ROGÉRIO DE CARVALHO VELOSO

EFFECTS OF NUTRITIONAL PLANS AND GENETIC GROUPS ON QUANTITATIVE TRAITS OF PIGS

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

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BIOGRAPHY

ROGÉRIO DE CARVALHO VELOSO, son of Mário Veloso Filho and Edneuda Maria Ferreira de Carvalho Veloso, was born February 02th, 1987 in Almenara, Minas Gerais, Brazil.

In August, 2006, he started his undergraduate in Animal Science at Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), in Diamantina. He graduated in December, 2010.

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A seleção de genótipos para alto desempenho teve como consequência redução do teor gordura e perda na qualidade da carne. Uma alternativa para melhorar a qualidade da carne é o cruzamento de raças locais, como o Piau, com raças melhoradas como Duroc, Pietrain e Large White. Contudo, as diferenças nas taxas de crescimento e de deposição de gordura apresentadas pelos suínos indicam que os grupos genéticos tenham diferentes necessidades nutricionais. A genética e o ambiente são importantes na expressão das características de desempenho e qualidade da carne, principalmente quando há variações nas taxas de deposição de tecido magro e gordo. Essas variações podem causar interferência no perfil de expressão dos genes que atuam nas vias metabólicas ou hormonais das características relacionadas à qualidade da carne. Objetivou-se, com este trabalho, avaliar o efeito do grupo genético paterno (Piau, Base-Duroc e Pietrain) e dos planos nutricionais contendo diferentes níveis de lisina digestível (Baixo, Médio e Alto) sobre as características de desempenho, carcaça e qualidade da carne; o padrão de expressão gênica das isoformas da cadeia pesada da miosina (*MyHC*) nos músculos Longissimus e Psoas; e a expressão diferencial dos genes relacionados com o metabolismo lipídico (*FAS*, *H-FABP*, *SCD*, *PRKAG1*, *PRKAG3* e *HSL*) no músculo Longissimus de suínos. Os machos castrados e as fêmeas cruzadas Pietrain e Duroc foram similares nas características de desempenho e carcaça e tiveram os melhores valores em comparação aos suínos cruzados Piau. Com relação à qualidade da carne, a perda por gotejamento foi maior nos machos castrados e fêmeas cruzadas Pietrain em comparação com os suínos cruzados Duroc e Pietrain. O consumo diário de lisina digestível dos animais foi maior no plano nutricional Alto, em relação aos planos nutricionais Médio e Baixo. A maioria das características de desempenho, carcaça e qualidade da carne avaliadas nos machos castrados e fêmeas não foram afetadas pelos planos nutricionais. Os níveis de expressão do *H-FABP* e *SCD* foram maiores nos suínos Duroc e Pietrain. Maior expressão do gene *PRKAG3* foi observada nos machos castrados cruzados alimentados com o plano nutricional Baixo em comparação com os suínos alimentados com plano nutricional Médio e Alto. Houve maior expressão do gene *MyHC IIb* no músculo Longissimus dos suínos

alimentados com os planos nutricionais contendo maiores níveis de lisina digestível em relação aos suínos cruzados que receberam plano nutricional contendo menores níveis de lisina digestível. Os níveis de expressão do gene *MyHC IIb* no músculo Longissimus foi maior nos machos castrados cruzados Duroc e Pietrain em comparação aos machos castrados cruzados Piau ($P < 0,05$). Em geral, os suínos cruzados Duroc e Pietrain tiveram melhor desempenho e rendimento de carcaça em comparação aos cruzados Piau. O atual estudo indica que o genótipo tem forte influencia sobre a deposição do conteúdo de gordura intramuscular, principalmente pela regulação da expressão gênica lipogênica intramuscular. A expressão diferencial das isoformas *MyHC* nos músculos podem ser os fatores mais importantes que influenciam o conteúdo de gordura intramuscular e a perda por gotejamento em suínos. Estes resultados podem fornecer informações valiosas para o entendimento das diferenças na qualidade da carne de diferentes grupos genéticos de suínos.

ABSTRACT

VELOSO, Rogério de Carvalho, D.Sc., Universidade Federal de Viçosa, March, 2016. **Effects of nutritional plans and genetic groups on quantitative traits of pigs.** Advisor: Paulo Sávio Lopes. Co-Advisers: Fabyano Fonseca e Silva and Márcio de Souza Duarte.

Selection of genotypes for high performance led to a fat level reduction and loss of meat quality as consequence. An alternative to improve meat quality is the crossbreeding of non-selected local pig breed, such as Piau, with improved breeds such as Duroc, Pietrain and Large White. However, growth rate and fat deposition differences in pigs indicate that genetic groups have different nutritional needs. Genetic and environment are important in expression of performance and meat quality traits, mainly when variations in deposition of lean and fat tissue rates are observed. These variations may affect genes expression profile that acts in metabolic or hormonal pathways of meat quality related traits. In this work, we aimed to evaluate the effects of paternal genetic group (Piau, Duroc-based and Pietrain) and nutritional plans with different digestible lysine levels (Low, Medium and High) on performance, carcass and meat quality traits; gene expression pattern of myosin heavy chain isoforms (*MyHC*) in Longissimus and Psoas muscles; and differential expression of lipid metabolism-related genes (*FAS*, *H-FABP*, *SCD*, *PRKAG1*, *PRKAG3* and *HSL*) in Longissimus muscle of pigs. Pietrain and Duroc crossbred barrows and gilts were similar in performance and carcass traits and had greatest values compared to Piau crossbred pigs. Regarding the meat quality, drip loss was greater in Pietrain crossbred barrows and gilts compared to Duroc and Piau crossbred pigs. Daily digestible lysine intake was greater in High nutritional plan animals in relation to Medium and Low nutritional plans. Most of performance, carcass and meat quality traits evaluated in barrows and gilts were not affected by nutritional plans. Expression levels of *H-FABP* and *SCD* were higher in Duroc and Pietrain pigs. Higher expression of *PRKAG3* gene in barrows fed with Low nutritional plan compared with pigs fed with Medium and High nutritional plan was observed. There was a higher expression of *MyHC IIb* gene in Longissimus muscle of crossbred barrows fed with greater digestible lysine levels compared to crossbred barrows that received nutritional plan with lower digestible lysine levels. The mRNA expression levels of *MyHC IIb* in Longissimus muscle was higher in Duroc and Pietrain crossbred compared to Piau crossbred barrows ($P < 0.05$). In general, Duroc and Pietrain crossbred pigs had greater performance and carcass rate

compared to Piau crossbred pigs. The present study indicated that genotype has a strong effect on intramuscular fat content deposition mainly by up-regulation of intramuscular lipogenic gene expression. The differentially expression of *MyHC* isoforms in muscles may be the most important factors affecting intramuscular fat content and drip loss in pig. These results may provide valuable information to the understanding of meat quality differences in divergent genetic groups of pigs.

1. GENERAL INTRODUTION

For many years, one of the main aims of pig industry has been to increase the lean-to-fat ratio of pig carcasses (CAMERON et al. 1990) having several factors that can be used to modified this ratio, such as genetic (WOOD et al., 2004) and diet (MADEIRA et al., 2013). Important improvements in body composition of pigs have been made through genetic selection (LATORRE et al., 2008).

On the other hand, such a great emphasis on animal production has also affected meat quality. Lower meat quality of highly productive pigs is frequently associated with excessive meat exudation, and decreased amount of fat (intramuscular fat and rib thickness fat) (KIM et al., 2008; WOOD et al., 2004).

Pietrain breed is known for low carcass fat content and higher drip loss percentage compared to other lean type breed such as Duroc (EDWARDS et al., 2003). Local breeds such as Piau, exhibits decreased growth rate and higher fat (SERÃO et al., 2011; VERONEZE et al., 2014). Among different strategies that have been used to improve pork quality, the use of crossbreeding between genetically improved pig breeds and local pig breeds is a potential alternative to improve pork quality (WOJTYSIAK; POŁTOWICZ, 2014), mainly by fat increase, with the aim of add value to the product for *in natura* consumption as well as processed products (BERTOL et al., 2010).

However, due to discrepancy of lean growth rate of non-selected compared to a high growth rate of selected pig breed, different feeding strategies for production of improving pork quality is expected (KATSUMATA, 2011). Dietary lysine reduction promoted accumulation of intramuscular fat content in Longissimus muscle of pigs (KATSUMATA et al., 2012). However, apparently a reduction of dietary lysine decreases lean meat percentage in pig carcasses with a consequent increase in fat tissue (SZABO et al., 2001). Lysine is the first limiting amino acid in most feeding strategies for pigs and the most abundant amino acids in body proteins (BIKKER et al., 1994).

However, up to now, little is known about molecular mechanisms underlying these differences in pork. Molecular mechanisms responsible for meat quality are often associated with postmortem metabolism, being the skeletal muscle fibers one of the main factors determining postmortem biochemical pathways (GUO et al., 2011).

Skeletal muscle is a heterogeneous tissue comprising different types of fibers. In postnatal growing pigs, skeletal muscles are categorized into four fiber types, based on isoforms of myosin heavy-chain (MyHC) ATPase: *MyHC I* (slow-oxidative),

MyHC IIa (fast-oxidative), *MyHC IIx* (intermediary to *MyHC IIa* and *MyHC IIb*) and *MyHC IIb* (fast-glycolytic) (PETTE; STARON, 2000). Myosin is the most abundant protein in skeletal muscle (LEFAUCHEUR et al., 2002), which is composed by several myofiber types (SCHIAFFINO; REGGIANI, 1994).

Each muscle fiber type has different biochemical and biophysical characteristics such as oxidative and glycolytic capacities, contraction speed, glycogen content and triglycerides content (LEFAUCHEUR, 2010). Previous studies have revealed a considerable association of muscle fiber type composition and pork quality traits due to effects of postmortem metabolic rate in conversion of muscle to meat (RYU; KIM, 2005).

Additionally, muscle fiber type composition could also be affected by diets. It was reported that isoleucine excess alone or combined with leucine excess in wheat-based diets lead to increased *MyHC IIb* expression in Longissimus muscle of pigs (CERVANTES-RAMÍREZ et al., 2013), suggesting that amino acids differentially affects myosin expression in skeletal muscles. BRODSKY et al. (2004) reported that restricting protein ingestion in human produces a change in myosin isoform distribution via post-transcriptional mechanisms. However, there are no reports in pigs showing the effect of dietary lysine on expression of *MyHC* isoforms that would indicate a role of lysine in the regulation of protein synthesis in pigs (MORALES et al., 2015).

