MARCOS HENRIQUE SOARES

BETAINE AND RACTOPAMINE FOR PIGS FROM 90 TO 140 KG

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.*

Adviser: Alysson Saraiva

VIÇOSA – MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
S676b
2021

Soares, Marcos Henrique, 1992-
Betaine and ractopamine for pigs from 90 to 140 kg / Marcos Henrique Soares. – Viçosa, MG, 2021.
1 tese eletrônica (73 f.): il.

Texto em inglês.
Inclui anexo.
Orientador: Alysson Saraiva.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Zootecnia, 2021.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2021.264
Modo de acesso: World Wide Web.

1. Suínos - Alimentação e rações. 2. Lipídios - Metabolismo. 3. Carne de porco - Qualidade. 4. Suínos - Registros de desempenho. 5. Expressão gênica. I. Saraiva, Alysson, 1975-. II. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 22. ed. 636.4085

Bibliotecário(a) responsável: Alice Regina Pinto CRB6 2523

MARCOS HENRIQUE SOARES

BETAINE AND RACTOPAMINE FOR PIGS FROM 90 TO 140 KG

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*.

APPROVED: October 8, 2021.

Assent:

Marcos H. Soares

Marcos Henrique Soares
Author

Alysson Saraiva
Adviser

ACKNOWLEDGMENTS

To God for His graces and strength from the beginning of my academic life.

To Federal University of Viçosa and the Department of Animal Science for the opportunity to complete my Doctor Scientiae program.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for granting the scholarship and Instituto Nacional de Ciência e Tecnologia - Ciência Animal (INCT-CA) for financial support.I must not fail to acknowledge the PIF-PAF industry for donate the animals for the experiments.

My sincere gratitude to professor Dr. Alysson Saraiva, my advisor, for all the knowledge transmitted during almost eight years between graduation and doctoral, and for guidance in the elaboration and execution of this work. I would also like to thank my other thesis committee members, Dr. Gabriel Cipriano Rocha, Dr. Márcio de Souza Duarte and Dr. Fabrício Santos for their availability, attention and contributions to this work.

I would like to thank the help provided by the staff of UEPE - Suinocultura (UFV), Fernando, José Roberto, Valdeir, Leandro, Arlindo and undergraduate and post-graduate students working on the UEPE – Suinocultura - UFV.

To my work team, Gustavo, Dante and Lívia, for their friendship and help during the execution of this study. In particular to Gustavo for helping me since my pigs arrived until the end of laboratory work.

To my parents, José Maria and Ivone for the love, trust and immeasurable support they provide me.

To my sisters Fernanda and Amanda for all the teaching, advice and affection. I would also like to thank my nephew Luis Otávio for all the happy moments.

To my girlfriend Mayra for love, companionship and for encouraging me in each decision I make.

To the friends of “Zoo 2011”, "Lapada F.C.", "Abeia" for the great moments together and for the encouragement during the years at UFV.

To the Grupo de Discussão em Suinocultura (GDSUI) for enriching my professional career.

ABSTRACT

SOARES, Marcos Henrique, D.Sc., Universidade Federal de Viçosa, October, 2021. **Betaine and ractopamine for pigs from 90 to 140 kg**. Adviser: Alysson Saraiva.

Two experiments were carried out with female pigs from 90 to 140 kg. In experiment I, the objective was to evaluate the supplementation of ractopamine and betaine, alone or in association, on performance, carcass traits, meat quality and expression of genes related to lipid metabolism in skeletal glycolytic and oxidative muscle tissue and in the subcutaneous adipose tissue. In experiment II, to evaluate performance, carcass traits, pork quality and expression of genes related to lipid metabolism in glycolytic and oxidative muscle tissues of two divergent genetic lines fed ractopamine. In experiment I, a total of 72 female pigs (Agroceres PIC) with initial body weight of 88.96 ± 3.44 kg were assigned to a completely randomized design in four diets with nine replicates and two pigs per experimental unit, represented by the pen. Diets consisted of a control (CTRL) diet (without ractopamine and betaine); CTRL + 2.5 g/kg betaine (BET); CTRL+ 20 ppm ractopamine (RAC); and RAC + 2.5 g/kg de betaine (RAC + BET). In experiment II, eighteen female pigs Agroceres PIC 337 (Large White x Landrace x Duroc x Pietrain) x Camborough (Large White x Landrace) (HYB), with initial body weight of 88.96 ± 3.44 kg and 12 female pigs DB LQ 1250 (Duroc) x DB 90 (Large White x Landrace) (DUR), with initial body weight of 85.63 ± 1.55 kg were used. Pigs were allotted in a completely randomized design in two groups: HYB and DUR with nine and six replicates, respectively, and two pigs per experimental unit, represented by the pen. In experiment I, pigs fed RAC and RAC+BET diets had higher daily weight gain, lower feed conversion compared to pigs fed the other diets and higher hot carcass weight and carcass yield, compared to CTRL diet. Pigs fed BET, RAC and RAC+BET diets had greater loin muscle area. The backfat thickness was lower in pigs fed RAC+BET diet, compared to the CTRL. The meat from pigs fed BET diet had lower Warner-Bratzler shear force and higher percentage of intramuscular fat compared to CTRL. BET diet increased the expression of lipogenic factors in *Longissimus dorsi* and Soleus muscles compared to CTRL. In adipose tissue, RAC and BET diets increased the expression of genes related to lipolysis and β-oxidation. The results indicate that performance and carcass traits can be improved with ractopamine and betaine promotes increase in loin muscle area and pork quality. In experiment II, the performance of pigs from both genetic groups was similar. However, DUR pigs had lower backfat thickness. The meat from DUR pigs had higher L* (brightness), lower a* (redness) and higher percentage of intramuscular fat. DUR pigs had

higher expression of peroxisome proliferator-activated receptor gamma (PPARγ) and fatty acid protein translocase/cluster differentiation (FAT/CD36), in addition to higher abundance of hormone-sensitive lipase (HSL) and Adiponectin (ADIP) mRNA in the *Longissimus dorsi*. Greater abundance of PPARγ and FAT/CD36 mRNA was also observed in the Soleus muscle of DUR pigs with no change in the expression of enzymes related to lipid degradation compared to HYB pigs. DUR pigs had carcasses with lower backfat thickness and better meat quality, evidenced by the higher percentage of intramuscular fat compared to HYB pigs. Possibly the higher concentration of intramuscular fat is mainly due to the higher expression of transcription factors and enzymes related to adipogenesis and lipogenesis in glycolytic and oxidative skeletal muscle tissue in DUR pigs.

Keywords: Lipid metabolism. mRNA expression. Performance. Pork quality.

RESUMO

SOARES, Marcos Henrique, D.Sc., Universidade Federal de Viçosa, outubro de 2021. **Betaína e ractopamina para suínos dos 90 aos 140 kg**. Orientador: Alysson Saraiva.

Foram conduzidos dois experimentos com fêmeas suínas dos 90 aos 140 kg. No primeiro estudo (I), o objetivo foi avaliar a suplementação de ractopamina e betaína, isolada ou em associação, no desempenho, características de carcaça, qualidade de carne e na expressão de genes relacionados ao metabolismo lipídico no tecido muscular glicolítico e oxidativo e no tecido adiposo subcutâneo. No segundo experimento (II), objetivou-se avaliar o desempenho, características de carcaça, qualidade de carne e a expressão de genes relacionados ao metabolismo lipídico no tecido muscular glicolítico e oxidativo de duas linhagens genéticas distintas consumindo ractopamina. No primeiro experimento, foram utilizadas 72 fêmeas híbridas (Agroceres PIC), com peso médio inicial de 88,96 ± 3,44 kg, distribuídos em delineamento inteiramente casualizado, com quatro tratamentos, nove repetições e dois animais por unidade experimental. Os tratamentos foram constituídos por uma ração controle (CONT), CONT + 2,5 g/kg de betaína (BET), ração com 20 ppm de ractopamina (RAC), RAC + 2,5 g/kg de betaína (RAC + BET). No segundo experimento, foram utilizadas 18 fêmeas Agroceres PIC 337 (Large White x Landrace x Duroc x Pietrain) x Camborough (Large White x Landrace) (HIB), com peso médio inicial de 88,96± 3,44 kg e 12 fêmeas DB LQ 1250 (Duroc) x DB 90 (Large White x Landrace) (DUR), com peso médio inicial de 85,63± 1,55 kg, distribuídos em delineamento inteiramente casualizado com dois tratamentos (Híbrido) ou (Duroc), com 9 e 6 repetições, respectivamente, e dois animais por unidade experimental. No experimento I, suínos consumindo RAC e RAC+BET apresentaram maior ganho de peso diário, menor conversão alimentar comparado às demais dietas e maior peso de carcaça quente e rendimento de carcaça em relação à dieta CONT. Suínos que consumiram as dietas BET, RAC e RAC+BET apresentaram maior área de olho de lombo, enquanto a espessura de toucinho foi menor nos animais que receberam a dieta RAC+BET em comparação ao CONT. A carne de animais que receberam a dieta BET apresentou menor força de cisalhamento e maior percentual de gordura intramuscular comparado ao CONT. A BET aumentou a expressão de fatores lipogênicos no *Longissimus dorsi* e no músculo Sóleo em relação ao CONT. Em relação ao tecido adiposo, RAC e BET aumentaram a expressão de genes relacionados a lipólise e β-oxidação. Os resultados indicam que o desempenho e as características de carcaça podem ser aumentados com o uso da ractopamina, enquanto a betaína promove aumento na área de olho de lombo e na

qualidade da carne suína. No experimento II, o desempenho dos suínos de ambos os grupos genéticos foi semelhante. No entanto, os suínos DUR apresentaram menor espessura de toucinho. A carne dos suínos DUR apresentou maior L* (luminosidade), menor a* (intensidade de vermelho) e maior percentual de gordura intramuscular. Suínos DUR apresentaram maior expressão do receptor ativado por proliferadores de peroxissoma gama (PPARγ) e da proteína ácido graxo translocase/cluster de diferenciação (FAT/CD36), além de maior abundância do mRNA da lipase hormônio sensível (HSL) e Adiponectina (ADIP) no *Longissimus Dorsi*. Maior abundância do mRNA do PPARγ e FAT/CD36 também foi observado no músculo Sóleo dos animais DUR, mas sem alteração na expressão de enzimas relacionadas à degradação lipídica comparado a suínos HIB. Suínos DUR apresentam carcaça com menor espessura de toucinho e melhor qualidade de carne, evidenciado pelo maior percentual de gordura intramuscular em relação aos suínos HIB. Possivelmente a maior concentração de gordura intramuscular seja devido principalmente a maior expressão de fatores de transcrição e enzimas relacionados a adipogêneses e lipogêneses no tecido muscular esquelético glicolítico e oxidativo nos suínos DUR.

Palavras-chave: Desempenho. Qualidade de carne. Expressão de mRNA. Metabolismo lipídico.

SUMMARY

CHAPTER 1: Betaine and ractopamine for pigs from 90 to 140 kg

Marcos Henrique Soares [a, b], Dante Teixeira Valente Júnior [a, b], Gustavo de Amorim Rodrigues[a, b], Ronaldo Lopes Cunha Júnior [a], Gabriel Cipriano Rocha [a, b], Marcio de Souza Duarte [a, b], Alysson Saraiva[a, b*]

[a]Department of Animal Sciences, Universidade Federal de Viçosa, Viçosa, MG, 36570-900, Brazil;

[b] Muscle Biology and Nutrigenomics Laboratory, Universidade Federal de Viçosa, Viçosa, MG, 36570-900, Brazil;

Corresponding author: Alysson Saraiva**,** alysson.saraiva@ufv.br

1. ABSTRACT

We aimed to evaluate the effects of ractopamine and betaine, supplemented alone or in association, on performance, carcass and meat quality traits, and expression of genes related to lipid metabolism in skeletal muscle and subcutaneous adipose tissue of pigs. Seventy-two pigs averaging 88.96 ± 3.44 kg of body weight were assigned to a control diet (CTRL - without ractopamine and betaine); CTRL + 2.5 g/kg betaine (BET); CTRL + 20 ppm ractopamine (RAC); and RAC + 2.5 g/kg de betaine (RAC+BET). Pigs fed RAC and RAC+BET had greater average daily gain and carcass yield compared. Pigs fed BET, RAC and RAC+BET had greater loin muscle area, while backfat thickness was lower in pigs fed RAC+BET compared to CTRL. Pork from BET had lower shear-force and greater intramuscular fat content compared to CTRL. Regarding adipose tissue, RAC and BET increased expression of genes related to lipolysis and β-oxidation. Our data indicate that performance and carcass traits of pigs can be improved by ractopamine, whereas betaine promotes an increase in loin muscle area and pork quality.

Keywords: Betaine; Lipid metabolism; Performance; Pork quality; Ractopamine.

2. INTRODUCTION

Slaughter weight is an important factor affecting the profitability of pig production (Wu et al., 2017). However, the increase in slaughter weight is accompanied by an enhancement in carcass fatness (Hwang et al., 2020), which is negatively correlated to feed efficiency and

consequently increase the production costs (Wu et al., 2017). Although harvesting heavier weights increases the overall carcass fatness, it also increases the probability to improve the intramuscular fat (IMF) deposition, which is a highly desirable meat quality trait. Therefore, the increase of carcass weight and marbling without an excessive increase on overall fatness has become one of the main challenges faced by the pork industry. In such a context, the use of pig genetics with greater potential for lean deposition associated with the use of additives that stimulate skeletal muscle growth and act to reduce the deposition of subcutaneous fat can enable the slaughter of heavy pigs.

Ractopamine, a β-adrenergic agonist, acts on the metabolism mediated by β-adrenergic receptors in cell membranes, which modulate enzymatic activity and gene expression (Parr et al., 2016) improving weight gain and feed efficiency in pigs (Apple et al., 2007; Marcolla et al., 2017). In adipose tissue, ractopamine reduces backfat thickness by increasing the rate of lipolysis and reducing lipogenesis (Mills, 2002; Halsey et al., 2011; Ferreira et al., 2017). In skeletal muscle tissue, ractopamine stimulates protein synthesis (Adeola et al., 1992; Apple et al., 2007) and the increase in the proportion of glycolytic muscle fibers, allowing greater muscle hypertrophy (Gunawan et al., 2007; Almeida et al., 2015). However, ractopamine increases the use of fatty acids as energy source in muscle tissue, which may negatively impact pork IMF content (Reiter et al., 2007; Martins et al., 2015; Ferreira et al., 2017).

Betaine, a tri-methyl derivative of glycine, seems to be a promising feed additive. Betaine is described as being able to improve pig performance (Huang et al., 2009; Bustillo et al., 2018), mainly due to its effect on osmoregulation and increase in nutrient digestibility (Lipiński et al., 2012; Wang et al., 2020). Betaine also acts to reduce backfat thickness (Dunshea et al., 2008), which may be due to an impairment of lipogenesis and increased β-oxidation in adipose tissue (Huang et al., 2009; Lothong et al., 2016). However, previous studies have shown that pigs fed betaine present an improved pork quality, mainly by increasing the concentration of IMF (Huang et al., 2009; Albuquerque et al., 2017; Li et al., 2017a).

Despite the current data regarding the use of betaine in pig's diet and its association with carcass and pork quality traits, little is known about the effect of betaine supplementation to heavy pigs. Moreover, information about the effects of its association with ractopamine and how the use of these additives (betaine and ractopamine) isolated or in association acts on molecular mechanisms underlying lipid metabolism in different muscle types and in subcutaneous adipose tissue is scarce.

Therefore, it was hypothesized that betaine and ractopamine, used alone or in combination, improve performance and carcass traits of pigs slaughtered at 140 kg and also

improve meat quality through modulation of lipid metabolism in different ways in muscle and fat tissues. The objective of this study was to evaluate the supplementation of ractopamine and betaine, alone or in association, on performance, carcass traits, meat quality and expression of genes related to lipid metabolism in skeletal glycolytic and oxidative muscle tissue and in the subcutaneous adipose tissue of female pigs from 90 to 140 kg.

3. MATERIAL AND METHODS

All methods involving the handling of pigs followed the ethical principles of animal research (CONCEA) and were approved by the Commission of Ethics in the Use of Production Animals (CEUAP) of the *Universidade Federal de Viçosa* (protocol 013/2018).

3.1. Animals, experimental design and diets

A total of 72 female pigs PIC 337 (Large White x Landrace x Duroc x Pietrain) x Camborough (Large White x Landrace) with initial body weight of 88.96 ± 3.44 kg were assigned to a completely randomized experimental design with four diets, nine replicates and two pigs per experimental unit, represented by the pen. Diets consisted of a control (CTRL) diet (without ractopamine and betaine); CTRL + 2.5 g/kg betaine (BET); CTRL + 20 ppm ractopamine (RAC); and RAC + 2.5 g/kg de betaine (RAC + BET).

Pigs were housed in 2.30 × 2.10 m concrete-floored pens with a dry feeder and a nipple drinker each. Pigs had free access to feed and water throughout the 45-d feeding trial. Temperature and humidity inside the barn were recorded every 3 h using a data logger. During the experimental period, average temperature inside the barn was 22.04± 2.66ºC and an average humidity was 79.47 ± 0.08%.

The experimental diets (Tables 1 and 2) were formulated based on corn and soybean meal and supplemented with minerals and vitamins to meet the requirements of the pigs, according to Rostagno et al. (2017), except for SID lysine in the diets with ractopamine (RAC and RAC + BET). SID lysine was increased by 20% considering the increase in the protein requirement of pigs fed diets with ractopamine (Xiao et al., 1999, Marcolla et al., 2017). Industrial amino acids were added maintaining the ratios with SID lysine recommended by Rostagno et al. (2017).

In the specific case of the RAC and RAC + BET groups, the pigs were fed the CTRL diet during the 17 initial days, and then fed the respective diets for the remaining 28 days. The difference in the number of days of BET (45 days), RAC and RAC + BET (28 days) supply was established according to previous research (Moody, 2000; Huang et al., 2009; Li et al., 2017a; Marcolla et al., 2017).

During the trial, feed was weighed before feeding and feed wastage and leftovers were manually collected and daily weighed to determine average daily feed intake (ADFI). At the end of the experimental period, piglets were individually weighed to determine final body weight (BW), average daily gain (ADG), and feed:gain (F:G).

3.2. Blood sampling and serological analysis

At the end of the trial pigs were submitted to a 14-hour fasting with free access to water. After fasting, blood was collected in 10 mL tubes without anticoagulants by puncturing the orbital sinus using hypodermic needles (40 x 1.6 mm) to determine the concentration of serum urea nitrogen (SUN), triglycerides (TG) and total cholesterol (CHO). Samples were immediately sent at room temperature to the Viçosa clinical laboratory. SUN concentration was evaluated using the automated enzymatic method, whereas the concentration of TG and CHO was determined using the enzymatic colorimetric method (Ureal Cobas c 311, Roche Diagnostics GmbH, Basel, Switzerland).

