PRISCILLA DUTRA TEIXEIRA

QUALITATIVE CHARACTERISTICS OF BEEF FROM NELLORE, ANGUS AND THEIR CROSSBRED FED WHOLE SHELLED CORN DIETS

LAVRAS - MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Zootecnia, área de concentração em Nutrição e Produção de Ruminantes, para obtenção do título de Doutor.

Orientador

Dr. Márcio Machado Ladeira

LAVRAS - MG

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APROVADA em 24 de agosto de 2018.

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LAVRAS – MG

2018

Aos meus pais que sempre me apoiaram e

não mediram esforços para me ajudar nesta conquista

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É por vocês que seguirei em frente!!!

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ABSTRACT

The objective of this study was to evaluate qualitative characteristics of beef from Nellore, Angus and their crossbreed fed whole shelled corn diets. *Experiment 1:* Seventeen Nellore and 17 Angus bulls with initial body weight of 381 ±12 kg were used in a completely randomized design using a 2 × 2 factorial arrangement (2 breeds and 2 diets). Experimental diets included: 1) a ground corn diet (GC) containing 30% corn silage and 70% GC and soybean meal-based concentrate, and 2) a whole shelled corn (WSC) diet containing 85% WSC and 15% of a soybean meal and mineral-based pelleted supplement. Twenty-four hours after slaughter, samples were taken from the *longissimus thoracis* muscle of the left half carcass between 12th and 13th ribs to analyze TBARS, color, cooking loss, shear force, myofibrillar fragmentation index, sarcomere length and fatty acid profile. *Experiment 2:* Sixteen Nellore and 16 Angus x Nellore steers with 353 ±25.3 kg were allotted in a completely randomized design using a 2 × 2 factorial arrangement (2 breeds and 2 diets). One diet contained 80% whole shelled corn and 20% of a protein-mineral pellet (WSC). The other diet had 74% whole shelled corn, 20% protein-mineral pellet, and 6% of bagasse sugarcane (WSCB). After slaughter, samples were taken from LT muscle of the left half carcass between 12th and 13th ribs for expression gene involved in lipid metabolism. Twenty-four hours after slaughter, samples were taken from the LT muscle to analyze chemical composition, color, myoglobin chemical forms, cooking loss, and shear force. In the *Experiment 1,* Angus beef was more tender compared to Nellore ($P < 0.01$). Angus bulls had greater concentration of polyunsaturated fatty acid (PUFA) and omega-6 ($P < 0.01$). Muscle of bulls fed WSC tended to have greater concentration of CLA C18:2 *cis*-9 *trans*-11 ($P = 0.10$), greater CLA C18:2 *trans*-10 *cis*-12 ($P = 0.01$) PUFA ($P = 0.05$), and consequently, greater oxidation. Regarding *Experiment 2*, there was no effect ($P > 0.05$) of breed and diet on

chemical composition. Muscle from Nellore x Angus steers had greater expression levels ($P < 0.05$) of *LPL*, *FASN* and *CPT2*. There was a tendency ($P = 0.10$) of the beef from Nellore steers be lower tender than Nellore x Angus steers, before aging. Muscle from steers fed the WSC diet had greater expression of *ACOX1* and grater lipid oxidation. In conclusion, Angus beef has greater marbling and tenderness, however Nellore and Nellore × Angus has similar marbling and tenderness after 14 days aging. Regarding diets, despite WSC diet not increases intramuscular fat, it has more PUFA that increase lipid oxidation.

Keywords: *Bos indicus*, CLA, crossbred, lipogenesis, tenderness.

RESUMO

O objetivo do presente estudo foi avaliar características qualitativas da carne de Nelore, Angus e seu cruzamento, alimentados com dietas de grão milho inteiro. *Experimento 1:* Foram utilizados 17 Nelore e 17 Angus com peso vivo médio inicial de 381,2 ±11,8 kg, em um delineamento inteiramente casualizado, em arranjo fatorial 2 x 2 (2 raças e 2 dietas). A dieta tradicional (SMC) continha 30% de silagem de milho e 70% de um concentrado à base de milho e farelo de soja. A dieta com grão de milho inteiro (GMI) continha 85% de grão de milho inteiro e 15% de um pellet comercial a base de farelo de soja e minerais. 24 horas após o abate, foram coletadas amostras do músculo LT entre 12ª e 13ª costelas para análise de perda de peso por cozimento, força de cisalhamento, índice de fragmentação miofibrilar, comprimento de sarcômero, TBARS, cor e perfil de ácidos graxos. *Experimento 2:* Foram utilizados 16 Nelore e 16 Nelore x Angus, com peso vivo médio inicial de 353 ± 25,3 kg, em um delineamento experimental inteiramente casualizado com arranjo fatorial 2 x 2 (2 raças e 2 dietas). As dietas continham 80% grão de milho inteiro e 20% pellet (GMI) ou 74% grão de milho inteiro, 20% pellet comercial proteico-mineral e 6% de bagaço de cana (GMIB). Após o abate, foram coletadas amostras do músculo LT entre 12ª e 13ª costelas para análise de expressão genica e 24 horas após o abate amostras para análise de composição centesimal, perda de peso por cozimento, força de cisalhamento, TBARS, cor e formas químicas da mioglobina. No *Experimento 1*, carne de tourinhos Angus apresentaram menor força de cisalhamento comparado com Nelore ($P < 0.01$). Angus apresentaram maior concentração de ácidos graxos poli-insaturados e ômega 6 ($P < 0.01$). Músculo de animais alimentados com GMI tenderam a ter maior concentração de CLA C18:2 *cis*-9 *trans*-11 ($P = 0.10$), maior concentração de CLA C18:2 *trans*-10 *cis*-12 ($P = 0.01$), ácidos poli-insaturados ($P = 0.05$), e consequentemente maior oxidação lipídica. Em relação ao *Experimento 2*, a

composição centesimal não foi afetada pelas raças e dietas ($P > 0.05$). Músculo de novilhos Nelore x Angus apresentaram maior expressão dos genes *LPL*, *FASN* e *CPT2*. Novilhos Nelore tenderam a ter maior força de cisalhamento (P = 0.10) após o abate. Músculo de novilhos alimentados com GMI tiveram maior expressão do gene *ACOX1* e maior oxidação lipídica. De forma geral, carne de tourinhos Angus possuem maior marmoreio e maciez, já carne de Nelore e Nelore × Angus não apresentam diferenças quanto ao marmoreio e possuem maciez semelhante após 14 dias de maturação. Em relação a dietas, apesar da dieta GMI não aumentar gordura intramuscular, apresenta maior concentração de AGPI causando maior oxidação lipídica.

Palavras-chave: *Bos indicus*, CLA, cruzados, lipogênese, maciez.

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FIRST CHAPTER

1. INTRODUCTION

Feedlot diets in Brazil undergoes change with increasing concentrate and grain inclusion level in their composition. According to Pinto and Millen (2017), the usual inclusion level of concentrate in Brazilian feedlot diets is between 81 to 90%. In another survey by Oliveira and Millen (2014), the authors reported that 9.4% of Brazilian feedlot has used whole shelled corn in their diets. In other words, diets with a high proportion of concentrate, or even diets without inclusion of forage, began to be extensively used in Brazilian feedlots.

It is expected that high grain diets provide greater amounts of intramuscular fat because lead to increased propionate production in the rumen that, in turn, increases glucose supply and, according to Smith (1984), intramuscular adipocytes prefer glucose as lipogenic substrates. Furthermore, intramuscular fat is more sensitive to insulin (GILBERT et al., 2003) and propiogenic diets can increase plasma insulin, increasing glucose uptake (SMITH, 2017; SMITH et al., 2018) which would also increase intramuscular fat deposition.

However, grain diets can reduce rumen pH that may change rumen microbiota and biohydrogenation, which can affect beef marbling (JENKINS; HARVATINE, 2014). To avoid this effect of high grain diets an alternative is the use of fiber sources in low quantities. Fiber sources can benefit intramuscular fat deposition, due to avoid ruminal pH drop, stimulate dry matter intake and provide greater weight gain (DE ALMEIDA CONTADINI et al., 2017; MARQUES et al., 2016).

Beef production in Brazil is largely based on *Bos indicus* such as Nellore breed, that represents the majority of feedlot cattle (OLIVEIRA; MILLEN, 2014) due to their superior adaptation to tropical conditions. However, it is known that there is a decline in beef quality when *Bos indicus* steers are used compared with *Bos taurus*. *Bos taurus* cattle have more intramuscular fat compared to *Bos indicus* (ELZO et al., 2012; SILVA et al., 2014) and have

greater tenderness (ANDRADE et al., 2010; BARCELLOS et al., 2017; DUARTE et al., 2013). Marbling and tenderness are one of the objectives of beef industry to satisfy consumer's desire because they are correlated with flavor, juiciness, and tenderness (EMERSON et al., 2013; STARKEY et al., 2016).

As mentioned before, breed affects meat fat content and fat content itself is a factor that influences fatty acid composition (MACEDO et al., 2008; PRADO et al., 2008). In this sense, breeds with a low concentration of lipid may have greater proportion of polyunsaturated fatty acid (PUFA) and lower saturated fatty acid (SFA) (WOOD et al., 2008), because of a reduced *de novo* synthesis. Bressan et al. (2016) detected that when animals are fed with grain, *Bos indicus* had greater concentration of SFA and 18:0, and lower amount of 18:2 n-6 and 18:3 n-3 fatty acids. On contrary, Lopes et al. (2012) showed greater contents of unsaturated fatty acid (UFA) and monounsaturated fatty acid (MUFA) in *Bos indicus*. In general, these divergences of fatty acid profile results among *Bos indicus*, *Bos taurus* and crossbred may be due to the diet used in the finishing phase (BRESSAN, M. et al., 2011; BRESSAN et al., 2016).

In addition, previous research has not demonstrated how WSC diets affect the expression of lipogenic genes and beef quality traits, mainly fatty acid profile in *Bos taurus*, *Bos indicus* and their crossbred. Therefore, the objectives of these studies were to evaluate the effect of diet (ground corn and whole shelled corn with or without sugarcane bagasse) and genetic group (*Bos indicus*, *Bos taurus* and crossbreed) on intramuscular fat, fatty acid composition and qualitative characteristics in two different trials.

2. LITERATURE REVIEW

2.1. Marbling and ruminal pH

Intramuscular fat is important for the development of meat flavor, juiciness and tenderness. The degree of marbling depends on the energy content in the diet (SMITH; CROUSE, 1984), and, therefore, for high intramuscular fat deposition is necessary that energy consumed must surpass requirements (LADEIRA et al., 2016).

Acetate and glucose are the major precursors used for biosynthesis of fatty acids in ruminants, where intramuscular adipocytes prefer glucose and subcutaneous adipocytes prefer acetate as lipogenic substrates (MAY et al., 1995; SMITH et al., 2018; SMITH; CROUSE, 1984). However, despite literature indicated glucose as carbon source of *de novo* fatty acid biosynthesis in vitro, recent researches suggesting that in high grain diet, glucose contributes only a small proportion of acetyl units to fatty acid biosynthesis relative to the contribution by acetate, regardless of adipose tissue (NAYANANJALIE et al., 2015; SMITH et al., 2018).

Approximately 61 to 76% of ground corn consumed is degraded in the rumen, where it is converted into volatile fatty acids (VFA), and the remainder undergoes post-ruminal digestion, mainly in the small intestine, resulting in free glucose for absorption (SMITH; CROUSE, 1984). Thus, greater passage of starch to the small intestine may be an alternative to increase the degree of marbling.

Furthermore, intramuscular fat is more sensitive to insulin than subcutaneous tissue (GILBERT et al., 2003). Additionally, propiogenic diets can increase plasma glucose, that in turn increase plasma insulin, promoting glucose uptake (SMITH, 2017; SMITH et al., 2018). In other words, ingredients that increase propionate production, such as corn, have greater glycogenic and insulinogenic capacity, that would provide more substrate for *de novo* lipogenesis, which could also promote greater deposition of intramuscular fat (GILBERT et al., 2003).

Corn diets increase production of propionate, a byproduct of ruminal fermentation (Figure 1A), and it is converted to glucose in the liver, which might subsequently enter the glycolytic pathway producing pyruvate, in adipocytes. The pyruvate is transported into mitochondria where it is decarboxylated in acetyl-CoA. As lipogenesis occurs in the cytosol and acetyl-CoA is not permeable through the mitochondrial membrane, acetyl-CoA is condensed to oxaloacetate to form citrate. After that, citrate is transported to the cytosol, where it is cleaved into oxaloacetate and acetyl-CoA (Figure 1B) (LADEIRA et al., 2016; PALMQUIST; MATTOS, 2011).

Finally, acetyl-CoA (product from acetate and/or glucose) undergoes action of the acetyl-CoA carboxylase (which is encoded by the gene *ACACA*) and fatty acid synthase (*FASN*) resulting in the synthesis of long-chain fatty acids (Figure 1C). The insulin stimulates peripheral tissue uptake of glucose and increases lipogenesis, increasing intramuscular fat (RHOADES et al., 2007). In addition, insulin activate acetyl-CoA carboxylase, citrate lyase and pyruvate dehydrogenase complex (BROWNSEY et al., 2006; POTAPOVA et al., 2000). Furthermore, the sterol regulatory element-binding protein 1c (SREBP-1c) is an important transcription factor of lipogenesis is regulated by insulin and their overexpression is required to increase lipogenic genes expression (SOYAL et al., 2015). First, insulin, via Akt, increases *SREBF1* transcripts encoding SREBP-1c. In addition, mammalian target of rapamycin complex 1 (mTORC1) and an autoregulatory positive feedback also activate SREBP-1c transcription (SOYAL et al., 2015).