The extent of fat deposition in skeletal muscles depends on balance between synthesis and degradation of triglycerides, which includes triglycerides synthesis, fat mobilization, fatty acid transport as well as fatty acid oxidation (ZHAO et al., 2009). Therefore, expression levels of lipid metabolism-related genes in muscle tissue could theoretically contribute to the difference in intramuscular fat content, which lead to different meat quality between pig genotypes.

Triacylglycerol (TAG) is the major component of fat and FAS (Fatty acid synthase) catalyze the entire pathway of palmitate synthesis from malonyl-CoA in mammals. Additionally, there is evidence that *SCD* (Stearoyl-CoA desaturase) participates in triglyceride syntheses by providing a better accessible pool of monounsaturated fatty acids. This suggests that the increased fat is probably due to increased expression of lipogenic genes (WANG et al., 2012). *HSL* (Hormone sensitive lipase), a lipolytic gene, cleaves fatty acids from intracellular triacylglycerol for oxidation and *H-FABP* (Heart fatty acid-binding protein) is involved in

intracellular targeting of fatty acids and facilitates fatty acids transport from membrane to sites of fatty acid oxidation or esterification into TAG or phospholipids (KRAG et al., 2007).

Intramuscular fat (IMF) content is an important factor on many aspects of meat quality traits, such as, pH and drip loss (CAMERON et al., 1990; GJERLAUG-ENGER et al., 2010). Negative genetic correlation between IMF content and drip loss, and positive genetic correlation between IMF and pH in meat were reported (GJERLAUG-ENGER et al., 2010).

Therefore, in this study we aimed to: (i) evaluate the effects of paternal genotype and nutritional plans with different digestible lysine contents on performance, carcass traits and meat quality in crossbred pigs from growth to finish; (ii) to evaluate gene expression pattern of myosin heavy chain isoforms in Longissimus and Psoas muscles and, (iii) to evaluate gene expression of lipid metabolism-related genes in Longissimus muscle in pigs.

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Chapter I

Effects of nutritional plans and genetic groups on performance, carcass and meat quality traits of finishing pigs

Abstract: We aimed to evaluate the effects of paternal genotypes (Piau, Duroc-based and Pietrain) and–nutritional plans with different digestible lysine contents (Low, Medium and High) on performance, carcass and meat quality traits of pigs. Pietrain and Duroc crossbred barrows and gilts were similar in performance and carcass traits and had greatest values compared to Piau crossbred pigs. Regarding to meat quality, drip loss was greater in pork from Pietrain crossbred barrows and gilts compared to Duroc and Piau crossbred pigs. The High nutritional plan had the greatest value of daily digestible lysine intake, followed by the Medium and Low nutritional plans. Most of performance, carcass and meat quality traits evaluated in barrows and gilts were not affected by nutritional plans. In general, Duroc and Pietrain crossbred pigs had greater performance and carcass yield compared to Piau crossbred pigs.

Keywords: crossbreeding, digestible lysine, Duroc, Piau, Pietrain

1. Introduction

Recently, meat quality has become a concern in the swine market due to the selection for high lean growth, which results in overall decrease of intramuscular fat deposition and tenderness (FABIAN et al., 2003; MADEIRA et al., 2013; WOJTYSIAK; POŁTOWICZ, 2014). Among different strategies that have been used to improve quality in swine production, the use of crossbreeding of genetically improved pig breeds with non-selected local pig breeds is a potential alternative to improve pork quality mainly through the increase of fat content. Fat increase quantity aims of add value to the product for consumption *in natura* as processed products (BERTOL et al., 2010). Several studies have identified the Piau local breed as a genetic source of meat quality traits such intramuscular fat (SERÃO et al., 2011), and thus may be used in programs aiming to improve the quality, resistance to disease and stress in crossbred swines. For these reasons, Piau pigs may be also successfully kept in organic farms.

However, because of the discrepancy of lean growth rate from non-selected compared to a high growth rate selected pig breed, it is expected a different nutritionals requirements for the crossbred pigs. In this context, dietary lysine requirement is one of the main concerns, since it is the first limiting amino acid in typical swine diets based on corn and soybean meal and also has an intrinsic relationship to muscle growth. Consequently, animal performance and lean tissue deposition are dependent of digestible lysine on dietary contents (BIDNER et al., 2004; KATSUMATA et al., 2012; YEN; COLE; LEWIS, 1986). Therefore, we aimed to evaluate the effects of the paternal genotypes and the nutritional plans with different digestible lysine contents on performance, carcass and meat quality traits in crossbred pigs from growth to finish.

2. Material and methods

2.1. Genetic groups and diets

The experiment was carried out at Pig Breeding Farm of *Universidade Federal de Viçosa,* Brazil. Animal handling was done in accordance with regulations approved by the institutional animal welfare and ethics/protection commission (Protocol nº 20/2014) of the *Universidade Federal de Viçosa*.

A total of 52 barrows averaging 25.44±3.27 kg of initial body weight and 50 gilts averaging 24.14±3.87 kg of initial body weight at 70 days of age were used and each animal was considered as an experimental unit. We used Piau Brazilian local

breed, Duroc-based line and Pietrain breed as sires. Thus, the barrows genetic group was composed of 18 Piau crossbred pigs [G1 = Piau male x (Pietrain x Large White female)], 18 Duroc crossbred pigs [G2 = Duroc-based male x (Pietrain x Large White female)], and 16 Pietrain crossbred pigs [G3 = Pietrain male x (Pietrain x Large White female)]. The gilt genetic group was composed of 16 Piau crossbred pigs (G1), 18 Duroc crossbred pigs (G2), and 16 Pietrain crossbred pigs (G3).

Pigs from each genetic group were randomly assign to three nutritional plans based on digestible lysine (DL) levels as follows: Low (7g DL fed from 70 to 98 days of age; 6g DL fed from 99 to 134 days of age; and 5g DL 135 to 156 days of age), Medium (9g DL fed from 70 to 98 days of age; 8g DL fed from 99 to 134 days of age; 7g DL 135 to 156 days of age), and High (11g DL fed from 70 to 98 days of age; 10g DL fed from 99 to 134 days of age; and 9g DL 135 to 156 days of age). Diets were formulated based on corn, soybean meal, supplemented with minerals, vitamins and amino acids to attend the nutritional requirements, of the animals with except of DL, according to ROSTAGNO et al. (2011). The proportions of essential amino acids and DL were met according to the ideal protein concept (ROSTAGNO et al., 2011). The different lysine levels of experimental diets were obtained from the inclusion of HCL L-lysine as a replacement of caulim. The description of the diets within each experimental phase is presented on Tables 1.

Table 1. Chemical and nutritional composition of experimental diets according to experimental treatments

Ingredient	Age of the animal								
	70 to 98 days			99 to 134 days			135 to156 days		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Corn meal	72.70	72.702	72.702	77.947	77.947	77.947	85.584	85.584	85.584
Soybean meal 45%	22.409	22.409	22.409	18.101	18.101	18.101	13.835	13.835	13.835
Soybean oil	1.159	1.159	1.159	0.686	0.686	0.686	0.441	0.441	0.441
Dicalcium phosphate	1.195	1.195	1.195	0.883	0.883	0.883	0.816	0.816	0.816
Caulim	1.100	0.696	0.035	1.100	0.734	0.069	1.100	0.770	0.111
Limestone	0.707	0.707	0.707	0.606	0.606	0.606	0.573	0.573	0.573
Salt	0.405	0.405	0.405	0.354	0.354	0.354	0.329	0.329	0.329
Vitamin mixture[1]	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Mineral mixture[2]	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
L-Lysine HCl 78%	-	0.260	0.520	-	0.260	0.520	-	0.260	0.520
DL-Methionine 99%	-	0.070	0.193	-	0.045	0.168	-	0.020	0.142
L-Threonine 98%	-	0.074	0.222	-	0.059	0.207	-	0.044	0.191
L-Tryptophan 98%	-	-	0.037	-	0.002	0.041	-	0.006	0.044
L-Valine 96,5%	-	-	0.093	-	-	0.095	-	-	0.092
L-Isoleucine 99%	-	-	-	-	-	-	-	-	0.042
Growth promotant[3]	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Butilhidroxitoluene	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Predicted nutritional composition									
ME (kcal/kg)	3,230	3,230	3,230	3,230	3,230	3,230	3,230	3,230	3,230
CD (%)	15.86	15.86	15.86	14.32	14.32	14.32	12.76	12.76	12.76
Dig. Lysine (%)	0.700	0.900	1.100	0.600	0.800	1.000	0.500	0.700	0.900
Dig. Met. + Cis. (%)	0.470	0.540	0.660	0.436	0.480	0.600	0.399	0.420	0.540
Dig. Threonine (%)	0.536	0.603	0.737	0.483	0.536	0.670	0.429	0.469	0.603
Dig. Tryptophan (%)	0.164	0.164	0.198	0.142	0.144	0.180	0.120	0.126	0.162
Dig.Valine (%)	0.672	0.672	0.759	0.604	0.604	0.690	0.535	0.535	0.621
Dig. Isoleucine (%)	0.000	0.000	0.000	0.523	0.523	0.523	0.452	0.452	0.495
Calcium (%)	0.635	0.635	0.635	0.512	0.512	0.512	0.474	0.474	0.474
Available P (%)	0.314	0.314	0.314	0.250	0.250	0.250	0.231	0.231	0.231
Sodium (%)	0.180	0.180	0.180	0.160	0.160	0.160	0.150	0.150	0.150

[1]For each kg of the product: Fe, 100 g; Cu, 10 g; Co, 1 g; Mn, 40 g; Zn, 100 g; I, 1,5 g; excipient q.s.p., 1.000 g.
[2]For each kg of the product: vitamin A, 6.000.000 UI; vitamin D3, 1.500.000 UI; vitamin E, 15.000.000 UI; vitamin B1, 1,35 g; vitamin B2, 4 g; vitamin B6, 2 g; Pantothenic acid, 9,35 g; vitamin K3, 1,5 g; Nicotinic acid, 20,0 g; vitamin B12, 20,0 g; Pholic acid, 0,6 g; Biotin, 0,08 g; Se, 0,3 g; excipient q.s.p., 1.000 g.
[3]Surmax® Elanco.

2.2. Animal performance, slaughter, carcass data collection and muscle sampling

The amount of feed provided and leftovers were daily recorded to obtain the average daily intake (g/d) of ration and DL (g/d). Additionally, to obtain the average

daily gain (g/d) and feed conversion rate (g/g), pigs were weighed (kg) at the beginning and at the end of the experimental period, which started with 70 and finished at 156 days of age.

