MAURICIO MIGUEL ESTRADA

METABOLOME AND BEEF QUALITY FROM NELLORE CULL COWS AS AFFECTED BY DAYS ON FEEDLOT OR BY DRY OR WET AGEING

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

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APPROVED: July 31, 2018.

Rafael Mezzomo

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Mario Luiz Chiofetti
(Adviser)

I dedicate this work to my eternal heroes: my parents and brothers. Without his company, even being far away, I could never have reached where I am, and without a doubt, I could never reach the proposed goals.

For you, all my thanks.

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BIOGRAPHY

MAURICIO MIGUEL ESTRADA, son of Felipe Miguel Soriano and María Martha Estrada Elizarraraz, was born in México City, Distrito Federal, México.

He started the undergrad in September of 1999 in Veterinary Medicine and Animal Science in the Universidad Nacional Autónoma de México, completing disciplines in February 2005 and became degree in Veterinary Medicine and Animal Science in November 2009.

From August 2005 to February 2009, he was a Veterinarian responsible in the "Hacienda San Jeronimo" farm in Guanacaste, Republic of Costa Rica.

From August 2009 to January 2011 he participated as assistant professor at the Department of Veterinary Medicine and Animal Science of UNAM.

From January 2011 to January 2012, I work as Veterinarian at the Secretaria de Agricultura, Ganaderia, Desarrollo Rural, Pesca y Alimentación of the State of Jalisco, México.

In February 2012 started the M.S. program with major on nutrition and production of ruminants in Animal Science Department of the Universidade Federal de Viçosa, became a M.S. in Animal Science in June 2014.

At the same year he started his D.S. program in Animal Science with major on nutrition and production of ruminants, defended his dissertation to obtain the Doctor Scientiae degree in Animal Science in July 2018.

INDEX

LIST OF TABLES

Chapter 1. Muscle metabolome and beef quality of Nellore cull cows submitted to different days of feedlot

Chapter 2. Metabolome and beef quality of Nellore cull cows wet or dry-aged

LIST OF FIGURES

Chapter 1. Muscle metabolome and beef quality of Nellore cull cows submitted to different days of feedlot

ABSTRACT

MIGUEL ESTRADA, Mauricio, D.Sc., Universidade Federal de Viçosa, July, 2018. **Metabolome and beef quality from Nellore cull cows as affected by days on feedlot or by Dry or Wet ageing.** Adviser: Mario Luiz Chizzotti.

The present work was based on two experiments. The objective of first experiment was to evaluate the metabolome and beef quality of zebu cull cows, slaughtered after three different periods of feedlot. A total of 20 Nellore cull cows, with an average age of 10.6 years (standard deviation, SD 2.5), fed with *Brachiaria decumbens* grass without supplementation two months before beginning the experiment were used. At the beginning of the experiment, the animals were weighed and wormed. The animals presented 442.4 kg (SD ± 41.6) of initial SBW, 55.87 cm^2 of REA and 4.23 mm of SFT. The animals were randomly assigned in three treatments: six cows slaughtered at the beginning as control group (C0), seven confined for 28 days (C28) and seven for 58 days (C58). The C28 and C56 treatments were fed with a feedlot diet according to the nutrients requirements for non-pregnant dry beef cows of BR-CORTE, aiming at an average daily gain of 1.2 kg. At the end of each experimental period, the animals were slaughtered and a sample of the *Longissimus lumborum* muscle was immediately obtained for metabolite analysis. Subsequently, after 24 hours of carcass cooling, six one-inch steaks of the same muscle were obtained for meat quality analyzes, being two steaks randomly distributed in three ageing times (0, 7, and 14 days). Performance data, carcass characteristics and meat quality were analyzed using the SAS GLM procedure, using weight and initial age as covariates; while metabolites in muscle and meat were analyzed by the TruTOF GC-MS system. Muscle of C28 and C56 showed a higher concentration of sugars (glucose, fructose and glycerol) and amino acids (leucine, alanine, isoleucine, valine, aspartate). The tenderness of the meat increased with the days in feedlot ($P=0.0305$) and aging time ($P<0.0001$). It is concluded that the feedlot increases the fatness of the cull cow´s carcass, which is reflected in a slow temperature drop and fast pH decline, improving the qualitative characteristics of the meat. The second experiment aimed to determine the metabolomic and the beef characteristics of cull Nellore cows from the grazing system, subjected or not to two different ageing techniques (Dry-aged versus Wet-aged) for 14 days, using analysis traditional as well as modern techniques to identify major changes in the metabolic profile of meat. For this, twelve carcasses of cull Nellore cows were used. Of each carcass, two sections were collected between the 9th and 11th ribs, one of each half

carcass, and randomly designated for one of the aging treatments (Dry-aged or Wed-aged). In addition, two *Longissimus lumborum* muscle steaks were removed immediately bellow the 11th rib as the treatments without aging (WA). Dry-aged samples were aged without any packaging in direct contact with the cooling room environment, while the Wet-aged samples were vacuum packed in plastic bags. The samples were aged for 14 continuous days at 4 °C and 85% humidity. The experimental design was completely randomized and the data analyzed using the SAS GLM procedure. It was determined that aging reduced the shear force ($P < 0.0001$) and increased metabolites such as amino acids, sugars and lipids, without differences between treatments. Dry-aged beef present greater losses ($P < 0.0001$) causing lower total salable yield. Whiles Wet samples have a higher concentration ($P = 0.01445$) of biogenic amines (e.g. Putrescine) which would reduce its shelf life. It is concluded that aging of cull cow beef is a viable option to improve the physicochemical characteristics of the product.

RESUMO

MIGUEL ESTRADA, Mauricio, D.Sc., Universidade Federal de Viçosa, julho de 2018. **Metaboloma e qualidade de carne de vacas de descarte Nelore afetada pelos dias em confinamento ou pela maturação a seco ou úmido.** Orientador: Mario Luiz Chizzotti.

O presente trabalho foi baseado em dois experimentos. O objetivo do primeiro experimento foi avaliar o metaboloma e a qualidade da carne bovina de vacas zebuínas de descarte abatidas após três diferentes períodos de confinamento. Foram utilizadas 20 vacas da raça Nelore, com idade média de 10,6 anos (desvio-padrão, DP 2,5), alimentadas dois meses antes do início do experimento com capim *Brachiaria decumbens* sem suplementação. No início do experimento, os animais foram pesados y vermifugados. Os animais apresentaram 442,4 kg (DP ± 41,6) de PCJ inicial, 55,87 cm^2 de AOL e 4,23 mm de EGS. Logo, os animais foram distribuídos aleatoriamente em três tratamentos: seis vacas abatidas no início como grupo controle (C0), sete confinadas por 28 dias (C28) e sete por 58 dias (C58). Os tratamentos C28 e C56 foram alimentados com uma dieta de confinamento de acordo com as exigências nutricionais para vacas de corte, secas não-gestantes segundo BR-CORTE, visando ganho médio diário de 1,2 kg. Ao final de cada período experimental, os animais foram abatidos e uma amostra do músculo *Longissimus lumborum* foi imediatamente obtida para analises dos metabólitos. Posteriormente, depois de 24 horas de resfriamento da carcaças, seis bifes de uma polegada do mesmo músculo foram retirados para analises de qualidade da carne, sendo dois bifes aleatoriamente distribuídos em três tempos de maturação (0, 7 e 14 dias). Os dados de desempenho, características da carcaça e qualidade da carne foram analisados mediante o procedimento GLM do SAS, usando o peso e idade inicial como covariáveis; em quanto os metabolitos no músculo foram analisados por meio do sistema TruTOF GC-MS. Os resultados indicaram que os músculos C28 e C56 apresentaram maior concentração de açúcares (glicose, frutose e glicerol) e aminoácidos (leucina, alanina, isoleucina, valina, aspartato). A maciez da carne aumentou com os dias em confinamento ($P = 0.0305$) e o tempo de maturação ($P < 0.0001$). Conclui-se que o confinamento aumenta a gordura da carcaça de vacas de descarte, o que se reflete em uma lenta queda de temperatura e um rápido declínio do pH, melhorando as características qualitativas da carne. O segundo experimento teve como objetivo determinar o metaboloma e as características da carne de vacas Nelore de descarte provenientes do sistema a pasto, submetida ou não a duas técnicas

diferentes de maturação (Dry-aged versus Wet-aged) por 14 dias, utilizando analises tradicionais assim como técnicas modernas para identificar as principais mudanças no perfil metabólico da carne. Para isso, foram utilizadas 12 carcaças de vacas Nelore de descarte. De cada carcaça, foram coletadas duas seções entre a 9ª e 11ª costelas, uma de cada meia carcaça, e destinadas aleatoriamente para um dos tratamentos de maturação (Dry-aged ou Wed-aged). Além disso, dois bifes do músculo *Longissimus lumborum* foram removidos imediatamente abaixo da 11ª costela como o tratamento sem maturação (WA). Amostras Dry-aged foram maturadas sem nenhuma embalagem e em contato direto com o ambiente da sala de refrigeração, enquanto as amostras Wet-aged foram embaladas a vácuo em sacos plásticos. As amostras foram maturadas por 14 dias contínuos a 4 °C e 85% de umidade. O desenho experimental foi completamente ao acaso e os dados analisados usando o procedimento GLM do SAS. Foi determinado que a maturação reduziu a força de cisalhamento ($P < 0.0001$) e aumentou os metabólitos, como aminoácidos, açúcares e lipídios, sem diferenças entre os tratamentos. As amostras Dry-aged apresentam maiores perdas ($P < 0.0001$), ocasionando um menor rendimento da carne comercializável. Em quanto amostras Wet-aged tiveram maior concentração ($P = 0.01445$) de aminas biogénicas (por exemplo Putrescina), o que reduziria a sua vida de prateleira. Conclui-se que a maturação da carne de vacas de descarte é uma opção viável para melhorar as características físico-químicas do produto.

INTRODUCTION

The increase in population and income of the middle class will lead to greater demand and consumption of beef globally in the coming years (Westcott, 2016), estimated that during the year 2024 are produced 75.4 million t carcass weight equivalent (CWE) of beef (FAO, 2015). Of this production, the meat of cull cows occupies an important percentage, generating important income to the livestock business (Boleman, Miller, Buyck, Cross, & Savell, 1996).

In that sense, it is estimated that in 2026, Brazil will have a herd of 234.2 million bovine (27.76% cows), reaching a production of 11.7 million t CWE, of which 2.2 million will be destined for the external market (Barioni, de Lima, Zen, & Ferreira, 2007; FAO, 2015; FIESP, 2016), representing 20% of world exports, which would make it the main exporter of beef (USDA, 2017).

To achieve this goal, 55.6 million cattle are required to be slaughtered (Barioni et al., 2007), of which 16.19% (9 million) will be finished in feedlot (Rasmussen, Fontes, & Cordingley, 2014), and 83.81%, mainly to pasture, which may mean a greater appreciation of the meat as considered healthy and economic (Carrillo et al., 2016; Lobato et al., 2014), since its cost represents 40% of the cost in Australia and 50% in the United States (Ferraz & Felício, 2010).

Nevertheless, about 80% of the Brazilian herd present influence of zebu cattle (Ferraz & Felício, 2010), mainly Nellore; causing, according to Wheeler, Savell, Cross, Lunt, & Smith (1990), reduction of tenderness by influence of *Bos indicus* breed; however, more recent results indicate that this theory cannot always be verified (Guelker et al., 2013), as it depends of various factors. In addition, due to the adaptability of livestock to the oscillating availability of nutrients in the tropics (Ferraz & Felício, 2010; Lobato et al., 2014), their meat is considered tough (Delgado, Aguiar, Ortega, Spoto, & Castillo, 2006).

In addition to the above, almost 35 to 40 % of the meat produced is obtained from females (Anualpec, 2016), and of these, 78% are cull cows (IBGE, 2016), which are considered to produce lower quality meat in relation to the males, reason why its price is on average, 9.2% less (Anualpec, 2016), without a consistent justification for such depreciation, since various works (Cattelam, J., de Menezes, L., Ferreira, J., Restle, J., Brondani, I., Arboitte, M., & de Paula, 2009; Coutinho, Peres, & Justo, 2006; Park et al., 2002; Santos et al., 2017; Schnell, Belk, Tatum, Miller, & Smith,

1997) have shown that in similar finishing conditions, there are no differences between the meat of these categories. In the same way, another factor to consider is that cull beef cows are discarded older compared to cull dairy cows (Vestergaard et al., 2007), increasing the shear force (SF) mainly by accumulation of the connective tissue (collagen), less palatable and with little stability of color (Boleman et al., 1996; Duarte et al., 2011), so it is marketed in food service, milled or to make sausages (Obuz, Akkaya, Gök, & Dikeman, 2014).