3.3. Slaughter procedures and tissue sampling

After blood sampling, one pig from each experimental unit with body weight closest to 140 kg, was stunned by electronarcosis and exsanguinated. At slaughter, samples of *Longissimus dorsi* (LD), soleus muscle (SM) and subcutaneous adipose tissue were collected for posterior gene expression analysis immediately after exsanguination, snap-frozen in liquid nitrogen and stored at -80°C for further nucleic acids extraction. All instruments used for sampling were sterilized and adequately cleaned with RNAse Exterminator (Protech Technology Enterprise Co., Ltd.) to avoid nucleic acid degradation.

After slaughter, carcasses were weighed to determine hot carcass weight (HCW) and carcass yield (CY), according to the formula: hot carcass weight divided by the final pre-slaughter weight multiplied by 100. The carcasses were divided longitudinally and refrigerated at 5°C for 24h. All measurements (pH, temperature, backfat thickness and loin muscle area) and sampling were performed on the left half of each carcass. The temperature and pH of LD were measured at 15 min, 45 min, 1h, 3h, 6h, 9h, 12h and 24h after slaughter, using a pH T meter with penetration probe and thermometer coupled (Testo SE & Co., Lenzkirch, FR, Germany) inserted into the LD at the level of the last lumbar vertebra.

For meat quality, a 20 cm sample was collected after a postmortem chill of 24 h. Samples were obtained from the LD of each right half of the carcasses, between the tenth rib and the first lumbar vertebra. Upon collection, LD samples received a craniocaudal identification and then vacuum packed, stored at -20°C for 24 hours and then sectioned into five 2.54 cm thick

chops. The 2.54 cm thick chops were individually vacuum packed, identified according to the pig and the position in the muscle from which they originated and stored at -20°C for further analysis (Bridi, 2006).

The LD and Soleus muscle samples were stored in duplicate in cryotubes with 1.8 mL capacity, instantly frozen in liquid nitrogen and stored in an ultra-freezer at -80ºC for further analysis.

3.4. Carcass traits

After 24h cooling half carcasses were divided at the height of the 10th rib, and the backfat thickness over the LD (6 cm away from the midline) was measured using a digital caliper. To determine loin muscle area (LMA), the muscular surface of the LD between the 10th and 11th rib was covered with a polyethylene sheet and contoured using a permanent fine-tipped marker. The sheets were digitally scanned and colored. Colored areas within the contour were measured using image analysis software (ImageJ version 1.49 t, National Institutes of Health, Bethesda, MD). HCW and CY were obtained soon after slaughter, as described above.

3.5. Pork quality

For water losses determination frozen LD samples were removed from the plastic bags, weighed on a semi-analytical scale and placed to thaw at 4°C for 16h. After thawing, the samples were gently wiped dry using a paper towel and weighed. Thaw water loss (TL) was considered to be the gravimetric difference between the steaks before and after thawing. For cooking water loss (CL), the thawed chops were vacuum packed and cooked in a digital water bath with a stirrer (WEALAB) at 71ºC for 40 minutes. After this time, the samples were removed from the water bath and placed in an ice bath for 10 minutes to stop cooking. At the end of cooking, the samples were weighed again and the cooking water loss was expressed as a percentage of the weight of the samples before and after cooking. The sum of water losses (SL) was estimated by the difference in weight between the frozen and cooked samples (Bridi, 2006).

After weighing, cooked chops were used to Warner-Bratzler shear force (WBSF) determination as proposed by American Meat Science Association (2016) with minor modifications. From each sample, 6 round cores measuring 1.27 cm in diameter were removed parallel to the longitudinal orientation of the muscle fibers, using a sharp stainless steel coring device. Care was taken to avoid sampling at areas containing visible fat and connective tissue. These round cores were sheared once through the center, perpendicularly to the longitudinal orientation of the muscle fibers, using a V-notch blade with 1.016 mm thickness and 60° angle

at fixed speed of 20 cm/min, coupled to a Warner-Bratzler Shear machine (G-R Electrical Manufacturing Company, Manhattan, KS). The WBSF was determined by the average of 6 measures and expressed in Newtons (N).

For color evaluation, the right half of each carcass were sectioned at the tenth rib and the first lumbar vertebra, and allowed to bloom for 30 min. After blooming period, meat color was determined using a handheld spectrophotometer (Hunter MiniScan EZ, 4500L; Hunter Associates Laboratory, Inc., Reston,VA), calibrated against a white and a black tile. The mean L* (lightness), a* (redness), and b* (yellowness) values of each chop were determined as the average from 3 readings on 3 different points of chop surface, using illuminant D65, a 31.8 mm port size and a 10° standard observer (Brewer et al., 2001; Karamucki et al., 2006).

To determine intramuscular fat (IMF) chops (2.54 cm thick) were thawed at 4ºC for 16 hand trimmed for visible fat and connective tissues, and then ground in a TURRAX CT-132 tissue homogenizer. A 100 g-sample of each chop was evaluated for IMF using near infrared spectrophotometry (FoodScan, FOSS NIRsystems Inc., Laurel, MD; AOAC, Official method 2007.04; AOAC, 2007).

3.6. RNA extraction and cDNA synthesis

Total RNA extraction was performed from 50 mg of LD and soleus muscle samples using Trizol® (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For the extraction of RNA from subcutaneous adipose tissue, Trizol® (Invitrogen TM) was also used and RNA washing and isolation was performed using RNase-free silica membrane columns (PureLink ™ RNA Mini Kit – Invitrogen TM). The final precipitate was rehydrated with 30 µL of UltraPure® DNase/RNase-Free water.

The RNA concentration was estimated using a NanoDropTM Lite spectrophotometer (ThermoFisher Scientific, Beverly, MA, USA), observing the A260/A280 ratios between 1.8 and 2.04 as purity control. The quality and integrity of extracted RNA was verified using 1% agarose gel.

The samples were then treated with DNase and reverse transcribed into cDNA using the Go Script Reverse Transcription (RT) Kit (Promega, Madison, WI), following the manufacturer's recommendations. cDNA samples were stored at -20°C until use in the real-time quantitative PCR reaction.

3.7. Gene expression analysis

Primers (Table 2) for amplification of target and endogenous gene fragments were designed using Primer Quest software (Integrated DNA Technologies Inc., Coralville, IA,

USA) from nucleotide sequences obtained from the GenBank database (www.ncbi.nlm.nih.gov). Optimization for each primer was done to calculate efficiency (Livak and Schmittgen, 2001).

The target genes evaluated in LD and in SL were lipoprotein *lipase* (*LPL*), *fatty acid binding protein 3* (*FABP3*), tr*anslocase fatty acid/cluster differentiation* (*FAT/CD36*), *peroxisome proliferator activated gamma receptor* (*PPARγ*), *CAAT/enhancer binding protein α* (*C/EBPα*), *adiponectin* (*ADIP*), *adiponectin receptor 1* (*AdipR1*), *adiponectin receptor 2* (*AdipR2*), *peroxisome proliferator activated receptor alpha* (*PPARα*), *hormone sensitive lipase* (*HSL*) and *carnitine palmitoyl transferase-1*(*CPT-1*). As a reference gene for normalization, *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) was used due to the high amplification and stability of this gene among groups. In the subcutaneous adipose tissue, in addition to the same genes evaluated as for muscle tissues the *Acetyl-CoA carboxylase* (*ACC*) gene was also analyzed and the *β-2 microglobulin* gene (*B2M*) was used as a housekeeping gene.

The RT-qPCR reactions were performed in duplicate using the thermocycler Applied Biosystems™ QuantStudio Real-Time PCR Systems (Applied Biosystems ™ PowerUp ™ SYBR ™ Green Master Mix) (Applied Biosystems, Foster City, CA, USA) from the method of Relative Quantitation, using as detection fluorophore SYBR® Green from the GoTaq® qPCR Master Mix kit (Promega corporation, Madison, USA). The PCR reactions were submitted to the cycling protocol following the instructions of the SYBR ™ Green Master Mix: 50°C for 2 min, 95°C for 2 min, followed by 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds and 72°C for 1 min.

The threshold cycle (Ct) values obtained were normalized (ΔCt) based on the Ct value of the endogenous control gene in muscle (GAPDH) and in adipose tissue (B2M). The calculation of relative levels of gene expression was performed according to the $2^{-\Delta\Delta Ct}$ method, described by Livak and Schmittgen (2001).

3.8. Statistical analysis

For performance (FBW, ADFI, ADG and F:G) data analysis the pen was considered the experimental unit. Only one pig per pen was considered the experimental unit for the other analysis. For the analysis of carcass traits (HCW, CY, LMA and BF) FBW corrected for diet effect was used as covariate.

The normality and homogeneity of the residues were verified using the Kolmogorov Smirnov and Bartlett tests, respectively. Statistical analysis was performed using the R software

(version 3.4.4; R Core Team 2018). Data were subjected to analysis of variance (ANOVA) and means were compared using Tukey test. Differences were considered at $\alpha = 0.05$.

4. RESULTS

4.1. Performance and serological analysis

Pigs fed diets RAC and RAC + BET had greater ($P < 0.001$) ADG and FBW ($P < 0.001$) compared to pigs fed the other diets (Table 4). The dietary additives did not affect ADFI ($P = 0.752$, Table 4). Pigs fed diets RAC and RAC + BET had improved F:G ratio ($P < 0.001$; Table 4) compared to the pigs fed the other diets.

Total cholesterol ($P = 0.33$) and triglyceride ($P = 0.87$) concentrations were not affected by the diets (Table 5). Pigs fed CTRL diet presented the greatest value for serum urea nitrogen concentration ($P = 0.039$, Table 5), while the lowest value for the same trait was observed for pigs fed the RAC+BET diet.

4.2. Carcass traits

The RAC and RAC+BET diets enhanced hot carcass weight (HCW; $P < 0.01$) (Table 6). Carcass yield (CY) of pigs fed RAC and RAC+BET was greater ($P = 0.02$) than CY from pigs fed the CTRL diet (Table 6). Pigs fed BET, RAC and RAC+BET diets had greater ($P < 0.01$) loin muscle area (LMA) compared to those fed CTRL diet (Table 6). Backfat thickness (BF) was lower ($P < 0.01$) in pigs fed RAC+BET compared to those fed CTRL diet (Table 6).

4.3. Carcass pH and temperature

There was no effect of the dietary additives on meat pH ($P > 0.05$) in any of the evaluated moments (Figure 1).

Pigs fed RAC diet had greater ($P = 0.041$) carcass temperature at 15 min after slaughter compared to pigs fed BET (Figure 2). The animals that consumed the RAC+BET or CONT diets presented intermediate carcass temperature values.

The carcasses from pigs fed RAC had a greater ($P = 0.01$) temperature 6h after slaughter compared to pigs fed BET or RAC+BET diets (Figure 2). The pigs fed CTRL diet had intermediate carcass temperature values.

The RAC diet resulted in greater ($P = 0.04$) carcass temperature at 15 minutes after slaughter compared to pigs fed BET diet. The carcasses of animals fed RAC diets had a greater temperature 6h after slaughter compared to pigs fed BET or RAC+BET diets ($P = 0.01$).

4.4. Pork quality

There was no effect (P = 0.32) of additives on thaw water losses (TL). However, cooking losses (CL) were lower (P < 0.006) in the meat from pigs fed RAC or RAC+BET compared to CTRL (Table 7). The sum of water losses (SL) was not affected (P = 0.1) by the diets.

Pigs fed BET diet had lower (P < 0.01) Warner-Bratzler shear force (WBSF) compared to pigs fed the other diets (Table 7).

Dietary additives did not affect (P > 0.05) *L** (brightness), *a** (redness) and *b** (yellowness) values of meat (Table 7).

Intramuscular fat content (IMF) in *Longissimus dorsi* (LD) of pigs fed BET diet was greater (P = 0.02) compared to pigs from the CTRL group (Table 7).

4.5. mRNA expression in *Longissimus dorsi* muscle

The BET diet resulted in an increase (P < 0.01) in the mRNAexpression of *PPARγ* compared to CTRL and RAC diets (Table 8). The mRNA expression of *C/EBPα* in LD of pigs fed BET and BET+RAC diets was greater (P = 0.01) compared to CTRL and RAC diets (Table 8). Similarly, mRNA expression of *FABP3* was also greater (P = 0.03) in pigs fed BET and RAC+BET compared to CTRL diet (Table 8).

The BET diet increased (P = 0.01) *LPL* mRNA expression compared to RAC and RAC+BET diets (Table 8). The mRNA expression of *FAT/CD36* was greater (P = 0.01) in pigs fed BET compared to RAC (Table 8). No effects of feeding additives were observed for mRNA abundance of (P = 0.17; Table 8).

Pigs fed the BET diet had enhanced the mRNA expression of CPT-1 (P < 0.01) compared to those fed the other diets (Table 8). Pigs fed BET diet had lower (P = 0.001) *HSL* mRNA expression compared to those fed the other diets (Table 8).

The dietary additives did not alter (P = 0.30) the mRNA expression of *adiponectin* (Table 8) and *AdipR2* (P = 0.06, Table 8). The mRNA expression of *AdipR1* was greater (P = 0.01) in pigs fed BET diet compared to those fed CTRL and RAC+BET diet (Table 8).

4.6. mRNA expression Soleus muscle

A reduction (P = 0.01) in *PPARγ* mRNA expression was observed in *Soleus* muscle (SL) of pigs fed RAC compared to CTRL and BET diets (Table 9).

Pigs fed BET diet had greater (P = 0.03) mRNA expression of the *C/EBPα* compared to CTRL and RAC diets (Table 9).

The mRNA expression of *FABP3* was not influenced (P = 0.55) by the diets (Table 9). Only animals fed BET diet have shown an increase in mRNA expression of *LPL* in SL muscle compared to CTRL diet (P = 0.04; Table 9).

The BET diet increased (P = 0.03) mRNA expression of *FAT/CD36* compared to RAC (Table 9).

The RAC and RAC+BET diets resulted in greater (P = 0.01) mRNA expression of PPARα compared to BET (Table 9). On the other hand, experimental diets did not alter the mRNA expression of *CPT-1* (P = 0.339), *HSL* (P = 0.562) and *Adiponectin* (P = 0.221) genes (Table 9). The *AdipR1* receptor had greater (P = 0.03) mRNA expression in pigs fed BET diet compared to RAC (Table 9).

The RAC+BET diet was the only one effective in increasing (P < 0.001) mRNA expression of *AdipR2* (Table 9).

4.7. mRNA expression in subcutaneous adipose tissue

The mRNA expression of *PPARγ* in the subcutaneous adipose tissue (AT) was not influenced (P = 0.31) by any of experimental treatments (Table 10). On the other hand, pigs fed RAC+BET diet had lower (P = 0.02) mRNA expression of C/EBPα compared to CTRL and BET diets (Table 10). The BET, RAC or BET+RAC diets did not altered the mRNA expression of *FAT/CD36* (P = 0.08) and *FABP3* (P = 0.09) compared to the CTRL diet (Table 10). The gene encoding *ACC* had lower (P < 0.01) mRNA expression in pigs fed RAC and RAC+BET compared to CTRL and BET diets (Table 10).

The pigs fed RAC+BET diet had lower (P = 0.01) *LPL* expression compared to pigs fed BET (Table 10). The mRNA expression of the gene encoding *CPT-1* was greater (P = 0.04) in pigs fed BET compared to CTRL diet (Table 10). The BET, RAC and BET+RAC diets resulted in increased (P < 0.01) the mRNA expression of *HSL* in compared to CTRL diet (Table 10). The mRNA expression of *Adiponectin* did not change (P = 0.16) among diets (Table 10). Conversely, *AdipR1* mRNA expression was greater (P = 0.03) in pigs fed BET diet compared to those fed RAC (Table 10). A similar mRNA expression of *AdipR2* (P = 0.07) was observed among the diets (Table 10). The BET, RAC and RAC+BET diets increased (P < 0.01) *PPARα* mRNA expression compared to CTRL diet (Table 10).

5. DISCUSSION

Our study has assessed the effect of betaine, ractopamine and the combination of both feed additives in diets of finishing pigs on performance, carcass and meat quality traits, as well

as on mRNA expression of lipid metabolism markers in skeletal muscle. Although we have hypothesized that betaine would improve animal performance, our results revealed supplementation of betaine was not able to promote major changes in pig's performance. Despite the fact that our results differ from previous studies (Huang et al., 2009; Bustillos et al., 2018), we have not assessed variables that would explain the lack of effects of betaine in overall performance of the finishing pigs.

It has been shown that ractopamine can increase the activity of several enzymes related to protein deposition in muscle tissue of finishing pigs (Ferreira et al., 2017). Adeola et al. (1992) reported an increase in the rate of myofibrillar protein synthesis with the supplementation of 20 ppm of ractopamine, suggesting that supplemented pigs had greater muscle deposition. Thus, in the present study, the higher ADG and FBW observed in pigs fed RAC and RAC+BET diets was likely a consequence of greater muscle deposition consequently resulting in a greater contribution to weight gain (Li et al., 2017b).

Serum urea nitrogen concentration is often used to assess the rate of nitrogen utilization and excretion in pigs (Bush et al., 2002; Kohn et al., 2005). High serum urea concentrations indicate low efficiency of dietary nitrogen utilization for protein synthesis (Waguespack et al., 2011). We have observed that only the group fed RAC+BET diet shows a lower serum urea concentration compared to CTRL, indicating that the association of RAC and BET was efficient in improving dietary nitrogen utilization for lean gain.

The effect of ractopamine on carcass traits of pigs is consistent with previous studies. It has been shown an increase in HCW (Rickard et al., 2012; Park et al., 2018; Brustolini et al., 2019) and CY (See et al., 2004; Souza et al., 2018) when pigs are fed ractopamine, being the increase of protein synthesis, the main mechanism causing a greater lean deposition and consequently, enhancement of HCW (Ferreira et al., 2017).

The supplemented additives, alone or in combination, resulted in similar increase in LMA compared to CTRL diet. The greater LMA observed in pigs fed RAC is likely explained by the effect of ractopamine in increasing AKT and p70S6K phosphorylation in muscle tissue (Ferreira et al., 2017). Increased activation of AKT is able to activate the mTOR complex, which in turn activates p70S6K increasing protein synthesis. The increased activity of AKT is also related to inhibition of transcription factors of the FOXO family, this inhibition reduces muscle proteolysis promoted by Muscle Atrophy F-box (MAFbx) and Muscle RING finger (MuRF) (Bodine et al., 2001; Kline et al., 2007; Southgate et al., 2007; Lynch & Ryall, 2008), resulting in increased muscle deposition.