The pigs were slaughtered at 156 days of age after 16 h of fasting. Pre-harvest handling and slaughtering procedures was in accordance with good animal welfare practices, following the Sanitary and Industrial Inspection Regulation for Animal Origin Products (BRASIL, 1997).

After the slaughter, each carcass was split into two identical longitudinal halves and chilled at 4°C for 24 h. Following the postmortem chill, carcasses were weighed to obtain the values of cold carcass yield. Rib fat thickness (RFT) and loin eye area (LEA) were measured in the cold carcass left, 6 cm from the mid-line on the last rib.

Carcass pH was recorded at slaughter (pH_0), at 45 min (pH_{45}) after slaughter and after 24 h postmortem chill (pH_{24}) using a pHmeter (Hanna-DIGIMED model DM-20) coupled to a penetration probe (DIGIMED, DME-CV1), inserted into the center of the *Longissimus* muscle in the left half-carcass between the 12th and 13th thoracic vertebra.

Trimmed cuts consisted of belly, trimmed ham with bones, picnic shoulder, loin, ribs, boston shoulder and tenderloin. Cuts were obtained in the left half of the carcass 24 h after postmortem, chill and yield were calculated as percentage of the left half of carcass weight.

At the end of carcass fabrication, a boneless Longissimus section 12 cm thick was collected. Longissimus samples were individually vacuum packaged and frozen at − 20 °C. Each frozen Longissimus sample was standardized into two 2.54 cm thick steak samples (AMSA, 1995). All steaks were then vacuum packaged and held at - 20 °C.

2.3. Instrumental color analysis

Instrumental color analysis was performed at the beginning of carcass fabrication after 24 h post mortem chill. Color measurements were taken at *Longissimus* muscle at the 10th rib after 30 min blooming. A total of three readings of L^*, a^* and b^* values were obtained for each carcass. Color coordinates (L^*, a^* and b^*) were recorded with a digital Minolta CR300 chromometer (Minolta Co., Osaka, Japan). Coordinate a^* ranged from red (+ a^*) to green (− a^*) and coordinate b^* from yellow (+ b^*) to blue (− b^*) (HUNTERLAB, 1996).

2.4. Drip loss

Drip loss was determined according the bag method described by HONIKEL (1987). Briefly, meat samples were collected 24 h after postmortem at 14th rib and trimmed from visible fat and connective tissue. Then, meat samples were divided into two slices weighing approximately 120 grams. Each slice was then hung in a hook under a lid of an airtight container and kept at 4 °C for 24 h. Following the 48 h chill, meat samples were weighed and the weight difference was divided by the initial sample weight.

2.5. Cooking loss

Cooking loss was evaluated on the meat samples that were also used for Warner-Bratzler shear force (WBSF) measurement. Cooking loss of each sample was recorded after steaks were oven-broiled. Total cooking loss was calculated as the difference between the weight of the steaks before and after oven-broiling.

2.6. Warner-Bratzler shear force measurement

For WBSF evaluation, steaks were thawed at 4 °C for 24 h and oven-broiled in an electric oven (Layr, Luxo Inox) preheated to 150 °C. Internal steak temperatures were monitored by 20-gauge copper–constantan thermocouples (Omega Engineering, Stamford, CT) placed in the approximate geometric center of each steak and attached to a digital monitor. When the internal steak temperature reached 35 °C, the steak was turned over and allowed to reach an internal temperature of 70 °C before removal from the oven. Cooked WBSF steaks were cooled for 24 h at 4 °C (AMSA, 1995). Eight round cores (1.27 cm diameter) were removed from each steak parallel to the long axis of the muscle fibers (AMSA, 1995). Each core was sheared once through the center, perpendicular to the fiber direction by a Warner-Bratzler shear machine (G-R Manufacturing Company, Manhattan, KS, USA).

2.7. Intramuscular fat content

Powdered lyophilizated meat samples were analyzed for moisture by the Method 934.01 (AOAC, 1990), and ether extract (EE) by the Method Am 5-04 (AOCS, 2009), to determine the amount of intramuscular fat content.

2.8. Statistical analysis

Statistical analyses were individually performed for each sex. Variables of animal performance and carcass traits (with exception of LEA and RFT) were analyzed as the following model:

$$y_{ijk} = m + G_i + N_j + GxN_{ij} + e_{ijk}$$

where:

G_i: ith level of the fixed effect of genetic group;

N_j: jth level of the fixed effect of nutritional plan;

e_{ijk}: random error associated with y_{ijk}.

The following model was used for LEA, RFT, and meat quality traits:

$$y_{ijk} = m + G_i + N_j + GxN_{ij} + b\left(W_{ijk} - \bar{W}_i\right) + e_{ijk}$$

where:

G_i: ith level of the fixed effect of genetic group;

N_j: jth level of the fixed effect of nutritional plan;

W_{ijk}: slaughter weight was used as a covariate within genetic group;

e_{ijk}: random error associated with y_{ijk}.

All analyses were performed using proc Mixed procedure of SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA). Means were compared by Tukey`s test and differences were considered at $P \leq 0.05$.

3. Results

3.1. Barrows

3.1.1. Performance

No interaction was observed between genetic group and nutritional plan for any performance variables evaluated ($P > 0.05$).

Final body weight (BWf) differed among genetic groups ($P = 0.0001$) where Pietrain and Duroc crossbred barrows had the greatest BWf, while Piau crossbred barrows had the least values of BWf (Table 2). Similarly, the average daily gain (ADG) was affected by genetic group ($P = 0.0001$) where Pietrain and Duroc crossbred barrows had similar values of ADG, but higher than the values observed for Piau crossbred barrows (Table 2). Consequently, feed conversion rate (FCR) was also

affected by genetic group (P = 0.0001), where Piau crossbred barrows showed greatest FCR values compared to Pietrain and Duroc crossbred (Table 2).

As expected, the daily digestible lysine intake (DLI) differed among nutritional plan groups (P = 0.0001) where barrows within the High nutritional plan group had the greatest value of DLI, followed by the Medium and Low nutritional plan groups (Table 2). These results show the effectiveness of the nutritional plans applied in the current experiment. Despite of the difference in DLI among the nutritional plan groups, no effects of nutritional plans were observed for BWf (P = 0.18), ADI (P = 0.84), ADG (P = 0.10), and FCR (P = 0.09).

Table 2. Effect of genetic group and nutritional plan of digestible lysine on performance of barrows from 70 to 156 days of age

Item	Genetic group			CV	P-value	Nutritional plan			CV	P-value
	G1	G2	G3	(%)		Low	Medium	High	(%)	
Initial body weight[1] (kg)	24.52	26.06	25.65	12.86	0.39	24. 64	26.24	25.35	12.84	0. 37
Final body weight[2] (kg)	90.86[b]	108.03[a]	104.28[a]	9.98	0.0001	97.43	102.91	102.83	12.04	0. 18
Average daily gain (g/d)	781[b]	971[a]	930[a]	10.14	0.0001	858	905	919	13.27	0.10
Average daily intake (g/d)	2527	2607	2455	10.06	0.23	2503	2555	2531	10.33	0.84
Feed conversion rate (g/g)	3.25[b]	2.68[a]	2.64[a]	7.61	0.0001	2.93	2.86	2.77	12.09	0.09
Digestible lysine intake (g/d)	21.82	22.09	21.19	24.88	0.57	15.91[c]	22.15[b]	27.05[a]	11.85	0.0001

CV: Coefficient of variation.
G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds.
Means with different letters differ at P≤0.05 by Tukey`s test.

3.1.2. Carcass traits

Among the carcass traits evaluated, an interaction between genetic group and nutritional plan was observed (P = 0.04) for hot carcass yield (HCY; Figure 1). For barrows within the Piau crossbred group, no differences were observed when barrows were fed different nutritional plans. However, a greater HCY was observed for Duroc crossbred barrows fed Medium nutritional plan compared to those fed High and Low nutritional plans, which also did not differ from each other. Differently, for Pietrain crossbred barrows a similar HCY was observed for barrows fed Medium and High nutritional plans and both were greater than HCY observed for barrows fed Low nutritional plan.

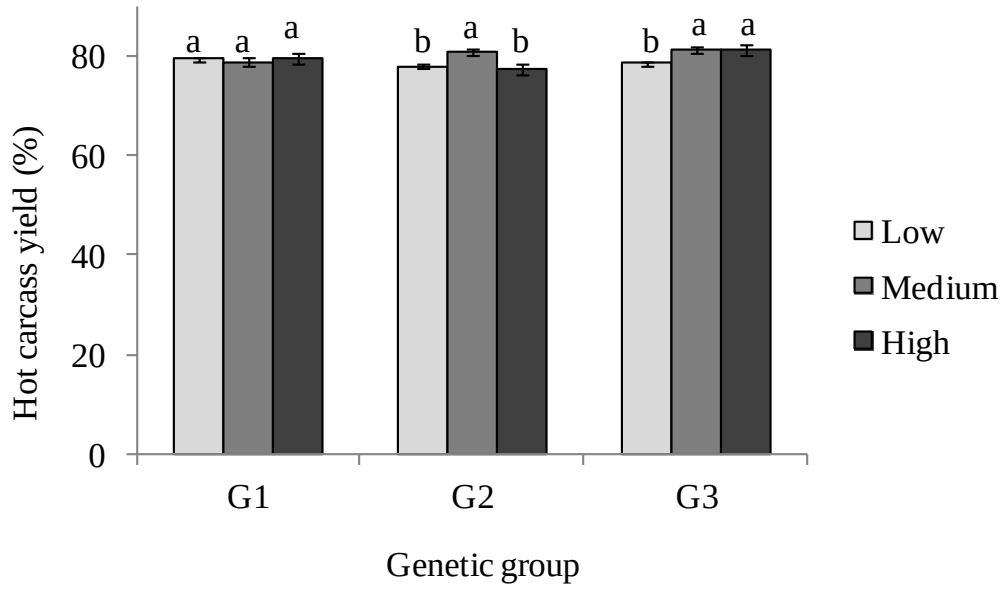

Figure 1. Comparison among nutritional plans (low, medium and high) within each genetic group (G1, G2, and G3) for hot carcass yield of barrows. Means with different letter within a genetic group differ at P ≤ 0.05 by Tukey`s test.