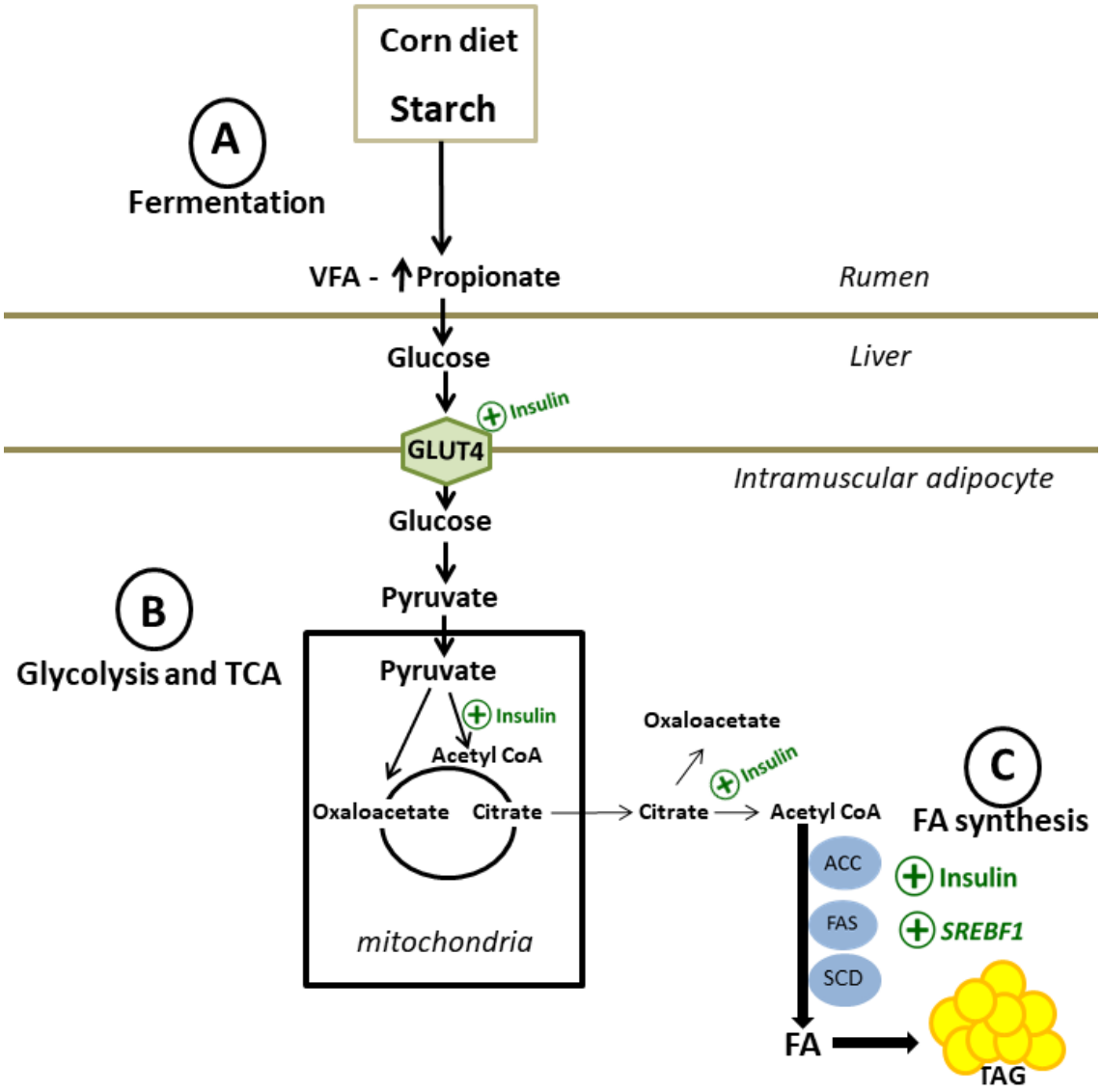

Figure 1. Synthesis of fatty acid from glucose on adipose tissue.

ACC: acetyl-CoA carboxylase; FA: fatty acid; FAS: fatty acid synthase; GLUT4: glucose transporter type 4; SCD: stearoyl-CoA desaturase; SREBF1: Sterol regulatory element binding transcription factor 1; TAG: triacylglycerol; TCA: citric acid cycle; VFA: volatile fatty acids.

Corroborating this assertion, Schoonmaker et al. (2003) found that starch fermentation increased propionate and insulin in steers fed a high-concentrate diet. Increased serum insulin leaded to increased glucose uptake and consequently marbling score.

According to Baldwin et al. (2006), abomasal infusion of glucose increased the transcription of the *ACACA* and *FASN* genes. The glucose stimulates the expression of these genes when the intracellular glucose-6-phosphate levels are elevated (GIRARD; FERRÉ; FOUFELLE, 1997), and by increasing the expression of transcription factors. SREBF expression increase with abomasal glucose infusion (BALDWIN et al., 2006), that, in turn, increase expression of *ACACA* and *FASN* (LADEIRA et al., 2016).

On the other hand, increasing starch supply to the rumen can cause acid accumulation (JENKINS; HARVATINE, 2014), reducing rumen pH, due to the rapid and excessive fermentation (OWENS et al., 1998) (figure 2A). The reduction in rumen pH changes the ruminal environment that can lead to a microbial population shift, accompanied by an increase of CLA C18:2 *trans*-10 *cis*-12 (JENKINS; HARVATINE, 2014) (figure 2B).

The increase in C18:2 *trans*-10 *cis*-12 can be responsible to reduce the expression of *SREBF1* (OBSEN et al., 2012; TEIXEIRA et al., 2017). This result is responsible for decreased lipogenesis, because of decreasing *ACACA* and *FASN* in muscle, thus reducing *de novo* fatty acid synthesis (HILLER; HERDMANN; NUERNBERG, 2011) (figure 2C).

Supporting these authors, Vyas et al. (2014) reported that a diet with greater concentration of CLA C18:2 *trans*-10 *cis*-12 reduced expression of *SREBF1* and peroxisome proliferator-activated receptor gamma (*PPARG*), which consequently reduced lipid synthesis by reducing *ACACA* and *FASN*. Similarly, in pre-adipocyte pigs, C18:2 *trans*-10 *cis*-12 decreased the synthesis of *SREBF1* mRNA (BRANDEBOURG; HU, 2005). Jenkins & Harvatine (2014) also found more C18:2 *trans*-10 *cis*-12 and lower *SREBF1* expression in the mammary glands when cows were fed diets rich in grains.

Additionally, C18:2 *trans*-10 *cis*-12 reduces insulin-stimulated glucose uptake due to suppress *PPARG* and glucose transporter type 4 (CHUNG et al., 2005). Moreover, this CLA

reduce total acyl-CoA levels and increased the amount of C18:0 acyl-CoA, which has been implicated in insulin resistance due to impaired oxidation (LOWELL; SHULMAN, 2005).

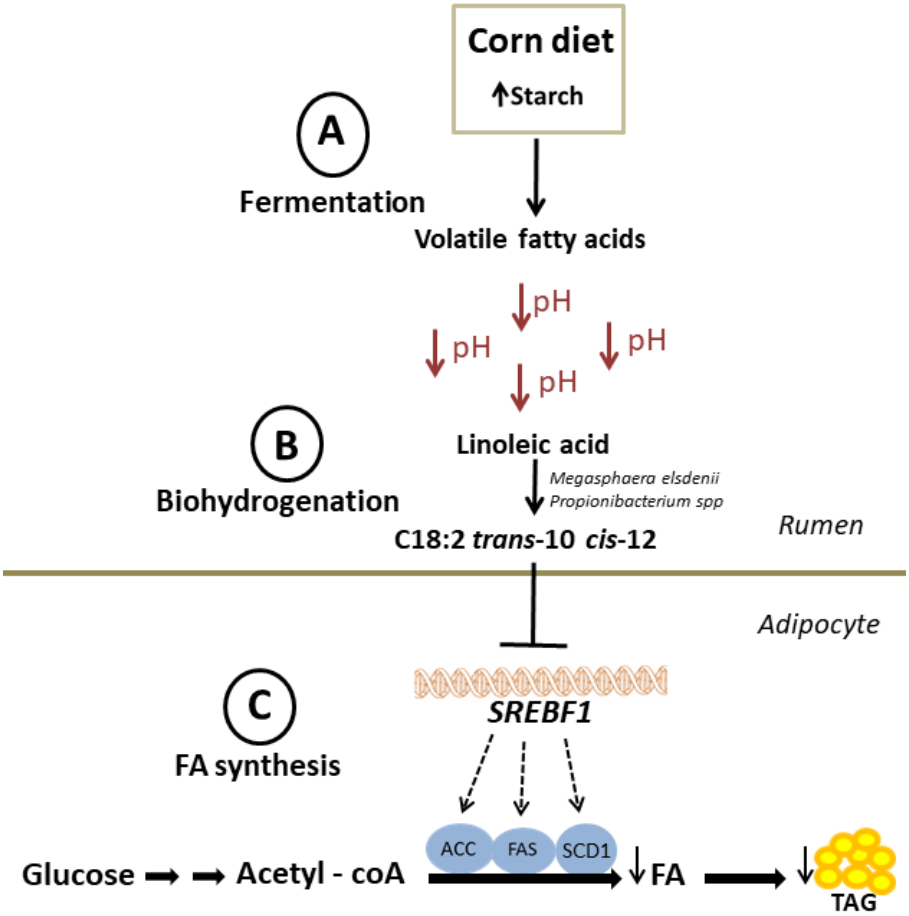

Figure 2. Effect of rumen pH on lipogenesis in bovine muscle. Adapted from Ladeira et al. (2018)

ACC: acetyl-CoA carboxylase; FA: fatty acid; FAS: fatty acid synthase; SCD: stearoyl-CoA desaturase; SREBF1: Sterol regulatory element binding transcription factor 1; TAG: triacylglycerol.

2.2. Effect of diet on fatty acid profile

There is interest in manipulating fatty acid profile of meat because many fatty acids have beneficial or detrimental actions in human metabolism. For example, PUFA participates in several biological processes relevant to human health, besides be precursors of a variety of lipid

regulators of cellular metabolism (BERTON et al., 2016; PILARCZYK; WÓJCIK, 2015). Additionally, CLA C18:2 *cis*-9 *trans*-11 has been suggested to be an anticarcinogenic and hypolipidemic and reduces the risk of diabetes (VAHMANI et al., 2015). On the other hand, SFA as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) fatty acids are hypercholesterolemic because of increase low-density lipoprotein (LDL) content in the blood (WOOD et al., 2003).

In cattle, as in other animals species, lipid tissue composition reflects and can be influenced by diet. In non-ruminant, dietary fatty acids can be incorporated directly into the tissues (Nuernberg et al., 2005). However, in ruminants, the deposition of dietary fatty acids is restricted due to ruminal biohydrogenation. Therefore, PUFA are obtained mainly from the diet and preferentially incorporated into phospholipids of the membranes, whereas SFA are mainly synthesized from glucose or acetate and deposited mainly in the triacylglycerol fraction, which increases with intramuscular fat (WOOD; ENSER, 2017).

In this sense, diets with a high proportion of concentrate (high starch) result in a lower biohydrogenation, allowing more UFA to reach the intestine (ROSSI et al., 2016), being absorbed and incorporated in the muscle tissue. Moreover, inhibition of the final step of biohydrogenation increase C18:2 *cis*-9 *trans*-11 and C18:1 *trans*-11. Additionally, greater concentration of MUFA may be due to an increase in expression and activity of stearoyl-CoA desaturase (SCD), known as delta-9-desaturase (DUCKETT; PRATT; PAVAN, 2009; SMITH et al., 2009), that convert C18:0 to C18:1 *cis*-9 (ROSSI et al., 2016)

On the other hand, as mentioned before, high grain diets can reduces rumen pH altering biohydrogenation pathways, increasing C18:1 *trans*-10 and C18:1 *trans*-10 *cis*-12 (BESSA et al., 2005; BRESSAN et al., 2016). Both fatty acids have negative actions to human health, where C18:1 *trans*-10 increasing plasma LDL cholesterol (BAUCHART et al., 2007) and C18:1

trans-10 *cis*-12 has linked to insulin resistance and inflammation in human adipocytes (KENNEDY et al., 2010).

Duckett et al. (2013), working with forage and grain diets, detected that steers fed grain diets had greater concentration of oleic, C18:1 *trans*-10 and MUFA, but lower concentration of C18:1 *trans*-11 and C18:2 *cis*-9 *trans*-11. Supporting this data, Faucitano et al. (2008) also showed greater MUFA and oleic acid concentrations in grain-fed versus grass-fed beef and Duckett et al. (2009) found greater concentration of C18:2 *cis*-9 *trans*-11 in forage-finished beef and greater concentration of C18:1 *trans*-10 and MUFA in concentrate finished beef. However, Bressan et al. (2016) found greater amount of SFA in beef from animals fed grain diet, due to greater amount of C14:0, C16:0 and C18:0. Likewise, Bressan at al. (2011) found a greater concentration of hypercholesterolemic fatty acids as C12:0, C14:0 and C16:0 in grain-finished animals when compared with pasture finishing.

Although high concentration of PUFA in the muscle is important, the n-6/n-3 ratio is even more crucial. Balance of n-6 to n-3 fatty acids is an important determinant in reducing risk of hypertension, heart disease, diabetes, arthritis and inflammatory disorders (VAHMANI et al., 2015). Wood et al. (2003) recommend maintain n-6/n-3 ratio below to 4.0, but current Western diets have ratios close to 15:1 (SIMOPOULOS, 2006). This occur because grain diets have high levels of 18:2 n-6 and lower concentration of 18:3 n-3. Consequently, beef from grain fed cattle has greater concentrations of omega-6 while grass-fed beef have greater concentrations of omega-3 (HWANG; JOO, 2017; LARICK; TURNER, 1990). In agreement with these studies, Chail et al. (2016) found greater n-6/n-3 ratio for animals fed grain diets, due to greater concentration of omega-6 and lower concentration of omega-3.

2.3. Effect of beef cattle breeds on quality meat

Attaining a high beef quality standard is important for consumer satisfaction and for repurchase (COLEMAN et al., 2016). Genetic is one of the main factors that influence beef quality, because breeds may exhibit distinct patterns of growth and, consequently, muscle tissue deposition and fatty acid profile, which might influence sensory characteristics, such as color, juiciness, flavor and tenderness (LADEIRA et al., 2014; MATSUISHI; FUJIMORI; OKITANI, 2001)

It is known that *Bos taurus* (e.g. Angus breed) cattle exhibit more marbling compared to *Bos indicus* (e.g. Nellore) (ELZO et al., 2012). This fact can be due to the frame of breeds, for example, Angus has a small-frame breed, and Nellore is a medium-sized breed. This characteristic means that Angus steers begin to deposit fat at an earlier age (Diniz et al., 2015). According to Paulino, Duarte & Oliveira (2013), the use of early maturity breeds is an alternative to stimulate intramuscular fat deposition.

Supporting these data, Silva et al. (2014) found that European animals had greater marbling than Zebu animals (4.99 vs. 3.95 points) when both were fed with diets with high concentrate. On the other hand, Bressan et al. (2011) showed that animals *Bos indicus* had 0.7% greater fat than *Bos taurus*. This result agrees with Moreira et al. (2003) that reported greater levels of intramuscular fat in Nellore cattle when compared with *Bos indicus* x *Bos taurus*. According to the authors, these can be due to Nellore bulls were more advanced maturity than crossbred steers.