Also, as mentioned above, in traditional handling of pasture finishing, the beef of cull cows has low tenderness, mainly due to the high content of insoluble collagen (Boleman et al., 1996) and the poor coverage of fat in the carcass (Lancaster, Krehbiel, & Horn, 2014) which causes low yield and cold shortening.

In addition to the above, grass-finished cows, usually present nutritional deficiencies (Devincenzi et al., 2012), which causes a low weight gain and in some cases, weight loss. These deficiencies cause that the metabolism of animals to be modified, reducing catabolism to a minimum, including muscle degradation (Hornick, Van Eenaeme, Gérard, Dufrasne, & Istasse, 2000).

Therefore, the enzymatic complex of calpains, considered as the main responsible for muscle degradation, reduces its activity, being inhibited by the calpastatin (Du, Zhu, Means, Hess, & Ford, 2004), preventing muscle loss. However, in extreme cases of nutritional deficiency, an increase in the activity of this enzyme has been reported (Leonardo, Delgado, Bagaldo, Lanna, & De Paz, 2008), aiming at the degradation of muscle cells with the objective of releasing amino acids that can be used as an energy source (Klinhom et al., 2006). Conversely, animals that are confined after a period of nutritional deficiency, present higher gains than those without restrictions (Silva et al., 2017; Vestergaard et al., 2007), being this increase in productivity known as compensatory growth.

In addition to surpassing animals without deficiency, animals with compensatory growth can rapidly regenerate tissues (Kristensen et al., 2002), mainly from the metabolic organs, as liver, intestine and spleen (Missio et al., 2013), but there is also a remodeling of muscle tissue (protein turnover), increasing the synthesis and degradation of the muscular fibers, reason why it is observed an increase of the activity of the calpain (Du et al., 2004).

It is in this phase, that the meat obtained from these animals can present high tenderness, in addition to being elevated, increases exponentially by reducing the pH

postmortem e release of intracellular calcium (D'Alessandro & Zolla, 2013). This has caused compensatory growth to be used as a way to improve productivity (Boleman et al., 1996; Lobley, 1998; Perry & Thompson, 2005a; Silva et al., 2017).

Due to the above, and due to the advantages of Brazil for grain production, mainly corn and soybean (Rasmussen et al., 2014), the finished in feedlot of cull cows is considered feasible, using compensatory growth as a productive technique for meat improvement, being that high rate of weight gain, contributes to improve the tenderness and palatability (Thompson, 2002).

Therefore, previous work them studied the performance of discarded cows in feedlot, including the quality of meat. However, most has used cull dairy cows (Bunmee, Jaturasitha, Kreuzer, & Wicke, 2014; Obuz et al., 2014; Therkildsen, Stolzenbach, & Byrne, 2011; Vestergaard et al., 2007) or European breeds (Boleman et al., 1996; Du et al., 2004; Shackelford, Koohmaraie, & Wheeler, 1995), without identifying the metabolic changes in muscle or the period of feedlot necessary to obtain maximum performance without reducing the quality of meat.

In the same sense, too there is a lack of information related to the use of other techniques to improve the meat of cull cows, such as aging (Vitale, Pérez-Juan, Lloret, Arnau, & Realini, 2014; Xiong et al., 2007). Since it is widely recognized that this process in addition to modifying the taste, reduces the shear force by degradation of muscle fibers through enzymatic systems (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002; Muchenje et al., 2009; Sitz, Calkins, Umberger, & Feuz, 2006). Regarding this methodology, currently three main methodologies for aging meat, which are dry aging, vacuum aging and a new dry aging technique that uses a highly moisture permeable bag (Ahnström, Seyfert, Hunt, & Johnson, 2006; Li et al., 2014; Stenström, Li, Hunt, & Lundström, 2014), minimally used in meat of cull cows.

In this context, aiming to study the meat of cull cows, two independent experiments were carried out. The first experiment aimed to evaluate the metabolism and beef quality of zebu cull cows, slaughtered after three different periods of feedlot. While the second experiment was proposed to determine the beef characteristics of cull Nellore cows from the grazing system, without or aged by two different techniques (Dry-aging versus Wet-aging) for 14 days, using analysis traditional as well as modern techniques to identify major changes in the metabolic profile of meat.

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Chapter 1

MUSCLE METABOLOME AND BEEF QUALITY OF NELLORE CULL COWS SUBMITTED TO DIFFERENT DAYS OF FEEDLOT

Abstract

The objective of this work was assess the muscle metabolome and beef quality of cull cows from a grazing system. Twenty Nellore cows were distributed in three treatments. Six cows slaughtered at the beginning as control group (C0), seven were fed 28 days (C28) and seven fed 58 days (C58) in feedlot. The meat was aged for 0, 7 or 14 days. Metabolites in meat were analyzed by the TruTOF GC-MS system. Meat of cull cows finished in feedlot showed a higher concentration of sugars (glucose, fructose and glycerol) and amino acids (leucine, alanine, isoleucine, valine, aspartate). The tenderness of the meat increased with the feedlot time and aging time. It is concluded that feedlot increases the fatness of the carcass, which is reflected in a slow temperature decline and a rapid drop of pH, improving the qualitative characteristics of the meat.

1. Introduction

Cull cows are important in the economy of the livestock business and the quality of the meat produced (Boleman et al., 1996; Therkildsen et al., 2011; Vestergaard et al., 2007). In Brazil, almost 35 to 40 % of the meat produced is obtained from cull cows (Anualpec, 2016), which 80% present influence of zebu cattle (Ferraz & Felício, 2010), mainly Nellore. However, due to the adaptability of livestock to the oscillating availability of nutrients in the tropics (Ferraz & Felício, 2010; Lobato et al., 2014), high content of insoluble collagen (Boleman et al., 1996) and the poor coverage of fat in the carcass (Lancaster et al., 2014), their meat is considered tough (Delgado et al., 2006).

In addition to the above, grass-finished cows, frequently undergo nutritional deficiencies (Devincenzi et al., 2012), which causes a low performance and in some cases, weight loss.

Thus, when the nutritional deficiency is intermediate, the enzymatic complex of calpains, considered as the main responsible for muscle degradation, reduces its activity mainly by the action of calpastatin (Du et al., 2004), preventing muscle loss. Conversely, animals that are confined after a period of nutritional deficiency, present

higher gains than those without restrictions (Vestergaard et al., 2007), being this increase in productivity known as compensatory growth. In the same sense, other researchers have concluded that the improvement in the body condition of ruminants is a strategy that can be manipulated to increase the quality of the meat (Muchenje et al., 2009).

So, previous work them studied the performance of discarded cows in feedlot, including the quality of meat. However, most evaluated dairy cull cows (Bunmee et al., 2014; Obuz et al., 2014; Therkildsen et al., 2011; Vestergaard et al., 2007) or European breeds (Boleman et al., 1996; Du et al., 2004; Shackelford et al., 1995), obtaining in most cases positive responses, but without identifying the metabolomic changes in muscle or the period of feedlot necessary to obtain maximum performance and quality of meat.

Thus, it is hypothesized that by improving the supply of nutrients in cows finished in feedlot, the quality of the meat is increased. Therefore, the objective of this study was to evaluate the muscle metabolome and beef quality of cull cows, slaughtered after three different periods of feedlot.

2. Materials and methods

2.1. Animals and nutritional management

The protocol used in this experiment was in accordance with the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil, approved all animal handling procedures (protocol number 036/2015) in accordance with ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010) and EU Directive 2010/63/EU (European Union, 2010). The assays were performed in the Feedlot Station of Animal Science Department, Universidade Federal de Viçosa, Viçosa, Brazil.

A total of 20 Nellore cull cows, with an average age of 10.6 years (SD ± 2.5), fed with *Brachiaria decumbens* grass without supplementation two months before beginning the experiment were used. The cows were discarded mainly by reproductive problems.

At the beginning of the experiment, the animals were weighed after previous solid deprivation for 16 hours (shrunk body weight, SBW), wormed and ultrasound measurements of rib-eye area of muscle *Longissimus lumborum* (REA) and subcutaneous fat thickness (SFT) between the 12th and 13th ribs using ultrasound

model Aloka 500 with linear housing transducer with 17.2 cm and frequency of 3.5 MHz were mad. The animals presented 442.4 kg (SD 41.6) of initial SBW, 55.87 cm^2 of REA and 4.23 mm of SFT.

Subsequently, the animals were divided into three groups: 6 cows conformed the control group (C0) that were slaughtered at the beginning of experiment; 7 were confined for 28 days (C28) and 7 for 56 days (C56). The animals remained in individual pens with an area of 20 m^2 each, with concrete floors, provided with shade, feeders and individual drinkers.

During the first 5 days, all animals received an adaptation diet and later, the animals of the groups C28 and C56 were fed with a feedlot diet (Table 1) according to the nutrients requirements for non-pregnant dry beef cows of BR-CORTE (Valadares Filho et al., 2016), aiming an average daily gain of 1.2 kg. The change from the adaptation diet to the final diet was performed gradually for 7 day, counting from the beginning of the experiment, to avoid ruminal disorders.

The diet was given twice daily, at 7:00 a.m. (60%) and 3:00 p.m. (40%). Daily the diet offered and orts of each animal were weights and sampled to estimate intake and feed conversion. Also, before the morning supply, the feeder was revised to maintain 5% of orts, attempt consumption *ad libitum*. At the end of each experimental period, an average daily gain (ADG) was calculated by the slaughter weight minus the initial weight between days of feedlot.

To determine the composition of the diet, weekly subsample representative of the total diet and orts were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE) and neutral detergent fiber (NDF) as described by Detmann et al. (2012). NDF analyzes were performed including the ash and protein corrections (NDFap) suggested by Mertens et al. (2002).

Table 1. Feedlot diet ingredients and chemical composition.

Ingredient	% DM Feedlot diet
Corn silage	35.64
Ground corn	56.11
Soybean meal	5.50
Urea	1.02
Comercial premix[1]	1.73
Chemical composition (g/kg of DM)[d]	
DM	384.6
OM	892.4
CP	132.2
EE	33.7
NDFap	281.0
NFC	563.2
TDN	756.9

[1]Contained per kg: 150 g of Ca, 17 g of P, 23 g of S, 45 g of K, 14 g of Mg, 57 g of Na,360 mg of Cu, 21.6mg of Co, 415mg of Fe, 21mg of I, 715 mg of Mn, 6 mg of Se, 397 g of CP (NPN) and 714 mg of sodium monensin.
[d] DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, NDFap = neutral detergent fiber corrected for ash and protein, NFC = non-fiber carbohydrate,
TDN = total digestible nutrients.

2.2. Slaughter procedure and carcass evaluation

At the end of each experimental period (0, 28 and 56 days), the animals were weighted after 16 h of fasting and slaughtered in accordance with humane slaughter practices following the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 2017).

The animal was desensitized by cerebral concussion followed by jugular section for total bleeding, the skinning and toilet being identical between treatments. Carcasses were not electrically stimulated. The carcass was divided into two half-carcasses (right and left), identified, washed and weighed.

The half-carcasses were hung from the pelvis (tenderstretch) and chilled in cold chamber for approximately 24 h at 4° C. At the start of the cooling and hourly for 24 h, the pH and temperature were measured between 12 and 13 ribs of the *Longissimus lumborum* muscle (LL), using a thermometer type K and a specific potentiometer (SevenGo, Mettler Toledo®). These values were used to estimate the decline in both pH and temperature as a function of time *pos-mortem* (Hwang & Thompson, 2001). The values of pH and temperature over time were estimated by a non-linear program (SAS 9.4) and used to predict the pH and temperature at specific times and the temperature when the pH was 6.0.

After refrigerated, the carcasses were weighed again to obtain the cold carcass weight (CCW) and losses by cooling (difference between HCW and CCW).

From the left half carcass, between the 12th and 13th ribs, was determined the REA with reticulated grating in cm^2 and SFT using a caliper at the reference point to ¾ parts of muscle length, expressing the value in millimeters.

2.3. Metabolome analysis

Immediately after the slaughter and bleeding of the animals from each experimental period, a 2x2x1 cm sample was taken from the LL muscle the 12th rib, clean of connective or fat tissues, rinsed with physiological saline and macerated in liquid nitrogen, placed in 2 ml tubes and stored at -80° C until the analyzes.

2.3.1. Sample preparation for metabolomic

Polar metabolites were extracted using the method of Lisec, Schauer, Kopka, Willmitzer, & Fernie (2006). A sample of 50 mg of ground muscle from each loin was homogenized in 2 ml tube with 700 µL methanol and 30 µL sugar stock (0.2 mg/ml Ribitol in water), shaked 15 min at 70 °C in a thermomixer at 1000 rpm and centrifuged for 10 min at 14000 rpm. After the aqueous phase was transferred to a new tube, and it was added 375 µL CHCl3, 750 µL of MilliQ water and centrifuged 15 min at 4000 rpm. 150 µL from the upper phase (polar phase) were transferred at new tube and the solvent was evaporated overnight using a Savant SpeedVac (Thermo Scientific).