No differences were observed among genetic groups for cold carcass yield (CCY; P = 0.35; Table 3). Regarding LEA, Pietrain crossbred barrows had the greatest LEA ($P < 0.01$) followed by Duroc and Piau crossbred barrows, which in turn had the least value of LEA among the genetic groups (Table 3). On the other hand, Piau crossbred barrows had the greatest value of RFT ($P < 0.01$) than Pietrain and Duroc crossbred barrows, which did not differed from each other.

Regarding trimmed cuts yield, ham and loin yields were lower in Piau crossbreds, compared to Duroc and Pietrain crossbred barrows, which did not differ from each other (P = 0.0001; Table 3). The yield of picnic shoulder (P = 0.0001) and boston shoulder (P = 0.0001) also differed among genetic groups, where the greatest value was observed for Pietrain, followed by Duroc and Piau crossbred barrows. For tenderloin yield, the least value was also observed in Piau compared to Pietrain crossbred barrows (P = 0.02), while no differences were observed for Duroc and Pietrain crossbreds genetic groups. On the other hand, Piau crossbred barrows had the greatest value of belly compared to Duroc and Pietrain crossbred barrows (P = 0.0004).

With regard to nutritional plans, barrows fed diets with Medium had a greater LEA followed by those fed High and Low nutritional plans, respectively (P = 0.003; Table 3). Effect of nutritional plan was also observed for loin yield (P = 0.04) where barrows fed the Low had the least value of loin yield compared to barrows fed Medium and High nutritional plans. The remaining carcass traits and trimmed cuts yield evaluated were not affected by nutritional plan ($P > 0.05$).

Table 3. Effect of genetic group and nutritional plan for digestible lysine on carcass traits of barrows

Item	Genetic group			CV	P-value	Nutritional plan			CV	P-value
	G1	G2	G3	(%)		Low	Medium	High	(%)	
Carcass traits										
Cold carcass yield (%)	78.90	77.84	79.01	3.19	0.35	77.94	79.39	78.41	3.10	0.23
Loin eye area (cm^2)	25.37^{c}	41.76^{b}	44.51^{a}	15.55	0.0002	33.86^{c}	40.01^{a}	37.77^{b}	26.35	0.003
Rib fat thickness (mm)	27.02^{a}	15.88^{b}	14.35^{b}	20.74	0.003	19.01	19.27	18.96	36.75	0.97
Trimmed cuts yield										
Ham (%)	22.46^{b}	26.28^{a}	26.87^{a}	5.46	0.0001	25.24	25.14	25.23	9.48	0.97
Picnic shoulder (%)	14.04^{c}	15.35^{b}	16.23^{a}	7.01	0.0001	15.04	15.18	15.40	9.18	0.61
Tenderloin (%)	0.89^{b}	0.98^{ab}	1.05^{a}	15.02	0.02	0.96	0.99	0.97	16.34	0.79
Boston shoulder (%)	1.12^{c}	1.31^{b}	1.46^{a}	9.86	0.0001	1.29	1.28	1.31	14.53	0.76
Belly (%)	8.36^{a}	7.78^{b}	7.41^{b}	8.06	0.0004	7.84	7.75	7.96	9.51	0.61
Ribs (%)	11.48	11.92	11.96	10.53	0.50	11.78	11.70	11.88	10.67	0.91
Loin (%)	3.65^{b}	5.32^{a}	5.52^{a}	9.44	0.0001	4.61^{b}	4.89^{a}	5.00^{a}	19.39	0.04

CV: Coefficient of variation.
G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds.
Means with different letters differ at P≤0.05 by Tukey`s test.

3.1.3. Meat quality traits

There was no interaction between genetic groups and nutritional plans for any meat quality traits evaluated ($P > 0.05$) in barrows.

No differences were observed for carcass initial pH, measured immediately after slaughter ($pH_{0;}$ $P = 0.65$), neither for pH measured at 45 min postmortem (pH_{45}; $P = 0.14$) nor final pH measured at 24 h postmortem (pH_{24}; $P = 0.64$) among genetic groups (Table 4). However, different values of drip loss were observed for pork from different genetic groups ($P = 0.02$) where barrows from Piau crossbred group had the least value for this variable, compared to Duroc and Pietrain crossbred barrows.

Similar values of L^* ($P = 0.61$), a^* ($P = 0.38$), and b^* ($P = 0.91$) were also observed among genetic groups (Table 4). Similarly, no differences of cooking loss (CL; $P = 0.64$), Warner-Bratzler shear force (WBSF; $P = 0.75$), and ether extract (EE; $P = 0.54$) were observed among genetic groups (Table 4).

Although the nutritional plan affected the carcass quality traits of barrows, it did not affect any of the meat quality variables evaluated in this study ($P > 0.05$).

Table 4. Effect of genetic group and nutritional plan of digestible lysine on meat quality traits of barrows

Item	Genetic group			CV	P-value	Nutritional plan			CV	P-value
	G1	G2	G3	(%)		Low	Medium	High	(%)	
pH_0	6.43	6.35	6.41	4.44	0.65	6.42	6.28	6.48	4.30	0.13
pH_{45}	6.34	6.25	6.11	5.05	0.14	6.24	6.23	6.24	5.56	0.99
pH_{24}	6.05	6.08	6.12	5.63	0.64	6.03	6.19	6.03	5.54	0.32
Drip loss (%)	7.77^{b}	9.61^{ab}	11.14^{a}	29.29	0.02	10.42	9.30	8.80	30.05	0.22
*L**	59.87	61.54	60.70	5.07	0.61	61.96	60.28	59.88	4.75	0.11
*a**	6.37	6.69	6.98	19.67	0.38	6.53	6.67	6.84	19.99	0.79
*b**	15.24	15.53	15.50	7.34	0.91	15.33	15.52	15.42	7.35	0.89
Cooking loss (%)	20.39	21.40	21.86	22.15	0.64	21.00	20.64	22.01	22.19	0.69
Shear-force (kgf)	2.97	2.92	2.88	17.53	0.75	2.86	2.86	3.04	17.25	0.53
Ether extract (%)	2.20	2.46	2.32	19.84	0.54	2.44	2.19	2.35	20.23	0.30

CV: Coefficient of variation.
G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds.
Means with different letters differ at P≤0.05 by Tukey`s test.

3.2. Gilts

3.2.1. Performance

No interaction was observed between genetic groups and nutritional plans for any of the performance variables evaluated in gilts ($P > 0.05$).

The initial body weight (BWi) differed among the genetic groups ($P = 0.0001$; Table 5), whereas Duroc had the highest value of BWi followed by Pietrain and Piau crossbred gilts, respectively. Similar results were observed for BWf ($P = 0.0001$) and ADG ($P = 0.0001$) where Duroc crossbred gilts had the greatest value of BWf and ADG followed by Pietrain and Piau crossbred gilts, respectively (Table 5).

The ADI ($P < 0.01$) and consequently the intake of DL ($P = 0.0002$) were both affected by genetic group where Piau and Pietrain crossbred gilts did not differ from each other but were both lower than Duroc crossbred gilts (Table 5). Feed conversion rate was affected by genetic groups ($P = 0.0001$), where Piau had the greatest value of FCR compared to Pietrain and Duroc crossbred gilts (Table 5). There was no effect of nutritional plan on any of the performance variables evaluated ($P > 0.05$; Table 5). As expected, the intake of DL differed among nutritional plan groups ($P = 0.0001$), which shows the effectiveness of dietary treatments also for the gilts groups (Table 5).

Table 5. Effect of genetic group and nutritional plan for digestible lysine on performance of gilts from 70 to 156 days of age

Item	Genetic group			CV	P-value	Nutritional plan			CV	P-value
	G1	G2	G3	(%)		Low	Medium	High	(%)	
Initial body weight[1] (kg)	21.68[c]	26.46[a]	24.10[b]	14.04	0.001	24.62	24.38	23.24	16.16	0.47
Final body weight[2] (kg)	77.93[c]	107.08[a]	91.88[b]	11.38	0.0001	91.88	92.88	92.13	17.51	0.96
Average daily gain (g/d)	659[c]	947[a]	814[b]	13.39	0.0001	793	813	813	20.12	0.82
Average daily intake (g/d)	2110[b]	2498[a]	2180[b]	11.22	0.0002	2282	2244	2262	13.68	0.73
Feed conversion rate (g/g)	3.25[a]	2.64[b]	2.68[b]	9.26	0.0001	2.93	2.78	2.86	13.32	0.25
Digestible lysine intake (g/d)	17.95[b]	20.88[a]	18.17[b]	25.21	0.003	14.28[c]	18.90[b]	23.82[a]	15.15	0.0001

Coefficient of variation.
G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds.
Means with different letters differ at P≤0.05 by Tukey`s test.

3.2.2. Carcass traits

No interaction between genetic group and nutritional plan was observed ($P > 0.05$) for any carcass traits evaluated in gilts (Table 6). No effects of nutritional plans were observed for any carcass traits evaluated in gilts ($P > 0.05$; Table 6).

The HCY was greater for Pietrain compared to Duroc and Piau crossbred gilts ($P < 0.01$), while similar values of CCY were observed among genetic groups ($P = 0.08$). The LEA observed in Piau was lower than the LEA observed in Pietrain and Duroc crossbred gilts ($P = 0.0001$). On the other hand, the greatest value of RFT was observed in carcasses of Piau followed by Duroc and Pietrain crossbred gilts, respectively ($P = 0.0001$; Table 6).

Piau crossbred gilts had the lowest value of ham ($P = 0.0001$), tenderloin ($P = 0.03$), and loin ($P = 0.0001$) compared to the remaining breeds (Table 6). The greatest value of boston shoulder was observed for Pietrain compared to Duroc and Piau crossbred gilts ($P = 0.0003$). Yields of picnic shoulder (P = 0.09), belly ($P = 0.16$), and ribs ($P = 0.61$) did not differ among genetic groups (Table 6).