Intramuscular fat content of meat is considered a highly desirable characteristic by consumers due to its correlation with flavor, juiciness, and tenderness (EMERSON et al., 2013; STARKEY et al., 2016). The fat affect flavor due to fatty acids, on oxidation, produce carbonyl compounds that are potent flavor contributors (HORNSTEIN, 1971). Additionally, these lipids present in meat stimulate flow of saliva that increase the meat's apparent juiciness

(SCHÖNFELDT et al., 1993). Furthermore, intramuscular fat may aid in meat tenderness due to the fat diluting the effects of tougher myofibrillar elements or reduce the rigidity of the muscle structure (WARRISS, 2010) or due to a decrease in the density of muscle (KARLSSON; KLONT; FERNANDEZ, 1999).

Along with marbling, tenderness is considered the main palatability characteristic by consumers (FONT-I-FURNOLS; GUERRERO, 2014). According to Koohmaraie (1992), 15% of the variation in meat tenderness is explained by differences in marbling and connective tissue and 85% is due to the mechanism of meat tenderization during *postmortem* storage.

It is known that zebu beef had lower tenderness due to a lower calpain and higher calpastatin activity compared with taurine cattle (DUARTE et al., 2013; KOOHMARAIE, 1994). The calpain-calpastatin system participates in breakdown during protein turnover and continues to be active in muscle *postmortem* affecting tenderization of meat (DUARTE et al., 2013). The calpains cause hydrolysis of intermediate filaments, weakening the muscle fibers' structure, making more tender meat (MUROYA et al., 2006). However, calpains may have activity inhibited by calpastatin (LAGE et al., 2009).

Martins et al. (2017) reported that Nellore bulls have higher calpastatin activity than Angus, resulting in a toughness. In this sense, Lage et al. (2012) found that Nellore had greater shear force than crossbreed animals (½ Angus × ½ Nellore), and it may happened because *Bos indicus* cattle reduce *postmortem* proteolysis resulting from elevated calpastatin activity. Barcellos et al. (2017) also found that Angus × Nellore had beef more tender than Nellore. This author also attributes this difference to higher calpastatin activity in Nellore, since they did not find a difference in total lipids and collagen.

Another important characteristic related to meat quality is its fatty acid profile, for affecting sensory characteristics of meat, as taste (WOOD et al., 2008). Moreover, as mentioned before, fatty acids profile has influence on consumer health, since some fatty acids have

beneficial and detrimental actions in human health. Breed affects meat fat content, and fat content itself is a factor in determining fatty acid composition (MACEDO et al., 2008; PRADO et al., 2008). As reported by Wood et al. (2008) and Rossato et al. (2010) differences in fatty acid profile between *Bos indicus* and *Bos taurus* also may be associated with genetic differences in the biohydrogenation process, linked to the microbial enzymatic processes and the time of permanence of the food particles in the rumen. In addition, muscle tissue metabolism affects fatty acid profile.

According to Mir et al. (2004), animals that had greater fat content had greater content of CLA per 100 g of meat. However, Wood et al. (2008) show that breeds with low concentration of lipid will have greater proportions of PUFA and lower SFA. This occurs due to the preferential incorporation of PUFA into the phospholipids associated within the muscle fiber membranes, whereas SFA and MUFA are deposited mainly in the triacylglycerol fraction, which increases with intramuscular fat content (DE SMET; RAES; DEMEYER, 2004).

The SCD is the main enzyme responsible to convert SFA into UFA (LADEIRA et al., 2016). Conforming to Bressan et al. (2011) differences among genetic groups explain between 17 and 24% of the variability observed in the estimated activity of delta-9-desaturase. In addition, crossbred steers has a behavior very close proximity with *Bos indicus* (GAMA et al., 2013).

Bressan et al. (2011), working with *Bos taurus* and *Bos indicus*, found that taurine tends to accumulate lower amounts of SFA when they are finished intensively. In this sense, Bressan et al. (2016) detected that when animals were fed with high grain diet, *Bos indicus* had greater concentration of SFA and 18:0, and lower amount of fatty acid synthesized from 18:2 n-6 and 18:3 n-3 than crossbred *Bos taurus* × *Bos indicus*, suggesting that Zebu may have lower capacity in biochemical pathways involved in the metabolism of omega-6 and omega-3 long chain fatty acids.

Similar, da Silva et al. (2014) found that Zebu had greater concentration of hypercholesterolemic fatty acids and European bulls had lower content of myristic, and gamma-linolenic. On the contrary, Lopes et al. (2012) showed greater contents of UFA and MUFA in Nellore than Red Norte bulls, a compound breed with 75% of *Bos taurus* blood. However, Red Norte had greater concentration of CLA. Rossato et al. (2010) found greater concentration of omega-3, CLA and C18:1 *trans* in Nellore than Angus, featuring nutritionally healthy Nellore beef. In general, these divergences of fatty acid profile results among *Bos indicus*, *Bos taurus* and crossbred may be due to the diets used in finishing phase (BRESSAN, M. et al., 2011; BRESSAN et al., 2016).

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SECOND CHAPTER

Fatty acid profile and beef quality of Nellore and Angus young bulls fed whole shelled corn

Article formatted according to the Meat Science guidelines

Fatty acid profile and beef quality of Nellore and Angus young bulls fed whole shelled corn

ABSTRACT: The objective was to evaluate the fatty acid profile and quality characteristics of beef from Nellore and Angus bulls fed whole shelled corn (WSC) and ground corn (GC) diets. Thirty-four young bulls with BW of 381 ±12 kg were used in a completely randomized design using a 2 × 2 factorial arrangement. Angus beef was more tender compared to Nellore ($P < 0.01$) and had greater concentration of polyunsaturated fatty acids (PUFA) and omega 6 ($P < 0.01$). Beef of bulls fed WSC tended to have greater concentration of CLA C18:2 *cis*-9 *trans*-11 ($P = 0.10$), greater CLA C18:2 *trans*-10 *cis*-12 ($P = 0.01$) and PUFA ($P = 0.05$), and consequently, greater oxidation. In conclusion, Angus bulls produce better quality beef showing more tenderness. Additionally, fatty acid profiles differed among Nellore and Angus bulls, but some differences between breed is dependent on the feedlot diet. In addition, WSC diet may reduce lipogenesis because increased C18:2 *trans*-10 *cis*-12 content.

Keywords: Bos indicus, CLA, PUFA, tenderness, rumenic acid.

1. INTRODUCTION

Promote a high beef quality and reduce human health risk are objectives of beef industry to satisfy consumer's desire. Genetic and nutrition are major factors that influence beef quality and, therefore, can be altered during the production system. Different breeds have distinct patterns of growth and, consequently, deposition of muscle and adipose tissue, which may affect juiciness, flavor and tenderness (Ladeira et al., 2014; Matsuishi, Fujimori, & Okitani, 2001). In this sense, as the percentage of *Bos indicus* genes increases, tenderness reduces (Andrade et al., 2010; Barcellos et al., 2017; Duarte et al., 2013). In addition, breeds with lower concentration of lipid have greater proportions of PUFA and lower concentration of saturated fatty acid (SFA)

and monounsaturated fatty acid (MUFA) (De Smet, Raes, & Demeyer, 2004). In this case, the preferential incorporation of PUFA is into phospholipids of the membranes, whereas SFA and MUFA are deposited mainly in the triacylglycerol fraction, which increases with intramuscular fat.

Furthermore, differences in fatty acid profile between *Bos indicus* and *Bos taurus* also may be associated with genetic differences in the biohydrogenation process, rumen volume and gastrointestinal tract (Bressan et al., 2011; Rossato et al., 2010). Bressan et. al. (2016) suggested that *Bos indicus* has a limited capacity in synthesizing n-3 and n-6 long-chain PUFA. Muscle metabolism and enzymes activities are also factors which may affect fatty acid profile. Conforming to Bressan et al. (2011) differences among genetic groups explain between 17 and 24% of the variability observed in the estimated activity of stearoyl-CoA desaturase (SCD), that is the main enzyme responsible to convert SFA into unsaturated fatty acids (UFA) (Ladeira et al., 2016).

These differences among breeds can also be highly influenced by finishing diet (Bressan et al., 2011; Bressan et al., 2016). Therefore, some diet components can change rumen microbial ecosystem, altering, for example, biohydrogenation. In this sense, increasing starch supply to the rumen can reduce rumen pH, due to the rapid and excessive fermentation (Owens, Secrist, Hill, & Gill, 1998), resulting in an incomplete biohydrogenation with an increase of CLA C18:2 *trans*-10 *cis*-12, instead of C18:2 *cis*-9 *trans*-11 (Jenkins & Harvatine, 2014). Moreover, the increase in C18:2 *trans*-10 *cis*-12 can be responsible for decrease the activity of key lipogenic enzymes (Obsen et al., 2012), reducing concentration of products from *de novo* fatty acid synthesis (Wu et al., 2011).

Therefore, we hypothesized that Angus bulls will have greater tenderness and greater *de novo* synthesis, increasing SFA. On the other hand, Nellore bulls will have lower intramuscular fat and consequently greater concentration of UFA. In addition, bulls fed a whole

shelled corn diet will have greater tenderness and lower biohydrogenation increasing UFA and decreasing SFA. Thus, the objective of this study was to evaluate the fatty acid profile, tenderness, color and oxidation of the beef from Nellore and Angus bulls fed a diet without forage and whole shelled corn or a diet with silage and ground corn.

2. MATERIAL AND METHODS

The experimental procedures were approved by the Ethics and Animal Welfare Committee of the Federal University of Lavras. The experiment was performed in the Beef Cattle facility of the Animal Science Department of the Federal University of Lavras (Lavras, Brazil).

2.1. Experimental Design, Animals, and Diets

Seventeen Nellore and 17 Angus young bulls with an age range of 18 to 22 mo and BW of 381 ±12 kg were housed in individual pens with individual feeders and automatic water drinkers. Experimental diets (DM basis) consisted of a ground corn (GC) diet with 30% corn silage and 70% of a GC, based concentrate, and a whole shelled corn (WSC) diet, with 85% WSC and 15% of a soybean meal and mineral-based pelleted supplement (Table 1). The same flint corn was used in both diets, being processing the only difference (whole or ground). The experimental design had a completely randomized design in a 2 x 2 factorial arrangement, consisting of the following treatments: Nellore fed the GC diet (n = 9), Nellore fed the WSC diet (n = 8), Angus fed the GC diet (n = 9), and Angus fed the WSC diet (n = 8). The experimental diets were offered for *ad libitum* intake, twice daily at 7:30 am and 3:30 pm.

The animals were given 28 days to adapt to the facilities and diets. At the beginning of the adaptation period, the animals were treated for internal and external parasites (Ivomec®, Paulínia, Brazil).

2.2. Slaughter, meat collection and analysis

After an experimental period of 81 days, Nellore bulls were slaughtered with a final BW of 451.5 kg and average daily gain (ADG) of 1.14 kg/day and Angus bulls with 545.5 kg of final BW and ADG of 1.89 kg/day (Carvalho et al., 2016). The technique of slaughter was concussion and exsanguination of the jugular vein followed by hide removal and evisceration. Subsequently, the carcasses were identified, washed, and divided into halves.

Twenty-four hours after cooling at 2°C, six 2.54 cm thick steaks were collected and sequentially identified for treatment in the following manner: Four steaks were individually packaged under vacuum (Packer model BS420, Ubá, MG, Brazil) in nylon-polyethylene packages to determine the color, cooking loss and shear force, being that each steak was used one aging time (0, 7, 14 and 21 days' *postmortem*). The fifth steak was divided into 4 parts, where one part was used for ether extract and fatty acid profile. The remaining three parts were packaged under vacuum (Packer model BS420, Ubá, MG, Brazil) in nylon-polyethylene packages to determine TBARS being that each steak was used one aging time (0, 14 and 42 days' postmortem). The last beef was used to analyze sarcomere length and myofibrillar fragmentation index at four aging times (0, 7, 14 and 21days' *postmortem*). The samples for aging time were stored under refrigeration (1 ± 0.5°C) in an environmental chamber (Model EL202, EletroLab, São Paulo, SP, Brazil).

2.2.1. Cooking loss

For determination of the cooking loss, steaks were weighed and grilled at 160–180°C until they reached an internal temperature of 71°C (AMSA, 1995) monitored by a digital thermometer (TD-880 with a K-type thermocouple; ICEL, Manaus, AM, Brazil) inserted into their geometric centers. Subsequently, each steak was conditioned to room temperature and

after temperature stabilization, the steak was weighed, and the result was expressed in a percentage.

2.2.2. Shear force

The shear force was obtained from the same samples used for the cooking loss, according to the Warner–Bratzler Square Shear Force method described by Silva et al. (2015). Six cores samples with 1.0 × 1.0 × 2.5 cm were obtained from each steak in the muscle fiber direction. The core was completely sheared perpendicularly to the muscle fibers with a Warner–Bratzler blade of 1.016 mm at a speed of 200 mm/min coupled to a TA.XT plus texture meter (Stable Micro Systems Ltd., Godalming, Surrey, UK). The maximum force (N) was measured, and the average value was calculated for each steak.

2.2.3. Myofibrillar fragmentation index

The myofibrillar fragmentation index was analyzed by the protocol described Olson & Stromer (1976), with modifications described by Culler, Parrish, Smith, & Cross (1978). An aliquot of the myofibril suspension was diluted with extraction solution to a protein concentration of 0.5 ±0.05 mg/mL and determined by the optical density at 540 nm in a Genesys 10 UV spectrophotometer (Thermo Scientific, Madison, WI, USA). Absorbance was multiplied by 200 to give a myofibrillar fragmentation index for each steak.

2.2.4. Sarcomere length

Sarcomere length analysis was carried out in the raw samples, which were fixed using the methodology described by Koolmees, Korteknie, and Smulders (1986), with minor modifications described by Aroeira et al. (2016). The sarcomere length was determined by

helium-neon laser diffraction (Model 05-LHR-073, Melles Griot, Carlsbad, USA) as described by Cross, West, & Dutson (1981), and the average was calculated.

2.2.5. Ether extract and fatty acids

To assess the ether extract, the meat samples were lyophilized to obtain homogeneous samples and was performed according to the method 920.85 of the Association of Official Analytical Chemists (AOAC, 1990).

The fatty acid profile was analyzed in high-resolution gas chromatography. Lipids were extracted according to the procedures established by Folch, Lees, & Sloane-Stanley (1957), using chloroform and methanol at a 2:1 ratio, and were esterified according to methods previously described by Hartman & Lago (1973).

