcaça. Foram utilizados 14 animais, com aproximadamente 450 ± 30 kg de peso corporal. A espessura de gordura subcutânea (EGS) dos animais foi avaliada no início do período experimental. Foram escolhidos 14 animais com EGS de 0-3 mm e 3,1-6 mm, formando-se dois grupos ao final do experimento: animais com acabamento entre 3-6 mm (sete animais) e entre 6,1-10 mm (sete animais). A cada 28 dias foram avaliados a EGS entre a 12-13ª costelas com ajuda de unidade ultrassonográfica. Os valores de coloração da carne, pH, perdas por cocção e força de cisalhamento não tiveram efeito de interação entre os tratamentos ($p > 0,05$). Não houve diferença significativa entre o GA sobre o comprimento de sarcômero ($p = 0,11$). Houve interação entre GA e maturação sobre a capacidade de retenção de água ($p < 0,01$). Os graus de acabamento avaliados não interferem na qualidade da carne, mas carcaças com acabamento entre 6,1-10 mm apresentam maior capacidade de retenção de água. A maturação do músculo longissimus em até 14 dias melhora a maciez da carne.

Palavra-chave: maturação, longissimus, gordura subcutânea.

Introduction

The search for quality food has increased in recent years, thus requiring professionalism in production, processing and marketing. In this sense, color, juiciness, texture, tenderness and flavor of the meat have been widely studied to know the factors responsible for these characteristics and how to improve them (Guerrero et al., 2013). Some factors can affect the quality of meat, for example, the thickness of subcutaneous fat in the carcass.

The subcutaneous fat thickness plays a key role in reducing the *cold shortening*, during carcass cooling processes (Dolezal et al., 1982), which is defined as a rapid drop in muscle temperature (less than 19-14°C) before reaching the stage of *rigor mortis*. When the muscle temperature is reduced (15°C) before *rigor mortis*, the sarcoplasmic reticulum does not work effectively and cannot bind to calcium, which leads to an abundance of calcium in the sarcoplasm. Given the presence of ATP, the muscle contracts at a maximum level, causing the filaments to slide over one another, essentially eliminating the I-band of

the sarcomere (Savell et al., 2005), which may reduce the meat tenderness.

In general, Brazilian slaughterhouses prefer carcasses with a minimum subcutaneous fat thickness of 3 mm, because below 3 mm, there is browning the outside of muscles exposed to cooling, giving undesirable visual appearance, among other factors (Rotta et al., 2009). Above 6 mm, losses imposed to the producer are related to cutting excess fat before weighing the carcass and to the slaughterhouse due to the increased operating cost of this process (Costa et al., 2002). Nevertheless, the backfat thickness of 6.2 mm has been suggested as the minimum to prevent *cold shortening* in beef cattle (Savell et al., 2005). Thus, the increase in the thickness of subcutaneous fat improves meat tenderness, as it allows the carcass to chill more slowly and increases enzyme activity (Smith et al., 1976), resulting in better quality meat.

In this sense, alternatives have been proposed to seek standardization in products, such as meat aging, which directly affects the tenderness of meat, obtaining homogeneous products, with quality and with the highest market value (Monsón et al., 2004; Prado et al., 2009). There is evidence that aging can improve by about 25% meat tenderness (Felício, 1997).

The consumer requires meat with lower contents of total lipids, saturated fatty acids and calories, as well as higher contents of polyunsaturated fatty acids, important in the prevention of cardiovascular disease (Scollan et al., 2006). However, besides protecting carcasses from the cold, fat is an important source of essential fatty acids and carriers of fat-soluble vitamins, and represents an energy source.

Given the above, there is a need to identify ways to improve the tenderness of the main commercial beef cuts, meeting consumer expectations (Brooks et al., 2000) and producing standardized meat cuts, especially regarding the qualitative characteristics. Therefore, this study evaluated the quality of the *Longissimus* muscle aged for 1, 7 and 14 days under two yield grades.

Material and methods

The experiment was conducted at the Department of Animal Science, FCAV/Unesp, Jaboticabal, São Paulo. There were 14 Nelore bulls from pasture supplementation system, with approximately 450 ± 30 kg body weight and 18 ± 2 months of age. Bulls were housed in individual pens of 8 m^2 (4 x 2 m), provided with concrete trough and drinker, where they remained for 77 days. The subcutaneous fat thickness of the animals was assessed at the beginning of the experimental period. Two groups were formed: seven animals with fat thickness of 0-3 mm and seven animals with fat thickness of 3.1- 6 mm, from a contemporary group. Bulls were given the same food and the increase in subcutaneous fat was assessed by ultrasound every 28 days.