Table 6. Effect of genetic group and nutritional plan for digestible lysine on carcass traits of gilts

Item	Genetic group			CV	P-value	Nutritional plan			CV	P-value
	G1	G2	G3	(%)		Low	Medium	High	(%)	
Carcass traits										
Hot carcass yield (%)	78.69^{b}	79.61^{ab}	80.49^{a}	2.01	0.009	78.89	79.86	80.04	2.13	0.08
Cold carcass yield (%)	76.57	77.62	78.10	2.48	0.08	76.66	77.91	77.72	2.53	0.13
Loin eye area (cm^2)	25.82^{b}	44.84^{a}	43.63^{a}	11.85	0.0001	36.93	38.39	38.97	25.82	0.40
Rib fat thickness (mm)	20.37^{a}	14.24^{b}	10.50^{c}	26.26	0.0001	14.93	15.33	14.85	37.94	0.94
Trimmed cuts yield										
Ham (%)	23.48^{b}	26.33^{a}	27.40^{a}	3.87	0.0001	25.66	25.79	25.75	7.52	0.93
Picnic shoulder (%)	14.80	15.22	15.57	6.25	0.09	15.22	15.13	15.24	6.61	0.94
Tenderloin (%)	0.94^{b}	1.04^{a}	1.07^{a}	12.57	0.03	1.02	1.02	1.01	13.57	0.96
Boston shoulder (%)	1.26^{b}	1.34^{b}	1.52^{a}	12.29	0.0003	1.34	1.37	1.41	14.55	0.53
Belly (%)	8.44	8.26	7.93	8.87	0.16	8.31	8.13	8.18	9.23	0.76
Ribs (%)	11.51	11.71	11.34	9.67	0.61	11.56	11.89	11.12	9.44	0.14
Loin (%)	4.54^{b}	5.62^{a}	6.02^{a}	12.38	0.0001	5.25	5.42	5.51	16.99	0.56

CV: Coefficient of variation.
G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds.
Means with different letters differ at P≤0.05 by Tukey`s test.

3.2.3. Meat quality traits

No interaction was observed ($P > 0.05$) between genetic groups and nutritional plans for any meat quality traits evaluated in crossbred gilts (Table 7).

No changes were observed for any meat quality variables evaluated among genetic groups ($P > 0.05$), with exception of drip loss ($P = 0.01$) which was greater in pork from Pietrain compared to Duroc and Piau crossbred gilts (Table 7).

The nutritional plans did not affect any of the meat quality variables evaluated ($P > 0.05$) with exception of ether extract ($P = 0.01$), which was greater in gilts fed Low compared to those fed High nutritional plan (Table 7).

Table 7. Effect of genetic group and nutritional plan of digestible lysine on meat quality traits of gilts

Item	Genetic group			CV	P-value	Nutritional plan			CV	P-value
	G1	G2	G3	(%)		Low	Medium	High	(%)	
pH_0	6.41	6.28	6.36	5.06	0.59	6.26	6.34	6.45	4.88	0.25
pH_{45}	6.31	6.27	6.14	4.95	0.22	6.21	6.25	6.25	4.96	0.55
pH_{24}	6.04	6.16	6.11	5.43	0.79	6.02	6.00	6.29	5.35	0.36
Drip loss (%)	6.85^{b}	7.37^{b}	10.24^{a}	34.04	0.01	8.07	8.16	7.60	36.27	0.31
*L**	59.76	59.91	59.46	4.59	0.75	60.33	59.65	59.16	4.66	0.25
a*	6.22	7.25	6.58	19.05	0.71	7.24	6.02	6.78	18.36	0.08
b*	14.97	15.62	14.93	7.01	0.74	15.28	15.14	15.10	6.97	0.29
Cooking loss (%)	20.00	21.82	22.12	13.08	0.33	22.13	20.47	21.34	13.80	0.72
Shear-force (kgf)	3.55	3.16	3.37	22.91	0.75	3.18	3.25	3.65	22.23	0.18
Ether extract (%)	2.35	2.23	1.94	25.59	0.49	2.53^{a}	2.01^{ab}	1.97^{b}	22.81	0.01

CV: Coefficient of variation.
G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds.
Means with different letters differ at P≤0.05 by Tukey`s test.

4. Discussion

4.1. Barrows

4.1.1. Performance

A lower final body weight observed in Piau crossbred barrows was expected, as these have a greater fat deposition compared to the other breeds evaluated. Moreover, Piau breed have a slowly growth compared to Duroc and Pietrain pigs, which in turn were selected for high growth rate, as wells as low feed conversion rate (WOOD et al., 2004). Low feed conversion rate observed in Duroc and Pietrain crossbred pigs may be explained by high ADG values compared to Piau crossbred, since the average daily intake was unaffected between genetic groups.

The nutritional plans did not affect any of the performance variables, only increased the digestible lysine daily intake, because pigs can tolerate a considerable excess of amino acids in the diets, including lysine, without change the voluntary feed intake (KERR et al., 2003).

4.1.2. Carcass traits

Analysis of the interaction between genetic group and nutritional plans for HCY (Figure 1) showed that Piau crossbred barrows may have met their nutritional requirements provided by the Low nutritional plan. This may have occurred due to slower growth rate, thus requiring lower supply of nutrients for deposition of lean meat (SERÃO et al., 2011; VERONEZE et al., 2014).

On the other hand, for Duroc and Pietrain crossbred barrows the Low nutritional plan, which provided low contents of digestible lysine, did not meet their nutritional requirements for maximum lean growth (HCY; Figure 1). Based on our findings, it seems that Duroc crossbred barrows have met their nutritional requirements when fed Medium nutritional plan, where the greatest HCY was observed. The decrease on HCY when Duroc crossbred barrows were fed High nutritional plan may be due to the excess on digestible lysine intake, which may cause an increase of energy expenditure, leading to decrease on energy availability for meat deposition in the carcass (VAN LUNEN; COLE, 1998). Thus, the decrease in the energy efficiency of High compared to the Medium nutritional plan, can be explained by higher maintenance requirement caused by the excess of amino acids associated with the deamination and urea excretion, therefore, a smaller efficiency for the deposition of lean meat (PERMENTIER et al., 2013).

The greater HCY observed on Pietrain crossbred barrows fed Medium and High nutritional plans compared to those fed Low nutritional plan (Figure 1) was likely due to their greater potential for lean growth and low deposition of adipose tissue (WOOD et al., 2004). Furthermore, this also may be attributed to the low lysine dietary limiting protein accretion and skeletal muscle synthesis (BIDNER et al., 2004), showing that lysine contents in pig diets are adjusted in relation to genotype. In this way, fast-growing genotypes, with a high capacity for skeletal muscle growth, such as Pietrain, may utilize high lysine dietary to greater meat deposition in carcass.

In general, Duroc and Pietrain crossbred barrows had greater trimmed cuts yield and lower values of RFT than Piau crossbred barrows. Such result may have occurred as a consequence of the intense selection process for lean growth and decrease of fatness that both Pietrain and Duroc has undergone (CAMERON et al., 2000; FABIAN et al., 2003).

Pigs fed both nutritional plans with Medium and High nutritional plans had greater LEA compared to those fed Low nutritional plan, confirming that the content of dietary digestible lysine directly affects protein deposition (KATSUMATA et al., 2012). It is noteworthy that greater values of LEA were observed concomitantly with the improvement of feed conversion rate barrows. This clearly demonstrates the change in the composition of BW gain of barrows due to a greater skeletal muscle deposition.

4.1.3. Meat quality traits

Differences observed for drip loss among genetic groups may be explained by the differences in muscle type fiber of the Longissimus muscle. According to CHOE et al. (2008) Longissimus muscle is mainly composed by type IIB fibers (glycolytic) and presents a low proportion of type I fibers (oxidative), leading to a high concentration of lactate within the first hour postmortem. Additionally, the frequency of type I fiber is positively associated to carcass pH at 45 min postmortem and negatively associated to drip loss (LEE et al., 2012; RYU et al., 2008; RYU; KIM, 2005; TRAORE et al., 2012). Thus, probably the Piau crossbred barrows had high frequency of type I and low frequency of type II muscle fiber in Longissimus muscle compared to the other genetic groups evaluated, since the frequency of glycolytic muscle fiber may vary among breeds (KIM et al., 2013; LEE et al., 2012; RUUSUNEN; PUOLANNE, 2004). It was observed a greater frequency of type I and

low type II muscle fiber in Longissimus muscle of local pig breed (Pulawska) compared to commercial pig breeds (WOJTYSIAK; POŁTOWICZ, 2014), which supports our hypothesis.

4.2. Gilts

4.2.1. Performance

Gilts were initially selected to have similar BWi among genetic groups and nutritional plans. However, BWi differed among genetic groups due to the discrepancy of their growth rate (CAMERON et al., 1999), which in turn affects the growth rate curve and the BW at maturity (FISHER et al., 2003). Therefore, the BWf was certainly affected by the BWi of the gilts. Moreover, the discrepancy from each paternal breed used in the present study to compose the genetic groups affected the performance traits. Duroc pigs have high BW gain as one of the main characteristics of the breed (WOOD et al., 2004) while Pietrain pigs have low feed intake (BERTOL et al., 2013) compared to other genetic improved breeds, and Piau breed is mainly characterized by its low growth rate (FARIA et al., 2009; PEIXOTO et al., 2009).

The low feed intake of Pietrain compared to Duroc crossbred gilts is due to the selection process for low feed conversion rate and high lean growth that Pietrain has undergone, which have negatively impact on their voluntary intake (BERTOL et al., 2013). Thus, it was expected that Duroc crossbred gilts pigs would present a greater feed intake values since Duroc has been reported to have greater feed intake compared to other breeds used in genetic improvement programs (AUGSPURGER et al., 2002).

As no difference was observed for average daily intake of gilts, the increase of digestible lysine intake was due to the different content of this amino acid in the nutritional plans evaluated.

4.2.2. Carcass traits

The greater HCY of Duroc and Pietrain compared to Piau crossbred gilts was due to their greater slaughter weight and carcass composition, since a greater RFT and lower LEA was observed in carcasses of Piau crossbred gilts compared to other genetic groups. The amount of subcutaneous fat is negatively correlated to the amount of lean tissue (SUZUKI et al., 2005) and consequently contributes to decrease the carcass yield. The greater LEA and trimmed cuts yield observed in Duroc and Pietrain crossbred gilts clearly demonstrates their greater ability for lean tissue deposition

compared to Piau crossbred gilts (EDWARDS; BATES; OSBURN, 2003; EDWARDS; TEMPELMAN; BATES, 2006; WOOD et al., 2004).

4.2.3. Meat quality traits

The drip loss from crossbred gilts was different among genetic groups, where meat from Duroc and Piau crossbred gilts had the least value of drip loss, compared to the other genetic groups. This difference was likely due to differences in frequency of muscle fiber type among breeds as previously discussed in barrows; where Pietrain crossbred gilts may have a higher frequency of glycolytic muscle.

The intramuscular fat measured by quantification of ether extract content on meat were higher with the decreasing of lysine in nutritional plans, similar to others observations (BIDNER et al., 2004; KATSUMATA et al., 2005). Collectively, these results indicate that intramuscular fat content may change according to the variation of digestible lysine on nutritional plan when the dietary content of this amino acid is lower than required by the animals.