With regard to results of LMA obtained when pigs were supplemented with betaine, its direct effect on protein synthesis pathways is poorly understood. However, Kathirvel et al. (2010) demonstrated that dietary betaine enhances the phosphorylation and consequent activation of insulin receptor 1 (IRS1), which is correlated with increased phosphorylation of AKT, a key signaling enzyme for protein synthesis. Furthermore, because betaine it is considered a zwitterionic molecule, it acts on water retention in the cell with reduced energy expenditure, when compared to the energy required for the activation of ion pumps to maintain the osmotic balance (Lipiński et al., 2012). Thus, betaine supplemented pigs may present a reduction in energy requirements for maintenance. The surplus of energy can be diverted to growth, mainly for muscle tissue (Schrama et al., 2003), since supplemented betaine affects the lipid/protein deposition ratio in pigs, increasing protein deposition in relation to lipid (Schrama et al., 2003). In this sense, these reports support the results of the present study where the BET diet increased LMA.

Both ractopamine and betaine are additives reported to reduce backfat thickness in finishing pigs (Huang et al., 2009; Rickard et al., 2012; Bustillos et al., 2018; Park et al., 2018). However, in the present study RAC and BET diets resulted in intermediate responses in reducing BF, with only RAC+BET diet being effective in reducing BF compared to CTRL diet, which demonstrates the synergistic effect between molecules in reducing BF is more intense than the use of the additives alone.

The decline of the binomial pH and temperature curve post-mortem are determinant for meat quality, with direct impact on meat color and on water retention capacity, which in turn influences juiciness and tenderness in addition to shelf life and consumer acceptance (Wismer-Pedersen, 1959; Lindahl et al., 2006).

In our study, although the additives influenced muscle deposition, and thus had the ability to change glycogen stores, there was no difference among diets on carcass pH, indicating that the use of the additives until the time of slaughter did not impairs the pH declining rate during postmortem period. Regarding carcass temperature, pigs fed RAC had higher values at 15 minutes and 6 hours after slaughter, compared to those fed BET, and higher carcass temperature 6 hours after slaughter compared to pigs fed RAC+BET diet. However, because this change was not accompanied by changes in postmortem pH decline, there was no changes in the pork quality traits that are intrinsically associated with the changes with pH x temperature interaction during the postmortem period.

In the present work, redness and yellowness were not influenced by the use of the dietary additives. Works carried out by Bahelka et al. (2020) and Madeira et al. (2015), supplementing

betaine for pigs also did not observe effect of the additive on meat color. Apple et al. (2007), in a meta-analytic study concluded that ractopamine has no deleterious effect on meat color, indicating a low relationship between the supplementation of these additives with negative changes in the color of pork.

The water loss of meat is considered an important indicator to assess its quality (Watanabe et al., 2018). Decreased water holding capacity of meat leads to increased loss of liquid during cooking, which negatively affects tenderness and juiciness (Aaslyng et al., 2003; Marcolla et al., 2017). Furthermore, increased water loss can negatively impact the slaughterhouse profitability, consequently, there is strong interest from the industry to reduce these losses (Warriss, 1982; Maribo et al., 1998). In the present study, none of the additives influenced thaw water loss and sum of water losses. However, interestingly, feeding the RAC and RAC+BET diets resulted in reduced cooking water loss compared to CTRL diet.

Different authors reported that there is no deleterious effect of the use of ractopamine on water loss in pork (Apple et al., 2007; Marcolla et al., 2017; Brustolini et al., 2019). However, our results corroborate those obtained by Uttaro et al. (1993), in which supplementation of 20 ppm ractopamine reduced cooking water loss of LD. Reduction in cooking water loss has a positive effect on the succulence of pork (Aaslyng et al., 2003), indicating the meat from pigs fed the RAC and RAC+BET diets may have greater juiciness.

One of the most important attributes for meat palatability is tenderness (Warner et al., 2010), which is measured by Warner-Bratzler shear force (WBSF). In the present study, the meat of pigs fed BET diet had lower WBSF compared to those fed other diets, an indication of greater tenderness. Previous studies of Yang et al. (2009) and Zhong et al. (2021) also reported betaine supplementation for pigs reduces WBSF resulting in increased tenderness, which is consistent with the results of the present study. Possibly, the lower WBSF observed in the meat of pigs fed BET was due to the increase in the percentage of IMF. According toVan Laack et al. (2001), there is a negative correlation between IMF content and WBSF. The use of betaine in our study resulted in a significant increase of 45.83% in the IMF content compared to CTRL, which likely explains the results observed for WBSF in our study.

Betaine is reported to act by modulating lipid metabolism differently in adipose and muscle tissue (Madeira et al., 2016; Albuquerque et al., 2017; Wu et al., 2018). In muscle tissue, betaine has a mechanism capable of increasing IMF (Yu et al., 2004; Huang et al., 2009). Albuquerque et al. (2017) reported the supplementation of 1 g/kg betaine for Alentejano pigs increased IMF concentration by 51.85%. Still, Li et al. (2017a), in a study with commercial hybrid pigs supplemented with 2.5 g/kg betaine, observed an increase in IMF concentration of

approximately 44.0%. In general, these reports indicate that none of the evaluated additives have negative effects on qualitative variables of meat and, particularly, betaine has shown to have effect on the modulation of lipid metabolism and on the increase of IMF of pigs, being an additive with the potential to improve pork quality.

Knowledge about the effect of betaine and ractopamine on the molecular mechanisms of lipid metabolism regulation in muscle and adipose tissue of commercial lineage pigs is limited. Thus, in the present study, gene expression was evaluated in muscle tissue with primarily glycolytic metabolism (LD), in muscle with primarily oxidative metabolism (SL), and in subcutaneous adipose tissue (AT) of pigs fed diets with betaine and ractopamine alone or in association.

Pigs fed BET diet had higher gene expression of *PPARγ* in LD. Similarly, Albuquerque et al. (2017) and Li et al. (2017a) also observed increased *PPARγ* gene expression in the glycolytic muscle of pigs supplemented with betaine, indicating consistency of the mechanism. The *PPARγ* is a key transcription factor in the control of adipogenesis and lipogenesis, which regulates the expression of several genes related to lipid metabolism by binding to its promoter region. Thus, its greater expression in muscle tissue may be related to increased IMF (Takahashi et al., 2009; Wu et al., 2018).

Results such as those obtained by Wu et al. (2018) provided relevant information on the regulation of lipid accumulation by betaine in muscle cells. Betaine strongly reduced the activity of extracellular signal-regulated protein kinase 1/2 (ERK 1/2). This protein is able to down-regulate gene expression and PPARγ activity during the adipogenesis process (Bost et al., 2005). The inhibition of ERK 1/2 allows the process of adipogenesis in skeletal muscle tissue. Wu et al. (2018) demonstrated that because BET inhibits the activity of ERK 1/2, there was an increase in the expression and activity of PPARγ and in the expression of genes related to adipogenesis and lipogenesis regulated by PPARγ.

In addition to PPARγ, the gene transcription factor CCAAT/enhancer binding protein α (C/EBPα) is another key regulator in the differentiation of preadipocytes into mature adipocytes, in addition to acting in lipogenesis (Jiang et al., 2006). The role of C/EBPα in the regulation of adipogenesis and lipogenesis is done in conjunction with PPARγ (Tontonoz et al. 1994; Lefterova et al., 2008).

In the present study, the pigs fed BET and RAC+BET diets had greater abundance in C/EBPα mRNA, indicating betaine enhances the molecular mechanisms of adipogenesis and lipogenesis in LD. Although PPARγ gene expression was not influenced by RAC+BET diet, the pigs fed this diet had higher FABP3 expression compared to CTRL and RAC. The FABP3

protein is involved in the uptake, utilization and intracellular transport of long-chain fatty acids and is strongly associated with increased IMF (Cho et al., 2011; Serão et al., 2011). Possibly, the increase in FABP3 is associated with greater expression of C/EBPα in those pigs, indicating the effect of betaine may not be exclusively mediated by PPARγ.

The increased expression of transcription factors PPARγ and C/EBPα in LD of pigs fed BET resulted in increased expression of FABP3. In addition, pigs fed BET also had an increase in LPL expression compared to RAC and RAC+BET and an increase in FAT/CD 36 expression compared to RAC fed pigs. LPL gene expression is also associated with increased IMF in pigs (Xue et al., 2015).

However, IMF accumulation is a result of the balance between lipid synthesis, transport and degradation. In this sense, the expression of genes related to lipolysis and β-oxidation in LD was evaluated. An increase in the expression of CPT-1 was observed in LD of fed BET compared to the other diets. CPT-1 is located in the outer region of the mitochondrial membrane and catalyzes the formation of the acyl-carnitine complex, which is transported to the interior of the mitochondria and acts in the β-oxidation of long-chain fatty acids. Thus, CPT-1 is characterized as a key regulator in controlling the rate of mitochondrial β-oxidation (Kerner et al., 2000; Li et al., 2007).

Pigs fed BET diets also increased concentration of AdipR1 mRNA compared to those fed CTRL and RAC+BET. The AdipR1 receptor is the main adiponectin receptor expressed in muscle tissue, and its activation is related to AMPK activation intensifying lipid degradation pathways (Patel, 2019). This could indicate BET diet favored the use of fatty acids as energy source in LD. However, pigs fed BET diet had lower HSL gene expression, the gene responsible for stimulating lipolysis.

Thus, with the increase in IMF concentration in pigs fed BET diet it is evident although betaine acts by modulating enzymes related to synthesis and degradation, the final balance is positive for IMF accumulation. Therefore, these molecular results of betaine signaling in lipogenesis, lipolysis and β-oxidation pathways can help to understand the IMF accumulation observed in those pigs. These results are supported by the reports of Li et al. (2017a) where pigs supplemented with betaine had increased expression of lipogenic enzymes related to lipid degradation, but phenotypically an accumulation of intramuscular lipids in LD was observed.

Betaine and ractopamine may have their effects altered according to the metabolism of the muscle fibers where they act. Thus, the expressions of the same genes as for LD were also assessed in the oxidative metabolism muscle tissue represented by the SL. In SL muscle BET diet increased gene expression of factor C/EBPα and LPL, indicating the effect of betaine may

be less intense in muscles with oxidative metabolism. On the other hand, pigs fed RAC had lower gene expression of the transcription factor PPARγ compared to CTRL and BET diets, showing different effect from that on the glycolytic muscle. Possibly the effect observed only in the oxidative muscle was due to the higher content of triglycerides in this muscle type, in addition to the higher concentration of β-adreno receptors present in the oxidative muscles (Parr et al., 2016).

According to Costa-Lima et al. (2015) ractopamine increases the abundance of carbonic anhydrase-3 in pig muscle. Carbonic anhydrase-3 decreases adipogenic and lipogenic activity through down-regulation of the PPAR-γ (Mitterberger et al., 2012). Indicating in muscles with oxidative metabolism, ractopamine can reduce adipocyte hyperplasia and hypertrophy.

Regarding lipolysis and β-oxidation, pigs fed RAC and the RAC+BET had higher PPARα gene expression than those fed BET. PPARα is a regulator of the transcription of genes involved in mitochondrial β-oxidation (Pawlak et al., 2015; Montagner et al., 2016) and its higher expression may reflect an increase in β-oxidation.

The greater expression of PPARα in the muscle of RAC+BET fed pigs may be related to the greater abundance of AdipR2 receptor mRNA observed in these pigs. The increase in the expression of AdipR2 is associated with PPARα activation, triggering β-oxidation signaling (Mao et al., 2006; Kim & Park, 2019).

The increased expression of PPARα in pigs fed RAC and RAC+BET diets may indicate a greater capacity to use lipids as energy source in this muscle tissue. Reiter et al. (2007) supplemented ractopamine for finishing pigs and reported that ractopamine increased the gene expression of acyl-CoA dehydrogenase (ACDH) in muscle. This enzyme is involved with the entry of fatty acids for mitochondrial β-oxidation, suggesting that ractopamine is able to increase the use of fatty acids as energy source in muscle tissue.

The modulation of energy metabolism by ractopamine, such as reduced lipogenesis and increased β-oxidation in muscle can improve the efficiency of energy use, increasing the energy available for muscle deposition. However, it can negatively impact IMF content (Reiter et al., 2007; Martins et al., 2015). However, in the present study ractopamine supplementation did not have a negative influence on IMF concentration.

Pigs fed BET had greater expression of AdipR1 compared to those fed RAC. AdipR1 is related to activation of AMPK, and consequently stimulates the lipolysis and β-oxidation pathways. This indicates that betaine can act in lipid metabolism regardless of the type of muscle fiber; however, apparently, the effect is more evident in glycolytic fibers.

In subcutaneous adipose tissue (AT) betaine and ractopamine are described as capable of reducing the thickness of porcine backfat. Thus, the present study sought to identify possible alterations in the expression of genes related to lipid synthesis and degradation pathways in subcutaneous AT.

The pigs fed RAC and RAC+BET diets had lower gene expression of the lipogenic enzyme ACC. RAC+BET diet also promotes decreased LPL expression in relation to BET diet. In addition, pigs fed RAC+BET diet had lower gene expression transcription factor C/EBPα in relation to CTRL, indicating a synergistic effect of the additives in reducing lipogenesis. This can be confirmed by the phenotypic results, where only the RAC+BET diet reduced the backfat thickness of pigs compared to CTRL diet.

Different reports in the literature indicate that ractopamine can reduce lipogenesis in pig subcutaneous AT. Reiter et al. (2007) and Halsey et al. (2011) supplemented pigs with ractopamine and reported reduced gene expression of the lipogenic fatty acid synthase (FAS) enzyme in subcutaneous AT. Halsey et al. (2011) also reported reduced gene expression of sterol regulatory element-binding transcription factor 1 (SREBP-1), a factor that is involved with the activity of lipogenic enzymes such as ACC and FAS (Kim & Spiegelman 1996; Shimano et al., 1999). However, the supply of the RAC diet in the present study, despite having reduced the expression of ACC, did not promote reduction in the backfat thickness compared to CTRL diet.

The BET diet, on the other hand, did not alter the expression of genes related to lipogenesis in subcutaneous AT, indicating its effect on backfat thickness reported in the literature may be due to pathways other than those analyzed in this study.

The variation in backfat thickness is orchestrated by lipid turnover, which is the balance between the rate of lipid synthesis and degradation (Chilliard, 1993). Thus, analyzing the effects of ractopamine and betaine on lipid degradation pathways in subcutaneous AT is essential to increase the understanding the action of these additives.

Regarding the genes related to lipolysis and β-oxidation, the pigs fed BET had higher expression of CPT-1 compared to the CTRL. Furthermore, BET, RAC and RAC+BET diets were efficient in increasing the abundance of HSL mRNA in AT of pigs compared to CTRL. The increase in expression of genes encoding enzymes related to β-oxidation and lipolysis may have been mediated by the higher expression of PPARα observed in AT of pigs fed BET, RAC and RAC+BET diets.

Specifically, the increase in CPT-1 expression observed in the subcutaneous AT of pigs fed BET diet may be due to an alteration in the expression of other genes, such as the increased

expression of PPARα which can induce greater activity of CPT-1 (Song et al., 2010) and the greater expression of the ADIP R1 receptor compared to pigs fed RAC, indicating greater potential of response to adiponectin binding. According to Li et al. (2007) the binding of adiponectin to ADIP R1 through AMPK signaling cascade may increase CPT-1 gene expression. This could explain the greater gene expression of CPT-1 observed in BET fed pigs.

The HSL enzyme acts in the hydrolysis of triglycerides, releasing free fatty acids and glycerol. Huang et al. (2006) also reported the effect of betaine on HSL activity in pig subcutaneous AT, with supplemented pigs having higher enzyme activity, indicating consistency in the lipolytic effect of betaine in subcutaneous AT of pigs.

Overall, the results indicate that the effect of betaine on lipogenesis is of slight relevance and its effect in adipose tissue is primarily related to increased lipolysis and β-oxidation. On the other hand, ractopamine and the association of both additives showed a potential effect in reducing lipogenesis and increasing the gene expression of lipolytic and β-oxidative enzymes, indicating greater potential of pathways for the action of these additives.

6. CONCLUSION

Ractopamine improves growth rate, feed conversion rate, loin muscle area and carcass yield of pigs from 90 to 140 kg. When combined, 20 ppm of ractopamine and 2.5 g/kg of betaine elicit a significant decrease on backfat thickness. Betaine improves the loin muscle area and pork quality by decreasing WBSF and increasing intramuscular fat content. The accumulation of intramuscular fat may be due, in part, by the increase of mRNA expression of enzymes related with adipogenesis and lipogeneses in muscle tissue with more evident effect in glycolytic muscle. Regarding adipose tissue, ractopamine and betaine increase mRNA expression of genes related to lipolysis and β-oxidation, and their association resulted in a more evident effect in decrease mRNA expression of genes related to adipogenesis and lipogenesis.

7. ACKNOWLEDGEMENTS

This work was supported by CNPq – *Conselho Nacional de Desenvolvimento Científico e Tecnológico*, CAPES – *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*, INCT-CA - *Instituto Nacional de Ciência e Tecnologia de Ciência Animal.*

8. REFERENCES

Aaslyng, M. D., Bejerholm, C., Ertbjerg, P., Bertram, H. C., & Andersen, H. J. (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. Food quality and preference, 14(4), 277-288.

Adeola, O., Ball, R. O., & Young, L. G. (1992). Porcine skeletal muscle myofibrillar protein synthesis is stimulated by ractopamine. The Journal of nutrition, 122(3), 488-495.

Albuquerque, A., Neves, J. A., Redondeiro, M., Laranjo, M., Felix, M. R., Freitas, A., ... & Martins, J. M. (2017). Long term betaine supplementation regulates genes involved in lipid and cholesterol metabolism of two muscles from an obese pig breed. Meat Science,124, 25-33.

Almeida, V. V., Nuñez, A. J. C., Schinckel, A. P., Ward, M. G., Andrade, C., Sbardella, M., ... & Miyada, V. S. (2015). Gene expression of beta-adrenergic receptors and myosin heavy chain isoforms induced by ractopamine feeding duration in pigs not carrying the ryanodine receptor mutation. Livestock Science, 172, 91-95.

AMSA. (2015). Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. 2 ed. American Meat Science Association, Champaign, IL.

AOAC. (2007). Official Methods of Analysis 18th ed. rev. 2. Association of Official Analytical Chemists. Washington, DC USA.

Apple, J. K., Rincker, P. J., McKeith, F. K., Carr, S. N., Armstrong, T. A., & Matzat, P. D. (2007). Meta-analysis of the ractopamine response in finishing swine. The Professional Animal Scientist, 23(3), 179-196.