Diets were prepared according to the requirements of the animals and supplied at a 40:60 forage concentrate ratio; corn silage was the source of forage and the concentrate consisted of corn, soybean meal, urea and mineral supplement (Table 1).

Table 1. Percentage composition and characteristics of the experimental diet.

Ingredients	(%DM)
Corn silage	40.00
Ground corn	46.40
Soybean meal	10.00
Urea	0.60
Mineral supplement1	3.00
Chemical composition2, %	
Dry matter	67.08
Crude protein	14.45
NDF	25.19
Ether extract	3.18
Non-fiber carbohydrates	53.93

[1]Composition (Calcium: 210 g; Phosphorus: 20 g; Sulfur: 37 g; Sodium: 80 g; Copper: 490 mg; Manganese: 1.424 mg; Zinc: 1.830 mg; Iodine: 36 mg; Cobalt: 29 mg; Selenium: 9 mg; Fluorine (max): 333 mg). [2]Analysis according to AOAC (1995).

Bulls were weighed every 28 days and evaluated by ultrasound (Aloka 500, linear carcass probe with 17.2 cm and 3.5 MHz frequency) for subcutaneous fat thickness on the left side of the animal between the 12nd and 13rd ribs. The images were obtained by a technique certified by Ultrasound Guideline Council and interpreted using certified software (Biosoft Toolbox® II for Beef/Biotronics, Inc., Ames, Iowa, USA). Backfat thickness was evaluated again at the end of the experimental period (77 days), classifying animals into two groups according to the yield grade of 3-6 mm BFT (seven animals) and 6.1-10 mm BFT (seven animals).

Animals were killed in a commercial slaughterhouse (80 km away from the university), with average shrunk body weight of 598 ± 41.7 kg. Carcasses were divided and chilled in cold storage at 0°C for 24 hours. After this, a sample of the *Longissimus* muscle was taken, with approximately 10 cm thick, from the cut made between the 10th and the 13rd ribs of the left half carcass for further analysis of meat. Of each carcass, three samples were taken to evaluate each variable of meat quality in its respective time (1, 7 and 14 days post-mortem), thus different steaks were used to evaluate the same variable. Samples were individually vacuum-packed. Samples for the day one were frozen at -20°C for

further analysis of meat quality. Samples for the days 7 and 14 were aged in cold storage between 0 and 2°C and subsequently stored at -20°C until quality analysis.

Meat was analyzed for color, pH, cooking losses, shear force, water holding capacity, lipid oxidation, sarcomere length and myofibril fragmentation index.

Meat color was determined as described by Houben et al. (2000), using a colorimeter (CR 300, Minolta Camera Co. Ltd., Osaka, Japan), evaluating the lightness (L★) 0 = black; 100 = white, the intensity of the red color (a★) and the intensity of the yellow color (b★). Thirty minutes before the evaluations at different points of the meat sample, a cross section of the muscle was made to expose the myoglobin to oxygen. The instrument calibration was performed, before the reading of the samples, with a standard white and black in different points of each steak or portion of subcutaneous fat. After 30 minutes of exposure to air, L★, a★, b★ values were determined according to the Cielab system. Values of L★, a★, b★ were obtained from five readings taken at different points in each steak.

The values of pH were measured after 24 hours of chilling (final pH) using a portable pH meter with a penetration electrode by introducing it into a cut 2 to 4 cm depth, made in the *Longissimus* muscle, left half carcass. On days 7 and 14, after exposure to air, pH readings were taken.

Warner–Bratzler shear force (WBSF) steaks were thawed at 4°C for 24h and oven-broiled in an electric oven (Layr, Luxo Inox) preheated at 150°C. Internal steak temperatures were monitored by 20-gauge copper-constantan thermocouples (Omega Engineering, Stamford, CT) placed in the approximate geometric center of each steak and attached to a digital monitor. When internal steak temperature reached 35°C, the steakwas turned over and allowed to reach an internal temperature of 70°C before removal from the oven. Cooked WBSF steaks were cooled for 24h at 4°C (AMSA, 1995). Eight round cores (1.27 cm diameter) were removed fromeach steak parallel to the long axis of themuscle fibers (AMSA, 1995). Each corewas sheared once through the center, perpendicular to the fiber direction by a Warner-Bratzler shear machine (G-R Manufacturing Company, Manhattan, KS, USA). Cook loss was evaluated on the steaks that were also used for WBSF measurement. Total cooking loss was calculated as the difference between the weight of the steaks before and after oven-broiling.

