











# Effect of castration and days on feed on body composition, glucose tolerance, and muscle gene expression in Nellore cattle

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Received: January 2, 2025

Accepted: April 10, 2025

**How to cite:** Silva, L. H. P.; Ramírez-Zamudio, G. D.; Silva, W.; Assis, D. E. F.; Assis, G. J. F.; Miguel Estrada, M.; Guimarães, S. E. F.; Duarte, M. S.; Dahlen, C. R. and Chizzotti, M. L. 2025. Effect of castration and days on feed on body composition, glucose tolerance, and muscle gene expression in Nellore cattle. *Revista Brasileira de Zootecnia* 54:e20250001.  
<https://doi.org/10.37496/rbz5420250001>

## Editors:

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**ABSTRACT** - The objective of this study was to evaluate the effect of castration and days of feed (DOF) on glucose sensitivity and muscle expression of genes related to glucose metabolism, muscle growth, and fat deposition. Thirty-six Nellore bull calves were either surgically castrated or left intact, then harvested at 0, 100, or 200 DOF. Glucose tolerance tests were performed at 100 and 200 DOF. *Longissimus dorsi* (LD) samples were taken just after stunning for gene expression. Data were analyzed as a complete randomized design following a two (sexual condition) × three (DOF) factorial arrangement of treatments. An interaction effect was found for carcass fat gain ( $P = 0.01$ ), showing that steers gained more fat than bulls from 100 to 200 DOF, but both sexual classes had a similar fat gain from 0 to 100 DOF. Basal blood glucose and the area under the curve post-infusion were not affected by castration or DOF ( $P > 0.05$ ). Castration tended ( $P = 0.08$ ) to upregulate LD expression of *Acetyl-CoA carboxylase alpha* (*ACACA*). Interaction effects ( $P < 0.05$ ) were found for LD expression of *IGF-1 receptor* (*IGF1R*) and *F-box protein 32* (*FBXO32*) with steers having greater relative abundance for both genes at 100 DOF, while bulls had greater relative abundance at 200 DOF. Despite the greater carcass fattening enhancement by castration and longer DOF, whole-body sensitivity to glucose does not change.

**Keywords:** carcass composition, castration, cattle, glucose tolerance test

## 1. Introduction

Castration is widely applied in beef production, mainly due to the marbling enhancement, improving carcass value, and aggressiveness reduction (Jeong et al., 2013; Dahlen et al., 2014). It is well established that castration affects growth performance and body composition, and steers present greater carcass fatness but a reduced growth rate than bulls (Seideman et al., 1982; Bretschneider, 2005). Nonetheless, the difference between bulls and steers varies with the length of the feeding period since

gain composition shifts to less lean and more fat after castration (Marcondes et al., 2012). Similarly, muscle growth decreases while adipose tissue increases with the extension of the feeding period (Marcondes et al., 2012). Thus, bulls usually require more days on feed (DOF) to reach the desired fat deposition (Moreira et al., 2017). Therefore, castration and DOF may interact affecting cattle body composition.

Body composition variations impact insulin sensitivity and glucose metabolism, which has been investigated mainly through glucose tolerance tests (GTT) to evaluate the whole-body sensitivity to a high exogenous glucose dose (González-Grajales et al., 2017). For instance, high-muscled Angus steers (myostatin mutation) exhibited greater glucose clearance after an intravenous GTT compared with low-muscled steers (McGilchrist et al., 2011). Similarly, heavier beef steers show lower insulin sensitivity, likely due to higher body fat (Eisemann et al., 1997). Regarding DOF, Radunz et al. (2012) observed that beef cattle subjected to a GTT at 41 DOF showed a faster blood glucose clearance rate than those evaluated at 111 DOF. In sheep, increasing body fat has been related to decreasing insulin responsiveness (Bergman et al., 1989). These results indicate that body insulin sensitivity and consequently body glucose uptake decrease as body fat percentage increases.

Although the GTT assesses whole-body glucose uptake, the muscle tissue has a high glucose uptake capacity, accounting for around 80% of the exogenous dose in mice and humans (Xia et al., 2013), and muscle glucose sensitiveness is mainly controlled by the amount of insulin-sensitive glucose transporter (GLUT4) at the sarcolemma (Fitzsimons et al., 2014). Thus, the lower glucose clearance in fatter cattle may result from decreased muscle GLUT4, leaving more glucose available for other tissues, potentially increasing marbling, as intramuscular fat (IMF) synthesis in cattle primarily uses glucose (Smith et al., 2009).

We hypothesized that steers with extended DOF would exhibit greater body fat and lower glucose clearance during a GTT. Therefore, the objective of this study was to evaluate the effects of castration and time on feed on cattle performance, glucose sensitivity, and muscle gene expression related to glucose metabolism, muscle growth, and fat deposition.

## 2. Material and methods

This experiment was conducted at the experimental feedlot of the Animal Laboratory of the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, MG, Brazil. All the experimental procedures were reviewed and approved by the Institutional Animal Care and Ethics Committee for Use of the Universidade Federal de Viçosa (CEUAP-UFV, protocol #035/2015).

### 2.1. Animals, experimental design, and feed management

Thirty-six Nellore bull calves averaging  $256.1 \pm 3.05$  kg body weight (BW) and  $8.2 \pm 0.07$  months old were used. Bulls were randomly assigned to one of two treatments before weaning: surgical castration ( $n = 18$ ; steers), or remained with testicles intact ( $n = 18$ ; bulls). The castration surgery was performed one week before weaning by a veterinarian with bulls restrained in a commercial squeeze chute. The castration site was cleaned with neutral soap and 2% iodine solution prior to surgery. One new scalpel blade was used per animal, and after the surgery, silver sulfadiazine and zinc oxide were placed on the surgery site, and oxytetracycline hydrochloride was intramuscularly administered (10 mg/kg of BW). The health of steers was checked daily for three weeks post-surgery, and silver sulfadiazine and zinc oxide were applied topically until complete healing.

At weaning, calves were ear-tagged, dewormed, and penned in two groups, according to sexual condition (bulls or steers). Calves were gradually adapted to the diet over four weeks. Six calves from each sexual condition were randomly assigned to be harvested after 0 (right after adaptation), 100, or 200 DOF. Therefore, this study was carried out as a complete randomized design in a  $2 \times 3$  factorial arrangement of treatments with main factors of sexual condition (steer or bull) and DOF (0, 100, or 200).