For the phase of derivatization, the remaining residue was reconstituted and 40 µL Methoxyamination reagent (20 mg methoxyamine hydrochloride/ml pure Pyridin) was added, shaked 2 h at 37 °C at 950 rpm and after centrifuged 1 min at 10000 rpm. Later 70 µL MSTFA mix (1 ml + 20 µL FAME) was added, shake 30 min at 37 °C at

950 rpm and centrifuged 1 min at 10000 rpm. Finally, 90 µL were transferred into glass vials suitable for GC-MS analysis.

2.3.2. Metabolite identification and quantitation

For the analysis of the samples it was used the GC-MS TruTOF system, Agilent Chroma tograph Technologies 7890A and TruTOF® HT TOFMS spectrometer Leco, equipped with a 30-m capillary column (DB-35 MS, Agilent Technologies) as suggested by Lisec, Schauer, Kopka, Willmitzer, & Fernie (2006). In splitless mode at 230 °C, 1 µl of each sample was injected charged by the helium gas, with continuous flow of 2 ml/min. Initially the temperature of oven was keeped at 80 °C and then increased 15 °C/min until reaching 330 °C keeping this temperature for 6 min. Chromatograms had the baseline corrected and their deconvolution was performed through the Chroma TOF software, Leco. Afterwards, the peaks were identified through the deconvoluted spectra obtained using the TagSearch software (Cuadros-Inostroza et al., 2009). The areas of the Chromatographic peaks of the fragmented ions were normalized and corrected for cell density (DO_{600nm}).

2.4. Meat samples preparation and analyzes

Of the LL muscle from left carcass, six 2.54 cm thick steaks were removed, identified and vacuum packed in plastic bags. The steaks were divided into three groups of two steaks each, each group was aged for 0, 7 and 14 days in a cold chamber at 4 ° C. At the end of aging time, the steaks were frozen and stored at -18° C until analysis.

2.4.1. Chemical composition of meat

One steak of aging time zero from each animal and treatment (C0, C28 and C56) was minced, weighed and lyophilized for 72 h, obtaining partial dry matter. After, they were partially defatted by washing with petroleum ether (Fernandes, Tedeschi, Paulino, & Paiva, 2010) and amount of fat lost was recorded. Later were ground with ball mill and analyzed for proximate analysis to determine dry matter (DM; method 934.01), ash (method 930.05), total nitrogen (N; method 981.10) and ether extract (EE; method 920.85) according to the AOAC, (2012) methods of analysis protocols. The crude protein (CP) content was obtained by multiplying 6.25 by the

total nitrogen content. The total EE was corrected by adding the fat lost during the partial defatting process.

2.4.2. Analysis of meat color

Another steak from each aging time was thawed overnight at 4 °C, removed from vacuum bags and after of minimum 30 min of bloom time. The objective color was measured using a Hunter MiniScan EZ (4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA), which was calibrated just before use. The mean at five locations on the displayed surface of each steak avoiding large marbling flecks, connective tissue and edge, was evaluated for lightness (L^*), redness (a^*) and yellowness (b^*) using illuminant D65, a 31.8 mm port size and 10 ° standard observer. Also using the above indices, it was calculated indicator Chroma $(a^{*2} + b^{*2})^{1/2}$ and Hue angle (tan -1(b^*/a^*)). Subcutaneous fat samples were analyzed in a similar way.

2.4.3. Cook losses and Shear force

In this analysis was used the same steaks of color determination. Was weighted raw, repacked to vacuum and broiled for 30 min in the water bath pre-heated at controlled temperature of 70 °C with constant agitation (Wealab, Model BC1020). After cooking, the steak was transferred to ice-water for 10 min, removed from the vacuum bag, weighted roast, packed in foil and cooled overnight at 4 °C. The percentage of cook losses was calculated with the steak weighted before and after cook with equation: weight before cooking − weight after cooking /weight before cooking ×100.

For the Shear force (SF), six cores (1.57 cm diameter) were obtained each steak parallel to the longitudinal orientation of the muscle fibers, discarding cores with apparent deposits of connective tissue or fat (AMSA, 1995). Each core was sheared once through the center, perpendicular to the muscle fiber direction using a Warner-Bratzler shear machine (G-R Electrical Manufacturing Company, Manhattan, KS, USA). The average of six cores was used to determine SF values expressed in Newton (N). The remaining portions of cores were used later to determine the sarcomere length.

2.4.4. Sarcomere length (SL)

The remaining portions of cores for determination of SF were used for obtain o SL. Were obtained seven images of myofibrils in sucrose buffer solution (0.2M sucrose in 0.1M NaHPO4, buffer at pH 7.2) and the SL was measured using helium neon laser diffraction (Model 05-LHR-021, Melles Griot, Carlsbad, CA, USA) and calculated as described by (Cross, West, & Dutson, 1981).

2.4.5. Myofibrillar fragmentation index measurements

Myofibrillar fragmentation index (MFI) was measured according to Culler, JR, Smith, & Cross (1978), with modifications described by Hopkins, Martin, & Gilmour (2004). In duplicate, 0.5 g of raw meat were removed along the direction of myofibrils, discarding samples with connective tissue or fat. Samples were homogenized twice (2 × 30 s) in 30 ml of ice-cold buffer using an Ultra Turrax with a 10 mm diameter shaft at 4,043 xg. The buffer used was composed of 0.1 M KCl, 1 mM EGTA, 1 mM NaN3, 1 mM MgCl2, and 20 mM potassium phosphate (10 mM KH2PO4 and 10 mM K2HPO4 adjusted to pH of 7.0 at 4 °C). The mix was filtered through a 1 mm² mesh into 50 ml centrifuge tubes, and myofibrils extraction was performed via centrifugation of the filtrate at 2 °C at 1000xg for 10 min (model: Avanti™J-25, Beckman Coulter, Inc., Fullerton, CA, USA) three consecutive times, and the pellet was re-suspended in 10 ml of ice-cold buffer. The protein concentration of the final suspension was determined using the biuret method (Gornall A. G., Bardawill C. L., 1949). Duplicate aliquots of the myofibril suspension were diluted in buffer to achieve a protein concentration of 0.5 mg/ml. The absorbance of the diluted myofibril suspension was measured immediately at 540 nm. The mean of the duplicate absorbance was multiplied by 150 to yield the MFI value.

2.4.6. Analysis of collagen content in meat

For the determination of the collagen content, the method proposed by Woessner (1961) with modifications Latorre, Lifschitz, & Purslow (2016) was used. Duplicate samples of 1.5 g of lyophilized muscle was placed in 50 ml centrifuge tubes and was added 12 ml of phosphate buffered saline (NaCl 137 mM, KCl 2,7 mM, Na2HPO4 10 mM, KH2PO4 1,8 mM, pH 5.6). The tubes were placed in a water bath at 80 °C for 60 minutes. Subsequently, the tubes were transferred to an ice bath for 10 minutes. The samples were centrifuged 2 times (6000 x g, 2 °C and 10 minutes) to

separate the supernatant and solid residues. Then each fraction was hydrolyzed in 6N HCl at 110 °C for 16 h. Subsequently, 200 and 900 mg of activated carbon were added to the supernatant and residue, respectively, agitated and filtered through filter paper No. 2. The pH was adjusted between 6.5-7.0 with 2N NaOH. The supernatant samples were diluted in 100 ml of distilled water and the residue 250 ml. To determine the content of hydroxyproline, one ml of the filtrate was placed in a test tube, in addition to 2 ml of Isopropanol (2-Propanol solution, Sigma Aldrich) and then 1 ml of Oxidant solution (1.41 g of Chloramine T dissolved in 100 ml of buffer solution, 30 g of citric acid monohydrate, 15 g NaOH, 90 g of sodium acetate Trihydrate, dissolved in 500 ml of dH2O). The tubes remained at rest for 4 minutes and subsequently it was added 2 ml of Ehrlich reagent (2 g of P-dimethylbenzaldehyde, 2.5 ml of 70% Perchloric acid). The tubes were capped, covered with aluminum foil and placed in a water bath at 60 ° C for 25 minutes. Then the tubes were transferred to an ice bath for 4.5 minutes and finally the samples were placed to cuvettes and read by spectrophotometry at 558 nm. The collagen content was determined by multiplying hydroxyproline amount by 7.52 for the supernatant and 7.25 for the residue (Cross, Carpenter, & Smith, 1973).

2.5. Statistical analysis

The experiment was conducted in completely randomized design. The data of performance and carcass characteristics were analyzed using the weight and initial age as covariates in the GLM procedure of SAS version 9.4 (SAS Institute, Inc.). When the model showed significant ($P < 0.05$) differences between pairs of means were tested using the option PDIFF.

The metabolomics data were analyzed using the web-based tool MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/; (Xia & Wishart, 2016)). The metabolite concentration table was uploaded and the data was log-transformed and Pareto-scaled before analysis. T-test and Fold Change were used to find the metabolites that differed significantly in concentration between the C0, C28 and C56 treatment groups ($P< 0.05$). False discovery rate correction was used to minimize the risk of Type I errors in the t-test.

Beef quality were analyzed in a factorial design 3 x 3 (3 feedlot periods x 3 aging times), using animal as experimental unit. When the interaction was not significant was removed from the model and the parameter analyzed individually.

3. Results and discussion

3.1. Growth and development in feedlot

The slaughter weight was different between treatment C0 and C56, while C28 did not differ between them (Table 2). This difference was expected due to the longer time in feedlot of treatment C56.

On the other hand, the performance in feedlot was similar for C28 and C56, being that the ADG was 0.78 and 0.72 kg/d, respectively (P = 0.8822). This contrasts with that described by Owens, Gill, Secrist, & Coleman (1995), who indicated that animals with higher SBW are less efficient in weight gain during feedlot. Situation that was not found in the current paper, because of the short interval between slaughterings.

3.2. Carcass quality characteristics

Differences were found for REA between C0 and C56, meanwhile C28 was similar between them (Table 2). The REA is indicative of the percentage of lean tissue in the carcass, so that C56 animals would present more marketable cuts in relation to the other treatments, condition that favors the termination of cows in feedlot. On the other hand, due to the physiological age of the animals, a minimum increase in the REA was expected, since according to Wright & Russel (1991), mature animals without restriction have limited capacity to deposit muscle tissue. In the same sense, Pethick, Rowe, & Tudor (1995) indicates that LL muscle has little capacity to respond to improvement in nutrition when compared to other muscles, such as *Semitendinosus*.

In relation to the SFT, the C56 treatment was higher when compared with C0 and C28, which did not differ among themselves (Table 2). As indicated by Hornick et al. (2000), mature animals or with little genetic capacity, the excess nutrients is deposited in the body mostly as fat. However, this feature may increase the value of the carcass in the foreign market or, cause a penalty in the internal because commercial slaughterhouses demand a maximum of 6 mm (Kuss et al., 2005).

According to Di Marco (1998), in bovines the first fat to be deposited is the intermuscular fat, followed by the subcutaneous, situation that could be the cause of the similarity between C0 and C28 in relation to the SFT, since the interval between slaughterings of these treatments was short.

Although C56 showed higher SFT, this was not an impediment for the carcass to have higher losses due to cooling in relation to C28 (P = 0.045), being that C0 was

similar with both treatments (Table 2). This variable is negatively correlated with SFT, since SFT functions as a physical barrier that prevents losses due to cooling during the stay of the carcasses in the cold room; however, other investigations have found no correlation between these variables (Missio et al., 2013).

The ultimate pH of the carcass was lower in C56 (P = 0.0003), there being no differences between C0 and C28 (Table 2). Despite the previous differences, all treatments reached a pH lower than 5.7, considered the maximum critical point that meat should have to be considered of quality (Thompson, 2002).

In relation to the ultimate temperature of the carcass, there were no differences between treatments (Table 2). Using the non-linear equations, it was possible to verify that the C0 treatment carcasses cooled more quickly when compared with C28 and C56, which were not different between them (Table 2). This fact is different than expected, since the drop in the temperature of the carcasses is influenced mainly by the SFT (Duarte et al., 2011; Juárez et al., 2012), and in the current work, carcasses C0 and C28 had similar SFT.

On the other hand, the time in which the pH reaches 6.0 is inversely proportional to the drop in temperature, being that C56 carcasses take less than half the time in relation to C0 and 5 hours less than C28, without the latter having difference. This acidification of the muscle is caused by the use of most of the reserves of glycogen, ATP and accumulation of lactic acid (Graham et al., 2012; Juárez et al., 2012), which also causes loss of water retention capacity and intracellular calcium release (Paredi, Raboni, Bendixen, de Almeida, & Mozzarelli, 2012), causing the development of *rigor mortis* (decrease in muscle flexibility) and activating the enzymatic system of calpains (Lomiwes, Farouk, Wu, & Young, 2014), responsible for the degradation of key proteins during postmortem.