Water holding capacity was calculated by difference in weight of a meat sample, approximately 2 grams, before and after subjected to the pressure of 10 kg for five minutes.

Lipid oxidation of the meat was carried out by the methodology described by Vyncke (1970), in which is obtained the content of mg malonaldehyde kg^{-1} meat. First, meat was weighed in a homogenizer cup (20 g crushed sample) using an analytical balance (AUY 220, Shimadzu). We added 60 mL of 7.5% TCA solution, and homogenized in Sorwall/Omni mixer for 2 minutes. The mixture was then filtered through filter paper, similar to Whatman 1. We pipetted 5 mL of the distillate into a test tube with screw cap, added 5 mL TBA reagent, stirred the tubes and immersed in water bath for 45 minutes. After cooling in an ice water bath for 10 minutes, the value was obtained by reading the sample absorbance at 538 nm against a blank (BioSpectrophotometro basic, UV, Eppendorf).

Sarcomere length was determined by laser diffraction of muscle fibers from 1.0 cm cubes sliced in duplicate from different parts of the longissimus muscle (between the 12^{nd} and 13^{rd} ribs).

Myofibril fragmentation indices (MFI) were determined on fresh muscle according to the procedures of Olson et al. (1976) and modified by Culler et al. (1978). Four grams of minced muscle were homogenized for 30 s in 10 vol (v w^{-1}) of a 2°C isolating medium consisting of 100 mM KCl, 20 mM K phosphate, 1 mM EDTA, 1 mM MgCl, and 1 mM sodium azide. The homogenate was centrifuged at 1000 x g for 15 min. and then the supernatant was decanted. The sediment was then resuspended in 10 vol (v w^{-1}) of isolating medium using a stir rod, centrifuged again at 1000 x g for 15 min. and the supernatant was decanted. The sediment was resuspended in 2.5 vol (v w^{-1}) of isolating medium and passed through a polyethylene strainer (18 mesh) to remove connective tissue and debris. An additional 2.5 vol (v w^{-1}) was used to facilitate passage of myofibrils through the strainer. The protein concentration of the myofibril suspension was determined by the biuret method as described by Gornall et al. (1949). An aliquot of the myofibril suspension was diluted with an isolating medium to reach a protein concentration of 0.5 ± 0.05 mg mL^{-1}. Protein concentration was determined by the biuret method. The diluted myofibril suspension was stirred and poured into a cuvette; absorbance of this suspension was measured immediately at 540 nm. Absorbance was multiplied by 200 to give a MFI for each sample.

Data were analyzed using a general linear model - Proc Mixed (SAS, Inst. Inc., Cary, NC) using the animal as an experimental unit. The model tested the fixed effects of yield grade, aging and their interaction. The animal was included as a random effect. Interaction was removed from the model when not significant. The means of the least squares were

generated for the main effects and significant interactions were separated ($p < 0.05$) and a tendency was considerate to 0.10 using the yukey test.

Results and discussion

Color, pH, cooking losses and shear force of meat were not affected by any interaction between treatments ($p > 0.05$), so the results were analyzed separately. However, there was an interaction between yield grade and aging for the water holding capacity (Table 2).

Despite a numerical increase in lightness values, there was no significant difference for lightness ($p > 0.05$) between the aging times. For the consumer, meat color is a quality parameter when buying the product

With longer aging time, there was an increase in the value of a★ of vacuum packaged meat ($p < 0.05$; Figure 1). According to Hood (1980), vacuum aged meat when exposed to air has a more intense redness. Although the meat is vacuum packed, the increase in the value of a★ was due to the oxidation of oxymyoglobin to metmyoglobin, resulting from the storage of meat for a long time.

This transformation into metmyoglobin gives rise to a brown color, which interferes with the values of a★, providing an increase in this value as observed in this study.

Values of pH directly affects the final quality of meat, such as tenderness, color, texture and water holding capacity. The other role of pH is related to the reduction in proliferation of microorganisms.

Rigor mortis occurs between 6 to 40 hours after slaughter, with decrease of pH from 7.0 to 5.3-5.8 (Savell et al., 2005). In this process, there is a shift in the metabolism to an anaerobic pathway (without oxygen), where glycogen is converted into pyruvate. Then, there is the conversion of pyruvate into lactic acid in the muscle, which is responsible for the adequate drop in pH.