Throughout the experiment, both groups (bulls and steers) were kept under the same experimental conditions and received the same diet (Table 1). Fresh total mixed ration was provided *ad libitum* twice a day, with 60% of the daily total delivered at 07:00 h and the remaining 40% at 14:00 h. The diet was formulated by averaging the nutrient requirements for bulls and steers gaining 1.4 kg/d of body weight, according to the BR-CORTE system (Valadares Filho et al., 2010). Each feedlot pen was equipped with three electronic feed bunk systems (AF 1000 Master; Intergado LTDA, Contagem, MG, Brazil) allowing to record individually the daily intake of feed.

**Table 1 - Ingredient and chemical composition of experimental diet**

Ingredient	% DM
Corn silage	40.68
Ground corn	44.37
Soybean meal	9.97
Urea	0.96
Ammonium sulfate	0.11
Sodium bicarbonate	1.50
Magnesium oxide	0.50
Mineral premix <sup>1</sup>	1.91
Chemical composition	
Dry matter (% of fresh matter)	47.1
Crude protein	13.8
Neutral detergent fiber	28.2
Digestible energy (MJ/kg of DM)	13.9

<sup>1</sup> Providing per kg: 120 g of Ca, 87 g of P, 12 g of S, 198 g of Na, 625 mg of Cu, 45 mg of I, 50 mg of Co, 1000 mg of Mn, and 7.5 mg of Se.

## 2.2. Cattle performance

Average daily gain (ADG) was calculated using cattle BW after 16 h of fasting, obtaining shrunk body weight (SBW). Body weight was recorded after the initial adaptation period (0 DOF) and at 100 and 200 DOF. Therefore, growth performance was calculated from 0 to 100 DOF and from 100 to 200 DOF.

Concentrate feedstuffs were sampled every week, while corn silage was sampled daily and pooled by week. The pooled corn silage samples were weighed, oven-dried at 55 °C for 72 h, and then weighed again to quantify the moisture loss. The ground corn, soybean meal, and dried corn silage samples were ground through a 1-mm screen using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA, USA). All ground feedstuffs were analyzed for DM content (method 934.01; AOAC, 2005). Therefore, DM intake (DMI) was obtained as the individual daily intake (as fed) obtained from the electronic feed bunk system multiplied by diet DM content. The relative DMI (%BW/d) was estimated using the average BW during the evaluated period. Furthermore, the gain to feed ratio (G:F) was calculated by dividing ADG by DMI.

## 2.3. Glucose tolerance test

The glucose tolerance tests (GTT) were performed one week prior to the harvest at 100 DOF, and one week before the harvest at 200 DOF. Due to facility limitations and to minimize variations within animals, these two GTT were performed using only the six bulls and the six steers selected to be harvested at 200 DOF. As the facility had two squeeze chutes, to minimize the difference in fasting before the GTT, cattle were divided into two groups of six (three bulls and three steers) to be subjected to GTT in two consecutive days. The animal sequence to GTT was balanced according to sexual condition, allowing simultaneous submission of one bull and one steer to GTT, with the same number of bulls and steers at each squeeze chute.

Cattle were weighed after 14 h of fasting to calculate the individual intravenous glucose dose of 200 mg/kg of BW. A sterile physiological saline solution with 50% glucose (Isofarma Industrial Farmacêutica Ltda, Eusébio, CE, Brazil) was used for this evaluation. The animal was carefully held and restrained in a squeeze chute, then a catheter was fitted into the right jugular vein. Blood samples to determine glucose basal level concentration were obtained from the catheter at -5 and 0 min relative to intravenous infusion. Then the glucose dose was injected into the left jugular vein, within 2 min. Blood samples were obtained from the catheter at 5, 10, 15, 20, 30, 45, 60, and 120 min post-infusion. The whole blood glucose concentration was determined in real-time using a glucometer (Accucheck performa nano, Roche Diagnostics GmbH, Mannheim, Germany). A natural logarithmic function was individually adjusted for blood glucose concentration post-infusion, and then the area under the curve (AUC) was obtained by integrating the function from 5 to 120 min post-infusion.

#### 2.4. Slaughter, *longissimus* muscle sampling, and carcass composition

Six bulls and six steers were harvested at 0, 100, and 200 DOF, totaling three slaughters of 12 cattle each. Before slaughter, the selected group was fasted for 16 h with free access to water. Due to the facility limitations, a balanced group of six cattle (three bulls and three steers) were harvested in each of two consecutive days. Procedures during the harvest followed humane slaughter practices, as described by the Brazilian standards of sanitary regulation for animal products (Brasil, 1997). Just after stunning (captive bolt) and bleeding, LD muscle samples were obtained from the right side of the carcass by making an incision through the hide at the 13th rib. The LD samples were trimmed of epimysium and subcutaneous fat, minced, and then snapped frozen in liquid nitrogen. Minced LD samples were ground using a mortar with liquid nitrogen, placed into labeled cryovial, and then stored at -80°C until RNA extraction.

After dressing, the empty body weight (EBW) was estimated by individually weighing all body components (e.g., blood, head, skin, legs, tail, organs, and hot carcass) including the emptied gastrointestinal tract. The mesenteric fat was physically separated from the gastrointestinal tract and weighed together with the kidney, pelvic, and heart fat (KPH) to yield total visceral fat (VF). Hot carcasses were weighed (HCW), suspended by the aitch bone (tenderstretch method), and chilled for 24 h at 2 °C. Afterward, the 9-11th rib section was removed from the left side of the cold carcass for carcass physical and chemical composition estimation (Hankins and Howe, 1946).

The 9-11th rib samples were dissected into lean, fat, and bone to estimate carcass physical composition according to Marcondes et al. (2012). The samples of lean, fat, and bone were freeze-dried and ground for analyses of crude protein (CP; method 984.13; AOAC, 2005), DM (method 934.01; AOAC, 2005), and ether extract (EE) in a XT15 extractor (Ankom, Macedon, NY, USA). Carcass chemical composition was estimated by equations suggested by Marcondes et al. (2012). To estimate carcass gain (CG), cattle harvested at 0 DOF were used as a reference group to assess initial carcass composition from 0 to 100 DOF, while cattle harvested at 100 DOF were a reference group to the period from 100 to 200 DOF. The gains of carcass physical and chemical components were then calculated from 0 to 100 and from 100 to 200 DOF. In addition, the carcass protein fractional accretion rate (FAR) was calculated by dividing the carcass protein gain by the average protein pool of the period.