Bahelka, I., Bučko, O., Stupka, R., Čítek, J., & Hozáková, K. (2020). Meat quality, chemical and fatty acids composition and oxidative stability of pork from entire males, surgical castrates and gilts after betaine supplementation to diet. Biotechnology in Animal Husbandry, 36(2), 139-154.

Bauman, D. E. (1976, September). Intermediary metabolism of adipose tissue. In Federation proceedings (Vol. 35, No. 11, pp. 2308-2313).

Bendall, J. R., & Swatland, H. J. (1988). A review of the relationships of pH with physical aspects of pork quality. Meat Science, 24(2), 85-126.

Bodine, S. C., Latres, E., Baumhueter, S., Lai, V. K. M., Nunez, L., Clarke, B. A., ... & Glass, D. J. (2001). Identification of ubiquitin ligases required for skeletal muscle atrophy. Science, 294(5547), 1704-1708.

Bost, F., Aouadi, M., Caron, L., Even, P., Belmonte, N., Prot, M., ... & Binétruy, B. (2005). The extracellular signal–regulated kinase isoform ERK1 is specifically required for in vitro and in vivo adipogenesis. Diabetes, 54(2), 402-411.

Brewer, M. S., Zhu, L. G., Bidner, B., Meisinger, D. J., & McKeith, F. K. (2001). Measuring pork color: effects of bloom time, muscle, pH and relationship to instrumental parameters. Meat Science, 57(2), 169-176.

Bridi, A. M. (2006). Métodos de avaliação da carcaça e da carne suína. Midiograf.

Brustolini, A. P. L., Rodrigues, L. A., Silva, F. C. O., Peloso, J. V., Aldaz, A., Junior, M. B. C., ... & Fontes, D. O. (2019). Interactive effects of feed allowance and ractopamine supplementation on growth performance and carcass traits of physically and immunologically castrated heavy weight pigs. Livestock Science, 228, 120-126.

Bush, J. A., Wu, G., Suryawan, A., Nguyen, H. V., & Davis, T. A. (2002). Somatotropin-induced amino acid conservation in pigs involves differential regulation of liver and gut urea cycle enzyme activity. The Journal of nutrition, 132(1), 59-67.

Bustillos, P., Joaquín, R., Olmedo, G., Eduardo, J., & Quisirumbay-Gaibor, J. R. (2018). Efecto de la inclusión alimenticia de betaína en cerdos en fase de finalización. LA GRANJA. Revista de Ciencias de la Vida, 28(2), 123-150.

Carr, J. (2006). The maintenance of health. In: I. Kyriazakis and C Whittemore, editors, Whittemore's science and practice of pig production. 3th ed. Blackwell Publishing, Oxford, UK, p. 263-316.

Chilliard, Y. (1993). Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: a review. Journal of Dairy Science, 76(12), 3897-3931.

Cho, K. H., Kim, M. J., Jeon, G. J., & Chung, H. Y. (2011). Association of genetic variants for FABP3 gene with back fat thickness and intramuscular fat content in pig. Molecular biology reports, 38(3), 2161-2166.

Costa-Lima, B. R., Suman, S. P., Li, S., Beach, C. M., Silva, T. J., Silveira, E. T., ... & Boler, D. D. (2015). Dietary ractopamine influences sarcoplasmic proteome profile of pork Longissimus thoracis. Meat Science, 103, 7-12.

Dunshea, F. R., Cadogan, D. J., & Partridge, G. G. (2008). Dietary betaine and ractopamine combine to increase lean tissue deposition in finisher pigs, particularly gilts. Animal Production Science, 49(1), 65-70.

Dunshea, F. R., King, R. H., Campbell, R. G., Sainz, R. D., & Kim, Y. S. (1993). Interrelationships between sex and ractopamine on protein and lipid deposition in rapidly growing pigs. Journal of Animal Science, 71(11), 2919-2930.

Ferreira, M. S. S., Araújo, T. S., Alves, A. C., Porto, L. C. J., Schinckel, A. P., Rambo, Z. J., ... & Sousa, R. V. (2017). Ractopamine with dietary lysine concentrations above basal

requirements of finishing barrows improves growth performance, carcass traits and modifies the mTor signalling pathway. Animal Production Science, 57(8), 1682-1691.

Grunert, K. G., Sonntag, W. I., Glanz-Chanos, V., & Forum, S. (2018). Consumer interest in environmental impact, safety, health and animal welfare aspects of modern pig production: Results of a cross-national choice experiment. Meat Science, 137, 123-129.

Gunawan, A. M., Richert, B. T., Schinckel, A. P., Grant, A. L., & Gerrard, D. E. (2007). Ractopamine induces differential gene expression in porcine skeletal muscles. Journal of Animal Science, 85(9), 2115-2124.

Halsey, C. H. C., Weber, P. S., Reiter, S. S., Stronach, B. N., Bartosh, J. L., & Bergen, W. G. (2011). The effect of ractopamine hydrochloride on gene expression in adipose tissues of finishing pigs. Journal of Animal Science, 89(4), 1011-1019.

Huang, Q. C., Han, X. Y., Xu, Z. R., Yang, X. Y., Chen, T., & Zheng, X. T. (2009). Betaine suppresses carnitine palmitoyltransferase I in skeletal muscle but not in liver of finishing pigs. Livestock Science, 126(1-3), 130-135.

Huang, Q. C., Xu, Z. R., Han, X. Y., & Li, W. F. (2006). Changes in hormones, growth factor and lipid metabolism in finishing pigs fed betaine. *Livestock Science, 105*, 78-85.

Hwang, Y. H., Lee, S. J., Lee, E. Y., & Joo, S. T. (2020). Effects of carcass weight increase on meat quality and sensory properties of pork loin. Journal of Animal Science and Technology, 62(5), 753.

Jiang, J. F., Xu, Z. R., Wang, Y. Z., Han, X. Y., & Wang, L. (2006). Postnatal expression pattern of the C/EBP alpha gene in porcine subcutaneous adipose tissue. Journal of Animal and Feed Sciences, 15(1), 61.

Karamucki, T., Jakubowska, M., Rybarczyk, A., Szaruga, R., Gardzielewska, J., & Natalczyk-Szymkowska, W. (2006). Relationship between CIE L^* a^* b^* and CIE L^* C^* h scale colour parameters determined when applying illuminant C and observer 2^ o and illuminant D65 and observer 10^ o and proximate chemical composition and quality traits of porcine longissimus lumborum muscle. Polish Journal of Food and Nutrition Sciences, 15(2), 129.

Kathirvel, E., Morgan, K., Nandgiri, G., Sandoval, B. C., Caudill, M. A., Bottiglieri, T., ... & Morgan, T. R. (2010). Betaine improves nonalcoholic fatty liver and associated hepatic insulin resistance: a potential mechanism for hepatoprotection by betaine. American Journal of Physiology-Gastrointestinal and Liver Physiology, 299(5), G1068-G1077.

Kerner, J., & Hoppel, C. (2000). Fatty acid import into mitochondria. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1486(1), 1-17.

Kim, J. B., & Spiegelman, B. M. (1996). ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes & development, 10(9), 1096-1107.

Kim, Y., & Park, C. W. (2019). Mechanisms of adiponectin action: implication of adiponectin receptor agonism in diabetic kidney disease. International journal of molecular sciences, 20(7), 1782.

Kline, W. O., Panaro, F. J., Yang, H., & Bodine, S. C. (2007). Rapamycin inhibits the growth and muscle-sparing effects of clenbuterol. Journal of applied physiology, 102(2), 740-747.

Kohn, R. A., Dinneen, M. M., & Russek-Cohen, E. (2005). Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. Journal of Animal Science, 83(4), 879-889.

Koopman, R., Ryall, J. G., Church, J. E., & Lynch, G. S. (2009). The role of β-adrenoceptor signaling in skeletal muscle: therapeutic implications for muscle wasting disorders. Current Opinion in Clinical Nutrition & Metabolic Care, 12(6), 601-606.

Lefterova, M. I., Zhang, Y., Steger, D. J., Schupp, M., Schug, J., Cristancho, A., ... & Lazar, M. A. (2008). PPARγ and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. Genes & development, 22(21), 2941-2952.

Li, L., Wu, L., Wang, C., Liu, L., & Zhao, Y. (2007). Adiponectin modulates carnitine palmitoyltransferase-1 through AMPK signaling cascade in rat cardiomyocytes. Regulatory peptides, 139(1-3), 72-79.

Li, S., Wang, H., Wang, X., Wang, Y., & Feng, J. (2017a). Betaine affects muscle lipid metabolism via regulating the fatty acid uptake and oxidation in finishing pig. Journal of Animal Science and Biotechnology, 8(1), 1-9.

Li, Y. J., Li, J. L., Zhang, L., Gao, F., & Zhou, G. H. (2017b). Effects of dietary starch types on growth performance, meat quality and myofibre type of finishing pigs. Meat Science, 131, 60-67.

Lindahl, G., Henckel, P., Karlsson, A. H., & Andersen, H. J. (2006). Significance of early postmortem temperature and pH decline on colour characteristics of pork loin from different crossbreeds. Meat Science, 72(4), 613-623.

Lipiński, K., Szramko, E., Jeroch, H., & Matusevičius, P. (2012). Effects of betaine on energy utilization in growing pigs-A review. Annals of Animal Science, 12(3), 291-300.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods, 25(4), 402-408.

Lothong, M., Tachampa, K., Assavacheep, P., & Angkanaporn, K. (2016). Effects of dietary betaine supplementation on back fat thickness and serum IGF-1 in late finishing pigs. The Thai Journal of Veterinary Medicine, 46(3), 427.

Lynch, G. S., & Ryall, J. G. (2008). Role of β-adrenoceptor signaling in skeletal muscle: implications for muscle wasting and disease. Physiological Reviews, 88(2), 729-767.

Madeira, M. S., Alfaia, C. M., Costa, P., Lopes, P. A., Martins, S. V., Lemos, J. P. C., ... & Prates, J. A. M. (2015). Effect of betaine and arginine in lysine-deficient diets on growth, carcass traits, and pork quality. Journal of Animal Science, 93(10), 4721-4733.

Madeira, M. S., Rolo, E. A., Alfaia, C. M., Pires, V. R., Luxton, R., Doran, O., ... & Prates, J. A. (2016). Influence of betaine and arginine supplementation of reduced protein diets on fatty acid composition and gene expression in the muscle and subcutaneous adipose tissue of cross-bred pigs. British Journal of Nutrition, 115(6), 937-950.

Mao, X., Kikani, C. K., Riojas, R. A., Langlais, P., Wang, L., Ramos, F. J., ... & Dong, L. Q. (2006). APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. Nature cell biology, 8(5), 516-523.

Marcolla, C. S., Holanda, D. M., Ferreira, S. V., Rocha, G. C., Serão, N. V. L., Duarte, M. S., ... & Saraiva, A. (2017). Chromium, CLA, and ractopamine for finishing pigs. Journal of Animal Science, 95(10), 4472-4480.

Maribo, H., Olsen, E. V., Barton-Gade, P., Møller, A. J., & Karlsson, A. (1998). Effect of early post-mortem cooling on temperature, pH fall and meat quality in pigs. Meat Science, 50(1), 115-129.

Martins, D. D. S., Soares, M. A., & Steffens, J. (2015). Qualidade da carcaça e rendimento de cortes suínos com o uso de ractopamina. Ciência Rural, 45(8), 1503-1508.

Mills, S. E. (2002). Biological basis of the ractopamine response. Journal of Animal Science, 80(E-suppl_2), E28-E32.

Mitterberger, M. C., Kim, G., Rostek, U., Levine, R. L., & Zwerschke, W. (2012). Carbonic anhydrase III regulates peroxisome proliferator-activated receptor-γ2. Experimental cell research, 318(8), 877-886.

Montagner, A., Polizzi, A., Fouché, E., Ducheix, S., Lippi, Y., Lasserre, F., ... & Guillou, H. (2016). Liver PPARα is crucial for whole-body fatty acid homeostasis and is protective against NAFLD. Gut, 65(7), 1202-1214.

Moody, D. E., Hancock, D. L., & Anderson, D. B. (2000). Phenethanolamine repartitioning agents. Farm Animal Metabolism and Nutrition, 65-96.

O'Hea, E. K., & Leveille, G. A. (1969). Significance of adipose tissue and liver as sites of fatty acid synthesis in the pig and the efficiency of utilization of various substrates for lipogenesis. The Journal of nutrition, 99(3), 338-344.

Park, B. C., & Lee, C. (2011). Feasibility of increasing the slaughter weight of finishing pigs. Journal of Animal Science and Technology, 53(3), 211-222.

Park, J., Campbell, C. P., Squires, E. J., de Lange, C. F. M., & Mandell, I. B. (2018). Effects of pig genotype, immunological castration, and use of ractopamine on growth performance, carcass traits, and pork quality for entire male pigs. Canadian Journal of Animal Science, 99(1), 82-106.

Parr, T., Mareko, M. H., Ryan, K. J., Hemmings, K. M., Brown, D. M., & Brameld, J. M. (2016). The impact of growth promoters on muscle growth and the potential consequences for meat quality. Meat Science, 120, 93-99.

Patel, N. J. (2019). Role of adiponectin and its target receptors to control deposition of fat in obesity related disorders. Obesity Medicine, 16, 100148.

Pawlak, M., Lefebvre, P., & Staels, B. (2015). Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. Journal of hepatology, 62(3), 720-733.

Reiter, S. S., Halsey, C. H., Stronach, B. M., Bartosh, J. L., Owsley, W. F., & Bergen, W. G. (2007). Lipid metabolism related gene-expression profiling in liver, skeletal muscle and adipose tissue in crossbred Duroc and Pietrain pigs. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 2(3), 200-206.

Rickard, J. W., Wiegand, B. R., Pompeu, D., Hinson, R. B., Gerlemann, G. D., Disselhorst, R., ... & Allee, G. L. (2012). The effect of corn distiller's dried grains with solubles, ractopamine, and conjugated linoleic acid on the carcass performance, meat quality, and shelf-life characteristics of fresh pork following three different storage methods. Meat Science, 90(3), 643-652.

Rostagno, H.S.; Albino, L.F.T.; Hannas, M.I, Donzele J. L. ..., Brito C. O. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. Viçosa: UFV, 2017. 488p.

Sales, J. (2011). A meta-analysis of the effects of dietary betaine supplementation on finishing performance and carcass characteristics of pigs. Animal Feed Science and Technology, 165(1-2), 68-78.

Schrama, J. W., Heetkamp, M. J. W., Simmins, P. H., & Gerrits, W. J. J. (2003). Dietary betaine supplementation affects energy metabolism of pigs. Journal of Animal Science, 81(5), 1202-1209.

See, M. T., Armstrong, T. A., & Weldon, W. C. (2004). Effect of a ractopamine feeding program on growth performance and carcass composition in finishing pigs. Journal of Animal Science, 82(8), 2474-2480.

Serão, N. V. L., Veroneze, R., Ribeiro, A. M. F., Verardo, L. L., Braccini Neto, J., Gasparino, E., ... & Guimarães, S. E. F. (2011). Candidate gene expression and intramuscular fat content in pigs. Journal of Animal Breeding and Genetics, 128(1), 28-34.

Shimano, H., Yahagi, N., Amemiya-Kudo, M., Hasty, A. H., Osuga, J. I., Tamura, Y., ... & Yamada, N. (1999). Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. Journal of Biological Chemistry, 274(50), 35832-35839.

Song, S., Attia, R. R., Connaughton, S., Niesen, M. I., Ness, G. C., Elam, M. B., ... & Park, E. A. (2010). Peroxisome proliferator activated receptor α (PPARα) and PPAR gamma coactivator (PGC-1α) induce carnitine palmitoyltransferase IA (CPT-1A) via independent gene elements. Molecular and cellular endocrinology, 325(1-2), 54-63.

Southgate, R. J., Neill, B., Prelovsek, O., El-Osta, A., Kamei, Y., Miura, S., ... & Febbraio, M. A. (2007). FOXO1 regulates the expression of 4E-BP1 and inhibits mTOR signaling in mammalian skeletal muscle. Journal of Biological Chemistry, 282(29), 21176-21186.

Souza, M. F., Donzele, J. L., Silva, F. C. O., Donzele, R. F. M. O., Cardoso, E. F., Braga, D. A. M., ... & Maurício, T. V. (2018). Effect of ractopamine on digestible-lysine requirement for finishing barrows under thermoneutral conditions. Animal Production Science, 59(3), 531-542.

Takahashi, N., Goto, T., Taimatsu, A., Egawa, K., Katoh, S., Kusudo, T., ... & Kawada, T. (2009). Bixin regulates mRNA expression involved in adipogenesis and enhances insulin sensitivity in 3T3-L1 adipocytes through PPARγ activation. Biochemical and biophysical research communications, 390(4), 1372-1376.

Tontonoz, P., Hu, E., & Spiegelman, B. M. (1994). Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. Cell, 79(7), 1147-1156.

Uttaro, B. E., Ball, R. O., Dick, P., Rae, W., Vessie, G., & Jeremiah, L. E. (1993). Effect of ractopamine and sex on growth, carcass characteristics, processing yield, and meat quality characteristics of crossbred swine. Journal of Animal Science, 71(9), 2439-2449.

Van Laack, R. L. J. M., Stevens, S. G., & Stalder, K. J. (2001). The influence of ultimate pH and intramuscular fat content on pork tenderness and tenderization. Journal of Animal Science, 79(2), 392-397.

Waguespack, A. M., Powell, S., Roux, M. L., Fruge, E. D., Bidner, T. D., Payne, R. L., & Southern, L. L. (2011). Effect of determining baseline plasma urea nitrogen concentrations on subsequent posttreatment plasma urea nitrogen concentrations in 20- to 50-kilogram pigs. Journal of Animal Science, 89(12), 4116-4119.

Wang, H., Li, S., Xu, S., & Feng, J. (2020). Betaine improves growth performance by increasing digestive enzymes activities, and enhancing intestinal structure of weaned piglets. Animal Feed Science and Technology, 267, 114545.

Warner, R. D., Greenwood, P. L., Pethick, D. W., & Ferguson, D. M. (2010). Genetic and environmental effects on meat quality. Meat Science, 86(1), 171-183.

Warriss, P. D. (1982). The relationship between pH 45 and drip in pig muscle. International Journal of Food Science & Technology, 17(5), 573-578.

Watanabe, G., Motoyama, M., Nakajima, I., & Sasaki, K. (2018). Relationship between water-holding capacity and intramuscular fat content in Japanese commercial pork loin. Asian-Australasian journal of animal sciences, 31(6), 914.