In an extensive review by Goll, Thompson, Li, Wei, & Cong (2003), indicates that there are three types of calpains, identifying (so far) as the main responsible for the early postmortem muscle degradation calpain-1 or μ-calpain, which requires a concentration of between 3 and 50 μM of calcium for reach half of its maximum activity. This potential is reached when the pH reaches between 6.3 and 6.1 (Nowak, 2011), due to the release by the sarcoplasmic reticulum of calcium ions, increasing to 100 μM a few minutes after slaughter (Jeacocke, 1993).

Table 2. Performance and carcass traits of cull cows without (C0) or with 28 (C28) or 56 (C56) days of feedlot.

Trait	Treatment			SE	P value
	C0	**C28**	**C56**		
Ultimate SBW (kg)	439.77 b	466.46 ab	483.59 a	9.65	0.0144
Ultimate REA (cm²)	56.57 b	57.62 ab	64.32 a	2.27	0.0422
Ultimate SFT (mm)	3.85 b	3.59 b	7.92 a	0.97	0.0065
Cooling losses (%)	2.34 ab	2.16 b	2.60 a	0.11	0.0201
Ultimate pH	5.63 a	5.67 a	5.52 b	0.02	0.0003
Ultimate Temperature	7.36	7.30	7.05	0.21	0.4952
Time (h) / Temp18	6.07 b	8.59 a	8.30 a	0.392	0.0009
Time (h) / pH6	16.10 a	13.09 a	7.51 b	1.016	<0.0001
Temp (°C) / pH6	8.80 c	12.98 b	19.33 a	0.995	<0.0001
pH / Temp18	6.76 a	6.36 b	5.87 c	0.078	<0.0001

SE = Standard error.
SBW = shrunk body weight; REA = rib-eye area of muscle *Longissimus lumborum*; SFT = subcutaneous fat thickness.
Means with a different superscript within a row are different ($P < 0.05$).

In relation to temperature, with the use of similar equations, when determining the temperature at pH 6.0, it can be observed that this has a behavior inversely proportional to the feedlot, causing the C56 carcasses to have the highest temperature, followed by C28 and C0 with the lowest temperature at that pH (Table 2).

Finally, the pH value was determined when the carcasses had an 18 °C temperature, resulting in an inverse behavior to the feedlot time, with the C0 carcasses having the highest pH, intermediate C28 and C56 the lowest (Table 2).

Therefore, both the pH and temperature of the carcasses play an important role in the transformation of muscle into meat and the quality of it. However, there are divergences in relation to which is more important. So, according to Olsson, Hertzman, & Tornberg (1994), the temperature at which *rigor mortis* occurs is more important for tenderness than ageing time. On the other hand, Hwang & Thompson (2001), indicate that the pH has greater influence on meat tenderness than the temperature of the carcass, although both factors affect the tenderness when the meat is ageing.

Combining both factors, Marsh, Ringkob, Russell, Swartz, & Pagel (1987) induced or not the pH drop by means of electrical stimulation and different rates of

cooling in bovine carcasses, found the lowest values of shear force when the pH was between 6.1 and 6.3 at 3 h postmortem, indicating that the application of electricity or cooling speed individually was of minimum importance, but that the drop in pH is highly dependent on both factors.

Thus, analyzing the factor pH and temperature it is possible to infer that the *rigor mortis* and the activation of the calpains occurred earlier in carcasses C56, where also the temperature at the time of activation was higher, factors that result in a rapid and marked degradation of myofibrillar proteins, producing tender meat few hours after slaughter (Lomiwes et al., 2014), fact discussed later.

3.3. Metabolites in muscle

A total of 301 metabolites were identified. Of those, 20 presented differences between treatments (Figure 1). Of these, one was repeated and another presented inconsistency with the metabolic pathway in cattle. Then, finally 18 different metabolites were considered between treatments, being 5 sugars, 5 amino acids, 5 carboxylic acids, 2 lipids and one pyrimidine (Table 3).

As observed, animals that had a period of feedlot had a higher concentration of Glucose-6-phosphate and Fructose-6-phosphate, which indicates a high energy intake by diet and a recomposition of body tissues, causing an increase in muscle proteins and in the deposition of fatness (Carrillo et al., 2016; Owens et al., 1995; Smith & Crouse, 1984). Besides being essential for the drop of the pH in the carcass (especially the glycogen) being completely transformed to lactic acid 48 hours after the slaughter (Pethick et al., 1995).

In agreement with our results, Mori et al. (2007) comparing different bovine termination systems (feedlot versus grazing), reported higher glucose concentration and high quality beef score in cattle finished in feedlot.

However, in the current work the levels of glycerol were higher in cows of treatments C28 and C56, being that this metabolite increases during lipolysis in animals in energy deficit, since it is one of the main precursors of hepatic gluconeogenesis (DiMarco, Beitz, & Whitehurst, 1981; Herdt, 1988; Noro & Wittwer, 2012; Reynolds, Aikman, Lupoli, Humphries, & Beever, 2003). However, Huntington, Harmon, & Richards (2006), in an extensive review, indicated that hepatocytes *in vitro* have little sensitivity to insulin, and may cause the use of glycogen precursors, such as glycerol, even in animals consuming high starch diets.

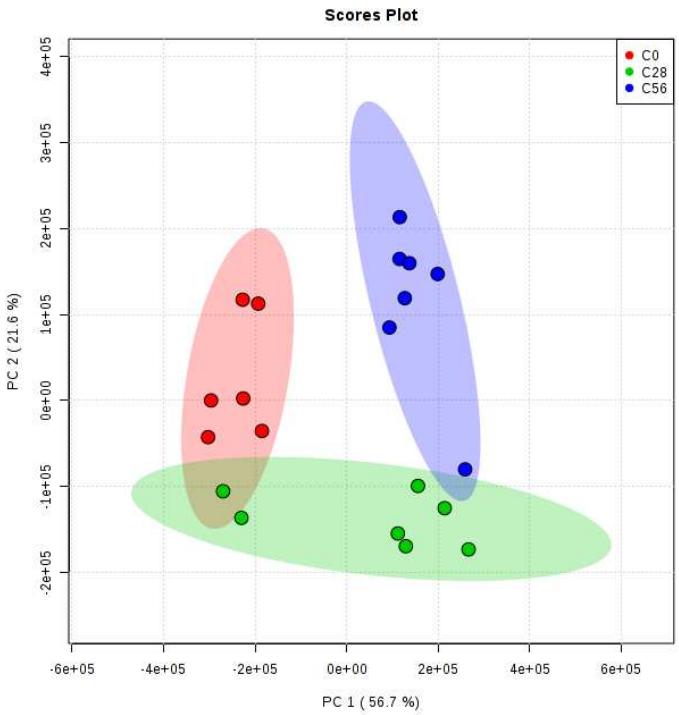

Figure 1. PLS-DA scores plots for Longissimus muscle of cull cows without (C0) or with 28 (C28) or 56 (C56) days of feedlot.
The numbers between parentheses in the axes refer to the variation between the samples.

In the same way, Peters (1986) evaluating cattle with accelerated gain or restricted feeding, verified higher concentrations of plasma glycerol in the first, explaining that this result was caused by an increase in adiposity turnover and therefore, there was an increase in glycerol to the bloodstream, a fact that could explain the cause of higher concentrations of this metabolite in muscle of animals confined in the present work.

In like manner, the concentration of amino acids was higher in the muscle of animals confined for 28 and 56 days, with the exception of Aspartate. In this sense, Lescoat, Sauvant, & Danfær (1996) indicated that muscle of animals with nutritional deficiencies has lower capacity of absorption of amino acids, indicating as factors changes in the oxidation rate of some amino acids, their use as a source of energy or reduction in the percentage net of absorption; results that can be observed in cows of the C0 treatment.

In relation to Aspartate, Hanigan et al. (2004) studying the metabolism in cows with negative energy balance, find a proportional increase in this acid when glucagon increased intracellularly, being widely recognized that this hormone is an indicator of

the alteration of hepatic metabolism (Boer, 2013; Brockman, 1978; Qaid & Abdelrahman, 2016), increasing their synthesis and concentration in the blood plasma mainly in ruminants with energy imbalance (Faramarzian, Haji Hajikolaei, Nouri, Mohebbi, & Shahriari, 2016; Gardner, Mcgilchrist, & Pethick, 2014).

Another possible explanation for the lower concentration of Aspartate in cows confined for 28 and 56 days is that this was indicated as one of the main metabolites responsible for the production of glucose in cows (Herdt, 1988; Mills, Beitz, & Young, 1986), so there would be a negative correlation between Glucose and Aspartate, as determined in the current study. In addition, this amino acid participates both in the urea cycle and in the Krebs cycle, reducing its availability in growing animals (Noro & Wittwer, 2012).

In relation to the carboxylic acids, these presented a variable behavior in the samples of analyzed muscle. In agreement with these results, Sahoo & Jena (2014) indicated that these compounds, although they are the main organic acids, have had a limited analysis due to inconsistent responses in the experiments.

For its part, in the case of thymine, it is considered an essential pyrimidine for the formation of RNA and DNA (Stentoft et al., 2015), participating in the structure of different genes, such as those that encode the synthesis of hormones such as leptin or proteins such as thyroglobulin (Carvalho et al., 2012), both of great importance in the formation of body fat and muscle development.

On the other hand, in addition to endogenous DNA, bovine plasma also transports products of the degradation of nucleic acids of ruminal microorganisms, as is the case of thymine, since these are absorbed in the bovine intestine and a minimum part escapes the liver metabolism, being able to be used in peripheral tissues (Stentoft et al., 2015).

Therefore, based on the previous work, we can infer that feedlot animals have greater flow and absorption of microbial protein, which represents an increase in the availability of byproducts such as thiamine, which can be used for the synthesis of leptin, causing an increase in the deposition of fat in the carcasses.

Table 3. Different metabolites identified in Longissimus muscle of cull cows without (C0) or with 28 (C28) or 56 (C56) days of feedlot.

Metabolite (arbitrary units)	Treatment			SE	FDR
	C0	C28	C56		
		Sugars			
Psicose	5455 c	58215 b	170018 a	14901	0.000241
Allose	187.21 b	141.08 b	208.25 a	21.43	0.006993
Glucose-6-phosphate	18946 b	46759 a	45847 a	4259	0.007927
Fructose-6-phosphate	36942 b	109595 a	119268 a	13740	0.017379
Glycerol	126276 b	283950 a	392890 a	41522	0.017379
		Amino acids			
Leucine	38568 b	89114 a	117195 a	10733	0.007927
Isoleucine	26195 b	53205 a	66738 a	5799	0.010104
Alanine	11229 b	21567 a	27512 a	2538	0.017379
Valine	59954 b	102954 a	118000 a	10258	0.033509
Aspartate	2282 a	615 b	295 b	363	0.033509
		Carboxylic acids			
Succinic acid	76405 b	380892 a	179316 b	139197	0.007927
Citric acid	2320 b	5517 a	2988 b	547	0.020436
3-Oxalomalate	5984 a	146 b	152 b	1119	0.031166
Malonic acid	11582 b	16328 b	26535 a	2681	0.033521
Quinic acid	57.56 b	73.82 a	78.86 a	11.58	0.033847
		Lipids			
Erucic Acid	2111 b	6118 b	14304 a	1215	0.000449
Epietiocholanolone	74.32 a	65.78 b	70.60 ab	15.16	0.048646
		Pyrimidines			
Thymine	62.89 b	71.40 b	86.38 a	3.51	0.01268

FDR = False discovery rate.
Means with a different superscript within a row are different ($P < 0.05$, FDR).

When analyzing the metabolic pathways in cattle through of MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/; (Xia & Wishart, 2016)), it was determined that metabolites with differences between treatments act mainly on four pathways: alanine,

aspartate and glutamate metabolism; valine, leucine and isoleucine biosynthesis; Aminoacyl-tRNA biosynthesis and, Citrate cycle (TCA cycle) (Figure 2).

Thus, it is observed that the amino acids valine and isoleucine have a great impact on the biosynthesis of other essential amino acids, mainly because the amount of amino acids is different during the muscle protein synthesis (Lescoat et al., 1996; Vopálenský et al., 2017).

On the other hand, alanine, aspartate and succinate present an important index because they are capable of modifying the metabolic pathway in which they are integrated (Figure 2), which would generate new metabolic products. With less impact, alanine, valine, isoleucine and aspartate participate in the biosynthesis of Aminoacyl tRNA. While Succinic Acid and citric acid are elements of the Krebs cycle (Figure 2).