#### 2.5. RNA extraction, cDNA synthesis, and RT-qPCR analysis

Fifty milligrams of powdered whole LD muscle were used for total RNA extraction with Trizol reagent (Invitrogen, Carlsbad, CA). To remove DNA contamination, total RNA samples were then treated with DNase I, Amplification Grade (Invitrogen, Carlsbad, CA, USA). The spectrophotometer NanoVue Plus (GE Healthcare, Freiburg, Germany) was used to estimate RNA concentration at 260 nm, and RNA quality was verified by the 260:280 nm ratio. Finally, RNA integrity was checked through the presence of 18s and 28s bands in 1% agarose gel electrophoresis. The cDNA synthesis was performed using the GoScript Reverse Transcriptase kit (Promega, Madison, WI, USA). Samples were stored at -20 °C until analysis.

The primers were designed by the web tool Primer-BLAST ([www.ncbi.nlm.nih.gov/tools/primer-blast](http://www.ncbi.nlm.nih.gov/tools/primer-blast)) using the *Bos taurus* reference sequences from GenBank database. The primer sequences of the 14 target genes and the 18S rRNA endogenous control are shown in Table 2. The real-time quantitative PCR was performed using a 7300 Real-Time PCR unit (Applied Biosystems, Carlsbad, CA, USA) and SYBR Green RT-PCR GoTaq Master Mix (Promega, Madison, WI, USA) following the cycle parameters: 95 °C for 3 min and 40 cycles at 95 °C for 10 s and 60 °C for 30 s. The cycle threshold (Ct) values were recorded for target and endogenous genes. Gene expression data were analyzed as proposed by Steibel et al. (2009), which consists of a linear mixed model that uses target and endogenous Ct to compute the relative quantification real-time PCR. The effects of castration, DOF, and their interaction were included as fixed effects, while the animal was included as a random effect. Gene expression is reported as fold-change calculated by the  $2^{-\Delta\Delta Ct}$  method (Livak and Schmittgen, 2001).

**Table 2 - Primer sequences of genes analyzed by qPCR**

Gene	Abbreviation	Accession code	Forward (F) and reverse (R) sequences
<i>Glycogen synthase kinase 3 beta</i>	<i>GSK3B</i>	NM_001101310.1	F: TACCAAATGGGCGAGACAC R: CCGAGCATGAGGAGGAATAAG
<i>Glycogen phosphorylase</i>	<i>PYGM</i>	NM_175786.2	F: CCCAAACAGCCTGACCTATT R: TCCACTCTCTCGGGTTCTT
<i>Phosphoglucomutase 1</i>	<i>PGM1</i>	NM_001076903.1	F: CACTGGAAGATGGAGGTTGAG R: AAGGCTCACCAAGGAGAAAG
<i>Insulin-regulated glucose transporter</i>	<i>GLUT4</i>	AY458600.1	F: CCTTGGTCCTTGCCGTATT R: CCAGCCAGTCTCATTGTAG
<i>Peroxisome proliferator activated-receptor gamma</i>	<i>PPARG</i>	NM_001098905.1	F: TGGAGACCGCCAGGTTTGC R: AGCTGGGAGGACTCGGGGTG
<i>Fatty acid transporter, member 1</i>	<i>SLC27A1</i>	NM_001033625.2	F: CTCGTGTCTGTGTCTGTGTC R: GGAGGAGAGATGGAGGAAGA
<i>Lipoprotein lipase</i>	<i>LPL</i>	NM_001075120.1	F: CCTGAAGACTCGTCTCAGATG R: AAGCCTGGTTGGTGTATG
<i>Acetyl-CoA carboxylase alpha</i>	<i>ACACA</i>	NM_174224.2	F: GAAGTGATGGGCTGCTTCT R: GGGACCTTGTCTTCGTCATAC
<i>Lipase, hormone-sensitive</i>	<i>LIPE</i>	NM_001080220.1	F: GAGGGTGATGAGAGGTAATTG R: AGGTGTGAAC TGAAACCC
<i>Myostatin</i>	<i>MSTN</i>	AF019620.1	F: CCACGGAGTCTGATCTTCTAAC R: TCCACAGTTGGGCTTTTAC
<i>Insulin like growth factor 1 receptor</i>	<i>IGF1R</i>	NM_001244612.1	F: CTCAACCCAGGGAACACTACAC R: GTCTTGGCCTGAACGTAGAA
<i>Caspase 3</i>	<i>CASP3</i>	NM_001077840.1	F: CGTCCCTTCTGCCATCC R: CAGACCATTAGGCCACACTC
<i>Serpin A3-6</i>	<i>SERPINA3-6</i>	NM_001146302.1	F: CTGGGCTGGTTCTGGTAAA R: TGACTGCTGTGCCATCTT
<i>F-box protein 32 (Atrogin-1)</i>	<i>FBXO32</i>	NM_001046155.1	F: CCCAGAGAGCTGTCCATTT R: CTCTGGATTCCCAACCATCC
<i>18 S ribosomal</i>	<i>18S</i>	DQ222453.1	F: CCTGCGGCTTAATTTGACTC R: AACTAAGAACGGCCATGCAC

## 2.6. Statistical analysis

Data were analyzed as a completely randomized design in a  $2 \times 3$  factorial arrangement of treatments using the MIXED procedure of SAS (Statistical Analysis System, version 9.4) according to the following statistical model:

$$Y_{ijk} = \mu + C_i + \text{DOF}_j + C \times \text{DOF}_{ij} + \beta \text{iBW}_{ijk} + e_{ijk}$$

in which  $Y_{ijk}$  is the dependent variable,  $C_i$  is the fixed effect of castration ( $i = \text{bull or steer}$ ),  $\text{DOF}_j$  is the fixed effect of DOF ( $j = 0, 100, \text{ or } 200$ ),  $C \times \text{DOF}_{ij}$  is the fixed effect of the interaction between  $i$ -th castration treatment and  $j$ -th DOF,  $\beta$  is the regression coefficient for the covariate initial body weight (iBW) of the  $k$ -th animal, and  $e_{ijk}$  is the residual error. The initial BW was included as a covariate in the model and then kept where  $P \leq 0.10$ . The GTT data were analyzed as a repeated measurement since both trials were performed using the same group of cattle. When ANOVA pointed out a significant ( $P \leq 0.05$ ) effect for DOF or castration by DOF interaction, treatment least squared means were compared using the PDIFF option adjusted with Turkey-Kramer.