The esterified samples were diluted in 1 mL of hexane and then centrifuged. A 1-μL aliquot was injected into Agilent 7890A gas chromatograph equipped with an automatic injector and flame ionization detector (Agilent Inc.; Santa Clara, CA). A 100 m SLB-IL111 column with 100 m × 0.25 μm internal diameter and 0.20 μm film thickness (Supelco Inc., Bellefonte, PA, USA). The samples were quantified according to the procedure described by Delmonte et al. (2011) to separate all long chain fatty acid isomers. Hydrogen was used as carrier gas at 1 mL/min constant flow with the linear velocity of 26 cm/s. The oven was maintained at 168ºC isothermal temperature, the injection port at 250ºC, and the detector at 250ºC. The split ratio was set to 1:100 and the typical injection volume was 1-μL. Reference mixtures (37-component FAME mix, conjugated (9Z,11E) linoleic, and conjugated (10E,12Z) linoleic acid; Sigma-Aldrich, St. Louis, MO, USA) were used to identify peaks. The molar percentage of fatty acids was calculated by dividing the concentrations of the individual fatty acid peak area by the total of all fatty acid peak areas, which gives grams of fatty acids per 100 grams of fatty acids (g/100g).

2.2.6. Thiobarbituric acid-reactive substances (TBARS)

TBARS was performed according to Tarladgis et al. (1960) and adapted by Carvalho et al. (2014). The absorbance was measured at 530 nm in a spectrophotometer (Bel Photonics, model SP 1105, Piracicaba, Brazil). The TBARS value expressed as the mg malonaldehyde/ kg meat was obtained multiplying absorbance for 7.8.

2.2.7. Color

Determination of the CIE L*, a*, and b* color components were taken after removing each sample from the vacuum package and exposing it to atmospheric air for 30 min for blooming for oxygenation of myoglobin. The color reading was performed on the surface of the steaks at each aging time using a CM-700 spectrophotometric colorimeter (Konica Minolta Sensing Inc., Osaka, Japan), with 8 mm aperture, illuminant A, and 10° observer angle.

Data were recorded using an average of five consecutive measurements representing the entire surface of each sample. The *L**, *a** and b* components were obtained from the SCE mode readings. The polar coordinates chroma (C*) and hue angle (h*) were also determined as: $C^*=[(a^*)^2+(b^*)^2]^{0.5}$ and $h^* = \tan^{-1}(b^*/a^*)$.

2.3. Statistical Analysis

The fatty acid profile was analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with diet, breed and diet*breed interaction as fixed effects and animals such as random effect.

Cooking loss, shear force, myofibrillar fragmentation index, sarcomere length, coloration and lipid oxidation were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effects of breed, diet, time and their interaction and animals as random effect. The covariance structure was chosen according to the

Bayesian information criterion, by comparing 4 covariance structures for each variable (compound symmetry, autoregressive order one, heterogeneous autoregressive order one, and unstructured), and the structure that yielded the smallest Bayesian information criterion was used. The least squares means (LSMEANS) statement was used to calculate the adjusted means for treatments. Statistical significance was declared at $P \leq 0.05$ and tendencies are discussed when $0.05 < P \leq 0.10$.

3. RESULTS

3.1. Cooking loss, shear force, myofibrillar fragmentation index and sarcomere length

There was no effect ($P > 0.05$) of diet and breed on cooking loss (Table 2). Beef from Angus bulls was more tender, had greater myofibrillar fragmentation index and sarcomere length than Nellore. However, there was no effect of diets on these quality characteristics (Figure 1).

Aging time affected all variables, regardless of diets and breeds. There were a quadratic effect on cooking loss, with greater loss on 17 days (Figure 2). Likewise, myofibrillar fragmentation index also had quadratic effect, with greater index for Nellore on 17 days and Angus on 16 days aging.

3.2. Fatty Acids Profile

A greater concentration of palmitic (C16:0) was observed in LT muscle of Nellore bulls, regardless of diet (Table 3). On the other hand, Angus bulls had greater content of linoleic (C18:2 n-6), PUFA and omega-6. There was a breed x diet interaction on concentration of five important fatty acid in *longissimus* muscle. Concentrations of myristic (C12:0) and stearic acid (C18:0) did not differ when Nellore was fed with WSC or GC diets, but when Angus bulls was fed with WSC, their muscle had greater concentration of these fatty acids. On the other hand,

concentration of α-linolenic (C18:2 n-3) did not differ with Angus fed WSC or GC but increased when Nellore was fed with WSC diet. Concentration of pentadecanoic (C15:0) and myristoleic (C14:1) were greater in Angus fed WSC diet, however in Nellore muscle, their concentrations had different effects, being C15:0 greater and C14:1 lower in Nellore fed WSC.

Regardless of breed, LT muscle of bulls fed WSC had greater concentration of linoleic acid and CLA C18:2 *trans*-10 *cis*-12 and tended to increase concentration of CLA C18:2 *cis*-9 t*rans*-11 and C18:1 *trans*. However, it was not possible to separate the C18:1 *trans* isomers. Furthermore, bulls fed WSC had greater concentration of PUFA and omega-6, and lower content of C16:0.

3.3. TBARS and Colors

Regardless of the breed, beef of animals fed WSC tended to have greater TBARS values than animals fed GC, which also increased with aging time (Figure 3). For color components, diets and breed did not affect L*, a*, b* and C*. However, Nellore beef tended to have (P = 0.06) greater hue value (Table 2). Furthermore, h* value was not affected by aging. On the other hand, L*, a*, b* and C* were affected by aging time with quadratic effect, regardless breed and diet. An increase in L*, a*, b* and C* was observed with aging time, but after 15 days ripening, a*, b* and C* started to decrease, whereas L* value decreased after 16 days aging (Figure 4).

4. DISCUSSION

In general, tenderness occurs by the fact that proteolytic enzyme activity, that participates in breakdown during protein turnover, continues to be active in muscle *postmortem* (Duarte et al., 2013). The enzyme activity of the proteolytic systems can be indirectly measured by myofibrillar fragmentation index and can predict over 50% of variations in beef tenderness (Warriss, 2010). According to Culler Jr, Smith & Cross (1978), myofibrillar fragmentation

index values above 60 characterize meat as very tender, values between 50 and 60 as moderate tenderness and values below 50 as a lack of tenderness. In this study, the beef of Angus and Nellore without aging time had myofibrillar fragmentation index equal to 35 and 36 respectively, meaning that the meat of Angus is classified with moderate tenderness and meat of Nellore classified with a lack of tenderness. However, with 14 of days aging time, Angus beef become very tender (index equal to 64.2), while Nellore beef approached the very tender level with only 21 days (index equal to 60.5). Moreover, evaluating shear force, Angus beef can be classified as tender on the 7st aging day (shear force equal 50.6 N), while the Nellore beef only approached the tender on the 21st aging day (shear force equal 52 N), considering 53 N threshold for tender meat (Silva et al., 2015).

In addition, sarcomere also contributes to explaining the variation in beef tenderness. A reduction in the sarcomere length overall lead to an increase in shear force, decreasing tenderness (Starkey, Geesink, Collins, Hutton Oddy, & Hopkins, 2016). Hwang, Park, Cho & Lee (2004) suggested that the effect of sarcomere length on beef tenderness is dependent on the sarcomere shortening, which in turn is dependent on the storage temperature of the carcass (Locker & Hagyard, 1963). Nellore bulls had less subcutaneous fat compared with Angus bulls (4.0 and 6.75; $P < 0.01$) (Carvalho et al., 2016), which may have to allow for a high rate of temperature decline of Nellore carcasses, causing sarcomere shortening and reducing the tenderness.

Besides these factors, for the *Longissimus* muscle, intramuscular fat is another significant factor that explain the variance in shear force (Starkey et al., 2016). Intramuscular fat may give benefit in beef tenderness due to the fat diluting the effects of tougher myofibrillar elements or reduce the rigidity of the muscle structure (Warriss, 2010) or due to a decrease in muscle density (Karlsson, Klont, & Fernandez, 1999). In this study, the Angus beef had more intramuscular fat (Table 2), which can support the better tenderness in this breed.

In addition to tenderness, another important characteristic related to beef quality is the fatty acid profile, as it plays an important role in oxidative stability during cooking process, affecting taste (Wood et al., 2008). Moreover, fatty acid profile has a direct influence on consumer health, since some fatty acids have beneficial or detrimental actions in human metabolism.

The WSC diet had a greater concentration of linoleic acid (14.6% more than GC diet, Table 1) that could be hydrogenated by microorganisms producing saturated end products. However, rumen pH of the bulls fed WSC diet was lower (5.74 and 6.22, $P = 0.03$) (Carvalho et al., 2016) and could change rumen microbiota, resulting in an incomplete biohydrogenation. This effect was proven since bulls fed WSC diet tended to increase C18:2 c*is*-9 *trans*-11 and C8:1 *trans*, the intermediates produced from linoleic acid biohydrogenation. In addition, the reduction in rumen pH was a factor that contributed to an increase in C18:2 *trans*-10 *cis*-12 content (Jenkins & Harvatine, 2014).

Moreover, in another paper from this study (Teixeira et al., 2017), it was demonstrated that the increase in C18:2 *trans*-10 *cis*-12 reduced *SREBF1* expression in muscle of bulls fed WSC (1.81 versus 5.48 $P < 0.01$). Consequently, it can decrease *de novo* fat synthesis by reducing the activity of key enzymes such as acetyl-CoA carboxylase and fatty acid synthase and explain the reduction in palmitic acid content, a final product of *de novo* synthesis.

The tendency to increase C18:2 c*is*-9 *trans*-11 in bulls fed WSC was important due the beneficial actions to human health of this fatty acid. To date, animal and human studies have shown C18:2 c*is*-9 *trans*-11 is associated with prevention of different types of cancer, diabetes, cardiovascular health, immune modulator and anti-inflammatory (Bassaganya-Riera et al., 2012; Castro-Webb, Ruiz-Narváez, & Campos, 2012; Dugan, Aldai, Aalhus, Rolland, & Kramer, 2011; Fuke et al., 2012; Scollan et al., 2014; Vahmani et al., 2015).

Angus fed WSC had greater concentration of C15:0 that might be due to diets with high levels of grains and starch can provide greater propionate production in the rumen, which is a precursor of this fatty acid. The odd-chain fatty acids, such C15:0, are originating from in the first step of *de novo* synthesis by rumen bacteria through elongation of propionate (Fievez, Colman, Castro-Montoya, Stefanov, & Vlaeminck, 2012), and later incorporated into the microbial lipids. C15:0 is the main fatty acid in the cell wall of *Megasphaera elsdenii*, bacteria that is increased when rumen pH is low (Jenkins & Harvatine, 2014) and it happened when WSC diet was used.

Muscle of Angus fed WSC diet had greater concentration of C14:0, a fatty acid considered atherogenic and undesirable for human health, because minimize activity of hepatic cholesterol receptors, increasing LDL concentrations on human blood vessels (Vitina et al., 2012). In addition, Angus fed WSC increased C18:0, that represented approximately 40% of the total saturated fatty acids in the *longissimus* muscle and has neutral effect on human health (Vitina et al., 2012). The greater amount of C18:0 could be obtained by elongation of palmitic acid (C16:0), main fatty acid products formed by *de novo* synthesis, as indicated by the numerical reduction of C16:0 on these animals. Additionally, alfa linolenic (C18:3 n-3) acid is a fatty acid exclusively from dietary origin. In this sense, Nellore fed WSC had greater concentration of C18:3 n-3 in beef, that can be due to the lower rumen volume of *Bos indicus* (Carvalho et al., 2016; Menezes et al., 2007), and consequently, different rumen microbial ecosystem that, apparent had less capacity to biohydrogenate C18:3 n-3. On the contrary, due to same reason, may Angus fed WSC diet had more capacity to biohydrogenation, increasing C18:0, an end product of the pathway.

One hypothesis of this study was that Nellore bulls would have more unsaturated fatty acid due to have low *de novo* synthesis and consequently lower intramuscular fat. However, this hypothesis was not verified, and an alternative explanation for this phenomenon can be due

to interactions between diet and breed found in this study, since among the individual main fatty acids, 30% were affected ($P < 0.05$) by the interaction between breed and diet.

Angus bulls had greater concentration of PUFA which is opposite from previous literature results (Aldai et al., 2006; De Smet et al., 2004; Nfor et al., 2014), which demonstrated that cattle breeds with greater levels of intramuscular fat produce beef with lower amount of PUFA. The greater deposition of PUFA and omega-6 in Angus may be due to greater content of C18:2 n-6 in muscle of these animals. The greater concentration of C18:2 n-6 the greater concentration may be due to lower biohydrogenation extent in Angus than Nellore, since Angus bulls had greater passage rate (4.74%/h and 3.20%/h vs, $P < 0.01$) (Carvalho et al., 2016) than Nellore, increasing linoleic acid in the muscle. Furthermore, Angus had lower concentration of palmitic acid, which also is beneficial for human health, because it is hypercholesterolemic (Vahmani et al., 2015).

Regarding to diet effects, bulls fed WSC diet had greater muscle concentration of PUFA, linoleic acid and omega 6, that could be due to WSC diet had greater PUFA and linoleic acid content. In addition, lower rumen biohydrogenation likely happened when WSC diet was used may be helped in the increase of these fatty acids.

The tendency of greater TBARS values in the muscle of animals fed WSC diet corroborates with fatty acids results. According to Wood et al. (2008), fatty acid composition of muscle affects its oxidative stability during processing and retail display, where PUFA in phospholipid being liable to oxidative breakdown at this stage.

Despite muscle of animals fed WSC diet had greater lipid oxidation, this effect may was not enough to affect taste and succulence of the beef. According to Tarladgis, Watts, Younathan & Dugan (1960) TBARS value of approximately 0.5 mg of malonaldehyde/kg of meat can be considered the threshold for acceptable meat, above this value may indicate a level of lipid oxidation products which produce a rancid odor and taste which can be detected by consumers.

Color indices corroborate this results that, despite fatty acid changes due to diet or breed used, these changes were not enough to have a negative effect on color myoglobin or lipid oxidation.

5. CONCLUSION

Fatty acid profiles differed among Nellore and Angus bulls, but it is important to emphasize that some differences in fatty acid profile between breed is dependent on the feedlot diet. Angus bulls had greater *de novo* synthesis, increasing marbling, and consequently tenderness, however, had lower biohydrogenation increasing concentration of PUFA. Regarding diet, WSC may reduce lipogenesis in *longissimus* muscle because it is favorable to increase C18:2 trans-10 cis-12 content, preventing the animals increases intramuscular

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Table 1. Percentage of ingredients, chemical composition and proportion (%) of the fatty acids of the experimental diets.