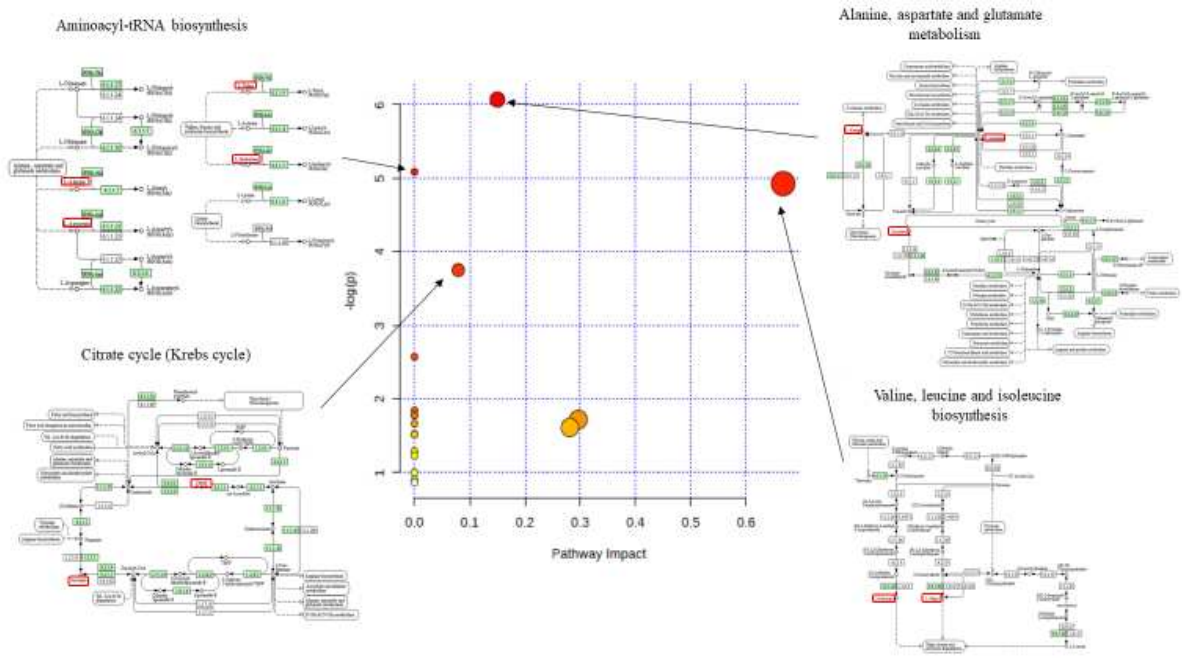

Figure 2. Analysis of the main metabolic pathways influenced by the metabolites that were different (indicated in red) in Longissimus muscle of cull cows without (C0) or with 28 (C28) or 56 (C56) days of feedlot.
X axis indicates the impact of the metabolite on the pathway, as the Y axis possible changes caused. The metabolic pathways were mapped in the KEGG pathway (accessible from http://www.genome.jp/kegg/pathway.html).

3.4. Meat quality characteristics

The nutritional composition of the meat between the treatments was similar, except for the percentage of moisture (Table 4). Meat from cows confined for 56 days showed higher dry matter (P = 0.0003), as well as trend in the ether extract content (P = 0.0598). This is correlated with the increase in SFT of the carcass, showing that, as mentioned above, in the order of deposition of fat tissue, intramuscular fat is the last (Di Marco, 1998).

In the same way, lower moisture content of the steak is in accordance with the greater weight loss in the carcass caused by the cooling (Table 2), which may affect the quality of the meat. So, according to Aalhus, Dugan, Robertson, Best, & Larsen (2004), a lower percentage of moisture can affect the tenderness of the meat by causing shrinkage of the sarcomere; however, these factors did not affect the shear force in this work (Table 5).

Table 4. Percentage of nutrient contents of meat of cull cows without (C0) or with 28 (C28) or 56 (C56) days of feedlot.

Trait (%)	Treatment			SE	P value
	C0	C28	C56		
Moisture	74.89 a	74.54 a	68.58 b	0.778	0.0003
Ash	3.80	3.26	3.72	0.168	0.0762
Crude protein	19.40	19.08	21.80	0.890	0.0993
Ether extract	1.93	3.16	4.11	0.621	0.0598

SE = Standard error.
Means with a different superscript within a row are different (P < 0.05).

In relation to the qualitative characteristics of meat (Table 5), it was determined the interaction between feedlot time and aging time for total losses in the roasted steak, this characteristic being described later.

On the other hand, no interactions were found for the variable shear force (SF) in the steaks (Table 5); however, there were differences between treatments and aging time, being discussed separately.

Feedlot time was inversely proportional to SF, determining that cows without feedlot had meat toughness in relation to C56, while C28 were similar to both treatments. In the same sense, Perry & Thompson (2005) analyzing the meat of bovines adapted to the tropics (Brahman, Belmont Red and Santa Gertrudis), found

that the SF showed quadratic behavior when correlated with the ADG, presenting reduction only when the ADG in feedlot was close to 2 kg/d; and a linear relationship when the model was adjusted for age.

The previous results, as well as the difference presented between the treatments C0 and C56 in relation to SF, could be caused by the early activation of the enzymatic system of Calpains-1, being that these are activated at a pH between 6.3 and 6.1 (Nowak, 2011), which was reached at 7.51 h postmortem with temperature of 19.3 °C in carcasses C56 (Table 2), causing the degradation of muscle cells and with it, an increase in the release of Ca ions, which concomitantly activate Calpains type 2 (Dransfield, 1994).

Contrary to the above, Lomiwes, Farouk, Wu, & Young (2014), determined lower SF for LL of Friesian or Friesian × Jersey bulls from 18 to 24 months of age, when present ultimate pH greater that 6.2 in relation to samples with pH lower to 5.79 (99.05 vs. 134.35 N), indicating as a possible cause, the early activation at high pH of calpain-1, which rapidly degrades high molecular weight proteins (titin, nebulin and filamin), but limiting its action to the first 48 hours postmortem due to rapid autolysis. However, these authors only measured the ultimate pH, so the onset of calpain-1 activity was not defined.

In another work (Hwang & Thompson, 2001), in carcasses of steers electrically stimulated and quickly cooled, reach pH of 6.0 to 1.5 (50%) and 3.0 (75%) h postmortem, provoking that calpain-1 were greatly influenced in the first 4 hours postmortem; determining that carcass with an intermediate rate of glycolysis had lower SF and that a slow decrease in temperature reduces to 3.92 N of SF on the first postmortem day.

Based on the above information and the results obtained in this work, we can say that the activation of enzymatic systems that cause degradation of muscle fibers after the slaughter, are early activated in animals with good body condition (sufficient reserves of energy to onset the *rigor mortis* and drop of the pH) and with adequate SFT (that prevents a rapid decrease of temperature in the carcass), conditions presented by the C56 treatment cows (Table 2); although the final pH is considered to be within acceptable levels in all treatments. This could invalidate the utility of the final pH in relation to SF.

Similarly, SF decreased 6.37 and 14.27 N from day 0 to day 7 and 14 of ageing, respectively (Table 5). The above was to be expected, since Aalhus, Dugan,

Robertson, Best, & Larsen (2004) assured that there is a continuous improvement in tenderness with the increase in ageing time, mainly due to the proteolytic degradation of the myofibrillar proteins in the LL muscle (Koohmaraie et al., 2002). In relation to this, Starkey, Geesink, Oddy, & Hopkins (2015) indicate that aging is responsible for 41% of the variation in the shearing force of sheep meat.

In a recent study, Colle & Doumit (2017) determined that the degradation of LL muscle fibers during the first hours after slaughter is the responsibility of calpain-1, since afterwards it self-degrades. However, with the increase in aging time, calpain-2 is activated and continues with muscle degradation until a maximum of 14 days after slaughter, so there is no improvement in tenderness after this period.

Thus, analyzing the results obtained in this work, we can observe an improvement in the tenderness of the meat of mature cows using both feedlot as aging of the meat, options that would depend on each particular situation.

On the other hand, there are works that indicate that the consumer of bovine meat from *Bos indicus* animals, give higher score to the flavor than to the tenderness (Delgado et al., 2006).

Differences were found in the sarcomere length (SL) in feedlots time, which resulted higher in the treatment C56 in relation to C0 and C28, which did not vary among themselves (Table 5); but this characteristic did not differ between maturation times. According to Pearson & Young (1989), Dransfield, (1994) and Hwang & Thompson (2001), for the sarcomere to have a shrinkage, the meat must have available energy to maintain the contraction, pH higher than 6.0 and a temperature lower than 12 °C.

Then, based on the previous statements and the results obtained in the current work by non-linear equations (Table 2), we can perceive that meat of the C0 treatment presented shrinkage, C28 had values very close to this and the C56 treatment there was no possibility for the pH and temperature values.

Another factor that may have modified the SL is the way in which the carcasses were hung, since in review by Juárez et al. (2012), carcasses hung by the obturator foramen in the pelvic bone (tenderstretch), methodology used in this work, they present longer sarcomeres.

In relation to the myofibrillar fragmentation index (MFI), related to myofibrillar degradation, it was similar between feedlot times; however, it increased as the days of aging increased (Table 5), as expected. This lack of difference between

feedlot times could be caused, according to Kristensen et al. (2002), by a short period of experimentation.

There were no differences in gross values of total collagen nor between periods of feedlot nor between aging times. However, the percentage of insoluble and soluble collagen showed differences between feedlot time and aging time, but no interaction (Table 5).

The total collagen (cross-links) and the percentage of soluble collagen are the main responsible for the toughness of meat in mature animals (Lepetit, 2008; Obuz et al., 2014; Stolowski et al., 2006; Vestergaard et al., 2007). However, Therkildsen, Stolzenbach, & Byrne (2011b) relate the improvement of the tenderness in LL muscle mainly to the increase in marbling, indicating that the percentage of soluble collagen has less impact. In that sense, Starkey, Geesink, Oddy, & Hopkins (2015) indicates that the solubility of the collagen alone is responsible for 8% of the variation in the shear force of lamb meat.

In the revision of Thompson (2002), it indicates that the speed of growth of the muscular tissue impacts the structure and collagen matrix. Likewise, Kopp & Valin (1981), showed that there are enzymes capable of degrading muscle collagen when the pH of the meat is around 5.5, as was the case of the carcasses of treatment C56 (Table 2), which would increase the percentage of soluble collagen both by increasing the feedlot time and by increasing the aging time. In addition to that animals of the treatment C28 and C56 to present increase and modification in body tissues, they also modified the synthesis and formation of new collagen, causing increase in the percentage of soluble collagen.

Similar results were reported by Boleman, Miller, Buyck, Cross, & Savell (1996a), who reported an increase in soluble collagen with the increase in the feedlot time of cull cows. In the same way Starkey et al. (2015) reported an increase in the percentage of soluble collagen in sheep meat aged for 14 days.

Table 5. Characteristics of meat of cull cows without (C0) or with 28 (C28) or 56 (C56) days of feedlot.

Trait	Feedlot time			Ageing time (days)			SE	P value		
	0	28	56	0	7	14		FT	AT	FT*AT
Total losses (%)	22.06	25.20	24.27	23.85	24.26	23.43	0.074	0.0129	0.6976	0.0001
Shear force (N)	52.29 a	49.45 ab	45.84 b	56.07 a	49.70 b	41.80 c	1.677	0.0305	<0.0001	0.4761
Sarcomere length (μm)	1.64 b	1.73 b	2.02 a	1.85	1.79	1.74	0.062	<0.0001	0.3958	0.7121
MFI	27.04	30.98	32.91	18.48 c	27.71 b	44.74 a	2.022	0.1059	< 0.001	0.7543
Total collagen (mg/g)	4.13	4.19	4.02	4.44	3.71	4.20	0.309	0.8708	0.1139	0.2198
lnsoluble collagen (%)	96.90 a	96.17 ab	95.39 b	95.57 b	96.67 a	96.22 ab	0.389	0.0095	0.0459	0.2457
Soluble collagen (%)	3.11 b	3.83 ab	4.61 a	4.43 a	3.33 b	3.78 ab	0.389	0.0095	0.0459	0.2457

SE = Standard error; MFI = myofibrillar fragmentation index; FT = Feedlot time; AT = Ageing time; FT*AT = interaction FT and AT. Means with a different superscript within a row are different (P < 0.05).

As mentioned above, there was interaction between feedlot time and aging time for total losses when the steak was roasted, with the least losses in steaks aged during 14 days of the C0 treatment (Table 6).

Table 6. Interaction feedlot time x aging time for total losses in steak of cull cows without (C0) or with 28 (C28) or 56 (C56) days of feedlot.

Aging time	Feedlot time		
	C0	C28	C56
0	24.77 a	23.95 a	22.81 ab
7	24.08 a	25.12 a	23.57 a
14	17.33 b	26.53 a	26.41 a

Means with a different superscript within in the table are different ($P < 0.05$).