### 3. Results

#### 3.1. Cattle performance

The final shrunk body weight for both sexual conditions at 0, 100, and 200 DOF averaged 276.4, 389.3, and 488.6 kg, respectively (Table 3). Regardless of DOF, bulls had greater SBW ( $P = 0.02$ ) and EBW ( $P = 0.03$ ) and tended to have greater HCW ( $P = 0.053$ ) than steers. An interaction was observed for VF ( $P < 0.01$ ), with no difference present at 0 and 100 DOF, but steers had a greater percentage of VF than bulls after 200 DOF. Castration did not affect DMI ( $P = 0.42$ ), but steers had greater DMI than bulls when expressed as a percentage of BW ( $P = 0.01$ ). Although DMI was greater from 100 to 200 DOF than from 0 to 100 DOF ( $P = 0.047$ ), the DMI as a percentage of BW markedly decreased ( $P < 0.01$ ) as DOF increased. Neither ADG nor CG were affected by castration ( $P > 0.05$ ). Increasing DOF decreased ADG ( $P = 0.03$ ), but CG was not affected ( $P = 0.17$ ). Bulls tended to have a greater G:F ratio than steers ( $P = 0.08$ ). Feed efficiency was negatively affected by increasing DOF ( $P < 0.01$ ).

**Table 3 - Growth performance of Nellore bulls and steers harvested at 0, 100, and 200 days on feed (DOF)**

Item	Treatment						SEM	P-value <sup>4</sup>		
	0 DOF		100 DOF		200 DOF			C	DOF	C×DOF
	Bull	Steer	Bull	Steer	Bull	Steer				
SBW (kg)	279	273	398	380	510	467	11.5	0.022	<0.01	0.251
EBW (kg)	258	252	371	353	476	438	11.6	0.033	<0.01	0.346
HCW (kg)	159	156	237	230	313	287	7.81	0.053	<0.01	0.259
VF (%EBW)	3.31c	3.06c	5.50b	5.08b	5.38b	7.82a	0.37	0.056	<0.01	<0.01
DMI (kg/d)	-	-	6.70	7.31	7.69	7.52	0.27	0.420	0.047	0.160
DMI (%BW/d)	-	-	2.04	2.27	1.68	1.75	0.06	0.015	<0.01	0.177
ADG (g/d)	-	-	1,170	1,077	983	891	80.6	0.266	0.031	0.997
CG <sup>1</sup> (g/d)	-	-	736	701	696	592	51.3	0.183	0.171	0.494
G:F (kg/kg)	-	-	0.173	0.143	0.130	0.121	0.01	0.080	<0.01	0.344
Glucose <sup>2</sup> (mg/dL)	-	-	92.6	94.4	87.1	91.5	3.92	0.449	0.310	0.753
Glucose AUC <sup>3</sup>	-	-	16,997	17,287	16,266	17,000	955	0.599	0.607	0.817

SBW - shrunk body weight; EBW - empty body weight; HCW - hot carcass weight; VF - visceral fat.

<sup>1</sup> CG - carcass gain. Cattle harvested at 0 and 100 DOF were used to estimate initial carcass weight to harvest at 100 and 200 DOF, respectively.

<sup>2</sup> Baseline whole blood glucose concentration.

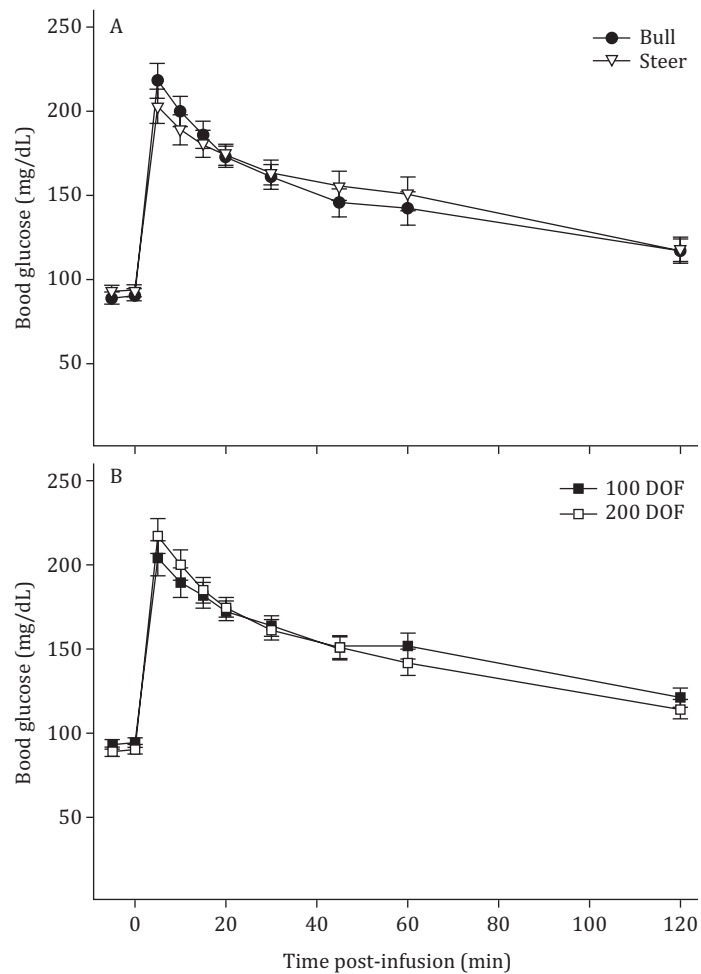
<sup>3</sup> Area under the curve post intravenous glucose infusion (200 mg/kg of BW).

<sup>4</sup> C - main effects of castration; DOF - main effect of days on feed; C×DOF - castration by DOF interaction effect.

a,b,c - Least square means with different letters differ by Tukey's test ( $P < 0.05$ ).

#### 3.2. Glucose tolerance test

Neither glucose baseline blood concentration nor glucose AUC response was affected by castration (Table 3 and Figure 1A) or DOF (Figure 1B;  $P > 0.05$ ).



The error bar represents SEM.

The blood glucose was not affected by castration ( $P = 0.78$ ), DOF ( $P = 0.96$ ), or castration by DOF interaction ( $P = 0.52$ ).

**Figure 1** - Whole blood glucose concentration prior (-5, and 0 min) and post intravenous glucose infusion (200 mg/kg of BW) of Nellore bulls and steers (A) at 100 and 200 days on feed (DOF; B).