It has been reported that pigs fed low contents of lysine have greater mRNA abundance of *PPARγ* and *SREBP-1* showing the greater lipogenesis in the skeletal muscle (SCHADINGER et al., 2005), which may have occurred in the present experiment. Additionally, the free L-carnitine content was lower in skeletal muscle of pigs fed low contents of digestible lysine (KATSUMATA et al., 2005). Thus, since L-carnitine plays a crucial role in lipid oxidation by transporting fatty acyl-CoA through mitochondrial membrane, the low contents of digestible lysine may have decrease the lipid oxidation allowing the deposition of fatty acid in the skeletal muscle.

5. Conclusions

Duroc and Pietrain crossbred barrows and gilts had greater performance and carcass yield compared to Piau crossbred pigs. There was no interaction genetic group and nutritional plans for most traits and few traits were affected by nutritional plan, however the genetic background of the pig during the growing-finishing phase seems to depend on the response of dietary lysine.

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Chapter II

Effect of nutritional plan and genotype on expression of lipid metabolism-related and myosin heavy chain isoform genes in skeletal muscle of pigs

Abstract: In this study, we aimed to evaluate the effects of paternal genetic group (Piau, Duroc-based and Pietrain) and nutritional plans with different digestible lysine contents (Low, Medium and High) on the expression of lipid metabolism genes and myosin heavy chain isoforms (*MyHC*) in skeletal muscle of pigs. Pigs from the three genetic groups were randomly assigned and barrows and gilts were slaughtered at 156 days of age. Subsequently, gene expression of *FAS*, *H-FABP*, *SCD*, *PRKAG1*, *PRKAG3*, *HSL*, *MyHC I,IIa, IIx* and *IIb* were assessed. The mRNA expression levels of *FAS* and *PRKAG1* in Longissimus muscle were higher in Piau crossbred pigs compared to the other genetic groups ($P < 0.05$). On the other hand, mRNA expression of *H-FABP* and *SCD* was higher in Duroc and Pietrain compared to Piau crossbred pigs ($P < 0.05$). Higher mRNA expression of *PRKAG3* in barrows fed with Low nutritional plan compared with pigs fed with Medium ($P = 0.03$) and High ($P = 0.04$) was observed. There was a higher mRNA expression of *MyHC IIb* in Longissimus muscle of crossbred barrows fed with High nutritional plan compared to crossbred barrows that received Low and Medium ($P < 0.05$). Expression levels of *MyHC IIb* in Longissimus muscle were higher in Duroc ($P = 0.01$) and Pietrain ($P = 0.004$) crossbred compared to Piau crossbred barrows. To evaluate the differential expression effect of lipid metabolism and myosin heavy chain related genes in skeletal muscle we also evaluated drip loss of pork. Drip loss was greater in pork from Pietrain crossbred barrows and gilts compared to Piau crossbred pigs ($P < 0.05$). Intramuscular fat ($P = 0.01$) was greater in Low fed gilts compared to those fed with High nutritional plan. The present study suggests that genetic group has a strong influence on deposition of intramuscular fat content mainly by up-regulation of intramuscular lipogenic gene expression. Differentially expression of *MyHC* isoforms in skeletal muscle may be also involved on fat content and drip loss. These results may provide valuable information for understanding the differences of meat quality in different pig genetic groups.

Keywords: drip loss, intramuscular fat, lysine, skeletal muscle

1. Introduction

Although it is well known that different pig breeds differ in carcass and meat quality traits (RUUSUNEN et al., 2012; SHEN et al., 2014) the molecular mechanisms underlying these differences remain unclear. Usually, differences in meat quality traits are smaller between commercial pig breeds, in terms that pig breeding goals such as fast growth and leanness of the carcass have been similar for a long time (RUUSUNEN et al., 2012). Conversely, local breeds such as Piau, exhibits decreased growth rate and greater fat content (SERÃO et al., 2011; VERONEZE et al., 2014) compared to commercial pig breeds. Pietrain is known for low carcass fat content and higher drip loss percentage compared to other lean type breed such as Duroc (EDWARDS et al., 2003).

It has been previously reported that fat accretion could be influenced by dietary lysine manipulation, in terms that lysine plays a pivotal role in regulating energy metabolism in skeletal muscle of pigs, without compromise growth rate (KATSUMATA, 2011; KATSUMATA et al., 2012). The extent of fat deposition in skeletal muscles depends on balance mechanism between synthesis and degradation of triglycerides, which includes triglycerides synthesis, fat mobilization, fatty acid transport as well as fatty acid oxidation (ZHAO et al., 2009). This balance mechanism includes lipogenic genes such as fatty acid synthase (*FAS*) and stearoyl-CoA desaturase (*SCD*) (DORAN et al., 2006) and lipolytic genes as hormone-sensitive lipase (*HSL*) (MORO et al., 2008). In addition, this process includes fatty acid transporting genes as heart fatty-acid binding protein (*H-FABP*) (JURIE et al., 2007).

Among molecular mechanisms underlying pork quality variation, the composition of skeletal muscle fibers appears as one of the main factors determining differences in biochemical pathways of the conversion of muscle into meat (GUO et al., 2011). In general, skeletal muscles fibers in pigs are categorized into four types, based on abundance of myosin heavy chain (*MyHC*) ATPase: *MyHC I* (slow-oxidative), *MyHC IIa* (fast-oxidative), *MyHC IIx* (intermediary to *MyHC IIa* and *MyHC IIb*) and *MyHC IIb* (fast-glycolytic) (PETTE; STARON, 2000).

There are limited reports regarding lysine intake effect on molecular mechanisms underlying the difference in fat content of genotypes with distinct body fat content and protein deposition rate remains unclear and the expression of *MyHC* isoforms would indicate a role of lysine in the regulation of protein synthesis in pigs and the effects on pork quality. In this study, we aimed to evaluate the effects of

paternal genetic group (Piau, Duroc-based and Pietrain) and nutritional plans with different digestible lysine contents (Low, Medium and High) on differential expression of lipid metabolism-related genes and pattern expression of *MyHC* isoforms in skeletal muscle.

2. Material and methods

2.1. Genetic groups and nutritional plans

Animal handling procedures were performed in accordance with the regulations approved by the Institutional Animal Welfare and Ethics/Protection commission (Protocol nº 20/2014) from the Universidade Federal de Viçosa, Brazil.

A total of 52 barrows averaging 25.44±3.27 kg of initial body weight and 50 gilts averaging 24.14±3.87 kg of initial body weight at 70 days of age were used and each animal was considered as an experimental unit. We used Piau Brazilian local breed, Duroc-based line and Pietrain breed as sires. Thus, the barrows genetic group was composed of 18 Piau crossbred pigs [G1 = Piau male x (Pietrain x Large White female)], 18 Duroc crossbred pigs [G2 = Duroc-based male x (Pietrain x Large White female)], and 16 Pietrain crossbred pigs [G3 = Pietrain male x (Pietrain x Large White female)]. The gilts genetic group was composed of 16 Piau crossbred pigs (G1), 18 Duroc crossbred pigs (G2), and 16 Pietrain crossbred pigs (G3).

Pigs from each genetic group (G1, G2 and G3) were randomly assign to one of the three nutritional plans based on digestible lysine (DL) levels as it follows: Low (7g DL fed from 70 to 98 days of age; 6g DL fed from 99 to 134 days of age; and 5g DL 135 to 156 days of age), Medium (9g DL fed from 70 to 98 days of age; 8g DL fed from 99 to 134 days of age; 7g DL 135 to 156 days of age), and High (11g DL fed from 70 to 98 days of age; 10g DL fed from 99 to 134 days of age; and 9g DL 135 to 156 days of age). Diets were formulated based on corn and soybean meal and supplemented with minerals, vitamins and amino acids to meet the nutritional requirements of the animals with except of DL, according to (ROSTAGNO et al., 2011).

Table 1. Chemical and nutritional composition of nutritional plans, for pig barrows and gilts, 70 to 98 days of age, 99 to 134 days of age and 135 to156 days of age

Ingredient	70 to 98 days of age			99 to 134 days of age			135 to156 days of age		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Corn meal	72.70	72.702	72.702	77.947	77.947	77.947	85.584	85.584	85.584
Soybean meal 45%	22.409	22.409	22.409	18.101	18.101	18.101	13.835	13.835	13.835
Soybean oil	1.159	1.159	1.159	0.686	0.686	0.686	0.441	0.441	0.441
Dicalcium phosphate	1.195	1.195	1.195	0.883	0.883	0.883	0.816	0.816	0.816
Caulim	1.100	0.696	0.035	1.100	0.734	0.069	1.100	0.770	0.111
Limestone	0.707	0.707	0.707	0.606	0.606	0.606	0.573	0.573	0.573
Salt	0.405	0.405	0.405	0.354	0.354	0.354	0.329	0.329	0.329
Vitamin mixture[1]	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Mineral mixture[2]	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
L-Lysine HCl 78%	-	0.260	0.520	-	0.260	0.520	-	0.260	0.520
DL-Methionine 99%	-	0.070	0.193	-	0.045	0.168	-	0.020	0.142
L-Threonine 98%	-	0.074	0.222	-	0.059	0.207	-	0.044	0.191
L-Tryptophan 98%	-	-	0.037	-	0.002	0.041	-	0.006	0.044
L-Valine 96,5%	-	-	0.093	-	-	0.095	-	-	0.092
L-Isoleucine 99%	-	-	-	-	-	-	-	-	0.042
Growth promotant[3]	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Butilhidroxitoluene	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Predicted nutritional composition									
ME (kcal/kg)	3,230	3,230	3,230	3,230	3,230	3,230	3,230	3,230	3,230
CD (%)	15.86	15.86	15.86	14.32	14.32	14.32	12.76	12.76	12.76
Dig. Lysine (%)	0.700	0.900	1.100	0.600	0.800	1.000	0.500	0.700	0.900
Dig. Met. + Cis. (%)	0.470	0.540	0.660	0.436	0.480	0.600	0.399	0.420	0.540
Dig. Threonine (%)	0.536	0.603	0.737	0.483	0.536	0.670	0.429	0.469	0.603
Dig. Tryptophan (%)	0.164	0.164	0.198	0.142	0.144	0.180	0.120	0.126	0.162
Dig.Valine (%)	0.672	0.672	0.759	0.604	0.604	0.690	0.535	0.535	0.621
Dig. Isoleucine (%)	0.000	0.000	0.000	0.523	0.523	0.523	0.452	0.452	0.495
Calcium (%)	0.635	0.635	0.635	0.512	0.512	0.512	0.474	0.474	0.474
Available P (%)	0.314	0.314	0.314	0.250	0.250	0.250	0.231	0.231	0.231
Sodium (%)	0.180	0.180	0.180	0.160	0.160	0.160	0.150	0.150	0.150

[1]For each kg of the product: Fe, 100 g; Cu, 10 g; Co, 1 g; Mn, 40 g; Zn, 100 g; I, 1,5 g; excipient q.s.p., 1.000 g.
[2]For each kg of the product: vitamin A, 6.000.000 UI; vitamin D3, 1.500.000 UI; vitamin E, 15.000.000 UI; vitamin B1, 1,35 g; vitamin B2, 4 g; vitamin B6, 2 g; Pantothenic acid, 9,35 g; vitamin K3, 1,5 g; Nicotinic acid, 20,0 g; vitamin B12, 20,0 g; Pholic acid, 0,6 g; Biotin, 0,08 g; Se, 0,3 g; excipient q.s.p., 1.000 g.
[3]Surmax® Elanco.