In the present study, on the day 1, pH values were normal (5.65), however, on the day 7, there was a sharper decline in pH, which increased again on the day 14 (Figure 2). This variation in pH may be the result of accumulation of lactic acid producing bacteria, since these organisms occur in the medium, due to the presence of lactic acid in the meat after slaughter. Meat with pH 5.3 to 5.8 is brightly red, whereas the meat with pH 6.0 or higher, is dark colored, due to increased enzymatic activity, improved water holding capacity and lower penetration of oxygen. .

Table 2. Color (L★, a★ and b★), pH, water holding capacity (WHC), shear force (SF), cooking losses (CL), sarcomere length (SL), malonaldehyde (MDA; mg kg^{-1} of meat) and myofibrillar fragmentation index (MFI) of the longissimus muscle as a function of yield grade and aging times.

Variables	Yield grade (YG) mm		p	Aging times (AT) days			p	GAxTM
	3 - 6	6,1 -10		1	7	14		
L★	40.99	41.55	0.674	40.67	41.62	41.52	0.811	0.995
a★	16.05	15.85	0.701	15.12b	15.87ab	16.87a	0.036	0.501
b★	14.39	14.36	0.994	13.68	14.47	14.94	0.358	0.752
pH	5.55	5.51	0.675	5.65a	5.23b	5.68a	0.001	0.524
WHC	71.02	71.07	0.954	72.83	68.06	72.23	<0.001	0.015
SF	4.41	4.16	0.607	5.07a	4.08b	3.72c	0.062	0.565
CL	29.54	29.97	0.736	28.49	29.59	31.19	0.231	0.543
SL, μm	0.88	1.01	0.110					
MDA, $mg.kg^{-1}$	0.90	0.94	0.675	0.83	0.88	1.05	0.142	0.527
MFI, %	38.52	40.23	0.571	36.15	38.90	43.08	0.185	0.573

★Mean values followed by different lowercase letters in the same row are significantly different at 5% probability.

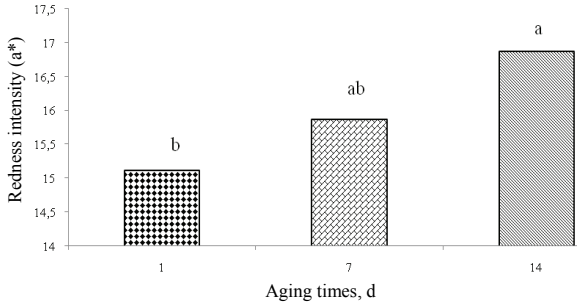

Figure 1. Redness values (a★) according to aging time (days). Different letters over the bars indicate significant differences at 5% probability level, using the tukey test.

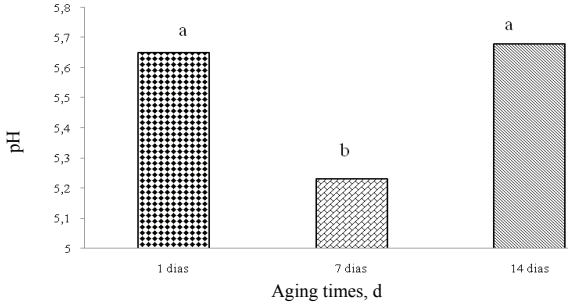

Figure 2. Values of pH according to different aging times (days). Different letters over the bars indicate significant differences at 5% probability level, using the tukey test.

There was an interaction (p = 0.015) between aging time and yield degree in relation to water holding capacity (WHC). Animals with more than 6.1 mm BFT (fat thickness) showed higher WHC at time 1 compared with meat from animals with BFT between 3.1 and 6 mm (Figure 3). According to Lawrie (1977), animals with higher amount of subcutaneous fat have higher water holding capacity. Carcasses with greater yield grade avoid excessive evaporation of liquid when exposed to low temperatures in the cold room, because the subcutaneous fat acts as a thermal insulator.

In the aging time 7, we found a decrease in water holding capacity when compared with time 1 for the two yield grades (Figure 3). According to Lawrie Lawrie (1977), lactic acid formation and consequent drop in pH are responsible for the decrease in water holding capacity of the meat during the application of forces, such as cutting and heating, whereas at pH 5.2 to 5.3 (isoelectric point of muscle protein, with balance of positive and negative charges), the meat has lower WHC. This was observed on aging day 7, where there was a drop in pH to 5.23, resulting in a lower WHC.