### 3.3. Carcass composition

Interactive effects of sexual condition and DOF were found ( $P < 0.05$ ) for carcass bone, carcass EE, fat gain, and EE gain (Table 4). Increasing cattle DOF improved ( $P < 0.01$ ) the weight of carcass lean, carcass fat, and lean:bone ratio. The quantity of carcass protein and water increased ( $P < 0.01$ ) with increasing DOF. The gain of carcass water ( $P < 0.01$ ) decreased from 100 to 200 DOF, but carcass gain of the other physical and chemical components was not impacted ( $P > 0.05$ ) by DOF.

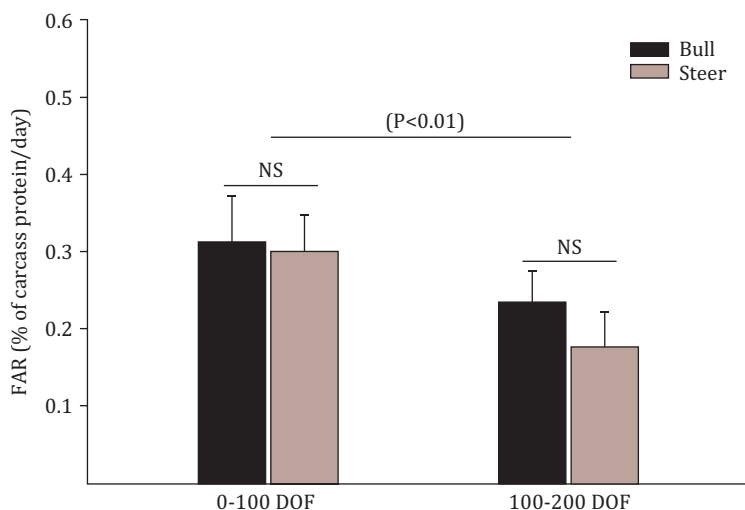
Regardless of time on feed at harvest, bulls had greater lean quantity than steers ( $P = 0.02$ ), but lean daily gain from 100 to 200 DOF did not differ between the sexual conditions (Table 4;  $P = 0.15$ ). Castration did not affect the carcass fat amount ( $P = 0.29$ ). Bulls had greater carcass bone gain than steers ( $P = 0.02$ ). Castration negatively affected ( $P < 0.05$ ) the carcass protein and water. Likewise, castration tended to decrease carcass gain of protein ( $P = 0.07$ ) and water ( $P = 0.07$ ). Castration did not affect the carcass protein fractional accretion rate ( $P > 0.05$ ), but it decreased ( $P < 0.01$ ) as DOF increased (Figure 2).

**Table 4** - Carcass physical and chemical composition of Nellore bulls and steers harvested at 0, 100, and 200 days on feed (DOF)

Item <sup>1</sup>	Treatment						SEM	P-value <sup>2</sup>		
	0 DOF		100 DOF		200 DOF			C	DOF	C×DOF
	Bull	Steer	Bull	Steer	Bull	Steer				
Carcass physical composition										
Lean (kg)	106	103	151	147	201	178	5.04	0.021	<0.01	0.090
Bone (kg)	34.2a	32.8a	42.7b	40.2b	54.6a	44.2b	1.55	0.001	<0.01	<0.01
Fat (kg)	15.7	16.9	39.2	38.3	53.2	59.2	2.45	0.293	<0.01	0.311
Lean:bone (kg/kg)	3.10	3.14	3.55	3.64	3.69	4.05	0.10	0.053	<0.01	0.219
Lean gain (g/d)	-	-	442	455	463	328	40.9	0.153	0.205	0.085
Bone gain (g/d)	-	-	88.2	77.4	111.5	50.8	14.6	0.024	0.908	0.103
Fat gain (g/d)	-	-	209a	190a	120b	217a	21.1	0.073	0.162	0.011
Carcass chemical composition										
Protein (kg)	28.9	28.8	42.6	40.1	55.2	48.2	1.76	0.013	<0.01	0.072
Ether extract (kg)	20.6d	18.7d	43.6c	41.6c	58.7b	67.8a	2.99	0.389	<0.01	0.039
Water (kg)	94.2	94.0	133.4	130.4	171.2	154.2	4.85	0.049	<0.01	0.088
Protein gain (g/d)	-	-	121	109	114	83.3	11.2	0.066	0.179	0.412
Ether extract (g/d)	-	-	205b	223ab	124c	267a	20.3	0.001	0.376	<0.01
Water gain (g/d)	-	-	382	372	342	242	28.8	0.071	<0.01	0.132

<sup>1</sup> Cattle harvested at 0 and 100 DOF were used to estimate initial carcass composition to harvest at 100 and 200 DOF, respectively.

<sup>2</sup> C - main effects of castration; DOF - main effect of days on feed; C×DOF - castration by DOF interaction effect.  
a,b,c - Least square means with different letters differ by Tukey's test (P<0.05).



The error bar represents SEM.

The protein FAR was not significantly (NS) affected by castration (P = 0.19). Castration by DOF interaction was not significant (P = 0.71).

**Figure 2** - Carcass protein fractional accretion rate (FAR) of Nellore bulls and steers from 0 to 100 days on feed (DOF), and from 100 to 200 DOF.

### 3.4. *Longissimus* muscle gene expression

Interaction effects were observed ( $P < 0.05$ ) for mRNA abundance of *IGF1R* and *FBXO32*, showing that steers had greater expression for both genes on 100 DOF compared with bulls, but no differences were observed between sexes at the other time points evaluated (Table 5). Sexual condition did not affect ( $P > 0.05$ ) LD gene expression, except for *ACACA*, which tended to increase in steers LD compared with bulls ( $P = 0.08$ ). Time on feed did not affect ( $P > 0.05$ ) LD gene expression, except for *SERPINA3-6*, which tended to be downregulated in the LD mRNA abundance as DOF increased ( $P = 0.08$ ).