2.2.Color, drip loss and ether extract

The pigs were slaughtered at 156 days of age after 16 h of fasting. Pre-harvest handling and slaughtering procedures was in accordance with good animal welfare practices, following the Sanitary and Industrial Inspection Regulation for Animal Origin Products (BRASIL, 1997). After the slaughter, each carcass was split into two

identical longitudinal halves and chilled at 4°C for 24 h. At the end of carcass fabrication, a boneless Longissimus section 12 cm thick was collected. Longissimus samples were individually vacuum packaged and frozen at − 20 °C. Each frozen Longissimus sample was standardized into two 2.54 cm thick steak samples (AMSA, 1995). All steaks were then vacuum packaged and held at - 20 °C.

Instrumental color analysis was performed at the beginning of carcass fabrication after 24 h post mortem chill. Color measurements were taken at Longissimus muscle at the 10th rib after 30 min blooming. A total of three readings of *L**, *a** and *b** values were obtained for each carcass. Color coordinates (*L**, *a** and *b**) were recorded with a digital Minolta CR300 chromometer (Minolta Co., Osaka, Japan). Coordinate *a** ranged from red (+ *a**) to green (− *a**) and coordinate *b** from yellow (+ *b**) to blue (− *b**) (HUNTERLAB, 1996).

Drip loss was determined according the bag method described by HONIKEL (1987). Briefly, meat samples were collected 24 h after postmortem at 14th rib and trimmed from visible fat and connective tissue. Then, meat samples were divided into two slices weighing approximately 120 grams. Each slice was then hung in a hook under a lid of an airtight container and kept at 4 °C for 24 h. Following the 48 h chill, meat samples were weighed and the weight difference was divided by the initial sample weight.

Powdered lyophilizated meat samples were analyzed for moisture by the Method 934.01 (AOAC, 1990), and ether extract (EE) by the Method Am 5-04 (AOCS, 2009), to determine the amount of intramuscular fat content.

2.3.RNA extraction and cDNA synthesis

Samples from Longissimus (LD) and Psoas muscles (PM) were collected in three pigs of each treatment (genetic group and nutritional plan) at slaughter, immediately immersed in tubes containing 15 ml of RNAHolder® (BioAgency, São Paulo, Brazil), kept at 4°C overnight and then stored at -20°C for subsequent RNA extraction. The total RNA from each LD and PM samples was isolated using ~40 mg of tissue previously stored in RNAHolder®. The samples were homogenized in buffer RLT containing 1% β-mercaptoethanol (RNeasy Mini Kit; Qiagen, Valencia, CA, USA) and lysed with a tissue ruptor (Qiagen) homogenizer. The total RNA from the LD muscle samples was extracted with the RNeasy Mini Kit following the

manufacturer's recommendations. Additional treatment with DNase was performed on the columns using the RNase-free DNase Set (Qiagen), according to the manufacturer's recommendations. RNA concentrations were checked by NanoVue Plus Spectrophotometer (GE Healthcare, Munich, Germany) with an optimal 260/280 ratio between 1.8 and 2.1. Purity and integrity were determined with an Agilent RNA 6000 Nano Kit using the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Ontario, Canada).

The ProtoScript M-MuLV First Strand cDNA Synthesis Kit (New England, BioLabs Inc., Ipswich, Massachusetts, USA) was used to produce cDNA immediately after the RNA extraction. The reactions were performed with 6 μg of total RNA and 2 μl of 50μM oligo(dT)23VN primer, following the manufacturer's recommended protocol. The cDNA concentrations from the samples were estimated on a NanoVue Plus spectrophotometer (GE Healthcare). Finally, the single-stranded cDNA samples were stored at -20ºC for analysis.

2.4.Quantitative real-time polymerase chain reaction (qRT-PCR)

From the 10 analyzed genes their sequences were obtained from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov).Genes involved in pork quality were studied *FAS* (Fatty acid synthase), *H-FABP* (Heart fatty acid-binding protein), *SCD* (Stearoyl-CoA desaturase), *PRKAG1* (Protein kinase, AMP-activated, γ1 subunit), *PRKAG3* (Protein kinase, AMP-activated, γ3 subunit), *HSL* (Hormone sensitive lipase) in Longissimus muscle. Genes involved in the four isoforms of myosin heavy chain also were studied *MyHC I*, *MyHC IIa*, *MyHC IIb* and *MyHC IIx* in Longissimus and Psoas muscles. The primers for the 10 genes were designed using PrimerQuest (www.idtdna.com/Scitools/Applications/PrimerQuest), provided by Integrated DNA Technologies Inc. (Coralville, IA, USA). *β-actin* gene was used as the reference gene for normalization, as it showed higher efficiency and less variation across treatments than *GAPDH* (data not shown). The nucleotide sequence of the primers and accession numbers is summarized in Table 2.

The qRT-PCR reactions were performed using the GoTaq® qPCR Master Mix (Promega Corporation, Madison, WI, USA) following the manufacturer's instructions in an ABI Prism 7300 Sequence Detection System thermocycler® (Applied Biosystems, Foster City, CA, USA). The reaction consisted of an initial step at 95ºC

for 10 min, a second step of 40 cycles with the same temperature for 15 s and a final extension step at 60ºC for 60 s. After the amplification cycles, an additional gradient step from 60ºC to 95ºC was used to obtain a melting curve. The measurement in qRT-PCR experiment is expressed in cycles to threshold (C_t) of PCR; a relative value that represents the cycle number at which the amount of amplified cDNA reaches the threshold level. The efficiency of each reaction was assessed in order to choose the best combination of cDNA and primer concentration in the subsequent reactions. Once the slope of a linear regression, established between the cDNA log and the C_t value, for each primer concentration was obtained, the PCR efficiency (E) was calculated using the following formula: E =10^(–1/slope). All reactions were done in duplicate and the coefficient of variation (CV) of C_t values from replicates within each sample was low, <5%, indicating acceptable accuracy and reproducibility (data not shown).

Table 2. Nucleotide sequence of the primers and accession number of the nucleotide sequences used in the qRT-PCR reactions identified as differentially expressed using the EST libraries analyzed

Gene symbol	Primer sequence (5'-3')[a]	Aces. number - NCBI
FAZ	Fw: GTACCCGGACCCAGAATA Rev: GTGGTGCAAGGGTTACAG	AJ001202.1
HFABP	Fw: CACTGGATGGAGGCAAAC Rev: GGGTGAGTGTCAGGATGA	JN646857.1
SCD	Fw: CACTGGGAAGCACAAGAG Rev: CCCAAGACTCAGCCAAAG	NM_213781.1
PRKAG1	Fw: GCATGAGACCCTGGAAAC Rev: CCCTTGACCACGTCATTC	NM_001001642.2
PRKAG3	Fw: GCTGACCATCACAGACTTC Rev: TCCCTCCAGGTCTCAATC	NM_214077.1
HSL	Fw: GCTCACGGTCACCATTTC Rev: CCTCACTGTCCTGTCCTT	AY559451.1
MyHC I	Fw: AAGGGCTTGAACGAGGAGTAGA Rev: TTATTCTGCTTCCTCCAAAGGG	AB053226 (WIMMERS et al., 2008)
MyHC IIa	Fw: AACACCCTGACCAAAGCTAAA Rev: TCCTCTTGGCTCTCTCTAAGTC	AB025256
MyHC IIb	Fw: ATGAAGAGGAACCACATTA Rev: TTATTGCCTCAGTAGCTTG	AB025261 (WIMMERS et al., 2008)
MyHC IIx	Fw: AGAAGATCAACTGAGTGAACT Rev: AGAGCTGAGAAACTAACGTG	AB025262 (WIMMERS et al., 2008)
β-actin[1]	Fw: GGACTTCGAGCAGGAGATGG Rev: TCTGCTGTCTTTGGAACTTTGTCT	NM_213978.1

[1]Reference gene.

[a]Fw= forward; Rev= reverse.

2.5.Statistical analysis

Statistical analyses were individually performed for each sex. The following model was used for meat quality traits:

$$y_{ijk} = m + G_i + N_j + GxN_{ij} + b(W_{ik} - \bar{W}_i) + e_{ijk}$$

where:

G_i: ith level of the fixed effect of genetic group; N_j: jth level of the fixed effect of nutritional plan; W_{ik}: slaughter weight was used as a covariate within genetic group; e_{ijk}: random error associated with $y_{ijk..}$

All analyses were performed using SAS 9.3 (Statistical Analysis System Institute, Inc., Cary, NC, USA). Contrasts were compared by Tukey`s test and differences were considered at $P \leq 0.05$.

Statistical analysis of C_t data in each growing phase was realized using %QPCR_MIXED macro SAS® (https://www.msu.edu/~steibelj/JP files/QPCR.html) developed to generate codes in SAS PROC MIXED suitable to analyze data from qRT-PCR, assuming independent random effects for reference and target genes in each biological replicate (STEIBEL et al., 2009). This statistical method is more accurate, powerful and flexible than existing alternatives for analysis of relative quantification qRT-PCR data, and it is especially useful in more complex experimental designs, involving more than two treatments or time points and multiple experimental factors. The following model was used:

$$y_{gikr} = GN_{gi} + C_{gik} + D_{ik} + e_{gikr}$$

where y_{gikr}: corresponds to the C_t value obtained from the thermocycler software for the gth gene (reference or targets) from the rth well which corresponds to the kth animal submitted to the ith treatment (genetic group and nutritional plans combination); GN_{gi}: the effect of the ith treatment on the expression of gene gth; $C_{gik} \sim N(0, \sigma_C^2)$: the gene-specific random effect of the kth animal; $D_{ik} \sim N(0, \sigma_D^2)$: the sample-specific random effect (common to reference and target genes); and $e_{gikr} \sim N(0, \sigma_e^2)$: the residual term.