Values of cooking losses (CL) increased with the aging times of 7 and 14 days (Table 2). According to Oliveira et al. (2012), cooking loss values can be influenced by WHC, which is considered a very important characteristic for consumption.

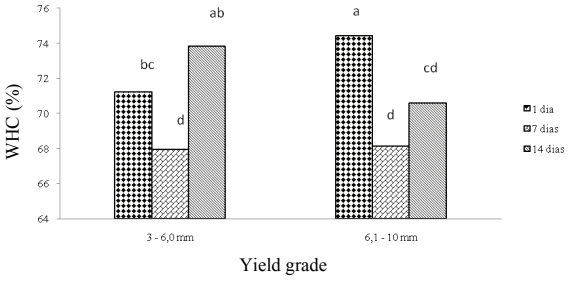

Figure 3. Water holding capacity (WHC) as a function of aging times (1, 7 and 14 days) and yield degrees (3 – 6.0 mm and 6.1 - 10 mm). Different letters over the bars indicate significant differences at 5% probability level, using the Tukey test.

There was no effect (p = 0.11) of the yield grade on the sarcomere length, but numerical increases of 0.88 and 1.01 were observed for 3-6 mm and 6.1-10 BFT, respectively. Heinemann et al. (2002) concluded that the difference in sarcomere length may be the result of carcass chilling, causing cold shortening; for this process to occur the intern temperature of the carcass should reach lower temperatures more rapidly. These authors mention that the sarcomere length increases with increasing fat content of carcasses of animals with the same age and different weights, according to Bouton et al. (1978), who verified that carcasses with greater fat thickness showed greater sarcomere length.

Additionally, no difference (p > 0.05) was detected for lipid oxidation, which was expected as the meats were vacuum packed. According to Kanner (1994), some factors accelerate the oxidation such as oxygen, unsaturated fatty acids, iron and temperature. The little available oxygen in the packaging and the constant storage temperature (0-2°C) did not promote lipid oxidation within 14 days. The value of MDA is a somewhat high, because the animals were slaughtered weighing more than those in other experiments with Nellore animals. In general, animals are killed with an average of 500 kg, which have little intramuscular fat. In this study, the animals were slaughtered with an average of 598 kg and probably had greater deposition of intramuscular fat, which interferes with the oxidation and consequently in MDA values.

There was no significant effect (p > 0.05) for SF, but a downward trend (p = 0.065) according to aging times (Figure 4). According to Felício (1997), temperature *post-mortem* has great effect on muscle properties and meat tenderness and can greatly accelerate the enzymatic activity. According to Shackelford et al. (1991), there is an influence of pH on the activity of the proteolytic enzymes, cathepsins and calpains. These enzymes are responsible for fiber fragmentation, promoting meat tenderness. For the same authors, values up to 4.5 kgf characterize a tender meat. In this way, meat aged for 1 day 1 (5.07 kgf) was not classified as tender, but those aged for 7 and 14 days were considered tender (4.08 and 3.72 kgf, respectively).

No effect of yield grade (p > 0.05) was found on the MFI, but there was an increase in MFI values according to aging times; this index is associated with the decreasing trend in SF (Figure 4). The MFI is responsible for 50% of meat tenderness, and values above 60% indicate a tender meat (Culler et al., 1978; Maggioni et al., 2012).

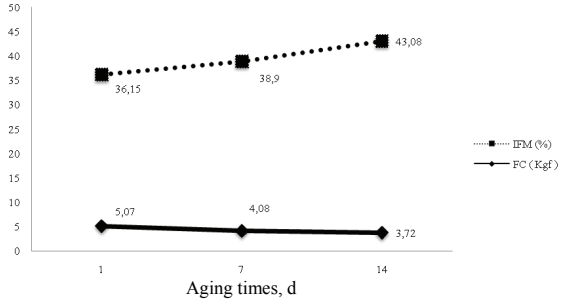

Figure 4. Myofibril fragmentation index (MFI) and shear force (SF) according to different aging times (days).

However, the values found herein were below 60%, but are consistent with the shear force, because the lower the shear force, the higher the myofibril fragmentation index.

Conclusion

Yield grades evaluated have no effect on meat quality, however, carcasses with 6.1-10 mm fat thickness present higher water holding capacity. The aging of the longissimus muscle for 14 days should be used to improve meat tenderness due to the tendency to decrease shear force in relation to beef aged for one or seven days.

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