**Table 5** - Normalized gene expression in the *longissimus dorsi* muscle of Nelore bulls and steers harvested at 0, 100, and 200 days on feed (DOF)

Gene	Treatment						SEM	P-value <sup>1</sup>		
	0 DOF		100 DOF		200 DOF			C	DOF	C×DOF
	Bull	Steer	Bull	Steer	Bull	Steer				
Glucose/Glycogen metabolism										
<i>GSK3B</i>	1.00	1.83	0.97	1.94	1.80	1.20	0.50	0.148	0.941	0.158
<i>PYGM</i>	1.00	1.12	0.71	1.24	1.01	0.66	0.48	0.686	0.549	0.354
<i>PGM1</i>	1.00	1.41	0.64	1.18	1.36	0.88	0.65	0.507	0.610	0.510
<i>GLUT4</i>	1.00	1.06	0.72	0.86	0.95	0.51	0.49	0.512	0.262	0.291
Adipogenesis and lipid metabolism										
<i>PPARG</i>	1.00	1.26	0.61	1.24	0.97	0.64	0.55	0.430	0.394	0.243
<i>SLC27a1</i>	1.00	0.87	0.82	1.26	1.24	0.67	0.51	0.596	0.903	0.450
<i>LPL</i>	1.00	1.60	1.01	1.71	1.67	0.92	0.55	0.542	0.978	0.338
<i>LIPE</i>	1.00	1.56	1.13	1.77	2.29	1.41	0.53	0.518	0.374	0.263
<i>ACACA</i>	1.00	1.71	0.81	1.27	0.89	1.03	0.53	0.086	0.458	0.394
Muscle protein turnover										
<i>MSTN</i>	1.00	1.29	1.08	2.78	2.12	1.72	0.77	0.289	0.353	0.356
<i>IGF1R</i>	1.00c	1.71abc	1.31bc	2.73a	2.41ab	1.32bc	0.48	0.252	0.258	0.035
<i>CASP3</i>	1.00	1.06	1.24	1.28	1.53	1.31	0.57	0.923	0.518	0.908
<i>SERPINA3-6</i>	1.00	1.03	0.75	0.62	0.30	0.18	1.34	0.692	0.083	0.364
<i>FBXO32</i>	1.00c	1.87bc	1.73bc	4.01a	3.50ab	2.25ab	0.55	0.126	0.018	0.008

*GSK3B* - glycogen synthase kinase 3 beta; *PYGM* - glycogen phosphorylase; *PGM1* - phosphoglucomutase 1; *GLUT4* - insulin-regulated glucose transporter; *PPARG* - peroxisome proliferator activated-receptor gamma; *SLC27A1* - fatty acid transporter, member 1; *LPL* - lipoprotein lipase; *LIPE* - lipase, hormone-sensitive; *ACACA* - acetyl-CoA carboxylase alpha; *MSTN* - myostatin; *IGF1R* - insulin like growth factor 1 receptor; *CASP3* - caspase 3; *SERPINA3-6* - serpin A3-6; *FBXO32* - F-box protein 32 (Atrogin-1).

<sup>1</sup> C - main effects of castration; DOF - main effect of days on feed; C×DOF - castration by DOF interaction effect.  
a,b,c - Least square means with different letters differ by Tukey's test ( $P < 0.05$ ).

## 4. Discussion

With increasing DOF, the BW of the cattle approached mature weight. Thus, the body composition changes as a result of the decreasing rate of muscle growth and simultaneous acceleration of fat tissue development (Owens et al., 1993). In addition, castration may speed up this body composition change, as steers have reduced mature body weight compared with bulls (Valadares Filho et al., 2016). Therefore, the degree of maturity and castration are two independent factors that can help us better understand the pathways related to economically important traits, such as carcass lean yield and fatness. Hence, in the current experiment, we evaluated the difference between bulls and steers on carcass composition, glucose tolerance, and gene expression in muscle as cattle progressed through the feeding period.

#### 4.1. Cattle performance

The FBW and EBW differences between bulls and steers increased from 6 kg at the start of the feeding period to 18 kg after 100 DOF, and up to 40 kg at 200 DOF. Likewise, the carcass weight difference between the sexual conditions increased from 3 kg up to 26 kg at harvests at 0 and 200 DOF, respectively. These results demonstrate a negative impact of castration on BW and carcass weight, with magnitude of difference increased with increased time on feed.

The weight loss post-surgery and the performance reduction due to the absence of androgens from testicles are the two main reasons for the reduced steers BW (Bretschneider, 2005). Castration-related weight loss is minimal close to birth but increases with age, especially post-puberty (Bretschneider, 2005). Likewise, the performance difference between bulls and steers increases after puberty due to the enhancement of testicular androgen production (Biagini and Lazzaroni, 2011). It has been reported that Nellore bulls reach puberty around 15 months of age (Freneau et al., 2006), with decreased time to puberty with enhanced nutrition (Renaville et al., 2000). Thus, in this study, bulls likely achieved puberty in the interval from 100 to 200 DOF when they reached 13 to 16 months of age, respectively.

The ADG and carcass gain were not observed to be different between sexual conditions. Previous studies reported that the growth rate of bulls is typically 7.5 up to 18.7% greater than for steers (Paulino et al., 2008; Marti et al., 2013). Castration did not affect DMI, corroborating Azevêdo et al. (2016). Nonetheless, bulls tended to have greater feed efficiency (i.e., G:F ratio) than steers, which agrees with previous reports (Seideman et al., 1982; Sales, 2014). Regardless of sexual condition, as cattle BW increased through the feeding period, the DMI increased accordingly. In addition, ADG decreased with increased time of feed, leading to a reduction in G:F ratio as the feeding period progressed. However, the DMI as a percentage of BW markedly decreased as cattle BW increased, indicating a reduction in feed intake capacity. Interestingly, although ADG decreased around 17% from 100 to 200 DOF, CG was reduced by only 10% during the same interval. These results indicate the later maturity of carcass compared with non-carcass components (Owens et al., 1993; Kern et al., 2014).

#### 4.2. Muscle growth

Skeletal muscle represents around 37% of EBW in Nellore bulls (Marcondes et al., 2012), and this tissue accounts for a great proportion of the animals' daily requirement of energy and protein (Goll et al., 2008; Wang et al., 2010). Previous studies have shown that castration negatively impacts muscle growth, as the anabolic effect of endogenous testosterone has direct and indirect effects on muscle hypertrophy (Gentile et al., 2010; Dayton and White, 2014). Furthermore, the rate of muscle accretion decreased with increasing cattle age and BW (Marcondes et al., 2015). The current experiment was designed to evaluate the interactive effects of removing the endogenous main source of testosterone and increasing body weight through the feeding period on muscle growth and gene expression.