In the same way, the instrumental analysis of the color of the meat and the subcutaneous fat showed interactions for all the indices (luminosity, redness and yellowness). So, the results are presented separately.

Previous studies have indicated that the main attributes considered by consumers when choosing beef are color and texture (Crouse, Cundiff, Koch, Koohmaraie, & Seideman, 1989). So, in relation to the brightness index (L*), a pattern was found in which the flesh of all treatments without aging presented the lowest values, while meat aged for 7 and 14 days had greater intensity (Table 7).

However, according to Duarte et al. (2011), normal values for this index are between 35 and 38 in mature cows, so in meat aged for 14 days in the current work is higher (on average 39.14). These values are also contrary to that expressed by Lobato et al. (2014), who indicate that *Bos indicus* breeds are more reactive so that muscle glycogen reserves are quickly depleted, causing their flesh to be dark, a situation that was not found in the present work.

With regard to the intensity of redness (a*), steaks aged for 14 days of cows without feedlot showed the highest intensity, and in the same treatment, steaks aged for 7 days or without aging had lower intensity (Table 7). In literature review by Muchenje et al. (2009), indicated that normal values are between 11.1 and 23.6, so the values found in the present work are within the expected values.

Table 7. Interactions between feedlot time and aging time for the color of the meat of cull cows without (C0) or with 28 (C28) or 56 (C56) days of feedlot.

Aging time	Feedlot time		
	C0	C28	C56
		L*	
0	31.80 e	34.17 cde	34.96 cde
7	39.15 ab	37.30 abc	36.35 bc
14	40.62 a	39.71 ab	37.10 abc
		a*	
0	14.96 bc	15.79 b	16.07 b
7	12.75 c	15.16 b	16.19 b
14	19.28 a	14.23 bc	15.94 b
		b*	
0	10.61 c	12.46 bc	12.02 bc
7	13.09 b	12.7 bc	13.13 b
14	16.25 a	12.02 bc	13.35 b
		Hue	
0	35.28 d	38.26 bd	36.72 cd
7	45.85 a	40.09 b	39.06 bc
14	40.13 b	40.09 b	39.88 b
		Chroma	
0	18.34 b	20.12 b	20.08 b
7	18.29 b	19.79 b	20.86 b
14	25.22 a	18.64 b	20.80 b

L* = brightness; a* = redness; b* = yellowness; HUE = hue angle.
Media with a different superscript within the table are different ($P < 0.05$).

Comparing the values of yellowness (b*) with the same literature cited above, we can observe that the beef in the present work is outside the recommended range (values between 6.1 and 11.3), whereas here the minimum value was 10.61, corresponding to steaks without aging of the C0 treatment, and 16.25 as maximum value in steaks aged for 14 days of the same treatment (Table 7).

With respect to the Hue angle, indicator of the discoloration rate (Kim, Kemp, & Samuelsson, 2016), was greater in steaks aged for 7 days and the lowest in steaks without aging, both of the C0 treatment (Table 7).

Regarding the Chroma value (color intensity), steaks aged for 14 days of the C0 treatment were different from all the others (Table 7), caused by the fact that these steaks presented the highest values in the previous indexes.

With respect to the brightness of the fat (L*), meat aged for 7 days of the C0 treatment presents the highest numerical value, and the meat without aging of the C56 treatment has the lowest (Table 8), and there is no pattern indicating proportional influence of aging time or days in feedlot. However, Boleman et al. (1996), found a significant reduction in fat color in mature cow carcasses as the feedlot time increased, the former being evaluated by trained personnel.

Table 8. Interactions between feedlot time and aging time for the color of the fat of cull cows without (C0) or with 28 (C28) or 56 (C56) days of feedlot.

	Feedlot time		
Aging time	C0	C28	C56
		L*	
0	68.1 ab	63.25 bc	61.38 c
7	72.24 a	66.54 abc	65.34 bc
14	65.02 bc	67.25 ab	68.09 ab

L* = lightness.
Media with a different superscript within the table are different (P <0.05).

Table 8. Continuation

		a*	
0	14.69 bc	20.30 a	18.77 ab
7	4.03 e	10.72 cd	11.40 cd
14	9.88 cd	10.13 cd	9.29 d
		b*	
0	40.65 a	36.88 ab	36.01 ab
7	22.15 c	27.04 c	29.10 bc
14	21.63 c	27.08 c	29.03 bc

a* = redness; b* = yellowness.
Media with a different superscript within the table are different (P <0.05).

While the intensity of a* was higher in meat without aging of treatments C28 and C56 (Table 8), and the lowest red intensity was in the meat matured for 7 days of the C0 treatment.

On the other hand, too steaks without aging had the highest values in the b* index (Table 8), indicating a possible drying of the fat, which could cause the final consumer to reject the cut by appearing a longer time of exposure on the shelf.

4. Conclusion

The results obtained in the current work allow us to conclude that Nellore cull cows finished in feedlot for 28 or 56 days increased the availability of nutrients, fatness and improved carcass quality, which is reflected in a slow drop in temperature and decline rapid of pH, causing that qualitative characteristics of the meat to be superior to meat produced by animals without feedlot. However, all treatments presented meat of acceptable quality.

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Chapter 2

METABOLOME AND BEEF QUALITY OF NELLORE CULL COWS WET OR DRY-AGED

Abstract

The present study aims identify the metabolome and beef quality of cows submitted to two different techniques of ageing (Dry-aged versus Wet-aged) of cull Nellore cows from grazing system. Dry-aging were performed without any packaging in direct contact with the cooling room environment, while the Wet-ageing samples were vacuum packed in plastic bags. The samples were aged for 14 continuous days at 4 °C and 85% humidity. Aging reduced the shear force and increased the metabolites such as amino acids, sugars and lipids, without differences between treatments. Dry samples present greater losses causing lower total salable yield, which would cause a higher price in the retail level. Wet samples have a higher concentration of biogenic amines (e.g. Putrescine) which would reduce its shelf life. It is concluded that aging meat cull Nellore cow is a viable option to improve the physicochemical characteristics of the product, regardless of the methodology used.

1. Introduction

The increase in the population and incomes of the middle class will lead to a greater demand and consumption of beef worldwide in the coming years (Westcott, 2016); it is estimated that during the year 2024 there will be 75.4 million t of meat equivalent (CWE) of beef (FAO, 2015), of which Brazil, with a herd of 234.2 million cattle (27.26% cows), It will produce 11.7 million t of CWE (Barioni et al., 2007; FAO, 2015; FIESP, 2016), becoming the main exporter of beef (USDA, 2017).

Of the animals slaughtered, between 30 and 40% are females (Anualpec, 2016), and of these, 78% are cull cows (IBGE, 2016). In addition, 23.28% of the income of the agricultural companies are obtained by the sale of category (Anualpec, 2011).

Despite its importance, there is limited information focused on the improvement of the quality of the meat of cull cows, with the rest being studies aimed mainly at metabolic changes (Boleman et al., 1996; Schnell et al., 1997; Therkildsen et al., 2011;

Vestergaard et al., 2007) and very few other techniques such as aging (Obuz et al., 2014; Vitale et al., 2014; Xiong et al., 2007), but studies using zebus cows are almost non-existent.

In relation to the aging of the meat, it is widely recognized that this process, in addition to modifying the flavor, reduces the cutting force due to the degradation of the muscle fibers through enzymatic systems (Koohmaraie et al., 2002; Muchenje et al., 2009; Bethany M. Sitz et al., 2006), once some studies have indicated that the action of fibrolytic enzymes is not significant in the first 24 hours postmortem (D'Alessandro et al., 2012). In addition, there are currently three main methodologies for aging meat, these being dry aging, vacuum aging and a novel dry aging technique that uses a bag highly permeable to moisture.

When these methodologies are compared, the results are variable in terms of factors of meat quality (Li et al., 2014; Vestergaard et al., 2007; Warren & Kastner, 1992), shelf-life (Ahnström et al., 2006; Colle et al., 2015; Obuz et al., 2014) and consumer preference (Parrish, Boles, Rust, & Olson, 1991; Bethany M. Sitz et al., 2006; Stenström et al., 2014).

However, a general consensus is that meat aged to dry presents lower percentages of salable meat and requiring greater control of the environmental conditions of aging. In spite of the above, it has also been reported that the flavor produced by this type of meat is valued by the consumer, being willing to pay an extra price (B. M. Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006; C. L. Wright, 2005).

Thus, we hypothesize that the quality of meat aged to dry for 14 days presents better characteristics of quality in relation to the traditional technique in plastic bags and meat without aging, in addition to producing a greater quantity of metabolites related to flavor.

Therefore, the present study aims to determine the beef characteristics of cull Nellore cows from the grazing system, without or aged by two different techniques (Dry-aging versus Wet-aging) for 14 days, using analysis traditional as well as modern techniques to identify major changes in the metabolic profile of meat.

2. Materials and methods

2.1. Animals and nutritional management

The Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil, approved all animal handling procedures (protocol 037/2017) in accordance with ARRIVE guidelines (Kilkenny et al., 2010) and EU Directive 2010/63/EU (European Union, 2010).

Twelve cull Nellore cows; with variable age in 6 to 15 years (standard deviation, SD 3.4) and 467 kg (SD 60.5) body weight were used. The animals remained in continuous grazing without supplementation for two months after weaning of their calves, and later were transferred to the Animal Science Department of the Universidade Federal de Viçosa where they remained in individual stalls for two or three days until the moment of the slaughter.

2.2. Slaughter procedure and carcass evaluation

The day of slaughter, the cows were weighted after 16 h of fasting. All procedures during the slaughters were performed in accordance with humane slaughter practices following the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 2017). The slaughter was carried out on two consecutive days, 6 cows at a time. During slaughter, all components of the animal's body were weighed to obtain empty body weight (EBW). Carcass were not electrically stimulated. The average weight of hot carcasses was 251.32 kg.

The carcass was divided longitudinally into two halves, weighted and suspended by the aitch bone (tenderstretch method) and chilled at 4° C for 22 at 24 h. Both at the entrance and at the exit of the cold chamber, pH and temperature of the *Longissimus lumborum* (LL) muscle were recorded at the 13th rib from the left carcass using an electrode (model: Inlab® Solids Pro, Mettler-Toledo AG, Schwerzenbach, Switzerland) connected to a portable Mettler-Toledo pH meter. Being the pH and final temperature of 5.8 and 4.9 °C, respectively.

After cooling, in the left carcass in the portion comprised between 12th and 13th ribs was measured the subcutaneous fat thickness (SFT) using a digital caliper.

2.3. Samples preparation

Of each carcass, two sections were collected between the 9th and 11th ribs, one of each half carcass, being weighed including bones, SFT, lean tissue and randomly destined for one of the aging treatments (Dry-aged or Wed-aged). In addition, two LL muscle steaks were removed immediately bellow the 11th rib, to conform the treatments without aging (WA).

2.3.1. Aging conditions

Samples assigned to the Dry-aging treatment (Dry) were placed on a tray with coarse salt covering the bottom of the same and suspended on a metal grid to avoid contact with the salt, placing the spinal process of the vertebra in contact with the grid. The salt will have the function to absorb the liquids lost by the piece and to avoid the rotting in the inferior surface. Wet-aging samples (Wet) were vacuum packed in commercial plastic vacuum bags and placed interposed with Dry samples.

The samples were placed on a stainless steel table in a cooling room where remained 14 continuous days (15 days post-mortem). During this period, the temperature and humidity were measured every minute using a Digital Datalogger (Instrutherm Data Loggers Ltd., UK), registering an average of 4.2 ° C and 80% relative humidity.

2.3.2. Losses by aging

After of aging, the Wet samples were removed from the plastic bag, the liquid residues were discarded and, both Wet and Dry treatment samples were reweighed to determine the drip losses, calculated as: (weight after aging/weight before aging) X 100. Subsequently, the Dry samples were trimmed and the surface exposed to the air was removed and weighed, considering this portion as non-consumable due to the dry appearance and dark color. Then the bones were removed, weighed and discarded, thus leaving the lean tissue clean. The weight of the bones was subtracted from the original weight to determine the weight of the lean tissue before aging, considered that the bones did not change their weight during this process. To obtain the total losses (%), the final saleable yield after aging was subtracted from the weight before aging, calculated as:

(weight of consumable lean tissue after aging/weight of total lean tissue before aging) X 100.

2.3.3. Steak preparation

To obtain WA samples, 24 h after slaughter two steaks from a 2.54 cm was removed between 12th and 13th ribs of each half carcass, which was packed in vacuum, frozen, and stored at −18° C until analysis, being considered these steaks samples without maturation. For aging samples, the LL muscle of both treatment (Dry and Wet) were separated from each piece and three steaks of 2.54 cm will be removed, vacuum-packaged, frozen and stored at -18° C until the meat quality analysis.