Wismer Pedersen, J. (1959). Quality of pork in relation to rate of pH change post mortem. Journal of Food Science, 24(6), 711-727.

Wood, J., & Whittemore, C. (2006). Pig meat and carcass quality. Whittemore's Science and Practice of Pig Production, 4-64.

Wu, F., Vierck, K. R., DeRouchey, J. M., O'Quinn, T. G., Tokach, M. D., Goodband, R. D., ... & Woodworth, J. C. (2017). A review of heavy weight market pigs: Status of knowledge and future needs assessment. Translational Animal Science, 1(1), 1.

Wu, W., Wang, S., Xu, Z., Wang, X., Feng, J., Shan, T., & Wang, Y. (2018). Betaine promotes lipid accumulation in adipogenic-differentiated skeletal muscle cells through ERK/PPARγ signaling pathway. Molecular and cellular biochemistry, 447(1), 137-149.

Xiao, R. J., Xu, Z. R., & Chen, H. L. (1999). Effects of ractopamine at different dietary protein levels on growth performance and carcass characteristics in finishing pigs. Animal Feed Science and Technology, 79(1-2), 119-127.

Xue, W., Wang, W., Jin, B., Zhang, X., & Xu, X. (2015). Association of the ADRB3, FABP3, LIPE, and LPL gene polymorphisms with pig intramuscular fat content and fatty acid composition. Czech Journal of Animal Science, 60(2), 60-6.

Yang, H. S., Lee, J. I., Joo, S. T., & Park, G. B. (2009). Effects of dietary glycine betaine on growth and pork quality of finishing pigs. Asian-Australasian Journal of Animal Sciences, 22(5), 706-711.

Yu, D. Y., Xu, Z. R., & Li, W. F. (2004). Effects of betaine on growth performance and carcass characteristics in growing pigs. Asian-australasian journal of animal sciences, 17(12), 1700-1704.

Zhong, Y., Yan, Z., Song, B., Zheng, C., Duan, Y., Kong, X., ... & Li, F. (2021). Dietary supplementation with betaine or glycine improves the carcass trait, meat quality and lipid metabolism of finishing mini-pigs. Animal Nutrition, 7(2), 376-383.

Table 1- Composition of experimental diets

Ingredient, %	CTRL	BET	RAC	RAC+BET
Corn	72.325	72.325	72.325	72.325
Soybean meal	20.000	20.000	20.000	20.000
Soybean oil	3.550	3.550	3.550	3.550
Dicalcium phosphate	0.950	0.950	0.950	0.950
Limestone	0.830	0.830	0.830	0.830
Inert clay filler	1.200	0.860	0.595	0.255
Salt	0.355	0.355	0.355	0.355
L-lysine HCl, 98.5%	0.230	0.230	0.435	0.435
DL – methionine, 99.0%	0.050	0.050	0.145	0.145
L – threonine, 98.5%	0.035	0.035	0.155	0.155
L – tryptophan, 98.0%	0.015	0.015	0.045	0.045
L – valine, 96.5%	---	---	0.055	0.055
Mineral premix[1]	0.200	0.200	0.200	0.200
Vitaminic premix[2]	0.200	0.200	0.200	0.200
Antibiotic[3]	0.050	0.050	0.050	0.050
Betaine HCl[4]	0.000	0.340	0.000	0.340
Ractopamine[5]	---	---	0.100	0.100
Antioxidant[6]	0.010	0.010	0.010	0.010

[1]Content per kilogram of product: Fe as iron sulfate (15.0g), Cu as copper sulfate (40.0g), I as calcium iodate (350.0 mg), Zn as zinc oxide (25.0 g), Mn as manganese sulfate (13.0 g).

[2]Content per kg of product: folic acid (125.00 mg), pantothenic acid (4,000 mg),bioin (12.50 mg),niacin (825.00 mg),selenium (75.00 mg),vitamin B6 (250.00 mg),vitamin B2 (1,350.00 mg),vitamin B1 (250.00 mg),vitamin A (2,100,000 U.I.),vitamin B 12 (6,000.00 mcg),vitamin D3 (350,000 U.I.),vitamin E (5.000 U.I.),vitamin K3 (850.00 mg).

[3]Provided per kilogram of diet: 3250 g tylosin (Tylan G250, Elanco, São Paulo, SP, Brasil).

[4]725.00 g betaine/kg (Shandong Aocter, Chemical Co., Ltd, Liaocheng, China).

[5]20 g ractopamine/kg (Ractomax, Sauvet, Campinas, SP, Brasil).

[6]865g butylated hydroxytoluene/kg, 15g ethoxyquin/kgand 7g butylated hydroxyanisole/kg(Banox 100, Alltech Inc., Nicholasville, KY).

Table 2 – Calculated nutritional composition of experimental diets[1]

Item[2]	Diet[3]			
	CTRL	BET	RAC	RAC + BET
ME, kcal/kg	3.350	3.350	3.350	3.350
Crude protein, %	15.00	15.00	15.44	15.44
SID Lys, %	0.811	0.811	0.973	0.973
SID Met + Cys, %	0.487	0.487	0.584	0.584
SID Thr, %	0.527	0.527	0.632	0.632
SID Trp, %	0.162	0.162	0.195	0.195
SID Val, %	0.623	0.623	0.671	0.671
SID Iso, %	0.546	0.546	0.546	0.546
Sodium, %	0.158	0.158	0.158	0.158
Calcium, %	0.545	0.545	0.545	0.545
Available phosphorus, %	0.269	0.269	0.269	0.269

[1] Values calculated according to Rostagno et al. (2017).

[2] SID = standardized ileal digestible

[3] CTRL = control diet; BET = CTRL + 2.5 g/kg de betaine; RAC = 20 ppm ractopamine diet; RAC + BET = RAC + 2.5 g/kg betaine.

Table 3 - Oligonucleotides used on gene expression analysis of female pigs fed different additives

Genes	GenBank no.	Sequence	Size, bp
B2M	NM_213978	F:5′-TATCTGGGTTCCATCCG-3’ R:5′-AACTATCTTGGGCTTATCG-3’	197
GAPDH	AF017079	F:5′-CCTTCCGTGTCCCTACTGC-3’ R:5′-CATCAAAGGTAGAAGAGTGAGTGTC-3’	195
CPT1	NM_001007191.1	F:5′-GGACGAGGAGTCTCACCACTATGAC-3’ R:5′- TCTTGAACGCGATGAGGGTGA-3’	128
PPARγ	NM_214379	F:5’-GTGGAGACCGCCCAGGTTTG-3’ R:5′-GGGAGGACTCTGGGTGGTTCA-3’	108
PPARα	NM_001044526.1	F:5′-GGCTTACGGCAATGGCTTCA-3′ R:5′-CGGTCTCCGCACCAAATGA-3′	168
C/EBPα	AF103944	F:5′-CGTGGAGACTCAACAGAAGG-3’ R: 5′-GCAGCGTGTCCAGTTCGCGG-3′	95
ACC	EF618729	F:5′- CTCCAGGACAGCACAGATCA-3 AGGACAGCACAGATCA-3 AGGACAGCACAGATCA-3 F: 5-ATG TTT CGG CAGTCC CTG AT-3’ R:5′-TGT GGA CCA GCTGAC CTT GA-3’	133
LPL	NM_214286.1	F:5’- CCCTATACAAGAGGGAACCGGAT-3’ R:5’- CCGCCATCCAGTCGATAAACGT-3’	138
FAT/CD36	NM_001044622.1	F:5’- CTGGTGCTGTCATTGGAGCAGT-3’ R:5’- CTGTCTGTAAACTTCCGTGCCTGTT-3’	160
FABP3	NM_001099931.1	F:5’- CCAACATGACCAAGCCTACCACA-3’ R:5′- ACAAGTTTGCCTCCATCCAGTGT-3’	176
ADIP	AY135647	F:5’- GGAGATACAGGTCTTACTGGTCCTA-3’ R:5′- CAGGAATGTTGCAGTGGAATTTGCCA-3’	262
AdipoR1	NM_001007193	F:5’-GACCATGCTCAGACCAAATATGTATTTCA-3’ R:5′-AACGAGTAATAGAGCCAGGGGACGAAGCT-3’	222
AdipoR2	NM_001007192	F:5′- CAAACATCTCCTTTGTGGCCC-3’ R:5′- CAAGTAGATGAAGCAAGGTTGCGGGTTA-3’	242
HSL	AJ000482	F:5′-GCT CCC ATC GTCAAG AAT C-3′ R:5′TAA AGC GAA TGCGGT CC-3′	112

β-2-Microglobulin (B2M); Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*); Carnitine palmitoyl transferase-1(*CPT-1*); Peroxisome proliferator activated gamma receptor (PPARγ); Peroxisome proliferator activated receptor alpha (PPARα); CAAT*/enhancer binding protein α* (C/EBPα); Acetyl-CoA carboxylase (ACC); Lipoprotein lipase (LPL); Translocase fatty acid/cluster differentiation (FAT/CD36);
fatty acid binding protein 3 (*FABP3*); Adiponectin (ADIP); Adiponectin receptor 1 (AdipR1); Adiponectin receptor 2 (AdipR2); Hormone sensitive lipase (HSL).

Table 4 – Performance of female pigs fed different additives

Item[2]	Diet[1]				SEM[3]	P-value
	CTRL	BET	RAC	RAC+BET		
IBW, kg	88.98	88.94	88.90	89.03	0.573	0.99
ADG, kg	1.025[b]	1.056[b]	1.238[a]	1.266[a]	0.035	<0.001
ADFI, kg	2.777	2.754	2.856	2.825	0.035	0.75
F:G	2.67[b]	2.69[b]	2.37[a]	2.24[a]	0.027	<0.001
FBW, kg	134.12[b]	135.86[b]	144.68[a]	146.00[a]	0.849	<0.001

[a,b]Means lacking a common uppercase superscript differ (P < 0.05) using Tukey test.

[1] IBW = initial body weight; ADG = average daily gain; ADFI = average daily feed intake; F:G = feed conversion ratio; FBW = final body weight.

[2] CTRL = control diet; BET = CTRL + 2.5 g/kg de betaine; RAC = 20 ppm ractopamine diet; RAC + BET = RAC + 2.5 g/kg betaine.

[3] Standard error of the mean.

Table 5 – Concentration of serum metabolites in female pigs fed different additives

Item[2]	Diet[1]				SEM[3]	P-value
	CTRL	BET	RAC	RAC+BET		
CHO, mg/dL	83.89	90.00	91.00	83.22	1.766	0.33
TG, mg/dL	31.67	33.33	31.00	30.33	1.262	0.87
SUN, mg/dL	25.44[a]	21.11[ab]	22.44[ab]	18.22[b]	0.812	0.04

[a,b] Means lacking a common uppercase superscript differ (P < 0.05) using Tukey test.

[1] CTRL = control diet; BET = CON + 2.5 g/kg de betaine; RAC = 20 ppm ractopamine diet; RAC + BET = RAC + 2.5 g/kg betaine.

[2] CHO = total cholesterol; TG = triglycerides; SUN = serum urea nitrogen.

[3] Standard error of the mean.

Table 6 – Carcass traits of female pigs fed different additives

Item[2]	Diet[1]				SEM[3]	P-value
	CTRL	BET	RAC	RAC+BET		
HCW, kg	113.08[b]	113.13[b]	120.71[a]	118.44[ab]	0.831	<0.001
CY, %	81.56[b]	82.65[ab]	83.74[a]	83.82[a]	0.263	0.02
LMA, cm²	58.71[b]	67.02[a]	73.33[a]	73.38[a]	1.045	<0.001
BF, mm	16.86[b]	13.83[ab]	14.54[ab]	10.61[a]	0.504	<0.01

[a,b] Means lacking a common uppercase superscript differ (P < 0.05) using Tukey test.

[1] CTRL = control diet; BET = CTRL + 2.5 g/kg de betaine; RAC = 20 ppm ractopamine diet; RAC + BET = RAC + 2.5 g/kg betaine.

[2] HCW = hot carcass weight; CY = carcass yield; LMA = loin muscle area; BF = backfat thickness.

[3] Standard error of the mean.

Table 7 – Pork quality parameters evaluated on *Longissimus dorsi* of female pigs fed different additives

Item[2]	Diet[1]				SEM[3]	P-value
	CTRL	BET	RAC	RAC+BET		
TL, %	5.98	7.22	7.40	6.85	0.50	0.32
CL, %	18.86[a]	17.69[ab]	15.46[b]	15.92[b]	0.76	<0.01
SL, %	23.70	24.28	22.28	21.69	0.97	0.10
WBSF, N	43.84[a]	35.99[b]	42.56[a]	42.36[a]	0.19	<0.001
*L**	54.22	53.39	54.00	54.71	0.24	0.32
*a**	7.28	7.35	6.84	6.59	0.16	0.33
*b**	13.77	13.58	13.26	13.34	0.12	0.51
IMF, %	1.20[b]	1.75[a]	1.42[ab]	1.28[ab]	0.06	0.02

[a,b] Means lacking a common uppercase superscript differ (P < 0.05) using Tukey test.

[1] CTRL = control diet; BET = CTRL + 2.5 g/kg de betaine; RAC = 20 ppm ractopamine diet; RAC + BET = RAC + 2.5 g/kg betaine.

[2] TL = thaw water losses; CL = cooking water losses; SL = sum of water losses; WSBF = Warner Bratzler shear force; IMF = intramuscular fat content.

[3] Standard error of the mean.

Table 8 – Gene expression in *Longissimus dorsi* of female pigs fed different additives

Item[3]	Diet[1]					
	CTRL	BET	RAC	RAC+BET	SEM[2]	P-value
PPARγ	1.947[b]	2.659[a]	1.885[b]	2.539[ab]	0.08	<0.01
C/EBPα	5.960[b]	10.294[a]	6.420[b]	9.801[a]	0.41	0.01
FABP3	1.088[b]	1.455[a]	1.253[ab]	1.459[a]	0.04	0.03
LPL	2.337[ab]	3.278[a]	1.507[b]	2.033[b]	0.16	<0.01
FAT/CD 36	2.222[ab]	2.705[a]	1.497[b]	1.936[ab]	0.11	0.01
PPARα	2.505	3.074	3.595	2.718	0.17	0.17
CPT-1	3.058[b]	4.500[a]	2.250[b]	3.070[b]	0.17	<0.001
HSL	14.899[a]	6.136[b]	12.944[a]	13.782[a]	0.49	<0.001
Adiponectin	13.024	15.364	14.499	13.420	0.45	0.30
ADIP R1	2.041[b]	4.184[a]	2.718[ab]	2.237[b]	0.21	0.01
ADIP R2	12.493	7.750	11.538	9.066	0.62	0.06

[a,b] Means lacking a common uppercase superscript differ (P < 0.05) using Tukey test.

[1] CTRL = control diet; BET = CON + 2.5 g/kg de betaine; RAC = 20 ppm ractopamine diet; RAC + BET = RAC + 2.5 g/kg betaine.

[2] Standard error of the mean.

[3]Peroxisome proliferator activated gamma receptor (PPARγ); CAAT/*enhancer binding protein α* (C/EBPα); Fatty acid binding protein 3 (*FABP3*); Lipoprotein lipase (LPL); Translocase fatty acid/cluster differentiation (FAT/CD36); Peroxisome proliferator activated receptor alpha (PPARα); Carnitine palmitoyl transferase-1(*CPT-1*); Hormone sensitive lipase (HSL); Adiponectin; Adiponectin receptor 1 (Adip R1); Adiponectin receptor 2 (Adip R2).

Table 9 - Gene expression in soleus muscle of pigs fed diets containing different additives

Item[3]	Diet[1]				SEM[2]	P-value
	CTRL	BET	RAC	RAC+BET		
PPARγ	1.978[a]	2.087[a]	1.044[b]	1.811[ab]	0.11	0.01
C/EBPα	7.856[b]	11.251[a]	7.097[b]	10.319[ab]	0.51	0.03
FABP3	2.092	2.608	2.380	2.184	0.13	0.55
LPL	2.532[b]	3.484[a]	2.577[ab]	3.008[ab]	0.12	0.03
FAT/CD 36	3.154[ab]	3.446[a]	2.107[b]	2.570[ab]	0.15	0.03
PPARα	7.607[ab]	4.660[b]	7.793[a]	8.239[a]	0.38	0.01
CPT-1	12.817	14.168	13.785	14.054	0.27	0.34
HSL	11.080	8.688	11.624	10.787	0.73	0.56
Adiponectin	11.340	14.221	11.356	11.891	0.53	0.22
AdipR1	14.652[ab]	15.440[a]	12.569[b]	13.890[ab]	0.32	0.03
AdipR2	6.697[b]	5.697[b]	4.430[b]	10.660[a]	0.46	<0.001

[a,b] Means lacking a common uppercase superscript differ (P < 0.05) using Tukey test.

[1] CTRL = control diet; BET = CON + 2.5 g/kg de betaine; RAC = 20 ppm ractopamine diet; RAC + BET = RAC + 2.5 g/kg betaine.

[2] Standard error of the mean.

[3]Peroxisome proliferator activated gamma receptor (PPARγ); CAAT/*enhancer binding protein α* (C/EBPα); fatty acid binding protein 3 (*FABP3*); Lipoprotein lipase (LPL); Translocase fatty acid/cluster differentiation (FAT/CD36); Peroxisome proliferator activated receptor alpha (PPARα); Carnitine palmitoyl transferase-1(*CPT-1*); Hormone sensitive lipase (HSL); Adiponectin; Adiponectin receptor 1 (Adip R1); Adiponectin receptor 2 (Adip R2).

Table 10 – Gene expression in adipose tissue of female pigs fed different additives

Item[3]	Diet[1]				SEM[2]	P-value
	CTRL	BET	RAC	RAC+BET		
PPARγ	1,247	1,499	1,345	1,308	0,05	0,31
C/EBPα	2,171[a]	2,171[a]	2,010[ab]	1,617[b]	0,07	0,02
FAT/CD 36	1,404	1,541	1,689	1,385	0,04	0,09
FABP3	1,575	1,933	1,980	1,880	0,06	0,08
ACC	3,884[a]	4,323[a]	2,659[b]	2,446[b]	0,14	<0,001
LPL	2,184[ab]	2,442[a]	1,949[ab]	1,765[b]	0,07	0,01
CPT-1	1,232[b]	1,748[a]	1,713[ab]	1,495[ab]	0,06	0,04
HSL	2,278[b]	2,852[a]	2,743[a]	2,766[a]	0,04	<0,001
Adiponectin	2,268	2,686	2,213	2,519	0,08	0,16
AdipR1	2,719[ab]	3,200[a]	2,645[b]	2,738[ab]	0,06	0,03
AdipR2	3,746	4,2852	3,735	3,420	0,11	0,07
PPARα	1,052[b]	1,709[a]	1,439[a]	1,430[a]	0,04	<0,001

[a,b] Means lacking a common uppercase superscript differ (P < 0.05) using Tukey test.