Although bulls had only around 3 kg greater lean carcass tissue than steers when harvested at 0 and 100 days of the feedlot, the magnitude of difference increased to 23 kg by 200 DOF. Regarding the carcass lean gain, both sexual conditions had similar muscle growth from day 0 to 100 on feed with an average of 448 g/d, whereas bulls maintained the lean growth rate (463 g/d) and steers tended to decrease (328 g/d) muscle accretion from day 100 to 200 on feed. In addition, carcass protein gain (mainly derived from muscle), tended to decrease following castration. These results indicate that castration decreased skeletal muscle growth as cattle BW increased. Similar results have been found in previous studies, in which muscle mass and metabolic characteristics did not differ between bulls and steers at five months post-surgery but differed at 13 months post-surgery (Picard et al., 1995). However, in the current experiment, the fractional accretion rate of carcass protein decreased as cattle became heavier, regardless of sexual condition, indicating that the difference between protein synthesis and breakdown decreases as cattle reach the end of the finishing period.

Myostatin has been reported as a biomarker for muscle mass, with increased muscle expression of *MSTN* being associated with muscle growth inhibition (Bouley et al., 2005). It has been proposed

that muscle upregulation of *MSTN* and *PPARG* are related to the reduction of muscle growth rate and accelerated fat deposition as the animal reaches the mature BW (Kern et al., 2014). However, in the current study, even though castration slightly decreased muscle growth and increased carcass adiposity in cattle that were fed for 200 days, muscle expression of *MSTN* and *PPARG* did not differ among treatments. Furthermore, muscle expression of *GSK3B* has been negatively related to muscle growth (Busato et al., 2016), whereas neither castration nor time on feed affected muscle mRNA abundance of *GSK3B*.

The GH/IGF1 axis has a key role in the regulation of skeletal muscle mass by stimulating protein synthesis (Velloso, 2008). The interactive effect observed for muscle expression of *IGF1R* pointed out that while this gene was upregulated as Nellore bulls increased the BW throughout the feeding period (from 0 to 200 DOF), in steers, the muscle *IGF1R* expression increased from day 0 to 100 and then decreased from 100 to 200 DOF. These findings indicate that the early inhibition of muscle growth in steers compared with bulls may be associated with the reduction of muscle sensitivity to IGF1 hormone. Furthermore, in addition to the direct effect of testosterone on muscle cells, it has been proposed that an indirect effect of testosterone on muscle anabolism is mediated by IGF1 (Gentile et al., 2010; Dayton and White, 2014).

Muscle protein turnover may affect muscle hypertrophy, growth efficiency, and meat quality (Koochmaria et al., 2002). Ubiquitin-proteasome is the main proteolytic system related to skeletal muscle protein turnover, and protein breakdown via this pathway may account for 80 to 90% of all *in vivo* muscle proteolysis (Goll et al., 2008). The *F-box protein 32* (*FBXO32*), also known as *Atrogin-1*, is an important subunit of the *E3 ubiquitin-protein ligase* (Foletta et al., 2011). Upregulation of muscle *Atrogin-1* has been related to muscle atrophy (Anthony, 2016). Muscle *FBXO32* expression enhanced as DOF increased, but steers had greater expression at 100 DOF compared with bulls with no differences between sexes at 200 DOF. These data suggest that the ubiquitin-proteasome system is associated with muscle growth and remodeling and may work to reduce muscle growth rate as cattle reach the mature weight, and a premature expression of atrophy-related genes may be partially responsible for the reduction in muscle-related measured in steers compared with bulls by the end of the feeding period.

In addition to proteasomes, caspases are another group of endogenous muscle proteases involved in muscle protein turnover and remodeling (Anthony, 2016). As DOF progressed, muscle mRNA abundance of *CASP3* did not change while a tendency was observed for decreasing *SERPINA3-6* abundance, indicating an increase in the caspase proteolytic system. These data corroborate previous observations showing that the protease inhibitor level is a better predictor of the proteolytic system activity than their respective target protease (Boudida et al., 2014).

Finally, the greater bone growth of bulls compared with steers may be due to the androgen stimuli, as previously confirmed in rats (Gentile et al., 2010; Xia et al., 2013). In agreement, others also reported a greater proportion of bone in bull carcasses regardless of the castration method (Marti et al., 2013; Moreira et al., 2017).

### 4.3. Adipose tissue growth

Castration markedly enhanced fat tissue growth, regardless of the body deposit. However, greater differences were observed when comparing bulls and steers at 200 DOF. An interaction effect was found for VF as a percentage of EBW, indicating that bulls and steers did not differ when harvested at 0 and 100 DOF, whereas steers had 45% greater VF than bulls when harvested at 200 DOF (7.82 vs. 5.38% of EBW). The same pattern was found for carcass EE yield, and steers had 15% greater carcass EE than bulls when harvested at 200 DOF (67.8 vs. 58.7 kg of EE). These results confirm that body composition is affected by castration only with additional time on feed with subsequent heavier body weights and likely puberty attainment.

As shown in our companion paper, the intramuscular fat differed between sexual conditions only at 200 DOF, with steers having 52% greater IMF than bulls (6.13 vs. 4.04%; Silva et al., 2019a). However,

regarding the expression of genes related to adipogenesis and lipid metabolism, only the *ACACA* gene tended to differ between sexual conditions, with steers having greater muscle mRNA abundance than bulls. The enzyme *ACACA* catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, a key step during fatty acid biosynthesis in ruminants (Shin et al., 2011). A high and positive correlation has been found between *ACACA* muscle expression and IMF, suggesting that *ACACA* plays an important role in the deposition of IMF in ruminants (Costa et al., 2013). Therefore, our observations confirm the enhancement of fat synthesis following cattle castration. In agreement, it has been reported that castration upregulated LD muscle *ACACA* expression in cattle, supporting the greater IMF found for steers (Bong et al., 2012). Our companion paper, reporting the muscle proteome of the same animals used in this study, shows that muscle glucose metabolism may have been modulated to support the *de novo* synthesis of fatty acids (Silva et al., 2019b).

The transcription factor *PPARG* promotes the expression of target genes related to adipocyte differentiation and lipid storage, and is therefore, a key biomarker for adipogenesis (Bionaz et al., 2013). Still, its regulatory mechanisms are complex and involve various inhibitory factors, such as the thioredoxin-interacting protein (Txnip), which is responsive to blood glucose levels and has been shown to exert a suppressive effect on *PPARG* expression (Dou et al., 2023). It was expected that an enhancement in muscle expression of *PPARG* would be observed as an effect of castration (Serra et al., 2013) and in response to increasing DOF (Kern et al., 2014). Nevertheless, in the current study, the muscle abundance of *PPARG* mRNA did not differ among treatments. In agreement, studying the muscle transcriptome of Nellore steers grouped as low and high IMF, Cesar et al. (2015) did not observe differences in LD muscle *PPARG* expression between the high and low IMF groups. Likewise, only a weak correlation was observed between muscle expression of *PPARG* target genes and IMF in Brahman, another zebu breed (De Jager et al., 2013).