Ingredients %	Diets (%DM)	
	Ground Corn	Whole Shelled Corn
Whole shelled corn[1]	-	85
Proteic and mineral supplement[2]	-	15
Corn silage[1]	30	-
Ground corn[1]	58	-
Soybean meal	10	-
Mineral supplement[3]	2	-
Nutrients. %		
Dry matter	57.3	87.8
Crude protein	12.7	14.6
Neutral detergent fiber	24.0	11.1
NDF from forage	14.7	0
Non-fiber carbohydrate[4]	56.2	67.1
Starch	49.0	61.8
Ether extract	2.2	2.6
Fatty acid		
Myristic (C14:0)	0.54	0.17
Palmitic (C16:0)	17.3	15.8
Stearic (C18:0)	6.13	3.35
Oleic (C18:1 c9)	27.32	29.4
Linoleic (C18:2)	42.16	48.3
Linolenic (C18:3)	3.3	2.97
ΣSaturated	22.63	16.8
ΣUnsaturated	74.95	83.2
ΣMonounsaturated	28.79	30.9
ΣPolyunsaturated	46.17	52.3

[1]Flint corn.

[2]Composition: corn, soybean meal, cottonseed meal, and soybean hulls (concentrations not provided by the company); CP: 32.0%, TDN: 50.0%, Ca: 45 g/kg, Mg: 7.5 g/kg, P: 11 g/kg, Cu 104 mg/kg, Zn: 344 mg/kg, Se: 0.83 mg/kg, 30,500 IU/kg of Vitamin A, 3,800 IU/kg of Vitamin D3, 134 IU/kg of Vitamin E (Nutronbeef Grano Entero; Nutron Alimentos, Campinas, Brazil).

[3]Assurance levels per kilogram of product: Ca: 170 g, P: 31 g, Na: 155 g, Zn: 2 mg, Cu: 396 mg, Mn: 515 mg, Co: 15 mg, I: 29 mg, Se: 5.4 mg, vitamin A: 111,000 IU, vitamin D3: 22,000 IU, vitamin E: 265 IU.

[4] Nonfiber carbohydrates calculated according to Sniffen, O'Connor, Van Soest, Fox, & Russell (1992).

Table 2. Cooking loss (CL), lightness index (L*), redness (a*), yellowness (b*) and chroma index (C*) of beef from Nellore and Angus young bulls fed ground corn (GC) or whole shelled corn (WSC) diets on different aging time.

	Angus		Nellore			*P* - Value			
Variables	GC	WSC	GC	WSC	SEM	Breed	Diet	B*D	B*D*A
CL (%)	26.6	27.6	27.8	26.0	0.82	0.80	0.59	0.11	0.55
L*	49.7	49.2	50.7	48.7	0.75	0.78	0.11	0.35	0.18
a*	13.9	14.0	13.8	13.9	0.47	0.76	0.84	0.94	0.89
b*	10.2	10.1	10.3	10.2	0.36	0.66	0.77	0.97	0.77
C*	17.3	17.3	17.2	17.3	0.58	0.97	0.98	0.95	0.84
h*	36.1	35.8	36.9	36.2	0.31	0.06	0.13	0.57	0.25

Table 3. Ether extract and composition of main fatty acids present in the muscle of Nellore and Angus young bulls fed ground corn (GC) or whole shelled corn (WSC) diets.

		Angus		Nellore			*P* – Value		
		GC	WSC	GC	WSC	SEM	Breed	Diet	B*D
Ether extract[1]		4.79	5.10	4.51	4.11	0.39	0.05	0.89	0.27
Fatty acids[1]									
Lauric	C12:0	0.56	0.54	0.56	0.63	0.08	0.56	0.71	0.55
Myristic	C14:0	3.98^{b}	7.53^{a}	4.97^{b}	4.06^{b}	0.79	0.12	0.09	<0.01
Myristoleic	C14:1	0.92^{c}	1.56^{ab}	1.74^{a}	1.10^{bc}	0.25	0.46	0.99	0.01
Pentadecanoic	C15:0	0.55^{d}	1.18^{a}	0.66^{cd}	0.85^{b}	0.10	0.24	<0.01	0.02
Palmitic	C16:0	25.9	14.6	30.8	24.7	2.19	<0.01	<0.01	0.22
Palmitoleic	C16:1	4.09	5.76	4.10	3.37	0.85	0.13	0.54	0.13
Margaric	C17:0	1.73	2.51	1.42	1.61	0.39	0.12	0.21	0.44
Stearic	C18:0	14.6^{b}	20.4^{a}	18.6^{ab}	14.8^{b}	2.66	0.63	0.58	0.04
Trans octadecenoic[2]	C18:1 *trans*	2.29	3.30	1.93	2.75	0.44	0.37	0.08	0.85
Oleic	C18:1 *cis*-9	29.6	27.0	29.3	34.7	3.65	0.21	0.64	0.18
Linoleic	C18:2 n-6	8.12	9.93	5.17	7.47	0.85	<0.01	0.02	0.76
CLA	C18:2 *cis*-9 *trans*-11	0.40	0.69	0.55	0.62	0.11	0.70	0.10	0.31
CLA	C18:2 *trans*-10 *cis*-12	0.14	0.17	0.14	0.18	0.01	0.96	0.01	0.68
α Linolenic	C18:3 n-3	0.43^{ab}	0.22^{b}	0.22^{b}	0.52^{a}	0.11	0.63	0.67	0.02
Arachidonic	C20:4 n-6	1.20	1.43	0.99	1.17	0.19	0.23	0.31	0.88
EPA	C 20:5 n-3	0.08	0.06	0.09	0.06	0.04	0.87	0.55	0.99
DHA	C22:6 n-3	0.22	0.24	0.19	0.23	0.04	0.72	0.46	0.79
∑ Saturated		49.74	48.02	55.29	47.87	4.40	0.51	0.26	0.48
∑ Unsaturated		50.35	51.43	45.34	52.28	4.28	0.60	0.31	0.46
∑ UFA/SFA		1.05	1.66	0.87	1.12	0.36	0.34	0.26	0.63
∑ Monounsaturated		37.65	35.62	35.76	40.91	4.57	0.69	0.71	0.40

∑ Polyunsaturated	12.70	15.81	9.58	11.36	1.29	<0.01	0.05	0.58
∑ Omega-3	0.93	0.88	0.82	0.77	0.14	0.40	0.69	0.98
∑ Omega-6	10.65	13.51	7.59	9.26	1.16	<0.01	0.03	0.57
∑Omega-6/∑Omega-3	16.22	16.39	10.58	13.31	3.26	0.15	0.63	0.67

[1] g/100 g

[2] Sum of C18:1trans (C18:1*trans*-9, C18:1 *trans*-10, C18:1 *trans*-11)

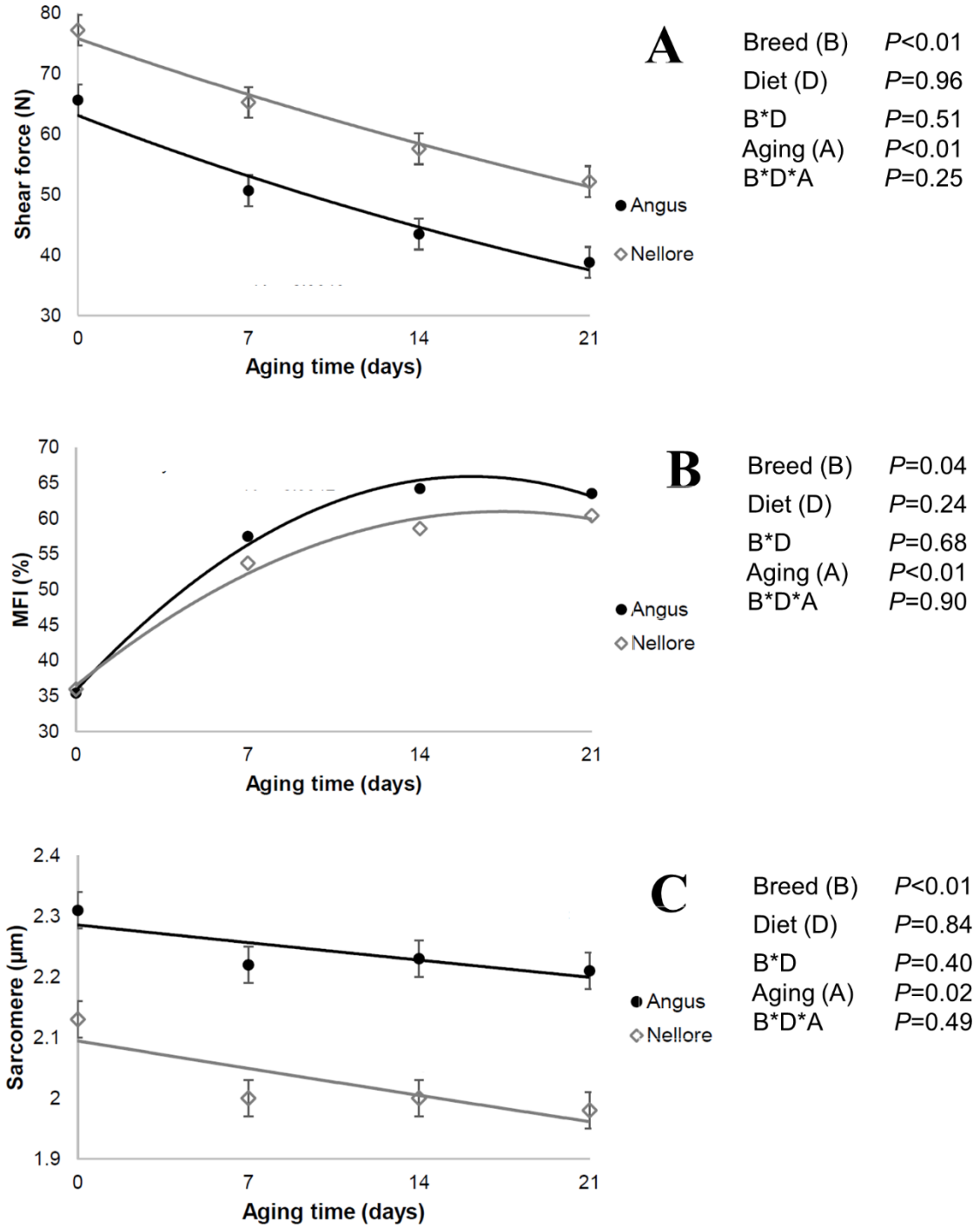

Figure 1. Shear force, sarcomere length and myofibrillar fragmentation index (MFI) of beef from Nellore and Angus young bulls fed ground corn (GC) or whole shelled corn (WSC) on different aging time.

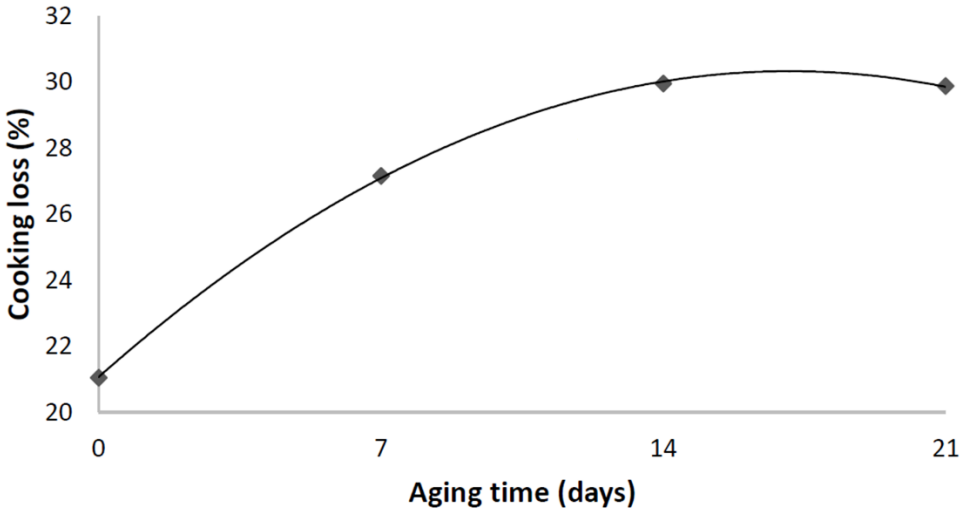

Figure 2. Cooking loss of beef from Nellore and Angus young bulls fed ground corn (GC) or whole shelled corn (WSC) on different aging time.

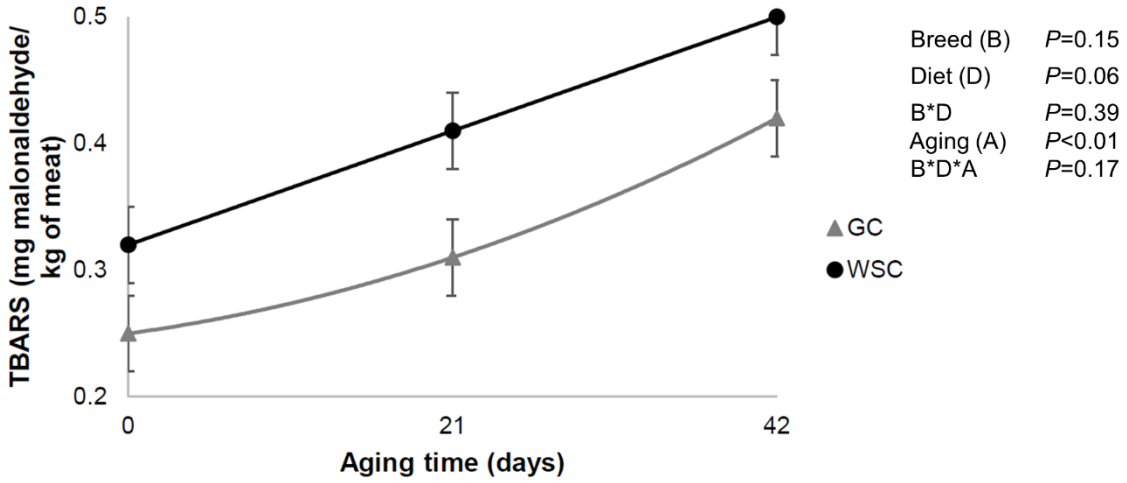

Figure 3. Lipid oxidation values of beef from Nellore and Angus young bulls fed ground corn (GC) or whole shelled corn (WSC) on different aging time.