[1]CTRL = control diet; BET = CON + 2.5 g/kg de betaine; RAC = 20 ppm ractopamine diet; RAC + BET = RAC + 2.5 g/kg betaine.

[2] Standard error of the mean.

[3]Peroxisome proliferator activated gamma receptor (PPARγ); CAAT/*enhancer binding protein α* (C/EBPα); Translocase fatty acid/cluster differentiation (FAT/CD36); Fatty acid binding protein 3 (*FABP3*); Acetyl-CoA carboxylase (ACC); Lipoprotein lipase (LPL); Carnitine palmitoyl transferase-1(*CPT-1*); Hormone sensitive lipase (HSL); Adiponectin; Adiponectin receptor 1 (Adip R1); Adiponectin receptor 2 (Adip R2); Peroxisome proliferator activated receptor alpha (PPARα).

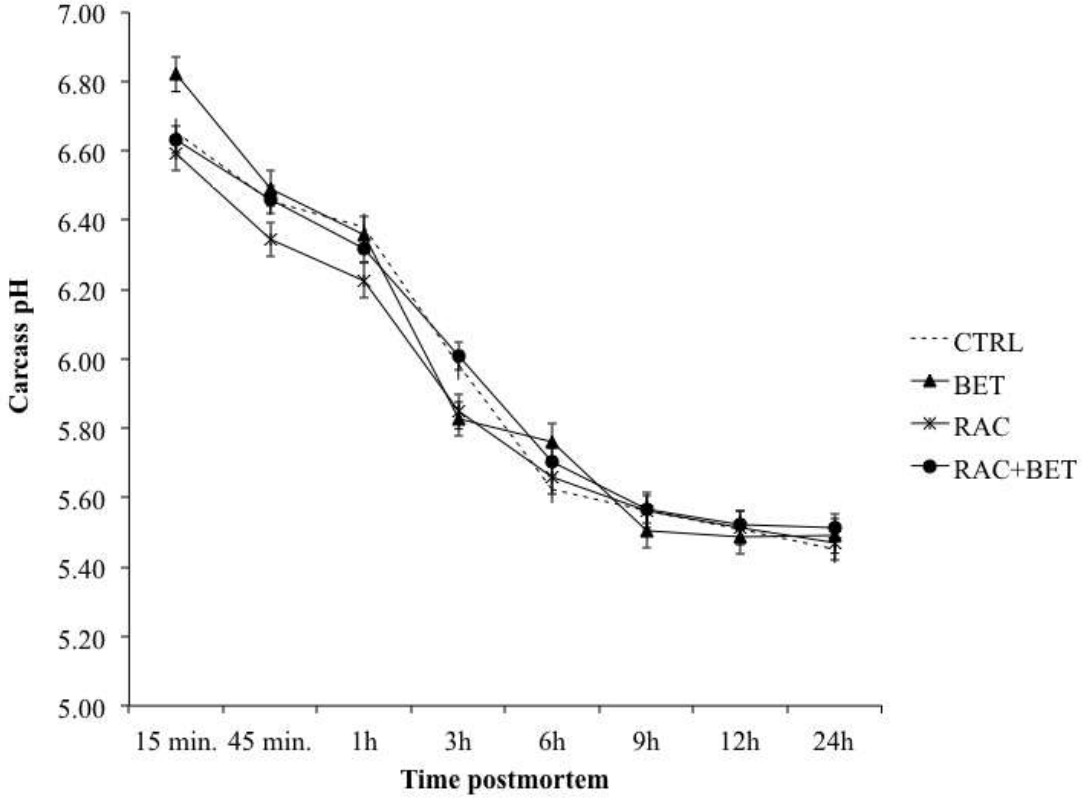

Figure 1- Average carcass pH values in the first 24 h after slaughter of female pigs fed different additives. CTRL = control diet, BET = CON + 2.5 g/kg de betaine, RAC = 20 ppm ractopamine diet, RAC + BET = RAC + 2.5 g/kg betaine.

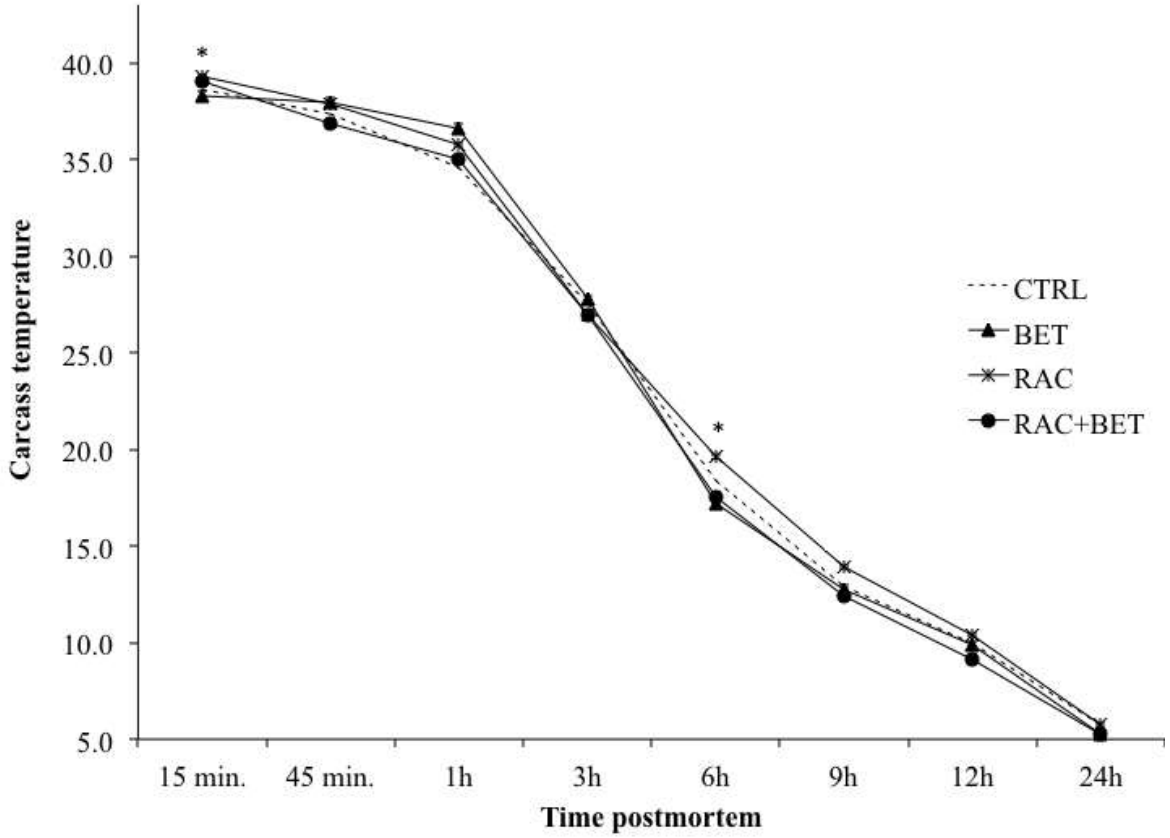

Figure 2 – Average temperature values in the first 24 h after slaughter of female pigs fed different additives. CTRL = control diet, BET = CON + 2.5 g/kg de betaine, RAC = 20 ppm ractopamine diet, RAC + BET = RAC + 2.5 g/kg betaine.

* $P < 0,05$.

CHAPTER 2: Performance, carcass traits, pork quality and expression of genes related to lipid metabolism in muscle tissues of two diverse genetic lines of pigs

Marcos Henrique Soares [a, b], Gustavo de Amorim Rodrigues [a, b], Dante Teixeira Valente Júnior [a, b], Ronaldo Lopes Cunha Júnior [a], Gabriel Cipriano Rocha [a, b], Marcio de Souza Duarte [a, b], Alysson Saraiva [a, b]

[a] Department of Animal Sciences, Universidade Federal de Viçosa, Viçosa, MG, 36570-900, Brazil;

[b] Muscle Biology and Nutrigenomics Laboratory, Universidade Federal de Viçosa, Viçosa, MG, 36570-900, Brazil;

Corresponding author: Alysson Saraiva**,** alysson.saraiva@ufv.br

1. ABSTRACT

To evaluate performance, carcass traits, pork quality and expression of genes related to lipid metabolism in glycolytic and oxidative muscle tissues of two divergent genetic lines of pigs. Eighteen female pigs Agroceres PIC 337 (Large White x Landrace x Duroc x Pietrain) x Camborough (Large White x Landrace) (Hybrid), with initial body weight of 88.96 ± 3.44 kg and 12 female pigs DB LQ 1250 (Duroc) x DB 90 (Large White x Landrace) (Duroc), with initial body weight of 85.63 ± 1.55 kg were used. Pigs were allotted in a completely randomized design in two groups: Hybrid and Duroc with nine and six replicates, respectively, and two pigs per experimental unit, represented by the pen. Performance of pigs from both genetic groups did not differ ($P > 0.05$). However, Duroc pigs had lower ($P < 0.03$) backfat thickness. The meat of Duroc pigs had higher ($P = 0.04$) L* value (brightness), lower ($P = 0.03$) a* value (redness) and higher ($P < 0.001$) intramuscular fat concentration. Duroc pigs had higher expression of the peroxisome proliferator-activated receptor gamma gene (PPARγ, $P = 0.008$) and fatty acid protein translocase/cluster differentiation (FAT/CD36, $P = 0.002$), and higher mRNA abundance of hormone sensitive lipase (HSL, $P = 0.01$) and Adiponectin (ADIP, $P = 0.002$) in *Longissimus Dorsi.* Greater expression of PPARγ ($P = 0.009$) and FAT/CD36 ($P = 0.02$) was observed in the soleus muscle of Duroc pigs, without changes in the expression of enzymes related to lipid degradation compared to Hybrid pigs. Duroc pigs had carcasses with lower backfat thickness and better meat quality, evidenced by the higher percentage of intramuscular fat content compared to the Hybrid pigs. Possibly, the higher concentration of intramuscular fat was mainly due to the higher expression of transcription factors and enzymes related to adipogenesis and lipogenesis in glycolytic and oxidative skeletal muscle tissues.

Keywords: Genetic line; Growth performance; Meat quality; Lipid metabolism.

2. INTRODUCTION

Over decades, the genetic improvement of pigs has focused on increasing weight gain, muscle deposition, reduction of feed conversion and backfat thickness (Van Wijk et al., 2005; Miar et al., 2014). However, in addition to the reduction in backfat thickness, genetic selection also resulted in a progressive decrease in intramuscular fat, which is directly related to pork quality (Wood & Warriss, 1992; Zappaterra et al., 2016).

High quality meat is perceived by consumers for its juiciness, tenderness, flavor and moderate concentration of intramuscular fat, associated with a pinkish-red color and stable pH (Ngapo & Gariépy, 2008; Ciobanu et al., 2011; Aaslyng & Meinert, 2017).

Thus, in order to add economic value to pork, meat quality-related traits began to be included in pig breeding programs, along with existing selection traits (Dransfield et al., 2005; Khanal et al., 2019).

The choice of the paternal line can determine carcass traits and meat quality (Aymerich et al., 2019). There are different strategies for the formation of paternal lines in order to maintain the zootechnical gains associated with improved meat quality and carcass traits, two of which are the use of selected pure breeds, such as Duroc or the use of hybrids, such as double hybrid composed by Large White, Landrace, Duroc and Pietrain breeds (Aymerich et al., 2019; Zhang et al., 2020).

The Duroc breed is commonly recognized for providing greater tenderness and marbling to the meat, being associated with high-quality meat (Jeleníková et al., 2008; Nakev & Popova, 2020), but with lower performance than hybrid pigs. Hybrids based on Large White, Landrace, Duroc and Pietrain breeds have high growth rate and muscle deposition (Augspurger et al., 2002), but with lower meat quality compared to Duroc. Possibly due to the negative correlation existing between high growth rate and meat quality (de Vries et al., 1994; Bidanel & Ducos, 1995).

However, there is little comparative information between the progeny of these genetic lines; in addition, little is known how the different lineages are related to lipid metabolism in skeletal muscle tissue, making it difficult to understand the molecular mechanisms related to deposition of intramuscular fat in these animals.

Therefore, it was hypothesized the use Duroc breed as paternal line provides performance and carcass traits similar to hybrid paternal line (Hybrid), but with better meat quality, and the evaluation of expression of genes related to lipid metabolism in muscle tissues can elucidate the possible mechanisms of action involved. Our objective was to evaluate performance, carcass traits, pork quality and expression of genes related to lipid metabolism in glycolytic and oxidative muscle tissues of two divergent genetic lines of pigs.

3. MATERIAL AND METHODS

All methods involving the handling of pigs followed the ethical principles of animal research (CONCEA) and were approved by the Commission of Ethics in the Use of Production Animals (CEUAP) of the *Universidade Federal de Viçosa* (protocol 013/2018).

3.1. Animals, experimental design and diets

Eighteen female pigs (Large White x Landrace x Duroc x Pietrain) x (Large White x Landrace) (HYB), with initial body weight of 88.96 ± 3.44 kg and 12 female pigs Duroc x (Large White x Landrace) (DUR), with initial body weight of 85.63 ± 1.55 kg were used.

Pigs were allotted in a completely randomized design in two groups: HYB and DUR with nine and six replicates, respectively, and two pigs per experimental unit, represented by the pen. Were housed in 2.30 × 2.10 m concrete-floored pens with a dry feeder and a nipple drinker each. Pigs had free access to feed and water throughout the 45-d feeding trial. Temperature and humidity inside the barn were recorded every 3 h using a data logger. During the experimental period, average temperature inside the barn was 22.04± 2.66ºC and average humidity was 79.47 ± 0.1%.

Diets, phase 1 and phase 2 (Tables 1 and 2) were formulated based on corn and soybean meal and supplemented with minerals and vitamins to meet the requirements of the pigs, according to Rostagno et al. (2017), except for SID lysine in phase 2, that were supplemented with ractopamine. SID lysine was increased by 20% considering the increase in the protein requirement of pigs fed diets with ractopamine (Xiao et al., 1999, Marcolla et al., 2017). Industrial amino acids were added maintaining the ratios with SID lysine recommended by Rostagno et al. (2017).

The experimental period was divided into two phases: a phase 1 (0 to 17 days) and phase 2 (18 to 45 days).

During the trial, feed was weighed before feeding and feed wastage and leftovers were manually collected and daily weighed to determine average daily feed intake (ADFI). At the

end of the experimental period, piglets were individually weighed to determine final body weight (BW), average daily gain (ADG), and feed:gain ratio (F:G).

3.2. Blood sampling and serological analysis

At the end of the trial pigs were submitted to a 14-hour fasting with free access to water. After fasting, blood was collected in 10 mL tubes without anticoagulants by puncturing the orbital sinus using hypodermic needles (40 x 1.6 mm) to determine the concentration of serum urea nitrogen (SUN), triglycerides (TG) and total cholesterol (CHO). Samples were immediately sent at room temperature to the Viçosa clinical laboratory. SUN concentration was evaluated using the automated enzymatic method, whereas the concentration of TG and CHO was determined using the enzymatic colorimetric method (Ureal Cobas c 311, Roche Diagnostics GmbH, Basel, Switzerland).

3.3. Slaughter procedures and tissue sampling

After blood sampling, one pig from each experimental unit with body weight closest to 140 kg, was stunned by electronarcosis and exsanguinated.

At slaughter, samples of *Longissimus dorsi* (LD) and soleus muscle (SM) were collected gene expression analysis were collected immediately after exsanguination, snap-frozen in liquid nitrogen and stored at -80°C for further nucleic acids extraction. All instruments used for sampling were sterilized and adequately cleaned with RNAse Exterminator (Protech Technology Enterprise Co., Ltd.) to avoid nucleic acid degradation.

After slaughter, carcasses were weighed to determine hot carcass weight (HCW) and carcass yield (CY), according to the formula: hot carcass weight divided by the final pre-slaughter weight multiplied by 100. The carcasses were divided longitudinally and refrigerated at 5°C for 24h. All measurements (pH, temperature, backfat thickness and loin muscle area) and sampling were performed on the left half of each carcass. The temperature and pH of Longissimus dorsi (LD) were measured at 15 min, 45 min, 1h, 3h, 6h, 9h, 12h and 24h after slaughter, using a pH T meter with penetration probe and thermometer coupled (Testo SE & Co., Lenzkirch, FR, Germany) inserted into the LD at the level of the last lumbar vertebra.

For meat quality, a 20 cm sample was collected after postmortem chill of 24 h. Samples were obtained from the LD of each right half of the carcasses, between the tenth rib and the first lumbar vertebra. Upon collection, LD samples received a craniocaudal identification and then vacuum packed, stored at -20°C for 24 hours and then sectioned into five 2.54 cm thick chops. The 2.54 cm thick chops were individually vacuum packed, identified according to the

pig and the position in the muscle from which they originated and stored at -20°C for further analysis (Bridi, 2006).

The LD and Soleus muscle samples were stored in duplicate in cryotubes with 1.8 mL capacity, instantly frozen in liquid nitrogen and stored in an ultra-freezer at -80ºC for further analysis.

3.4. Carcass traits

After 24h cooling half carcasses were divided at the height of the 10th rib, and the backfat thickness over the LD (6 cm away from the midline) was measured using a digital caliper. To determine loin muscle area (LMA), the muscular surface of the LD between the 10th and 11th rib was covered with a polyethylene sheet and contoured using a permanent fine-tipped marker. The sheets were digitally scanned and colored. Colored areas within the contour were measured using image analysis software (ImageJ version 1.49 t, National Institutes of Health, Bethesda, MD). HCW and CY were obtained soon after slaughter, as described above.

3.5. Pork quality

For water losses determination frozen LD samples were removed from the plastic bags, weighed on a semi-analytical scale and placed to thaw at 4°C for 16h. After thawing, the samples were gently wiped dry using a paper towel and weighed. Thaw water loss (TL) was considered to be the gravimetric difference between the steaks before and after thawing. For cooking water loss (CL), the thawed chops were vacuum packed and cooked in a digital water bath with a stirrer (WEALAB) at 71ºC for 40 minutes. After this time, the samples were removed from the water bath and placed in an ice bath for 10 minutes to stop cooking. At the end of cooking, the samples were weighed again and the cooking water loss was expressed as a percentage of the weight of the samples before and after cooking. The sum of water losses (SL) was estimated by the difference in weight between the frozen and cooked samples (Bridi, 2006).