2.4. Instrumental and chemical meat quality measurements

2.4.1. Chemical composition of meat

One steak from each treatment (WA, Dry and Wet) was freeze dried for 72 h, obtaining partial dry matter. After, samples were partially defatted by washing with petroleum ether (Fernandes et al., 2010) and amount of fat lost was recorded. Then the samples were ground with ball mill and analyzed for proximate analysis to determine dry matter (DM; method 934.01), ash (method 930.05), total nitrogen (N; method 981.10) and ether extract (EE; method 920.85) according to the AOAC, (2012) methods of analysis protocols. The crude protein (CP) content was obtained by multiplying 6.25 by the total nitrogen content. The total EE was corrected by adding the fat lost during the partial defatting process.

2.4.2. Color analysis

A second steak were thawed overnight at 4° C, removed from vacuum bags and allowed to bloom for 30 min prior to measurements. Objective color was measured using a Hunter MiniScan EZ (4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA), which was calibrated before use. The mean at five locations on the displayed surface of each steak avoiding large marbling flecks, connective tissue and edge, was evaluated for lightness (L^*), redness (a^*) and yellowness (b^*) using illuminant D65, a 31.8 mm port size and 10° standard observer. Similarly, using the above indices was

calculated or indicator Chroma $(a^{*2} + b^{*2})^{1/2}$ and Hue angle (tan -1(b^*/a^*)). Likewise, SFT samples were analyzed the day after slaughter, and after maturation in all treatments.

2.4.3. Cook losses and Shear force

The same steaks used for color determination was used in this analysis. The steak was weighted, repacked to vacuum and broiled for 30 min in the water bath pre-heated with constant agitation and controlled at 70° C (Wealab, Model BC1020). After cooking, the steak was transferred to ice-water for 10 min, after removed from the vacuum bag, weighted again and cooled for 16 h at 4° C. The percentage of cook losses was calculated with the steak weighted before and after cook with equation: (weight after cook/weight before cook) X 100. For the SF, six cores (1.27 cm diameter) were obtained each steak parallel to the longitudinal orientation of the muscle fibers, discarding cores with apparent deposits of connective tissue or fat (AMSA, 1995). Each core was sheared once through the center, perpendicular to the muscle fiber direction using a Warner-Bratzler shear machine (G-R Electrical Manufacturing Company, Manhattan, KS, USA). The average of six cores was used to determine SF values expressed in Newton (N). The remaining portions of cores were used later to determine the sarcomere length (SL).

2.4.4. Sarcomere length (SL)

The remaining portions of cores of the determination of SF were used for obtain o SL. Of the portions were obtained eight fiber bundles and placed in the slide with a sucrose buffer solution (0.2M sucrose in 0.1M NaHPO4, buffer at pH 7.2) and the SL was measured using helium neon laser diffraction (Model 05-LHR-021, Melles Griot, Carlsbad, CA, USA) and calculated as described by (Cross et al., 1981).

2.4.5. Myofibrillar fragmentation index measurements

Myofibrillar fragmentation index (MFI) was measured according to Culler, JR, Smith, & Cross (1978), with modifications described by Hopkins, Martin, & Gilmour (2004). In duplicate, the protein concentration of the final suspension was determined using the biuret method (Gornall A. G., Bardawill C. L., 1949). Duplicate aliquots of the myofibril suspension were diluted in buffer to achieve a protein concentration of 0.5

mg/ml. The absorbance of the diluted myofibril suspension was measured immediately at 540 nm. The mean of the duplicate absorbance was multiplied by 150 to yield the MFI value.

2.5. Metabolite analysis

2.5.1. Sample preparation

Polar metabolites were extracted using the method of Lisec, Schauer, Kopka, Willmitzer, & Fernie (2006). 50 mg of ground beef from each loin was homogenized in 2 ml tube with 700 µL cold methanol and 30 µL stock Ribitol (0.2 mg/ml Ribitol in water), shake 15 min at 70° C at 1000 rpm. At once, centrifuged 10 min at 14000 rpm. The aqueous phase was transferred to a new tube, add 375 µL CHCl3, 750 µL of MilliQ water and again centrifuged 15 min at 4000 rpm. 150 µL from the upper phase (polar phase) were transferred at new tube and the solvent was evaporated overnight using a Savant SpeedVac (Thermo Scientific).

For the phase of derivatization, the remaining residue was reconstituted add 40 µL Methoxyamination reagent (20 mg methoxyamine hydrochloride/ml pure Pyridin), shake 2 h at 37° C at 950 rpm, after centrifuged 1 min at 10000 rpm. Add 70 µL MSTFA mix (1 ml + 20 µL FAME), shake 30 min at 37° C at 950 rpm, centrifuged 1 min at 10000 rpm. Finally, transfer 90 µL at sample into glass vials suitable for GC-MS analysis.

2.5.2. Metabolite identification and quantitation

For the analysis of the samples it was used the GC-MS TruTOF system, Agilent Chroma tograph Technologies 7890A and TruTOF® HT TOFMS spectrometer Leco, equipped with a 30-m capillary column (DB-35 MS, Agilent Technologies) as suggested by Lisec, Schauer, Kopka, Willmitzer, & Fernie (2006). In splitless mode at 230° C, 1 µl of each sample was injected charged by the helium gas, with continuous flow of 2 ml/min. Initially the temperature of oven was maintained at 80° C and then increased 15° C/min until reaching 330° C maintaining this temperature for 6 min. Chromatograms had the baseline corrected and their deconvolution was performed through the Chroma TOF software, Leco. Afterwards, the peaks were identified through the deconvoluted spectra obtained using the TagSearch software (Cuadros-Inostroza et al., 2009). The areas of the

Chromatographic peaks of the fragmented ions were normalized and corrected for cell density (DO_{600nm}).

2.5.3. Metabolomics data analysis

The metabolomics data were analyzed using the web-based tool MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/; (Xia & Wishart, 2016)). The metabolite concentration table was uploaded and the data was log-transformed and Pareto-scaled before analysis. T-test and Fold Change were used to find the metabolites that differed significantly in concentration between the WA, Dry and Wet treatment ($P< 0.05$). False discovery rate correction was used to minimize the risk of Type I errors in the t-test.

2.6. Statistical analysis

The experiment was conducted completely randomized design considering the steak as the experimental unit. The data were analyzed using the GLM procedure of SAS version 9.1, (SAS Institute, Inc.) calculating least squares means (LSM) and standard errors (SE), when the model showed significant ($P < 0.05$) differences between pairs of means were tested using the option PDIFF.

3. Results and discussion

3.1. Losses by aging

Weight losses were affected by the treatment ($P <0.05$), being higher in the Dry pieces (Table 1). WA treatment samples did not present losses of any kind to be frozen immediately after obtained; in the case of the Wet treatment, have very small losses when packaged in vacuum, avoiding losses by evaporation and not needing cleaning. On the other hand, aging pieces without bag had high total losses, being more significant those caused by dripping and evaporation than those derived from trim.

Previous research reported similar results in treatments in relation to total losses (Ahnström et al., 2006; Li et al., 2014; Obuz et al., 2014; Parrish et al., 1991; Warren & Kastner, 1992), although the meat was obtained from animals of different breed, ages and systems of termination; indicating that regardless of the origin of the meat, the Dry treatment has a lower retail yield.

These results were expected because the external surface of the dry-aging meat is in direct contact with the air flow in the refrigerated room, causing dehydration and darkening of the outer layer, which has to be discarded to improve the appearance visual for the consumer.

Table 1. Percentage of weight loss in samples without aging (WA), Dry-aged (Dry) and Wet-aged (Wet) for 14 days.

Trait	Ageing method			SE	P-value
	WA	Dry	Wet		
Drip loss[1] (%)	-	11,59	0,54	0,37	<0,001
Trim loss (%)	-	10,30	0,00	0,48	<0,001
Total loss (%)	-	21,89	0,54	0,58	<0,001

[1] Losses caused for dripping and evaporation.
Means with a different superscript within a row are different ($P < 0.05$)

This difference may be the main limitation for the adoption of dry-aging by the industry, being necessary compensate the lowest percentage of retail yield with an increase in quality and, consequently, the price of the same.

3.2. Instrumental and chemical meat quality measurements

3.2.1. Chemical composition of meat

The percentage of DM and CP was not influenced by the treatment (Table 2). On the other hand, Dry-aging steaks presented lower percentage of ash ($P <0.05$) in relation to Wet-aging and WA treatments, which were not different (Table 2). Inverse results were found in EE, with the WA and Wet samples presenting lower percentages ($P <0.05$).

In spite of the DM not having been different, the lower moisture content in the Wet and WA treatments may have caused a lower concentration of EE, which would have an impact on the sensory analysis of the meat (Duarte et al., 2011).

Table 2. Chemical composition of samples without aging (WA), Dry-aged (Dry) and Wet-aged (Wet) for 14 days.

Trait	Ageing method			SE	P-value
	WA	Dry	Wet		
DM[1] (%)	23,25	24,07	22,83	0,46	0,162
Ashes (%)	4,56 a	4,18 b	4,56 a	0,11	0,024
CP[2] (%)	19,98	20,22	20,76	0,92	0,817
EE[3] (%)	2,87 b	4,17 a	2,65 b	0,42	0,028

[1] Dry matter, [2] crude protein; [3] ether extract.
Means with a different superscript within a row are different (P < 0.05).

3.2.2. Color analysis

In the present study, brightness (*L**) was altered by maturation (P <0.05), increasing 2.02 and 2.47 units in the treatments Dry and Wet, respectively, in relation to the WA treatment (Table 3). This increase can be caused by accumulation of liquid on the surface of the meat due to the loss of sarcoplasmic fluid by cell destruction (Aroeira et al., 2016), causing the incidence of light rays to be reflected with greater intensity, a characteristic that can be appreciated by the final consumer when finding bright meat on tray.

Regarding to intensity of yellow (*b**) aged meat have higher in relation to un-aged meat (P <0.05), and for both aging methods there were no differences (Table 3). This situation may be negative in retail sales because the final consumer prefers meat with cherry red intensity rather than yellow (Colle et al., 2015), associating the yellow color to meat of animals with greater maturity (Duarte et al., 2011) or sick (Muchenje et al., 2009).

In relation to the intensity of red (*a**), Chroma and Hue there were no differences between treatments (P> 0.05, Table 3).

According to a review published by Muchenje et al. (2009), the acceptable intensities for brightness (*L**) are between 33.2 and 41.0; for red (*a**) between 11.1 and 23.6; and for yellow (*b**) between 6.1 and 11.3. Based on the above, the indices found in

the present work are within the range, except for the *b**, mainly due to the termination system and the age of the animals.

Table 3. Instrumental evaluation of the color of meat and subcutaneous fat thickness (SFT) of samples without aging (WA), Dry-aged (Dry) and Wet-aged (Wet) for 14 days.

Trait		Ageing method			SE	P-value
		WA	Dry	Wet		
Meat	*L**	35,28 b	37,30 a	37,75 a	0,67	0,025
	*a**	15,33	16,64	16,11	0,65	0,344
	*b**	12,12 b	13,46 a	13,05 a	0,36	0,032
	Chroma	19,56	21,42	20,75	0,68	0,148
	Hue angle	38,32	39,18	39,23	0,82	0,662
SFT	*L**	63,93	61,09	65,10	2,00	0,356
	*a**	15,96 a	15,53 a	10,98 b	1,41	0,031
	*b**	31,76 a	32,20 a	25,10 b	2,02	0,030
	Chroma	35,73 a	35,83 a	27,49 b	2,29	0,020
	Hue angle	64,00	64,20	66,72	1,61	0,414

Means with a different superscript within a row are different ($P < 0.05$).

In SFT, lightness and Hue were similar in all treatments ($P > 0.05$; Table 3); characteristics unchanged by the lesser amount of water present in the fat, which reduces dehydration. On the other hand, intensity of red, yellow and Chroma were similar in WA and Dry, but in Wet treatment showed lower values ($P < 0.05$).

We can confirm, in general, that the aging causes changes in the color of the meat, changes that may be positive when increasing the brightness or negatives to increasing the intensity of the yellow both in the lean tissue and in the SFT.

3.2.3. Cook losses

There were no differences in the losses caused during the cooking of the meat between treatments ($P > 0.05$; Table 4), finding lower percentages compared to other

studies (Ahnström et al., 2006; Kim et al., 2016); in addition, other studies have reported higher losses in WA meat (32.63%), intermediate in Wet-aging (30.84%) and lower in Dry-aging (26.31%) (Warren & Kastner, 1992).

Table 4. Cooking variables of meat of samples without aging (WA), Dry-aged (Dry) and Wet-aged (Wet) for 14 days.

Trait	Ageing method			SE	P-value
	WA	Dry	Wet		
Thawing loss (%)	3,90	2,71	3,54	0,54	0,277
Cooking loss (%)	15,17	15,89	17,45	0,99	0,232
Total loss (%)	18,91	18,15	20,37	1,14	0,370

Means with a different superscript within a row are different ($P < 0.05$).