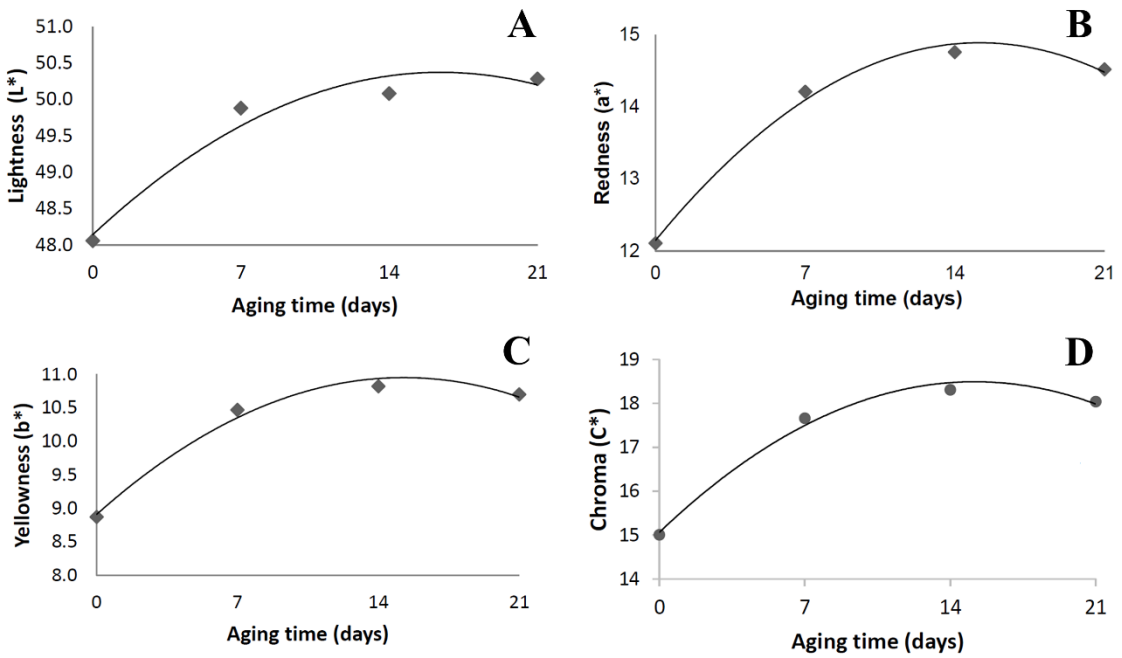

Figure 4. Color components of beef from Nellore and Angus young bulls fed ground corn (GC) or whole shelled corn (WSC) diets in different aging time.

THIRD CHAPTER

Beef quality and expression of genes involved in lipid metabolism in the muscle of Nellore and Nellore × Angus steers fed whole shelled corn diets

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Beef quality and expression of genes involved in lipid metabolism in the muscle of Nellore and Nellore × Angus steers fed whole shelled corn diets

ABSTRACT: Sixteen Nellore and Sixteen Nellore × Angus steers with 353 kg ±25.3 kg were randomly assigned into 2 feeding groups: whole shelled corn without forage (WSC) or WSC with sugarcane bagasse (WSCB), to evaluate chemical composition, expression of genes involved in lipid metabolism and beef quality. There was no effect ($P > 0.05$) of breed and diet on chemical composition. There was a tendency ($P = 0.10$) of Nellore beef be less tender 24 h after slaughter. Muscle from Nellore × Angus had greater expression ($P < 0.05$) of *LPL*, *FASN* and *CPT2*. Muscle from steers fed WSC diet had greater expression of *ACOX1* and lipid oxidation ($P < 0.05$). In conclusion, Nellore × Angus had greater lipid turnover that prevented to had greater intramuscular fat. In addition, animals fed whole shelled corn diet with sugarcane bagasse did not increase expression of *SREBF1* and lipogenic genes, and consequently did not increase marbling.

Keywords: *Bos indicus*, crossbreed, lipogenesis, sugar cane bagasse, tenderness.

1 INTRODUCTION

Diets with a high proportion of concentrate or even diets without inclusion of forage started to be used in Brazilian feedlots (Oliveira & Millen, 2014; Pinto & Millen, 2017). However, it is known that high grain diets can reduce rumen pH, which may alter rumen microbiota, and change biohydrogenation pathways (Jenkins & Harvatine, 2014). Therefore, this effect may increase C18:2 *trans*-10 *cis*-12 production and decrease lipid synthesis by reducing expression of transcription factors and other genes involved in lipogenesis (Obsen et al., 2012; Teixeira et al., 2017). In addition, it is expected that the use of fiber source in low quantities in whole shelled corn diets, as sugarcane bagasse,

can stimulate rumination and avoid metabolic disorders, helping animals to avoid rumen pH drop (Nagaraja & Lechtenberg, 2007; Owens, Secrist, Hill, & Gill, 1998), which consequently may increase muscle fat synthesis.

Bos indicus, such as Nellore, represents the majority beef breed in Brazil (Oliveira & Millen, 2014) due to their superior adaptation to tropical conditions. However, it is known that there is a decline in meat quality when *Bos indicus* are compared with *Bos taurus*, due to the lower tenderness and marbling in Zebu cattle. Therefore, crossbreeding *Bos indicus* with *Bos taurus* would be a possible way to improve meat quality, without losing adaptation characteristics from Zebu (Bressan et al., 2016). However previous researches have not demonstrated how diets without forage affect the expression of lipogenic genes and beef quality traits in crossbred from *Bos indicus* and *Bos taurus.*

The hypothesis of this study is that Nellore × Angus steers have a greater *de novo* synthesis that results in greater amounts of intramuscular fat and greater tenderness. In addition, steers fed a whole shelled corn diet with sugarcane bagasse increases expression of *SREBF1* which consequently increase expression of lipogenic genes and marbling.

Therefore, the aim of this study was to evaluate muscle chemical composition, expression of genes involved in lipid metabolism and qualitative characteristics of the beef from Nellore and Nellore × Angus fed whole shelled corn diet with or without sugarcane bagasse.

2. MATERIAL AND METHODS

The experiment was performed in the Beef Cattle facility of the Animal Science Department of the Federal University of Lavras (Lavras, Brazil). The experimental procedures were approved by the Ethics and Animal Welfare Committee of the Federal University of Lavras.

2.1 Experimental Design, Animals and Diets

Sixteen Nellore and Sixteen Nellore × Angus steers with 353 kg ±25.3 kg were housed in individual pens with individual feeders and automatic waterer drinkers. One experimental diet consisted of 80% whole shelled corn and 20% of a soybean meal and mineral-based pelleted supplement (WSC). The other diet had 74% whole shelled corn, 20% of the same protein-mineral pellet, and 6% of sugarcane bagasse (WSCB) (Table1). The experimental had a completely randomized design in a 2 x 2 factorial arrangement. Therefore, the treatments were: Nellore fed WSC diet, Nellore fed WSCB diet, Nellore ×Angus fed WSC diet, and Nellore ×Angus fed WSCB diet. The experimental diets were offered for *ad libitum* intake, 3 times daily at 7:30 am, 12:00 pm and 3:30 pm. The steers were given 20 days to adapt to the facilities and diets and 96 days of the experimental period.

2.2. Slaughter, meat collection, and analysis

Nellore steers were slaughtered at an average weight of 438 kg and Nellore × Angus steers with 456 kg by concussion technique and exsanguination of the jugular vein followed by hiding removal and evisceration. Subsequently, carcasses were identified, washed and divided into halves. After these procedures, muscle samples for expression analyses were taken from the *Longissimus thoracis* (LT) of the left half-carcass at the 13th rib. All instruments used for tissue collection were sterile. These muscle samples were washed with a 0.9% NaCl physiological solution and then frozen, transported in liquid nitrogen and stored at −80°C until gene expression analysis.

Twenty-four hours after cooling at 2°C, four 2.54 cm thick steaks were collected and sequentially identified for treatment in the following manner): First steaks divided,

and 2/3 of de beef was used for chemical composition analyses and 1/3 of the beef to fatty acid profile. The second and third beefs were individually packaged under vacuum (Packer model BS420, Ubá, MG, Brazil) in nylon-polyethylene packages and stored under refrigeration (1 ± 0.5°C) in an environmental chamber (Model EL202, EletroLab, São Paulo, SP, Brazil) to determine color, cooking loss and shear force, being that each steak was used for one aging time (3 and 14 days' *post mortem*). The last beef was used to TBARS. This procedure was analyzed in two *post mortem* aging (3 and 14 days) and two different time points (post mortem 3 = 0 and 4 days; post mortem 14 = 0 and 4 days), simulating supermarket display. For 4 days, the samples were identified and packed in polyethylene tray and overwrapped with PVC film at 2°C in a Bio-Chemical Oxygen Demand (BOD) incubator (Model EL202, EletroLab, São Paulo, SP, Brazil). For aging time 14, the samples were identified, and vacuum packed in nylon-polyethylene packages at 2°C in a BOD incubator.

2.2.1. Chemical composition

The 2/3 of the steak was thawed at room temperature, ground and used for chemical composition analyses using the FoodScan Meat Analyser TM® (FOSS, Hillerød, Denmark) with near-infrared spectrophotometer technology (analyzes AOAC method: 2007-04).

2.2.2. Cooking loss

For determination of the cooking loss, the whole steaks were weighed and grilled at 160–180°C until they reached an internal temperature of 71°C (AMSA, 1995) monitored by a digital thermometer (TD-880 with a K-type thermocouple; ICEL, Manaus, AM, Brazil) inserted into their geometric centers. The cooking loss was determined using

samples thawed at room temperature as the difference in the weight of a steak before and after cooking, and the result was expressed in a percentage.

2.2.3. Shear force

The shear force was obtained from the same steak used for the cooking loss, according to the Warner–Bratzler square Shear Force method described by Silva et al. (2015). Six slices samples with 1.0 × 1.0 × 2.5 cm were obtained from each steak in the muscle, free of visible fat and connective tissue with the direction of muscle fibers parallel to the length. Each slice was completely sheared perpendicularly to the muscle fibers with a Warner–Bratzler blade of 1.016 mm at a speed of 200 mm/min coupled to a TA.XT plus texture meter (Stable Micro Systems Ltd., Vienna Court, UK). The maximum force (N) was measured, and the average value was calculated for each steak.

2.2.4. Thiobarbituric acid-reactive substances (TBARS)

TBARS was performed according to Tarladgis et al. (1960) and adapted by Carvalho et al. (2014). The absorbance was measured at 530 nm in a spectrophotometer (Bel Photonics, model SP 1105, Piracicaba, Brazil). The TBARS value expressed as the mg malonaldehyde/ kg meat was obtained multiplying absorbance for 7.8.

2.2.5. Color

Determination of the CIE L*, a*, and b* color components were taken after removing each sample from the vacuum package and exposing it to atmospheric air for 30 min for blooming for oxygenation of myoglobin. The color reading was performed on the surface of the steaks from each aging time using a CM-700 spectrophotometric colorimeter (Konica Minolta Sensing Inc., Osaka, Japan), with 8 mm aperture, illuminant

A, and 10° observer angle. Data were recorded using an average of five consecutive measurements representing the entire surface of each sample. The *L**, *a** and b* components were obtained from the SCE mode readings. The polar coordinates chroma (*C**) and hue angle (h*) were also determined as: $C^*=[(a^*)^2+(b^*)^2]^{0.5}$ and $h^* = \tan^{-1}$ (b*/a*).The proportions of the myoglobin chemical forms were estimated by the Krzywicki (1979) mathematical method. Intermediate reflectance values (473, 525 and 572 nm) obtained on the SCI mode were determined by linear interpolation and the relative content of the heme pigments, expressed as a percentage of oxymyoglobin (OMb), deoxymyoglobin (DMb) and metmyoglobin (MMb).

2.2.6. Gene expression analyses

The design of target and reference primers was performed using sequences that are registered and published in the GenBank public data bank, a National Center for Biotechnology Information (NCBI) platform (Table 2). For gene characterization, the open reading frames (ORF) of the selected sequences were obtained using the ORFinder tool from NCBI, and the sequences of the codified proteins were obtained using the translate tool from the ExPASy protein bank. Primers were designed using OligoPerfect Designer software (Invitrogen, Karlsruhe, Germany) and synthesized (Invitrogen, Carlsbad, CA, USA).

Total RNA was extracted from muscle samples using QIAzol (QIAGEN, Valencia, CA) and treated with DNA-free DNase (Ambion, Austin, TX) according to the manufacturer's instructions. To analyse the 28S and 18S rRNA bands, the total RNA was electrophoresed in a 1.0% (m/v) agarose gel, stained with GelRed nucleic acid gel stain (Biotium, Hayward, CA) and visualized with a UVItec FireReader XS D-77Ls-20M (UVItec, Cambridge, UK). cDNA synthesis was performed using the High Capacity

cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions, and samples were stored at −20°C. Reverse-transcription quantitative PCR (RT-qPCR) was performed on Eppendorf Realplex system (Eppendorf, Hamburg, Germany) using SYBR Green detection system (Applied Biosystems, Foster City, CA, USA) according to Oliveira et al. (2014). The RT-qPCR analyses of each studied gene were performed using cDNA from 8 biological replicates, with 3 technical replicates per biological replicate.

The amplification efficiency of each reaction was measured using standard curves that were generated for the studied genes with the following dilutions: 1:5, 1:25, 1:125, 1:625 and 1:3125. Seven reference genes were tested and the best individual or combination of reference genes was chosen using the web-based tool RefFinder and the minimum number of genes was recommended by geNorm. RefFinder selected the *18s*, *β-actin* and *Cancer susceptibility candidate 3* (*CASC3*) genes as more stable to be used for muscle gene expression as a reference in the calculation of the comparative expression.

The Relative expression levels were calculated according to the method described by Pfaffl (2001), which is based on Ct values that are corrected for the amplification efficiency of each primer pair.

2.3. Statistical Analysis

Chemical compositions and gene expression were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with diet, breed and diet*breed interaction as fixed effects and animals such as random effect. A Shapiro-Wilk test was performed to assess the normality of all collected data. When data were not normally distributed, they were transformed using PROC RANK from SAS (SAS Inst. Inc., Cary, NC). Pearson correlation coefficients were calculated for gene expression using the

PROC CORR from SAS (SAS Inst. Inc., Cary, NC). The correlation analyses were based on individual values and were independent of the diets or treatments.

Cooking loss, shear force, color and lipid oxidation were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effects of breed, diet, time and their interaction and animals as a random effect. The covariance structure was chosen according to the Bayesian information criterion, by comparing 4 covariance structures for each variable (compound symmetry, autoregressive order one, heterogeneous autoregressive order one, and unstructured), and the structure that yielded the smallest Bayesian information criterion was used. The least squares means (LSMEANS) statement was used to calculate the adjusted means for treatments. Statistical significance was declared at $P \leq 0.05$ and tendencies are discussed when $0.05 < P \leq 0.10$.