After weighing, cooked chops were used to Warner-Bratzler shear force (WBSF) determination as proposed by American Meat Science Association (2016) with minor modifications. From each sample, 6 round cores measuring 1.27 cm in diameter were removed parallel to the longitudinal orientation of the muscle fibers, using a sharp stainless steel coring device. Care was taken to avoid sampling at areas containing visible fat and connective tissue. These round cores were sheared once through the center, perpendicularly to the longitudinal orientation of the muscle fibers, using a V-notch blade with 1.016 mm thickness and 60° angle

at fixed speed of 20 cm/min, coupled to a Warner-Bratzler Shear machine (G-R Electrical Manufacturing Company, Manhattan, KS). The WBSF was determined by the average of 6 measures and expressed in kgf.

For color evaluation, the right half of each carcass were sectioned at tenth rib and the first lumbar vertebra, and allowed to bloom for 30 min. After blooming period, meat color was determined using a handheld spectrophotometer (Hunter MiniScan EZ, 4500L; Hunter Associates Laboratory, Inc., Reston, VA), calibrated against a white and a black tile. The mean L* (lightness), a* (redness), and b* (yellowness) values of each chop were determined as the average from 3 readings on 3 different points of chop surface, using illuminant D65, a 31.8 mm port size and a 10° standard observer (Brewer et al., 2001; Karamucki et al., 2006).

To determine intramuscular fat (IMF) chops (2.54 cm thick) were thawed at 4ºC for 16h and trimmed for visible fat and connective tissues, and then ground in a TURRAX CT-132 tissue homogenizer. A 100 g-sample of each chop was evaluated for IMF using near infrared spectrophotometry (FoodScan, FOSS NIRsystems Inc., Laurel, MD; AOAC, Official method 2007.04; AOAC, 2007).

3.6. RNA extraction and cDNA synthesis

Total RNA extraction was performed from 50 mg of LD and SL muscle samples using Trizol® (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA concentration was estimated using a NanoDropTM Lite spectrophotometer (ThermoFisher Scientific, Beverly, MA, USA), observing the A260/A280 ratios between 1.8 and 2.04 as purity control. The quality and integrity of extracted RNA was verified using 1% agarose gel.

The samples were then treated with DNase and reverse transcribed into cDNA using the Go Script Reverse Transcription (RT) Kit (Promega, Madison, WI), following the manufacturer's recommendations. cDNA samples were stored at -20°C until use in the real-time quantitative PCR reaction.

3.7. Gene expression analysis

Primers (Table 3) for amplification of target and endogenous gene fragments were designed using Primer Quest software (Integrated DNA Technologies Inc., Coralville, IA, USA) from nucleotide sequences obtained from the GenBank database (www.ncbi.nlm.nih.gov). Optimization for each primer was done to calculate efficiency (Livak and Schmittgen, 2001).

The target genes evaluated in LD and in SL were lipoprotein lipase (LPL), fatty acid binding protein 3 (FABP3), translocase fatty acid/cluster differentiation (FAT/CD36), peroxisome proliferator activated gamma receptor (PPARγ), CAAT/enhancer binding protein α (C/EBPα), adiponectin (ADIP), adiponectin receptor 1 (AdipR1), adiponectin receptor 2 (AdipR2), peroxisome proliferator activated receptor alpha (PPARα), hormone sensitive lipase (HSL) and carnitine palmitoyl transferase-1 (CPT-1). As a reference gene for normalization, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used due to the high amplification and stability of this gene among groups.

The RT-qPCR reactions were performed in duplicate using the thermocycler Applied Biosystems™ QuantStudio Real-Time PCR Systems (Applied Biosystems ™ PowerUp™ SYBR™ Green Master Mix) (Applied Biosystems, Foster City, CA, USA) from the method of Relative Quantitation, using as detection fluorophore SYBR® Green from the GoTaq® qPCR Master Mix kit (Promega corporation, Madison, USA). The PCR reactions were submitted to the cycling protocol following the instructions of the SYBR ™ Green Master Mix: 50°C for 2 min, 95°C for 2 min, followed by 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds and 72°C for 1 min.

The threshold cycle (Ct) values obtained were normalized (ΔCt) based on the Ct value of the endogenous control gene in muscle (GAPDH). The calculation of relative levels of gene expression was performed according to the $2^{-\Delta\Delta Ct}$ method described by Livak and Schmittgen (2001).

3.8. Statistical analysis

For performance (FBW, ADFI, ADG and F:G) data analysis the pen was considered the experimental unit, the initial body weight was used as a covariate. Only one pig per pen was considered the experimental unit for the other analysis. For the analysis of carcass traits (HCW, CY, LMA and BF) FBW was used as covariate.

The normality and homogeneity of the residues were verified using the Kolmogorov Smirnov and Bartlett tests, respectively. Statistical analysis was performed using the R software (version 3.4.4; R Core Team 2018). Data were subjected to analysis of variance (ANOVA). Differences were considered at $\alpha = 0.05$.

4. RESULTS

4.1. Performance and serological analysis

There was no effect ($P > 0.05$) final body weight (FBW), average daily feed intake (ADFI) and feed conversion ratio (F:G) between genetic groups (Table 4).

Concentrations of total cholesterol (CHO), triglycerides (TG) and serum urea nitrogen (SUN) were no influenced ($P > 0.05$) by genetic groups (Table 5).

4.2 Carcass traits

There was no difference ($P > 0.05$) on hot carcass weight (HCW), carcass yield (CY) and loin muscle area (LMA) between genetic groups. However, DUR pigs had lower ($P = 0.03$) back fat thickness (BF) compared to HYB (Table 6).

4.3. Carcass pH and temperature

There was no difference ($P > 0.05$) in carcass pH values between genetic groups. Higher ($P = 0.01$) temperature at 6h was observed in carcass of HYB pigs (Table 7).

4.4. Pork quality

The meat of DUR pigs had the highest ($P = 0.04$) L* (brightness) and the lowest ($P < 0.03$) a* (redness) values. The b* (yellowness) value was not influenced ($P > 0.05$) by genetic groups (Table 8).

Warner-Bratzler shear force (WBSF) was not different ($P > 0.01$) between genetic groups. However, DUR pigs had a higher ($P < 0.001$) percentage of IMF.

Thaw water losses (TL), cooking losses (CL) and sum of water losses (SL) did not differ between genetic groups (Table 8).

4.5. mRNA expression in *Longissimus dorsi* muscle

Greater abundance of PPARγ ($P = 0.008$), FAT/CD 36 ($P = 0.002$) and the HSL ($P = 0.01$) and ADIP mRNA ($P = 0.002$) was observed in DUR pigs (Table 9).

4.6. mRNA expression Soleus muscle

Greater abundance of PPARγ ($P = 0.009$) and FAT/CD 36 ($P = 0.02$) mRNA was observed in DUR pigs (Table 10).

5. DISCUSSION

In the present study, no differences were observed in performance and serum variables between genetic groups, however, DUR pigs had lower BF. Possibly this result is due to the

intense selection in modern Duroc breed genotypes, which have a high growth rate with low subcutaneous fat deposition.

The adequate behavior of pH and temperature decrease of the carcass is relevant for the transformation of muscle into quality meat, directly impacting on sanitary aspects such as shelf life and microbiological proliferation, as well as water retention capacity and perception of meat color by consumers (Wismer-Pedersen, 1959; Lindahl et al., 2006).

In this study, although the genetic groups may have different muscle deposition potentials, and thus, diverse concentration of glycogen stored in the muscle tissue no difference was observed in the reduction of carcass pH between both groups, indicating the use of DUR or HYB pigs does not affect the post mortem pH reduction curve.

The carcass temperature reduction curve differed between genetic groups only at 6h after slaughter. However, this variation did not change the pH values of the carcasses.

Although no effect on pH was observed, meat from DUR pigs had higher L* and lower a* values compared to pigs from HYB group, indicating greater luminosity and lower red intensity.

Pigs from both genetic groups consumed ractopamine in the final 28 days of the trial, which may have contributed to increase the proportion of glycolytic muscle fibers in relation to oxidative ones (Almeida et al., 2015). The increase in the proportion of glycolytic fibers is correlated with the increase in meat luminosity values (Ryu and Kim, 2005). Furthermore, glycolytic fibers have lower concentration of myoglobins, resulting in a less intense red color than muscles with high concentration of myoglobins (Bekhit & Faustman, 2005). This fact may be an indication that DUR pigs may be more sensitive to ractopamine supplementation in relation to meat color. In addition, previous studies indicate that meat color is influenced by breed (Li et al., 2013; Zhang et al., 2018; Kim et al., 2020) which could explain the differences observed in the present study.

The quality of meat is perceived by consumers for its visual and sensory traits. Among sensory traits, flavor, juiciness and tenderness are essential to determine meat quality, and a visual trait that is directly related to sensory ones is the intramuscular fat content. Thus, intramuscular fat is very important for the quality of meat, because it mainly intensifies juiciness and flavor (Jeleníková et al., 2008; Hocquette et al., 2010).

In the present study, the meat of DUR pigs had a higher concentration of intramuscular fat. This is in agreement with other studies that showed greater potential of Duroc breed in deposition of intramuscular fat, compared to other strains of pigs (Jeleníková et al., 2008; Kim et al., 2020; Nakev et al., 2020).

Another important sensory variable for meat palatability is tenderness (Warner et al., 2010), measured by the Warner-Bratzler shear force (WBSF). In the present study, WBSF of meat was similar between HYB and DUR pigs, however, there was a trend towards a reduction of WBSF in DUR pigs. This trend may be due to the higher concentration of intramuscular fat observed in DUR pigs, since there is a negative correlation between intramuscular fat content and meat tenderness (Van Laack et al., 2001).

In this work, greater deposition of intramuscular fat was evidenced in DUR pigs compared to HYB pigs. Other studies also indicate the Duroc breed has greater potential for intramuscular fat deposition than other breeds and strains (Suzuki et al., 2003; Jeleníková et al., 2008; Choi et al., 2014).

However, little is known about the differences in the molecular mechanisms associated to intramuscular fat deposition in DUR and HYB pigs.

A greater abundance of PPARγ transcription factor mRNA was observed in the LD in DUR pigs compared to the HYB group. PPARγ is crucial for the processes of adipogenesis and lipogenesis. Thus, its greater abundance in muscle tissue may be associated with the increase in the number and hypertrophy of adipocytes, resulting in the accumulation of intramuscular fat (Takahashi et al., 2009; Wu et al., 2018).

There was also an increase in the gene expression of FAT/CD36 in DUR pigs. FAT/CD36 is a trans-membrane fatty acid transport protein with an important role in lipid accumulation. Possibly, this increase was caused by the increased expression of PPARγ, since the expression of FAT/CD36 is regulated by PPARγ (Glatz & Luiken, 2016).

DUR pigs also showed greater abundance of HSL and ADIP mRNA in the LD, both related to lipolytic and oxidative processes.

Intramuscular fat can be seen as an energy reservoir in muscle tissue (Zhang et al., 2017) and when necessary to mobilize this energy, lipolytic enzymes such as HSL become active and initiate the lipolysis process. Thus, the increased expression of HSL and ADIP may be related to greater use of intramuscular fat as a source of energy in muscle tissue.

The CPT-1 enzyme catalyzes the formation of the Acyl-carnitine complex, which acts on the β-oxidation of long-chain fatty acids. Thus, CPT-1 is characterized as a key regulator in controlling the rate of mitochondrial β-oxidation (Kerner et al., 2000; Li et al., 2007). In the present study, there was a trend ($P = 0.06$) of increased expression of the CPT-1 enzyme in DUR pigs, indicating in these animals, despite the accumulation of intramuscular fat, there was also a higher expression of enzymes related to β-oxidation, which suggests that possibly these pigs use a greater proportion of intramuscular fat as energy source compared to HYB pigs.

The increase of ADIP concentration in skeletal muscle tissue is often associated with increased β-oxidation (Fang & Judd, 2018). However, interestingly, Wang et al. (2020) demonstrated a positive correlation between ADIP mRNA abundance and intramuscular fat content in Chinese Laiwu pigs, which is consistent with what was observed in the present study, suggesting that in DUR pigs the greater expression of ADIP in muscle tissue is due to the increase in intramuscular fat, since ADIP can be produced by adipocytes including intramuscular ones.

In the soleus muscle, mainly oxidative, the gene expression of enzymes and transcription factors related to lipogenesis followed a behavior similar to that observed in LD. Duroc pigs had greater abundance of PPARγ and FAT/CD 36 mRNA in the soleus muscle, indicative of greater lipogenic potential compared to the HYB group.

6. CONCLUSION

Pigs from both genetic groups had similar performance; however, DUR pigs had lower backfat thickness and higher intramuscular fat content. Possibly, the higher concentration of intramuscular fat is mainly due to the higher expression of transcription factors and enzymes related to adipogenesis and lipogenesis in glycolytic and oxidative skeletal muscle tissues.

7. ACKNOWLEDGEMENTS

This work was supported by CNPq – *Conselho Nacional de Desenvolvimento Científico e Tecnológico*, CAPES – *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*, INCT-CA - *Instituto Nacional de Ciência e Tecnologia de Ciência Animal.*

8. REFERENCES

Aaslyng, M. D., & Meinert, L. (2017). Meat flavour in pork and beef–From animal to meal. Meat Science, 132, 112-117.

Almeida, V. V., Nuñez, A. J. C., Schinckel, A. P., Ward, M. G., Andrade, C., Sbardella, M., ... & Miyada, V. S. (2015). Gene expression of beta-adrenergic receptors and myosin heavy chain isoforms induced by ractopamine feeding duration in pigs not carrying the ryanodine receptor mutation. Livestock Science, 172, 91-95.

AMSA. (2015). Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. 2 ed. American Meat Science Association, Champaign, IL.

Augspurger, N. R., Ellis, M., Hamilton, D. N., Wolter, B. F., Beverly, J. L., & Wilson, E. R. (2002). The effect of sire line on the feeding patterns of grow-finish pigs. Applied Animal Behaviour Science, 75(2), 103-114.

Aymerich, P., Gasa, J., Bonet, J., Coma, J., & Solà-Oriol, D. (2019). The effects of sire line, sex, weight and marketing day on carcass fatness of non-castrated pigs. Livestock Science, 228, 25-30.

Bekhit, A. E. D., & Faustman, C. (2005). Metmyoglobin reducing activity. Meat Science, 71(3), 407-439.

Bidanel, J. P., & Ducos, A. (1995). Variabilite et evolution genetique des caracteres mesures dans les stations publiques de controle de performances chez les porcs de race Pietrain. In 27. Journees. ITP.

Brewer, M. S., Zhu, L. G., Bidner, B., Meisinger, D. J., & McKeith, F. K. (2001). Measuring pork color: effects of bloom time, muscle, pH and relationship to instrumental parameters. Meat Science, 57(2), 169-176.

Bridi, A. M. (2006). Métodos de avaliação da carcaça e da carne suína. Midiograf.

Carr, J. (2006). The maintenance of health. In: I. Kyriazakis and C Whittemore, editors, Whittemore's science and practice of pig production. 3th ed. Blackwell Publishing, Oxford, UK, p. 263-316.

Choi, J. S., Lee, H. J., Jin, S. K., Choi, Y. I., & Lee, J. J. (2014). Comparison of carcass characteristics and meat quality between Duroc and crossbred pigs. Korean journal for food science of animal resources, 34(2), 238.

Ciobanu, D. C., Lonergan, S. M., & Huff-Lonergan, E. J. (2011). Genetics of meat quality and carcass traits. The genetics of the pig, 2, 355-389.

De Vries, A. G., Van der Wal, P. G., Long, T., Eikelenboom, G., & Merks, J. W. M. (1994). Genetic parameters of pork quality and production traits in Yorkshire populations. Livestock Production Science, 40(3), 277-289.

Dransfield, E., Ngapo, T. M., Nielsen, N. A., Bredahl, L., Sjöden, P. O., Magnusson, M., ... & Nute, G. R. (2005). Consumer choice and suggested price for pork as influenced by its appearance, taste and information concerning country of origin and organic pig production. Meat Science, 69(1), 61-70.

Fang, H., & Judd, R. L. (2011). Adiponectin regulation and function. Comprehensive Physiology, 8(3), 1031-1063.

Glatz, J. F., & Luiken, J. J. (2017). From fat to FAT (CD36/SR-B2): Understanding the regulation of cellular fatty acid uptake. Biochimie, 136, 21-26.

Hocquette, J. F., Gondret, F., Baeza, E., Medale, F., Jurie, C., & Pethick, D. W. (2010). Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal, 4(2), 303-319.

Jeleníková, J., Pipek, P., & Miyahara, M. (2008). The effects of breed, sex, intramuscular fat and ultimate pH on pork tenderness. European Food Research and Technology, 227(4), 989-994.

Kamyk-Kamieński, P., Babicz, M., Lechowski, J., Soszka, M., & Krasucki, W. (2012). Comparative analysis of reproduction, fattening, and slaughter values of selected hybrid swine breeds. Annales UMCS, Zootechnica, 30(1).

Karamucki, T., Jakubowska, M., Rybarczyk, A., Szaruga, R., Gardzielewska, J., & Natalczyk-Szymkowska, W. (2006). Relationship between CIE L^* a^* b^* and CIE L^* C^* h scale colour parameters determined when applying illuminant C and observer 2^ o and illuminant D65 and observer 10^ o and proximate chemical composition and quality traits of porcine longissimus lumborum muscle. Polish Journal of Food and Nutrition Sciences, 15(2), 129.

Kerner, J., & Hoppel, C. (2000). Fatty acid import into mitochondria. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1486(1), 1-17.

Khanal, P., Maltecca, C., Schwab, C., Gray, K., & Tiezzi, F. (2019). Genetic parameters of meat quality, carcass composition, and growth traits in commercial swine. Journal of Animal Science, 97(9), 3669-3683.

Kim, J. A., Cho, E. S., Jeong, Y. D., Choi, Y. H., Kim, Y. S., woo Choi, J., ... & Sa, S. J. (2020). The effects of breed and gender on meat quality of Duroc, Pietrain, and their crossbred. Journal of Animal Science and Technology, 62(3), 409.

Li, L., Wu, L., Wang, C., Liu, L., & Zhao, Y. (2007). Adiponectin modulates carnitine palmitoyltransferase-1 through AMPK signaling cascade in rat cardiomyocytes. Regulatory Peptides, 139(1-3), 72-79.

Li, Y. X., Cabling, M. M., Kang, H. S., Kim, T. S., Yeom, S. C., Sohn, Y. G., ... & Seo, K. S. (2013). Comparison and correlation analysis of different swine breeds meat quality. Asian-Australasian Journal of Animal Sciences, 26(7), 905.