3.2.4. Warner-Bratzler shear force, MFI and sarcomere length

Meat WA presented greater resistance to cutting than Dry and Wet ($P < 0.001$), without any difference between the latter (Table 5). This result is caused by the longer time of action of the fibrolytic enzymes. Moczkowska, Półtorak, Montowska, Pospiech, & Wierzbicka (2017) confirmed the above with the Western blots technique, reporting a reduction in the percentage of troponin-T and desmin in aged meat.

Based on the above, we can say that the aging can be an option to improve the texture of the meat of culling cows, which is considered tough for coming from old animals (Ngapo, Braña Varela, & Rubio Lozano, 2017; Therkildsen et al., 2011).

Research with culling cows show divergences in the SF depending on the termination system. Vitale, Pérez-Juan, Lloret, Arnau, & Realini (2014), working with meat of Holstein cows with good conformation and SFT, reported a SF of 43.1 N in un-aged meat (similar value with the present study) and 24.0 N in beef aged under Wet-aging for 14 days. In system of pasture, meat removed from the carcass of old cows 7 days after the slaughter presented a SF of 69.63 N (Boleman et al., 1996), being less tenderness than that of animals finished in feedlots.

Table 5. Shear force (SF), myofibrillar fragmentation index (MFI) and sarcomere length (SL) of samples without aging (WA) or ageing by Dry-ageing (Dry) and Wet-ageing (Wet) for 14 days.

Trait	Ageing method			SE	P-value
	WA	Dry	Wet		
SF (N)	46,47 a	31,44 b	35,45 b	1,65	<0,001
MFI	15,69 b	35,42 a	33,48 a	2,68	<0,001
SL	1,93	2,10	2,04	0,07	0,216

Means with a different superscript within a row are different ($P < 0.05$).

The values of SF found in the present study are inferior to the studies cited above, including the un-aging meat. On the other hand, Aalhus et al. (2004a) mention that the exudate lost in the Wet treatment contains sarcoplasmic enzymes such as calpain, which may reduce degradation and increase SF. However, other studies minimize this fact by considering that calpain and cathepsin are not the main mechanisms of tenderness meat during maturation, indicating that glycolytic enzymes, oxidative stress, heat shock proteins (HSPs) are more important (D'Alessandro et al., 2012).

Thus, same as cull cow meat is considered tough, the samples used in this study can be classified as acceptable, even those without aging; something unusual in this type of animal. In addition, as reported by Aalhus, Dugan, et al. (2004a), the reduction of 9.81 N in SF is the minimum required for consumers to point out differences between meats. On the other hand, AMS (2012), considers that 5.0 N is sufficient to perceive sensorial differences. Using these references, we can affirm that the treatments of aging in this study would cause a better evaluation by consumers of the meat of culling cows, since the texture is the main limitation so that meat are appreciated (Bunmee et al., 2014), there being great variation in the results.

In that sense, Aalhus, Dugan, Robertson, Best, & Larsen (2004b) indicate that consumers consider as acceptable meat with SF less than 35.30 and unacceptable those with more of 62.76 N (tough) and less of 22.56 N (tender) (Savell et al., 1987). Other

values indicate acceptable meat with <40.21 N and extremely tenderness those with <37.74 N (Huffman et al., 1996). The previous classifications indicate that the perception of smoothness is inherent to the market where the study is done, and the majority of work is carried out in countries where *Bos taurus* cattle or their crosses predominate, and there is little literature regarding the sensorial perception of *Bos indicus*.

In relation to the IFM, the same pattern is found. WA meat expressed less fragmentation that aging meat (P <0.001), no differences between maturation methods (Table 5). This was to be expected when presenting the large correlation of MFI with the SF.

On the other hand, the SL did not differ between treatments (Table 5), result influenced by the SFT present in the carcasses (4.1 mm), what avoid shortening by cold (Pflanzer & Felício, 2009); in addition to that the methodology used to determine SL was made in cooked meat, and as mentioned before, there were no differences in weight loss during this process. Similar results were presented in meat of cows of different ages terminated in feedlots for 60 days (Shackelford et al., 1995). Thus, it can be assumed that aging also assists in the standardization of meat from animals with diverse characteristics, as was the case with the current report.

3.2.5. Metabolites

Differences (P<0.05, FDR) between 26 metabolites of WA, Dry-aging and Wet-aging were found (Table 6).

All amino acids determined by metabolomics were increased with aging, independent of the methodology. The butanoic acid increased with maturation (P <0.05) without deferring between the methods (Table 6). This amino acid and the lactic acid are positively related to aging time (Ercolini et al., 2011) and rancid odor (Stetzer, Cadwallader, Singh, Mckeith, & Brewer, 2008), so they are rejected by consumers who are not familiar with this type of smell.

Similarly, Methionine was altered with maturation (P <0.05), increasing 15 times in Dry-aging and 18 in Wet-aging in relation to WA treatment. This compound as an important contributor to the aroma of cooked meat, as was indicated by Ercolini et al. (2011); however, also their degradation generates bitter compounds. In other experiments

been related to the sweet taste and as precursor of some aromatic compounds (Brewer, 2007), hence, aged meat has a strong smell.

According Nishimura, T.; Rhue, M.R.; Okitani, A.; Kato (1988), the amino acids valine, isoleucine and leucine are related to the bitter taste; while alanine and methionine with the sweet taste. In the present study, these amino acids were increased in aged meat from both treatments, what would improve the taste.

In relation to sugars, four had a higher concentration in un-aged meat (Glucose, Glucose-6-phosphate, IMP and D-Ribulose-5-phosphate, being that Glucose varied among all the treatments); while of the remaining metabolites, only four were different among the maturation treatments (Melanic acid, Methionine and Putrescine more abundant in the aged meat in bags and Saccharopine in Dry-aging meat); nonetheless the metabolic profile of aged meat was similar.

As expected, the concentration of Glucose, Glucose-6-phosphate and D-Ribulose-5-phosphate was very low in the aged samples (Table 6). Post-mortem glycolysis has the potential to release sugars that participate in Maillard reactions during the cooking of the meat, provoking volatile flavorings like meaty-caramel (Kosowska, Majcher, & Fortuna, 2017; Koutsidis et al., 2008). In other species, these compounds have been used to improve the taste and aroma of meat (Meinert, Schäfer, Bjergegaard, Aaslyng, & Bredie, 2009), although they can also favor the deterioration of the meat because provide nutrients for bacterial growth (Ercolini et al., 2011).

The concentration in the meat of this sugar can be influenced, among other factors, by the type of diet, genetic group and factors prior to slaughter, such as transportation, management and fasting time (Komatsu, Shoji, Saito, & Suzuki, 2014), since grazing-finished cattle present a lower concentration of muscular glycogen in relation to those finished in feedlot (Pethick et al., 1995; Vestergaard, Oksbjerg, & Henckel, 2000).

In addition, *Bos taurus* cattle have a greater capacity to recover glycogen released in stress situations, such as during pre-slaughter, in relation to *Bos indicus* (Apaoblaza & Gallo, 2014; Rodrigues et al., 2017), and zebu animals have less glycogen storage capacity in muscle because they are temperamental (Lobato et al., 2014).

Table 6. Meat metabolites different between samples without aging (WA) or ageing by Dry-ageing (Dry) and Wet-ageing (Wet) for 14 days.

Metabolite (arbitrary units)	Tratament			SE	FDR
	WA	Dry	Wet		
		Amino acids			
Alanine	18803 b	55196 a	51402 a	2460	8.71E-10
β-Alanine	3520 b	7526 a	9893 a	1015	7.45E-08
Butanoic acid[1]	10582 b	14484 a	14708 a	323	1.14E-07
Oxobutanoic acid[2]	163 b	235 ab	274 a	20	1.58E-07
Citrulline	301 b	968 a	1252 a	87	3.66E-07
Asparagine	88 b	221 a	213 a	15	0.00179
Aspartate	1025 b	5231 a	4973 a	488	0.00306
Isoleucine	32169 b	124003 a	123384 a	8495	0.00331
Leucine	52109 b	269238 a	264740 a	19567	0.00516
Methionine	1147 c	16706 b	20409 a	8791	0.0088
Saccharopine	129 a	138 a	102 b	9	0.02304
Valine	65665 b	183903 a	182333 a	11256	0.04314
		Sugars			
Phosphogluconic acid[3]	67155 b	43997 a	42855 a	6034	7.98E-07
Glucose	24068 a	0 c	5890 b	4281	1.14E-06
Glucose-6-phosphate	30368 a	20151 b	18236 b	2281	3.08E-06
D-Glucurono-3,6-lactone	64 b	1090 a	1247 a	362	7.03E-06
Acetylmannosamine[4]	73 b	112 a	114 a	6	0.00753
IMP	743 a	485 b	280 b	281	0.01433
D-Ribulose 5-phosphate	81 a	59 b	69 ab	5	0.0216

[1] = 4-amino-Butanoic acid; [2] = 4-methylthio-2-oxobutanoic acid; [3] = 6-Phosphogluconic acid; [4] = N-Acetylmannosamine
FDR = False discovery rate.
Means with a different superscript within a row are different ($P < 0.05$, FDR).

Table 6. Continuation

Metabolite (arbitrary units)	Tratament			SE	FDR
	WA	Dry	Wet		
		Phenolic compounds			
Melanic acid	75 b	82 b	143 a	14	0.00822
Homovanilic acid	109 a	81 b	91 b	6	0.00044
		Amines			
Putrescine	312 c	719 b	934 a	42	0.01445
3-methoxytyramine	129 b	649 a	726 a	99	0.03029
		Lipids			
Myristic acid	24309 b	32933 a	31184 a	1549	8.71E-10
		Vitamins			
Pyridoxamine	73	-	-	21	0.01983
		Pyrimidines			
Uracil	334 b	429 a	444 a	17	0.03581

FDR = False discovery rate.
Means with a different superscript within a row are different ($P < 0.05$, FDR).

Also, IMP was reduced in aged meat in relation to those without maturation ($P < 0.05$), being in accordance with previous works in aging meat for 14 d (Ercolini et al., 2011; Graham et al., 2012; Nishimura, T.; Rhue, M.R.; Okitani, A.; Kato, 1988). On the other hand, in aged meat for 21 days, a higher concentration of IMP was reported (Kim et al., 2016; Koutsidis et al., 2008).

The concentration of IMP is related to the "umami" flavor, described as savory, brothy or beefy (Brewer, 2007), making him an important contributor to the taste of meat (Ercolini et al., 2011). However, when its relevance in meat prepared in soup was

analyzed, it did not affect taste improvement (Nishimura, T.; Rhue, M.R.; Okitani, A.; Kato, 1988).

In turn, the putrescine is the principal biogenic amine present in meat, produced by the decarboxylation of free amino acids and therefore, increases with storage time (Ruiz-Capillas & Jiménez-Colmenero, 2005); however, there are reports where the concentration remained constant over 60 days of maturation in Wet-aging (Marquezini, Orlando, Yotsuyanagi, & Bromberg, 2016); but it can be an indicator of the deterioration of the meat, mainly by accumulation of lactic bacteria (Ruiz-Capillas & Jiménez-Colmenero, 2005). In the present work (Table 6), the concentration of this metabolite was higher in Wet meat, followed by Dry treatment and lower in un-aged meat ($P < 0.05$), indicating a better preservation of the product when it is Dry-aging for 14 days.

As can be seen, the concentration of amino acids increases with aging (Table 6). Khan, Jo, & Tariq (2015), indicate that enzymatic degradation releases precursors that have a positive impact on the taste of meat through Strecker degradation and Maillard's reaction.

So, in aging meat there is a mixture of released or transformed compounds (Meinert et al., 2009), that causing flavor intensification (Khan et al., 2015), since the raw meat has a slight taste, but is a store of intensifying compounds of the same (Kosowska et al., 2017).

Based on the above, we can infer that the products generated by the various treatments of aging of the meat do not present a standard of production, being influenced by several factors such as the production system, race, age and aging conditions, which would cause a wide range of sensory characteristics related to the metabolites. However, in the current study, dry aged pieces would present a longer shelf life compared to the aged to humid, due to lower production of amines related to bacterial load.

4. Conclusion

The ageing for 14 d of meat from Nellore cull cows from the pasture system is a viable option to improve the physicochemical characteristics of the meat, especially the shear force and the metabolites related to the taste, there being no difference between the two methods analyzed (Dry versus Wet). Aging by the dry method has the disadvantage of reducing salable yields, so it is necessary to increase the selling price, in addition to studying the consumer acceptability for this type of meat. Wet aging has a higher concentration of biogenic amines (e.g. Putrescine), which would reduce the shelf life of aging meat by this methodology in relation to aged to dry.

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