It has been shown that the increased IMF of steers is supported by enhancement of lipid uptake and decreasing lipolysis (Jeong et al., 2013; Baik et al., 2017). However, in the current study, neither castration nor DOF affected the expression of genes related to lipid uptake (*LPL* and *SLC27A1*) or lipolysis (*LIPE*).

#### 4.4. Glucose/glycogen metabolism

Glucose is an important source of energy, with some body tissues such as the brain and red blood cells using it as their only source of energetic fuel (Huntington and Richards, 2005). However, even though muscle can use other energy sources, it is estimated that the skeletal muscle accounts for approximately 20–40% of glucose uptake in ruminants (Dijkstra et al., 2005). Others reported that in cattle the skeletal muscle metabolizes 50–55% of the blood glucose (Ortigue-Marty et al., 2003). Furthermore, in rats, 80–90% of exogenous glucose dose is taken up by skeletal muscle (Xia et al., 2013). These results indicate that muscle plays an important role in blood glucose homeostasis. Uptake of glucose by the skeletal muscle can be affected by castration (Xia et al., 2013) and body composition changes (Huntington and Richards, 2005). In the current experiment, the GTT data indicates that neither sexual condition nor DOF affected basal concentrations of blood glucose and clearance of exogenous glucose. The GTT results are in line with the lack of difference among treatments for muscle expression of *GLUT4*. In agreement, it has been reported that cattle with different residual feed intake (RFI) had similar glucose AUC, and the muscle expression of *GLUT4* did not differ among groups of RFI (Fitzsimons et al., 2014). Further, a similar glucose clearance has been found for Angus steers with different muscling genotypes (McGilchrist et al., 2011). It has also been reported that concentrations of non-esterified fatty acids (NEFA) in blood are negatively correlated to glucose sensitivity (Burdick Sanchez et al., 2016). In the current experiment, concentrations of NEFA were not evaluated, but the mRNA abundance of the lipase, hormone-sensitive (*LIPE*) in the LD, did not differ among treatments, suggesting a similar triacylglycerol hydrolysis between bulls and steers regardless of the time on feed.

Soon after uptake into the muscle cell, glucose molecules are phosphorylated to glucose-6P, which can subsequently follow the glycolysis pathway or be stored as glycogen. Glucose-6P must be converted

to glucose-1P to be stored as glycogen, and this reaction is catalyzed by phosphoglucomutase (*PGM1*). The glycogen synthase kinase (*GSK3B*) modulates the glycogen synthase activity and is a key enzyme in glycogen metabolism. Finally, glycogen phosphorylase (*PYGM*) catalyzes the first step of glycogen breakdown. In the current experiment, the mRNA abundance of *PGM1*, *GSK3B*, and *PYGM* were not affected, suggesting that neither castration nor increasing DOF affect glycogen metabolism at the gene expression level, suggesting post-translational events as key regulators. In contrast, muscle proteome analysis showed a greater abundance of PGM1 protein in the muscle of steers than in bulls (Silva et al., 2019b).

Decreased muscle glucose sensitivity may impair muscle glycogen storage and subsequent postmortem carcass pH decline (Aoki et al., 2007). However, in the current experiment, no differences were observed among cattle groups for GTT, suggesting that muscle glucose metabolism is not affected by castration or time on feed. Moreover, the postmortem pH decline did not differ among treatments in the current experiment (data previously published by Silva et al., 2019a).

## 5. Conclusions

The interaction effect found for carcass composition, especially fat gain, indicates that while bulls and steers showed similar carcass composition at the start of feeding, steers accumulated significantly more fat than bulls as the feeding period progressed. Nellore whole-body glucose sensitivity is not affected by castration or days on feed. In addition, the upregulation of *longissimus dorsi* muscle *ACACA* by castration suggests that the greater intramuscular fat observed in steers may be supported by enhanced *de novo* fatty acid synthesis. Finally, the tendency for downregulation of muscle *serpin A3-6* in longer-fed cattle (i.e., 200 days on feed) indicates that reducing the protein fractional accretion rate as cattle became heavier might be supported by greater caspase activity, as serpin is a caspase inhibitor.

## Data availability

The data supporting the findings of this study are available from the corresponding author upon request.

## Author contributions

**Conceptualization:** Silva, L. H. P.; Silva, W.; Duarte, M. S. and Chizzotti, M. L. **Data curation:** Silva, L. H. P.; Assis, D. E. F.; Miguel Estrada, M. and Dahlen, C. R. **Formal analysis:** Silva, L. H. P.; Ramírez-Zamudio, G. D.; Silva, W.; Assis, G. J. F.; Duarte, M. S. and Chizzotti, M. L. **Funding acquisition:** Chizzotti, M. L. **Investigation:** Silva, L. H. P.; Ramírez-Zamudio, G. D.; Assis, D. E. F.; Assis, G. J. F. and Miguel Estrada, M. **Methodology:** Silva, L. H. P.; Silva, W.; Guimarães, S. E. F.; Duarte, M. S. and Chizzotti, M. L. **Project administration:** Silva, L. H. P.; Assis, D. E. F.; Assis, G. J. F.; Miguel Estrada, M. and Chizzotti, M. L. **Resources:** Guimarães, S. E. F.; Duarte, M. S. and Chizzotti, M. L. **Supervision:** Silva, L. H. P.; Ramírez-Zamudio, G. D. and Miguel Estrada, M. **Visualization:** Silva, L. H. P. and Ramírez-Zamudio, G. D. **Writing – original draft:** Silva, L. H. P.; Ramírez-Zamudio, G. D.; Dahlen, C. R. and Chizzotti, M. L. **Writing – review & editing:** Silva, W.; Assis, D. E. F.; Assis, G. J. F.; Miguel Estrada, M. and Guimarães, S. E. F.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgments

The authors thank the Laboratório de Ciência da Carne (LCC) and Laboratório de Biotecnologia Animal (LABTEC) of the Universidade Federal de Viçosa (Viçosa, MG, Brazil) for their helpful support during the gene expression analyses.

## Financial support

This Research was partially supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Grant# APQ-02577-14), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Grants 459912/2014-3 and 307241/2013-0), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Estágio Sênior #88881.119281/2016-01).

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