Lindahl, G., Henckel, P., Karlsson, A. H., & Andersen, H. J. (2006). Significance of early postmortem temperature and pH decline on colour characteristics of pork loin from different crossbreeds. Meat Science, 72(4), 613-623.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods, 25(4), 402-408.

Marcolla, C. S., Holanda, D. M., Ferreira, S. V., Rocha, G. C., Serão, N. V. L., Duarte, M. S., ... & Saraiva, A. (2017). Chromium, CLA, and ractopamine for finishing pigs. Journal of Animal Science, 95(10), 4472-4480.

Miar, Y., Plastow, G., Bruce, H., Moore, S., Manafiazar, G., Kemp, R., ... & Wang, Z. (2014). Genetic and phenotypic correlations between performance traits with meat quality and carcass characteristics in commercial crossbred pigs. PloS one, 9(10), e110105.

Nakev, J., & Popova, T. (2020). Quality of meat in purebred pigs involved in crossbreeding schemes. I. Chemical composition and quality characteristics of m. Longissimus thoracis. Bulgarian Journal of Agricultural Science, 26, 894-898.

Ngapo, T. M., & Gariepy, C. (2008). Factors affecting the eating quality of pork. Critical Reviews in Food Science and Nutrition, 48(7), 599-633.

Rostagno, H.S.; Albino, L.F.T.; Hannas, M.I, Donzele J. L. ..., Brito C. O. (2017). Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. Viçosa: UFV. 488p.

Ryu, Y. C., & Kim, B. C. (2005). The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. Meat Science, 71(2), 351-357.

Suzuki, K., Shibata, T., Kadowaki, H., Abe, H., & Toyoshima, T. (2003). Meat quality comparison of Berkshire, Duroc and crossbred pigs sired by Berkshire and Duroc. Meat Science, 64(1), 35-42.

Takahashi, N., Goto, T., Taimatsu, A., Egawa, K., Katoh, S., Kusudo, T., ... & Kawada, T. (2009). Bixin regulates mRNA expression involved in adipogenesis and enhances insulin sensitivity in 3T3-L1 adipocytes through PPARγ activation. Biochemical and biophysical research communications, 390(4), 1372-1376.

Van Laack, R. L. J. M., Stevens, S. G., & Stalder, K. J. (2001). The influence of ultimate pH and intramuscular fat content on pork tenderness and tenderization. Journal of Animal Science, 79(2), 392-397.

Van Wijk, H. J., Arts, D. J. G., Matthews, J. O., Webster, M., Ducro, B. J., & Knol, E. F. (2005). Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. Journal of Animal Science, 83(2), 324-333.

Wang, H., Wang, J., Yang, D. D., Liu, Z. L., Zeng, Y. Q., & Chen, W. (2020). Expression of lipid metabolism genes provides new insights into intramuscular fat deposition in Laiwu pigs. Asian-Australasian Journal of Animal Sciences, 33(3), 390.

Warner, R. D., Greenwood, P. L., Pethick, D. W., & Ferguson, D. M. (2010). Genetic and environmental effects on meat quality. Meat Science, 86(1), 171-183.

Wismer-Pedersen, J. (1959). Some Observations on the Quality of Cured Bacon in Relation to Ante-Mortem Treatment: I. Results of a sugar feeding experiment. Acta Agriculturae Scandinavica, 9(1), 69-90.

Wood, J. D., & Warriss, P. D. (1992). The influence of the manipulation of carcass composition on meat quality.

Wu, W., Wang, S., Xu, Z., Wang, X., Feng, J., Shan, T., & Wang, Y. (2018). Betaine promotes lipid accumulation in adipogenic-differentiated skeletal muscle cells through ERK/PPARγ signaling pathway. Molecular and cellular biochemistry, 447(1), 137-149.

Xiao, R. J., Xu, Z. R., & Chen, H. L. (1999). Effects of ractopamine at different dietary protein levels on growth performance and carcass characteristics in finishing pigs. Animal Feed Science and Technology, 79(1-2), 119-127.

Zappaterra, M., Deserti, M., Mazza, R., Braglia, S., Zambonelli, P., & Davoli, R. (2016). A gene and protein expression study on four porcine genes related to intramuscular fat deposition. Meat Science, 121, 27-32.

Zhang, J., Chai, J., Luo, Z., He, H., Chen, L., Liu, X., & Zhou, Q. (2018). Meat and nutritional quality comparison of purebred and crossbred pigs. Animal Science Journal, 89(1), 202-210.

Zhang, R., Lin, Y., Zhi, L., Liao, H., Zuo, L., Li, Z., & Xu, Y. (2017). Expression profiles and associations of adiponectin and adiponectin receptors with intramuscular fat in Tibetan chicken. British Poultry Science, 58(2), 151-157.

Table 1- Diets composition

Ingredient, %	Phase 1	Phase 2
Corn	72.325	72.325
Soybean meal	20.000	20.000
Soybean oil	3.550	3.550
Dicalcium phosphate	0.950	0.950
Limestone	0.830	0.830
Inert clay filler	1.200	0.595
Salt	0.355	0.355
L-lysine HCl, 98.5%	0.230	0.435
DL – methionine, 99.0%	0.050	0.145
L – threonine, 98.5%	0.035	0.155
L – tryptophan, 98.0%	0.015	0.045
L – valine, 96.5%	---	0.055
Mineral premix[1]	0.200	0.200
Vitamin premix[2]	0.200	0.200
Antibitotic[3]	0.050	0.050
Ractopamine[4]	---	0.100
Antioxidant[5]	0.010	0.010

[1]Content per kilogram of product: Fe as iron sulfate (15.0g), Cu as copper sulfate (40.0g), I as calcium iodate (350.0 mg), Zn as zinc oxide (25.0 g), Mn as manganese sulfate (13.0 g).

[2]Content per kg of product: folic acid (125.00 mg), pantothenic acid (4,000 mg), bioin (12.50 mg), niacin (825.00 mg), selenium (75.00 mg), vitamin B6 (250.00 mg), vitamin B2 (1,350.00 mg), vitamin B1 (250.00 mg), vitamin A (2,100,000 U.I.), vitamin B 12 (6,000.00 mcg), vitamin D3 (350,000 U.I.), vitamin E (5.000 U.I.), vitamin K3 (850.00 mg).

[3]Provided per kilogram of diet: 3250 g tylosin (Tylan G250, Elanco, São Paulo, SP, Brasil).

[4] 20 g ractopamine/kg (Ractomax, Sauvet, Campinas, SP, Brasil).

[5]865g butylated hydroxytoluene/kg, 15g ethoxyquin/kg and 7g butylated hydroxy anisole/kg (Banox 100, Alltech Inc., Nicholasville, KY).

Table 2 – Diet calculated nutritional composition [1]

Item	Phase 1	Phase 2
ME, kcal/kg	3.350	3.350
Crude protein, %	15.00	15.44
SID Lys, %	0.811	0.973
SID Met + Cys, %	0.487	0.584
SID Thr, %	0.527	0.632
SID Trp, %	0.162	0.195
SID Val, %	0.623	0.671
SID Iso, %	0.546	0.546
Sodium, %	0.158	0.158
Calcium, %	0.545	0.545
Available phosphorus, %	0.269	0.269

[1] Values calculated according to Rostagno et al. (2017).

[2] SID = standardized ileal digestible

Table 3 - Oligonucleotides used on gene expression analysis

Gene	GenBank no.	Sequence	Size, bp
GAPDH	AF017079	F:5′-CCTTCCGTGTCCCTACTGC-3’ R:5′-CATCAAAGGTAGAAGAGTGAGTGTC-3’	195
CPT1	NM_001007191.1	F:5′-GGACGAGGAGTCTCACCACTATGAC-3’ R:5′- TCTTGAACGCGATGAGGGTGA-3’	128
PPARγ	NM_214379	F:5’-GTGGAGACCGCCCAGGTTTG-3’ R:5′-GGGAGGACTCTGGGTGGTTCA-3’	108
PPARα	NM_001044526.1	F:5′-GGCTTACGGCAATGGCTTCA-3′ R:5′-CGGTCTCCGCACCAAATGA-3′	168
C/EBPα	AF103944	F:5′-CGTGGAGACTCAACAGAAGG-3’ R: 5′-GCAGCGTGTCCAGTTCGCGG-3′	95
LPL	NM_214286.1	F:5’- CCCTATACAAGAGGGAACCGGAT-3’ R:5’- CCGCCATCCAGTCGATAAACGT-3’	138
FAT/CD36	NM_001044622.1	F:5’- CTGGTGCTGTCATTGGAGCAGT-3’ R:5’- CTGTCTGTAAACTTCCGTGCCTGTT-3’	160
FABP3	NM_001099931.1	F:5’- CCAACATGACCAAGCCTACCACA-3’ R:5′- ACAAGTTTGCCTCCATCCAGTGT-3’	176
ADIP	AY135647	F:5’- GGAGATACAGGTCTTACTGGTCCTA-3’ R:5′- CAGGAATGTTGCAGTGGAATTTGCCA-3’	262
AdipoR1	NM_001007193	F:5’-GACCATGCTCAGACCAAATATGTATTTCA-3’ R:5′-AACGAGTAATAGAGCCAGGGGACGAAGCT-3’	222
AdipoR2	NM_001007192	F:5′- CAAACATCTCCTTTGTGGCCC-3’ R:5′- CAAGTAGATGAAGCAAGGTTGCGGGTTA-3’	242
HSL	AJ000482	F:5′-GCT CCC ATC GTCAAG AAT C-3′ R:5′TAA AGC GAA TGCGGT CC-3′	112

Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*); Carnitine palmitoyl transferase-1(*CPT-1*); Peroxisome proliferator activated gamma receptor (PPARγ); Peroxisome proliferator activated receptor alpha (PPARα); CAAT*/enhancer binding protein α* (C/EBPα); Lipoprotein lipase (LPL); Translocase fatty acid/cluster differentiation (FAT/CD36); fatty acid binding protein 3 (*FABP3*); Adiponectin (ADIP); Adiponectin receptor 1 (AdipR1); Adiponectin receptor 2 (AdipR2); Hormone sensitive lipase (HSL).

Table 4 – Performance of pigs of different genetic lines

Item[1]	HYB	DUR	SEM[3]	P-value
IBW, kg[2]	88.90	85.63	0.73	0.06
FBW, kg	144.68	143.65	1.54	0.39
ADFI, kg	2.860	3.020	0.05	0.26
ADG, kg	1.238	1.289	0.03	0.38
F:G	2.37	2.37	0.03	0.88

[1] IBW = initial body weight, FBW = final body weight, ADFI = average daily feed intake, ADG = average daily gain, F:G = feed conversion ratio.

[2] IBW was used as a covariate.

[3]Standard error of the mean.

HYB = Hybrid; DUR = Duroc.

Table 5 – Concentration of serum metabolites of pigs of different genetic lines

Item[1]	HYB	DUR	SEM[1]	P-value
CHO, mg/dL	91.00	88.00	2.06	0.50
TG, mg/dL	31.00	27.17	2.15	0.41
SUN, mg/dL	22.44	23.83	1.19	0.59

[1] CHO = total cholesterol; TG = triglycerides; SUN = serum urea nitrogen.

[2] Standard error of the mean.

HYB = Hybrid; DUR = Duroc.

Table 6 – Carcass traits of pigs of different genetic lines

Item[1]	HYB	DUR	SEM[3]	P-value
HCW[2], kg	120.52	116.87	1.05	0.12
CY, %	83.74	82.74	0.38	0.24
BF, mm	13.86	11.49	0.68	0.03
LMA, cm²	73.33	68.89	1.46	0.19

[1] HCW = hot carcass weight; CY = carcass yield; LMA = loin muscle area; BF = backfat thickness.

[2]FBW was used as covariate for hot carcass traits.

[3] Standard error of the mean.

HYB = Hybrid; DUR = Duroc.

Table 7 – Carcass pH and temperature of pigs of different genetic lines

Item	HYB	DUR	SEM[1]	P-value
pH				
15mim	6.593	6.720	0.05	0.19
45h	6.353	6.513	0.04	0.09
1h	6.227	6.487	0.08	0.16
3h	5.848	6.048	0.08	0.28
6h	5.660	5.798	0.06	0.30
9h	5.564	5.532	0.03	0.65
12h	5.503	5.486	0.02	0.66
24h	5.461	5.471	0.01	0.69
Temperature				
15 mim	39.29	38.67	0.17	0.10
45 mim	37.90	36.48	0.38	0.10
1 h	35.77	36.90	0.63	0.41
3 h	26.92	26.18	0.73	0.64
6 h	19.55	17.38	0.37	0.01
9 h	13.92	12.68	0.44	0.21
12 h	10.41	9.52	0.32	0.21
24 h	5.74	5.30	0.19	0.30

[1] Standard error of the mean.

HYB = Hybrid; DUR = Duroc.

Table 8 – Pork quality parameters evaluated on *LD* of pigs of different genetic lines

Item[1]	HYB	DUR	SEM[1]	P-value
TL, %	7.399	6.360	0.43	0.28
CL, %	15.460	15.100	0.52	0.77
SL, %	22.285	20.489	0.47	0.09
WBSF, kgf	4.340	3.926	1.19	0.07
*L**	54.001	56.808	0.59	0.04
*a**	6.836	5.610	0.25	0.03
*b**	13.265	13.532	0.19	0.51
IMF, %	1.446	1.933	0.05	<0.001

[1] TL = thaw water losses; CL = cooking water losses; SL = sum of water losses; WSBF = Warner-Bratzler shear force; IMF = intramuscular fat content.

[2] Standard error of the mean.

HYB = Hybrid; DUR = Duroc.

Table 9 – Gene expression in *LD* of pigs of different genetic lines

Item	HYB	DUR	SEM[1]	P-value
PPARγ	1.236	2.034	0.12	0.008
CEBPα	5.489	5.375	0.52	0.92
FAT/CD 36	1.497	2.817	0.16	0.002
FABP3	1.405	1.198	0.09	0.27
LPL	1.507	2.444	0.26	0.11
HSL	3.471	5.782	0.37	0.01
PPARα	2.053	2.337	0.18	0.47
CPT-1	1.247	1.852	0.14	0.06
ADIP R1	2.336	1.758	0.31	0.39
ADIP R2	8.907	11.608	0.99	0.22
ADIP	1.224	3.475	0.28	0.002

[1] Standard error of the mean.

HYB = Hybrid; DUR = Duroc.

Peroxisome proliferator activated gamma receptor (PPARγ); CAAT/*enhancer binding protein α* (C/EBPα); Translocase fatty acid/cluster differentiation (FAT/CD36); Fatty acid binding protein 3 (*FABP3*); Lipoprotein lipase (LPL); Hormone sensitive lipase (HSL); Peroxisome proliferator activated receptor alpha (PPARα); Carnitine palmitoyl transferase-1(*CPT-1*); Adiponectin receptor 1 (AdipR1); Adiponectin receptor 2 (AdipR2); Adiponectin (ADIP).

Table 10 - Gene expression in SM muscle of pigs of different genetic lines

Item	HYB	DUR	EPM[1]	P-value
PPARγ	1.243	2.310	0.17	0.009
CEBPα	6.008	4.728	1.17	0.61
FAT/CD 36	1.941	2.936	0.18	0.02
FABP3	8.270	9.472	0.32	0.10
LPL	1.603	2.096	0.21	0.28
HSL	8.407	7.026	1.08	0.56
PPARα	13.448	9.345	1.01	0.08
CPT-1	2.768	2.394	0.43	0.69
ADIP R1	13.278	14.956	0.72	0.29
ADIP R2	5.213	7.930	1.18	0.30
ADIP	14.505	11.671	1.32	0.39

[1] Standard error of the mean.

HYB = Hybrid; DUR = Duroc.

Peroxisome proliferator activated gamma receptor (PPARγ); CAAT/*enhancer binding protein α* (C/EBPα); Translocase fatty acid/cluster differentiation (FAT/CD36); Fatty acid binding protein 3 (*FABP3*); Lipoprotein lipase (LPL); Hormone sensitive lipase (HSL); Peroxisome proliferator activated receptor alpha (PPARα); Carnitine palmitoyl transferase-1(*CPT-1*); Adiponectin receptor 1 (AdipR1); Adiponectin receptor 2 (AdipR2); Adiponectin (ADIP).

ANEXO

UNIVERSIDADE FEDERAL DE VIÇOSA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS DE PRODUÇÃO
CEUAP/UFV

Campus Universitário – Viçosa, MG – 36570-900 – Telefone: (31) 3899.3275 – e-mail: ceuap@ufv.br – site: www.ceuap.ufv.br

Viçosa, 06 de Abril de 2018

CERTIFICADO

Certificamos que o projeto intitulado **"Efeito da suplementação da ração com betaína e ractopamina no desempenho, características de carcaça e qualidade de carne de suínos machos castrados em terminação"**, protocolo **nº 013/2018**, sob a responsabilidade de **Alysson Saraiva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo chordata, subfilo vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 8 de outubro de 2008, do decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo conselho nacional de controle da experimentação animal (concea), e foi aprovado pela comissão de ética no uso de animais de produção da universidade federal de viçosa (ceuap-ufv) em reunião de **26 de Mar. de 2018.**

Finalidade: **(X)Pesquisa ( )Ensino**
Vigência do Projeto: de **04 de Jun. de 2018 a 08 de Ago. de 2018**
Espécie/linhagem: **Suínos** ***(Sus scrofa domesticus)*** Nº de animais: **90**
Peso: **70 Kg** Idade: **110 dias** Sexo: **Macho** Origem: **Setor de Suinocultura/DZO/UFV - CNPJ/CPF: 25.944.455/0001-96**

CERTIFICATE

We certify that the project entitled **"Effects of betaine and ractopamine on growth performance, carcass traits, and pork quality of finishing pigs"**, protocol **nº 013/2018**, under the responsibility of **Alysson Saraiva** - which involves the production, maintenance and/or use of animals belonging to the phylum chordata, subphylum vertebrata (except man), for scientific research purposes (or education) - is in accordance with the law nº. 11.794, of October 8, 2008, Decree nº. 6899 of July 15, 2009, and the rules issued by the Brazilian National Council for Animal Experimentation Control (CONCEA), and was approved by the Ethics Commission on the use of farm animals of Universidade Federal de Viçosa (CEUAP-UFV) in its meeting on **Mar, 26th, 2018.**

Finality: **(X)Research ( )Education**
Duration of the Project: from **Jun, 04th, 2018 to Aug. 08th, 2018.**
Species / strain: **Swine** ***(Sus scrofa domesticus)*** Nº of animals: **90**
Weight: **70 Kg** Age: **110 days** Sex: **Male** Source: **Setor de Suinocultura/DZO/UFV - CNPJ/CPF: 25.944.455/0001-96**

Luciana Navajas Rennó
Coordenadora da CEUAP/UFV