The expression of transcription factors (*PPARA*, *PPARG*, and *SREBF1*) was compared between them and in function of diet, breed and their interactions. This analysis was performed using the MANOVA statement (Multivariate Analysis of Variance) of the GLM procedure of SAS 9.4. This was possible because the correlation among the three dependent variables was not greater than 0.37 (Cohen, 1988).

3. RESULTS

3.1. Quality characteristics

There was no effect ($P > 0.05$) of diet on chemical composition (Table 3). Nevertheless, LT muscle of Nellore steers tended to have a greater amount of protein ($P = 0.10$). Beef from Nellore steers had lower cooking loss and tended ($P = 0.10$) to be less tender than Nellore × Angus steers, only 24 h after slaughter (Figure 1). However, there was no effect of diet on these quality characteristics. Regardless of the breed, steers fed

WSC diet had greater TBARS values than steers fed WSCB after 4 days of display in both aging time (Figure 2). Furthermore, aging time increased TBARS value.

For color components, breed, diet and post mortem aging did not affect L*, a*, b* and C*. However, Nellore × Angus tended (P = 0.10) to have a greater hue value (Table 4). Furthermore, shear force (Figure 1A), cooking loss (Figure 1B) and color components (Table 5) were affected by aging time, where all variables increased after aging process. Regarding myoglobin pigments, there was interaction between breed and aging time for OMb (Figure 3A) and MMb (Figure 3B), where Nellore steers reduced MMb and increased OMb after 14 days' post mortem and Nellore × Angus steers increased MMb and reduced OMb after 14 days' post mortem.

3.2. Gene expression

An interaction among expression of transcription factors was found, with *SREBF1* being lower expressed than *PPARA* and *PPARG* in the muscles of all animals regardless of treatment (Figure 4). Additionally, there was no effect of diet and breed on gene expression of the transcription factors *SREBF1* and *PPAR* isomers (Table 6). Nellore × Angus steers had greater expression of *LPL*, *FASN* and *CPT2*. The expression of *SCD1* gene tended to increase in muscle of Nellore steers fed WSCB diet, while in Nellore × Angus muscle, this diet decreased *SCD1* expression. Regarding diet, steers fed WSC had lower expression of *FABP4*, and greater expression of *ACOX1*.

4. DISCUSSION

Nellore × Angus steers had greater dry matter intake (DMI) (8.24 and 7.31 kg/day P = 0.01), and consequently, had more energy intake for growth that could result in greater intramuscular fat. It could happened because, according to Smith and Crouse (1984), to

occur intramuscular fat deposition is necessary that energy consumed exceed requirement. However, in the present study there was no difference in intramuscular fat content (Table 3). An explanation for this result is the light slaughter weight (456 and 438 kg $P = 0.02$) of the steers which happened due to the low average daily gain (1.07 and 0.88 kg/day $P = 0.01$) and DMI. Steers had low DMI by the fact they have been castrated and housed in individual pens. According to Marcondes et al. (2008) steers housed in individual pens may have more stress, which causes reduction in DMI and consequently decrease performance of these animals.

In addition, gene expression results also explain the lack of difference in marbling. Lipid deposition results from the balance among fatty acid uptake from the diet, through lipoprotein lipase (LPL) and fatty acid binding protein 4 enzyme (FABP4); *de novo* synthesis, that depends on the action of the acetyl CoA carboxylase and fatty acid synthase; and lipid catabolism by β-oxidation (Ladeira et al., 2018). Therefore, changes in the balance between synthesis and degradation can cause an increase or decrease in intramuscular fat content. Nellore × Angus had greater expression of genes related to uptake *(LPL*) and synthesis (*FASN*), but, also, increased gene expression of oxidation *(CPT2*), characterizing a higher lipid turnover in these animals, which prevented marbling deposition. Besides, *LPL* gene exhibited positive correlations with *FASN* and *CPT2* (Table 7).

Despite there was no effect on transcription factors expression, *SREBF1* had a strong positive correlation with the genes *FABP4*, *ACACA* and *SCD1* and a negative correlation with *ACOX* (Table 7). In other words, *SREBF1* acts regulating positively expression of genes involved in lipogenesis and down regulate β-oxidation. On the other hand, the correlation analysis shows that *PPARA* was associated with lipid oxidation,

since it had negative correlation with genes from de novo synthesis (*ACACA* and *SCD1*) (Table 7).

Additionally, *SREBF1* had lower expression in the muscle when compared with *PPAR* isomers (Figure 4) that explain the lower synthesis in Nellore × Angus. Therefore, we speculate that animals with light slaughter weight had lower intramuscular fat due to lower expression of *SREBF1* that reduces expression of genes involved in synthesis. Supporting this assertion Teixeira et al. (2017) found that Angus bulls had greater slaughter weights, greater expression of *SREBF1* and consequently greater intramuscular fat than Nellore bulls.

In another paper from this study, Nellore steers fed WSCB tented to spend more time ruminating ($P = 0.07$) and had greater rate of rumination/DMI ($P = 0.03$) (Rodrigues, 2018) that may have contributed to increase salivary flow and ruminal motility, that in turn, controlled more efficiently ruminal pH. The higher rumen pH in this condition may have caused indirectly a greater expression of *SCD1*. It happened because of reduction in rumen pH contribute to an increase in C18:2 *trans*-10 *cis*-12 that cause inhibition of *SCD* (Park et al., 2000; Teixeira et al., 2017).

Additionally, *SCD1* gene was positively correlated with *FABP4* and *ACACA*, showing that *SCD1* follow synthesis and uptake by adipocytes, inserting double bounds in the chain of saturated fatty acids to convert them to their respective monounsaturated fatty acids (Ladeira et al., 2016).

After 3 days *postmortem*, beef of Nellore steers tended to be less tender, that can be due to a higher calpastatin activity in this breed. Several studies have indicated that the reduced tenderness of meat from *Bos indicus* cattle is due to lesser *postmortem* proteolysis resulting from elevated calpastatin activity (Duarte et al., 2013; Koohmaraie, 1994; Martins et al., 2017). The calpastatin inhibits the activity of calpain, that is responsible to

hydrolyze the intermediate filaments, weakening the muscle fibers' structure, making meat more tender (Muroya, Nakajima, Oe, & Chikuni, 2006).

According to Silva et al. (2015), shear force values for meat above 69 N is considered tough, between 53 N and 69 N intermediate tenderness, and below 53 N, tender. Thus, 24 h after slaughter, beef of Nellore and Nellore × Angus steers could be considered with intermediate tenderness (65 N and 57 N, respectively). However, with 14 days' post mortem, the differences between breeds on tenderness in the study disappeared, being considered for both breeds, a tender beef. The lack of difference after 14 days of aging between breed could be due to Nellore be more responsive to aging time process. This result suggests that the enzymes present in Nellore were more active in degrading the muscle tissue during aging (Johnson, Huffman, Williams, & Hargrove, 1990), since calpastatin activity declined during aging allowing calpains to work (Cramer, Penick, Waddell, Bidwell, & Kim, 2018; Geesink & Koohmaraie, 1999). Geesink & Koohmaraie (1999) evaluating calpain and calpastatin, reported that calpastatin was more degraded during *postmortem* storage.

As the beef samples was subjected to freezing prior shear force analyses, beef from Nellore steers had likely more thawing loss, and consequently less water to lose during cooking. This phenomenon can be explained by the fact that Nellore had lower final weight (437.5 and 455.5 kg; $P = 0.02$) and, according to Pacheco et al. (2011), slaughter weight and loss of thawing have negative correlation. In other words, steers with light slaughter weight has greater thawing loss. Another explanation for this phenomenon can be related to fat thickness. Nellore × Angus steers had more subcutaneous fat than Nellore steers (4.77 and 3.28; $P < 0.01$) that protect the carcass to lose water during cooling. However, water losses during cooking can be higher, since

Bruce at al. (2004) showed positive correlation between subcutaneous fat and cooking loss (r = 0.61).

With 14 days' *postmortem*, beef from Nellore × Angus was less stable, with lower OMb and greater MMb. In addition, these steers had greater expression of *CPT2*, that may be due to greater concentration of PUFA in Nellore × Angus. Since Bressan et al. (2016) detected that when bulls were fed with grain diets, crossbred *Bos taurus* × *Bos indicus* had greater concentration of PUFA than *Bos indicus*. In turn, PUFA increase the expression of *CPT* (Bionaz, Thering, & Loor, 2012; Johnson et al., 2015; Totland et al., 2000).

According to Faustman, Sun, Mancini, & Suman (2010) oxidation of OMb to MMb generates intermediates capable of enhancing oxidation of fatty acids, and at the same time, products of lipid oxidation compromise meat color by accelerating myoglobin oxidation (Faustman, Liebler, McClure, & Sun, 1999). Fatty acids are oxidized primarily in the mitochondria in a pathway called β-oxidation and carnitine palmitoyltransferase 2 enzyme is involved in the entry of long-chain fatty acids into the mitochondria (Adami, Nakamura, de-Oliveira, & da Silva Gevaerd, 2006). In other words, lipid oxidation and OMb oxidation occur concomitant and each process appears capable to induce the other (Chan, Faustman, Yin, & Decker, 1997).

When MMb is produced, cause a brown coloration in the beef, which is undesirable to the consumer (Gupta, Bower, Cavender, & Sullivan, 2018; Mancini & Hunt, 2005). However, considering that consumers reject beef when the MMb reaches values on the surface close to 40% of the myoglobin chemical forms (Greene, Hsin, & Zipser, 1971), regardless of breed, ours steaks would not be rejected by color.

The greater lipid oxidation (TBARS) in steers fed WSC diet can be explained by the greater expression of *ACOX1* in these animals. Whilst, this greater expression of

ACOX1 may be due to the greater proportion of PUFA, since Chail et al. (Chail et al., 2016) found greater concentration of PUFA in grain-fed animals. According to Baillie, Takada, Nakamura, & Clarke (1999) PUFA induces genes involved in peroxisomal lipid oxidation as acyl-CoA oxidase, increasing whole body fat oxidation (Chorner et al., 2016).

The acyl-coenzyme A oxidase (ACOX) is a rate-limiting enzyme in peroxisomal beta-oxidation and beef containing high levels of very long chain fatty acid is more prone to lipid peroxidation (Li & Liu, 2012). The ACOX enzyme transfers two hydrogens from the substrate to its FAD cofactor and then to O_2, which is reduced to H_2O_2 (Schulz, 1996). According to Li and Liu (2012) presence of oxidative agents, O_2 and H_2O_2 in meat is recognized for initiating of lipid oxidation. In other words, high *ACOX1* gene expression indicates a greater production of H_2O_2, a substrate to lipid oxidation in the tissue.

5. CONCLUSION

Nellore × Angus had greater lipid turnover that prevented to had greater intramuscular fat. In addition, animals fed whole shelled corn diet with sugarcane bagasse did not increase expression of *SREBF1* and lipogenic genes, and consequently did not increase marbling.

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Table 1. Percentage of ingredients and chemical composition of the experimental diets.

Ingredients %	Diets (%DM)	
	Whole Shelled Corn	Whole Shelled Corn and bagasse
Whole shelled corn	80	74
Sugarcane bagasse	-	6
Protein and mineral supplement*	20	20
Nutrients %		
Dry matter[1]	89.0	88.0
Crude protein[2]	15.0	14.7
Neutral detergent fiber[2]	15.2	19.0
Non-fiber carbohydrate[2]	60.0	56.7
Starch[2]	57.2	52.9
Ether extract[2]	3.17	3.03
Metabolizable energy[2] (Mcal)	3.0	2.65

*Assurance levels per kilogram of product: CP: 32.5%; NDF: 21.6%, Ca: 45g; Mg: 7.5g/kg; Cu: 104 mg/kg; Zn: 344 mg/kg; Se: 0.83mg/kg; Vit. A: 30.500 UI/kg; Vit. D: 3800 UI/kg; Vit. E: 134 UI/kg; Rumensin: 140 mg/kg.

[1] - natural matter basis. [2] - dry matter basis

Table 2. Sequência (5’ para 3’) e eficiência dos primers que foram usados na PCR quantitativa em tempo real.

Symbol	Name	Forward (F) and Reverse (R)	Access Number	Amplicon (bp)	R^2	Efficiency
PPARA	*Peroxisome proliferator-activated receptor α*	F CAATGGAGATGGTGGACACA R TTGTAGGAAGTCTGCCGAGAG	NM_001034036.1	95	0.992	99.2
PPARG	*Peroxisome proliferator-activated receptor gamma*	F GCAATCAAAGTGGAGCCTGT R CCATGAGGGAGTTGGAAGG	NM_181024.2	94	0.973	100
SREBF1	*Sterol regulatory element-binding protein-1c*	F GAGCCACCCTTCAACGAA R TGTCTTCTATGTCGGTCAGCA	NM_001113302.1	88	0.985	94.6
LPL	*Lipoprotein lipase*	F CTCAGGACTCCCGAAGACAC R GTTTTGCTGCTGTGGTTGAA	NM_001075120.1	98	0.99	96.7
FABP4	*Fatty acid binding protein 4*	F GGATGGAAAATCAACCACCA R GTGGCAGTGACACCATTCAT	NM_174314.2	84	0.991	99
ACACA	*Acetyl CoA carboxylase alfa*	F TGAAGAAGCAATGGATGAACC R TTCAGACACGGAGCCAATAA	NM_174224.2	88	0.994	96.6
FASN	*Fatty acid synthase*	F ATCAACTCTGAGGGGCTGAA R CAACAAAACTGGTGCTCACG	U34794.1	83	0.974	99.5
SCD1	*Stearoyl-CoA desaturase*	F ACCATCACAGCACCTCCTTC R ATTTCAGGGCGGATGTCTTC	NM_173959.4	95	0.991	98
ACOX	*Acyl-coenzyme A oxidase 1*	F GCTGTCCTAAGGCGTTTGTG R ATGATGCTCCCCTGAAGAAA	BC102761.2	83	0.994	99
CPT2	*Carnitine palmitoyl transferase 2*	F CTATTCCCAAACTTGAAGAC R TTTTCCTGAACTGGCTGTCA	NM_001045889.2	81	0.952	98
β-actin	*β-actin*	F GTCCACCTTCCAGCAGATGT R CAGTCCGCCTAGAAGCATTT	NM_173979.3	90	0.996	105

CASC3	*Cancer susceptibility candidate 3*	F GGACCTCCACCTCAGTTCAA R GTCTTTGCCGTTGTGATGAA	NM_001098069.1	85	0.976	98
18S	*Ribossomal Protein 18S*	F CCAGTAAGTGCGGGTCATAA R CCATCCAATCGGTAGTAGCG	NM_001033614	84	0.999	99

Table 3. Chemical composition (%) of meat Nellore and Nellore × Angus steers fed whole shelled corn (WSC) or whole shelled corn with sugarcane bagasse (WSCB) diets.

Item	Nellore		Nellore × Angus		SEM	P - Value		
	WSC	WSCB	WSC	WSCB		Breed	Diet	B*D
Moisture	72.0	71.0	72.0	72.0	0.38	0.36	0.41	0.62
Ashes	1.87	1.69	1.87	2.01	0.17	0.34	0.90	0.34
Protein	22.0	23.0	22.0	22.0	0.18	0.10	0.60	0.29
Ether extract	2.78	3.34	2.74	2.99	0.34	0.54	0.22	0.63

Table 4. Color and myoglobin pigments of meat Nellore and Nellore × Angus steers fed whole shelled corn (WSC) or whole shelled corn with sugarcane bagasse (WSCB) diets.

	Nellore		Nellore × Angus			*P* - Value			
	WSC	WSCB	WSC	WSCB	SEM	Breed	Diet	B*D	B*D*A
L*	41.5	41.7	42.7	41.5	0.86	0.59	0.58	0.44	0.36
a*	20.0	20.0	19.0	20.2	0.62	0.83	0.66	0.61	0.76
b*	13.3	13.5	13.9	13.7	0.45	0.38	0.94	0.72	0.57
C*	24.0	24.1	24.0	24.4	0.72	0.88	0.74	0.82	0.68
h*	33.5	34.0	35.2	34.3	0.58	0.10	0.67	0.24	0.76
MMb	29.8	30.9	31.2	32.0	0.77	0.11	0.20	0.87	0.83
DMb	9.26	8.50	8.00	8.50	0.51	0.25	0.82	0.25	0.83
Omb	60.9	60.5	60.8	59.4	0.91	0.47	0.34	0.60	0.94

Table 5. Color and myoglobin pigments of meat Nellore and Nellore × Angus steers fed whole shelled corn (WSC) or whole shelled corn with sugarcane bagasse (WSCB) diets on different aging time.

	Aging time			
	0	14	SEM	*P* - Value
L*	41.3	42.4	0.54	<0.01
a*	19.0	20.8	0.44	<0.01
b*	12.4	14.7	0.32	<0.01
C*	22.8	25.5	0.53	<0.01
h*	33.2	35.3	0.38	<0.01
MMb	30.8	31.1	0.56	0.70
DMb	8.90	8.32	0.32	0.14
Omb	60.2	60.6	0.68	0.62

Table 6. Gene expression in the longissimus thoracis muscles of Nellore and Nellore × Angus steers fed whole shelled corn (WSC) or whole shelled corn with sugarcane bagasse (WSCB) diets.

Item	Nellore		Nellore × Angus		SEM	*P* - Value		
	WSC	WSCB	WSC	WSCB		Breed	Diet	B*D
SREBF1	1.20	1.68	1.33	1.00	0.34	0.20	0.76	0.27
PPARA	1.27	1.09	1.00	1.14	0.32	0.73	0.44	0.52
PPARG	1.00	1.10	1.24	1.52	0.21	0.15	0.27	0.57
LPL	1.00	1.15	3.23	2.38	0.31	<0.01	0.36	0.18
FABP4	1.00	2.38	1.02	1.70	0.48	0.66	0.03	0.69
ACACA	1.00	1.90	1.61	1.54	0.39	0.41	0.22	0.49
FASN	1.26	1.00	1.80	1.55	0.18	<0.01	0.14	0.74
SCD1	1.00	2.47	2.21	1.70	0.51	0.19	0.06	0.06
CPT2	1.08	1.01	2.34	1.66	0.44	0.04	0.66	0.64
ACOX	1.40	1.00	1.40	1.29	0.13	0.20	0.04	0.37

Table 7. Correlation coefficients among gene expression in the longissimus thoracis muscles of Nellore and Nellore × Angus steers.

Gene	*ACOX*	*CPT2*	*SCD1*	*FASN*	*ACACA*	*FABP4*	*LPL*	*PPARG*	*PPARA*
SREBF1	**-0.49***	-0.25	**0.83***	0.04	**0.41***	**0.55***	0.15	0.35	-0.32
PPARA	0.29	-0.02	**-0.42***	-0.16	**-0.43***	-0.16	-0.19	-0.19	
PPARG	-0.31	-0.04	**0.53***	0.26	0.28	0.21	0.26		
LPL	0.28	**0.47***	0.34	**0.64***	0.31	0.03			
FABP4	**-0.39***	-0.25	**0.49***	-0.14	**0.68***				
ACACA	-0.10	-0.18	**0.53***	0.04					
FASN	0.33	0.38	0.09						
SCD1	**0.47***	-0.06							
CPT2	0.40								

* $P < 0.05$.

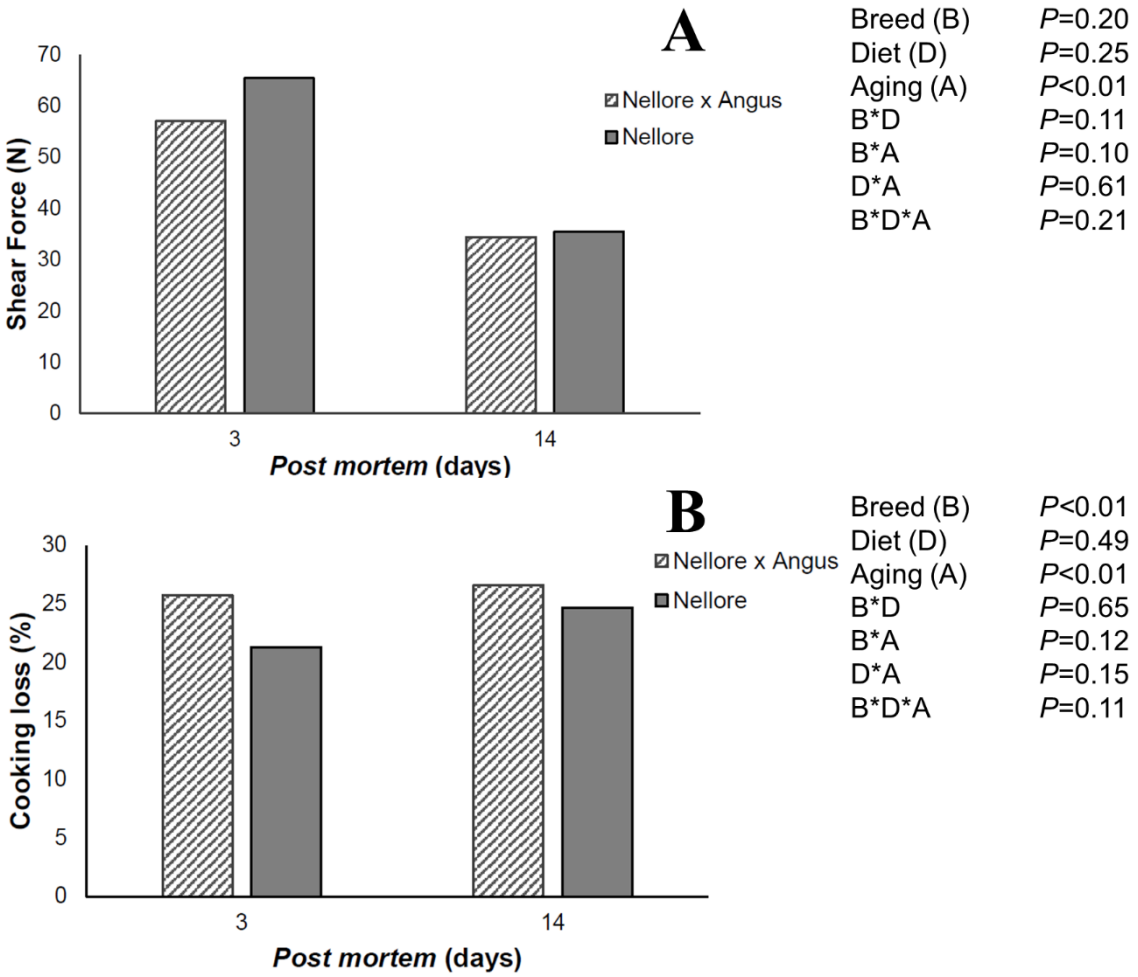

Figure 1. Shear force (A) and cooking loss (B) of beef from Nellore and Nellore × Angus steers fed whole shelled corn (WSC) or whole shelled corn with sugarcane bagasse (WSCB) diets on different aging time.

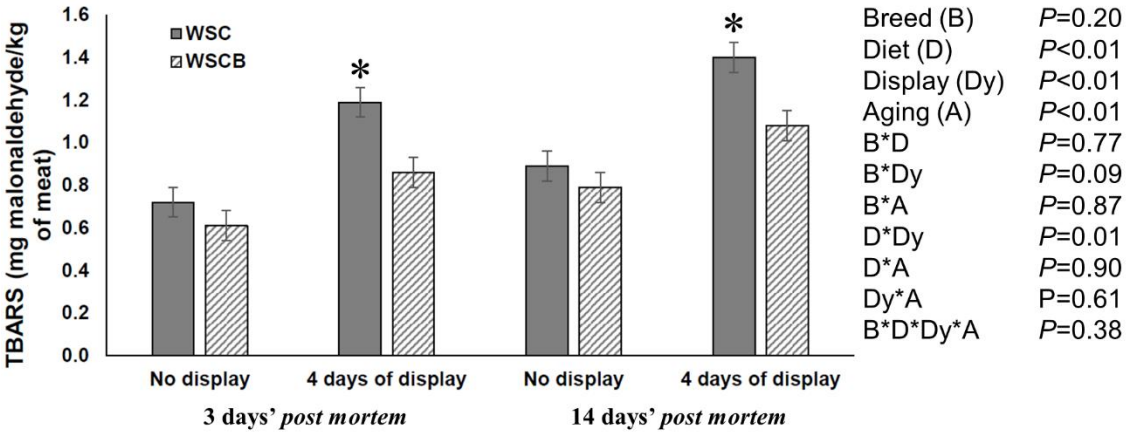

Figure 2. Lipid oxidation values of beef from Nellore and Nellore × Angus steers fed whole shelled corn (WSC) or whole shelled corn with sugarcane bagasse (WSCB) diets on different aging time.

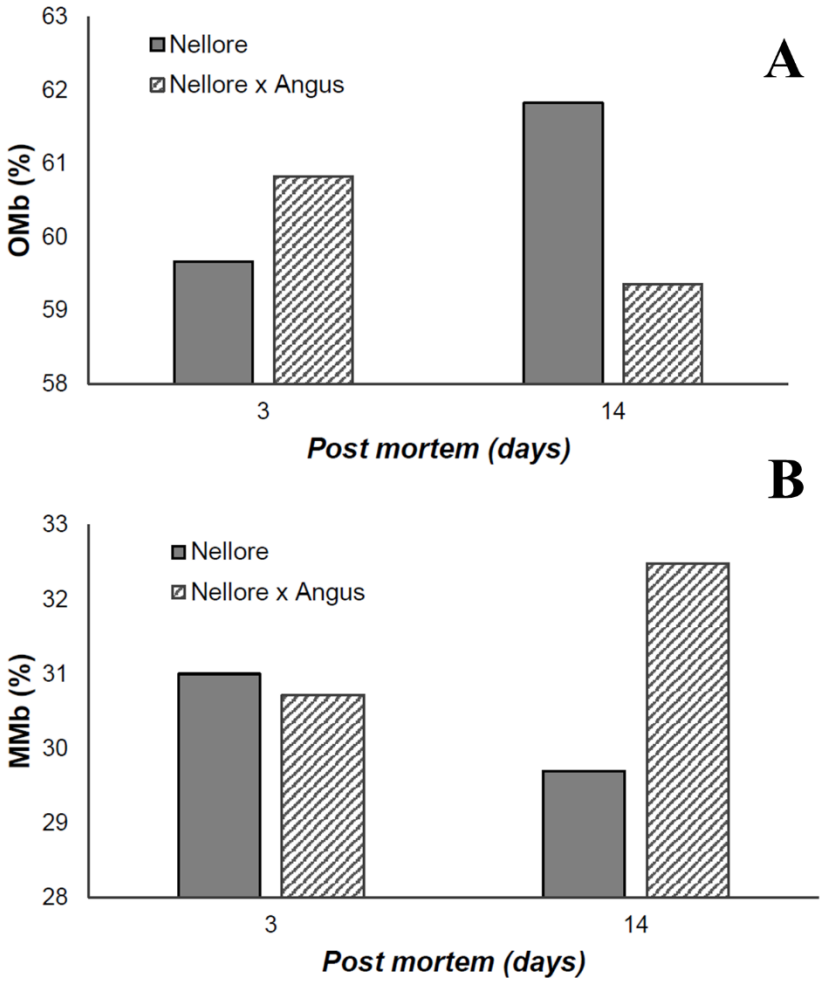

Figure 3. Oxymyoglobin - OMb (A) and metmyoglobin – MMb (B) of beef from Nellore and Nellore × Angus steers fed whole shelled corn (WSC) or whole shelled corn with sugarcane bagasse (WSCB) diets on different aging time.

Breed*Aging – OMb $P = 0.01$; MMb $P = 0.02$.

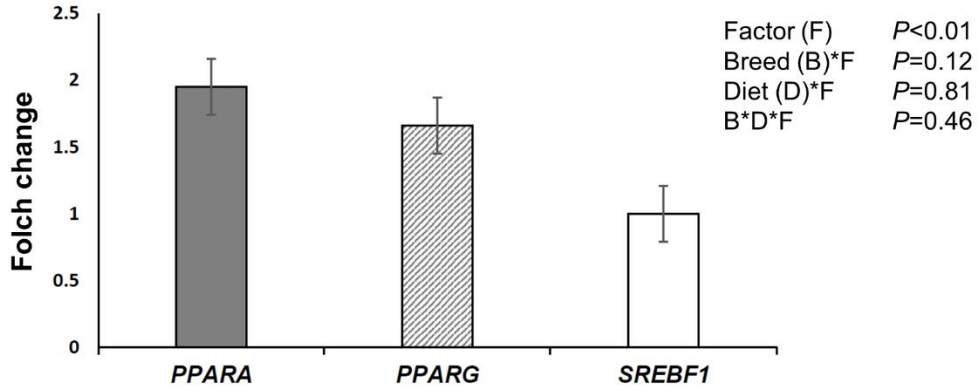

Figure 4. Relative expression of PPARA, PPARG and SREBF1 in the longissimus thoracis muscles of beef from Nellore and Nellore × Angus steers fed whole shelled corn (WSC) or whole shelled corn with sugarcane bagasse (WSCB) diets.