The fold change values were estimated with the $2^{\wedge -\Delta\Delta C_t}$ method (Livak and Shmittgen, 2001). The ΔC_t are estimates of comparison of C_t values between treatments for the normalized target genes. For each target gene, the comparison of C_t values across treatments, inside each phase, was performed by CONTRAST statement of the GLM procedure (SAS software) using Student's t-test to the level of 5%. Once the efficiency (E) of the qRT-PCR reaction was close 100%, one PCR cycle of difference between two samples means twice as much expression in the first sample in comparison with the second.

3. Results

3.1.Barrows

3.1.1. Color, drip loss and ether extract

There was no interaction between genetic groups and nutritional plans for any meat quality traits evaluated ($P > 0.05$) in barrows.

Different values of drip loss were observed for pork from different genetic groups ($P = 0.02$) where barrows from Piau crossbred group had the least value for this variable, compared to Pietrain crossbred barrows.

No differences were observed for values of L^* ($P = 0.61$), a^* ($P = 0.38$), b^* ($P = 0.91$) and ether extract (EE; $P = 0.54$) among genetic groups (Table 3).

Nutritional plan did not affect any of the meat quality variables evaluated in this study ($P > 0.05$).

Table 3. Effect of genetic group and nutritional plan of digestible lysine on drip loss, color and ether extract of barrows

Item	Genetic group			CV	P-value	Nutritional plan			CV	P-value
	G1	G2	G3	(%)		Low	Medium	High	(%)	
Drip loss (%)	7.77[b]	9.61[ab]	11.14[a]	29.29	0.02	10.42	9.30	8.80	30.05	0.22
*L**	59.87	61.54	60.70	5.07	0.61	61.96	60.28	59.88	4.75	0.11
*a**	6.37	6.69	6.98	19.67	0.38	6.53	6.67	6.84	19.99	0.79
*b**	15.24	15.53	15.50	7.34	0.91	15.33	15.52	15.42	7.35	0.89
Ether extract (%)	2.20	2.46	2.32	19.84	0.54	2.44	2.19	2.35	20.23	0.30

CV: Coefficient of variation.
G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds.
Means with different letters differ at P≤0.05 by Tukey`s test.

3.1.2. Gene expression of lipid metabolism-related genes in Longissimus muscle

Relative expression changes (fold change) all statistical results for each contrast across genetic groups and nutritional plans are shown in the Appendix for barrows and gilts.

There was a higher expression of the *FAS* gene in skeletal muscle of Piau crossbred compared to Duroc and Pietrain crossbred barrows (Figure 1-A). However, there was a higher expression of the *H-FABP* gene in skeletal muscle of Duroc and Pietrain crossbred compared to Piau crossbred barrows (Figure 1-A). Piau crossbred had higher expression of *PRKAG1* compared to Duroc crossbred barrows (Figure 1-A). The expression of *SCD* gene was higher in Duroc crossbred compared to Piau crossbred barrows (Figure 1-A).

The nutritional plan did not affect the expression of the genes evaluated with exception of *PRKAG3*, which was greater in barrows fed Low nutritional plan compared to those fed Medium and High nutritional plan (Figure 1-B).

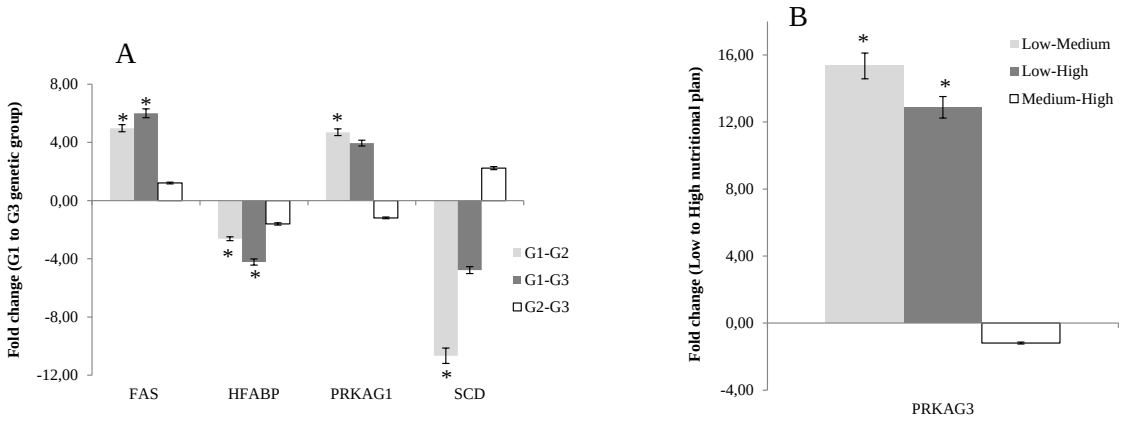

Figure 1. Fold change values for five genes that exhibited differential expression between G1, G2 and G3 (G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds) (A) or between nutritional plan Low, Medium and High (B) in barrows. Bars above the origin means higher expression at first level relatively to second. *P < 0.05.

3.1.3. Gene expression of muscle fiber type in Longissimus and Psoas muscle

There was a higher expression of the *MyHC IIb* gene in Longissimus muscle of Duroc and Pietrain crossbred compared to Piau crossbred barrows (Figure 2-A). The nutritional plan did affect the expression of the *MyHC IIb* gene in Longissimus muscle, which was greater in barrows fed Medium and High nutritional plan compared to those fed Low nutritional plan (Figure 2-B).

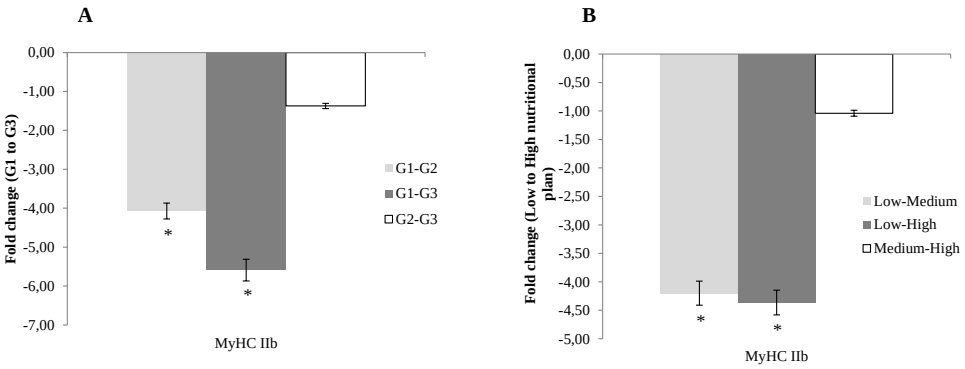

Figure 2. Fold change values for *MyHC IIb* gene that exhibited differential expression between G1, G2 and G3 (G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds) (A) or between nutritional plan Low, Medium and High (B) in Longissimus muscle. Bars above the origin means higher expression at first level relatively to second. *$P < 0.05$.

There was a higher expression of the *MyHC IIa* gene in Psoas muscle of Duroc crossbred compared to Piau crossbred barrows (Figure 3-A). Gene expression of the *MyHC IIa* was greater in barrows fed Low nutritional plan compared to those fed Medium and High nutritional plan (Figure 2-B).

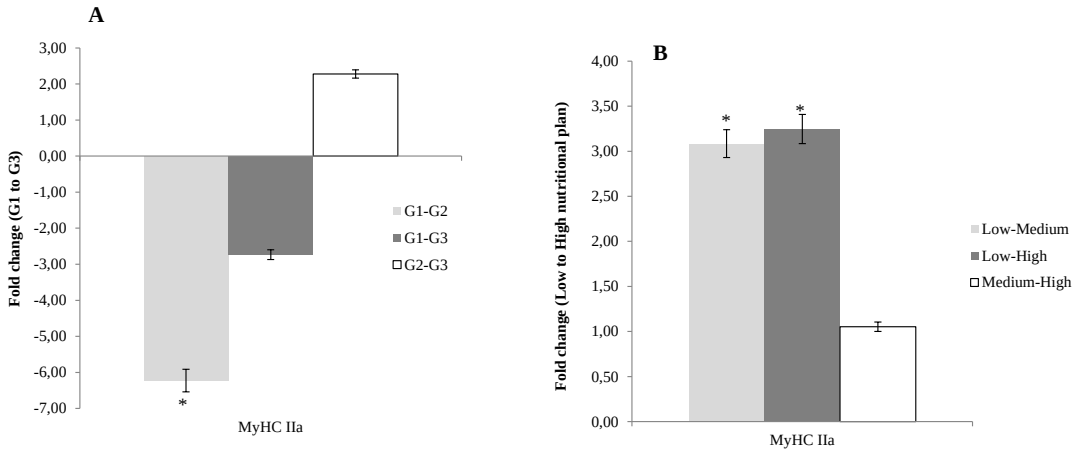

Figure 3. Fold change values for *MyHC IIa* gene that exhibited differential expression between G1, G2 and G3 (G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds) (A) or between nutritional plan Low, Medium and High (B) in Psoas muscle. Bars above the origin means higher expression at first level relatively to second. *$P < 0.05$.

3.2.Gilts

3.2.1. Color, drip loss and ether extract

No interaction was observed ($P > 0.05$) between genetic groups and nutritional plans for any meat quality traits evaluated in crossbred gilts (Table 4).

No changes were observed for any meat quality variables evaluated among genetic groups ($P > 0.05$), with exception of drip loss ($P = 0.01$) which was greater in pork from Pietrain compared to Duroc and Piau crossbred gilts (Table 4).

The nutritional plans did not affect any of the meat quality variables evaluated ($P > 0.05$) with exception of ether extract ($P = 0.01$), which was greater in gilts fed Low compared to those fed High nutritional plan (Table 4).

Table 4. Effect of genetic group and nutritional plan of digestible lysine on drip loss, color and ether extract of gilts

Item	Genetic group			CV	P-value	Nutritional plan			CV	P-value
	G1	G2	G3	(%)		Low	Medium	High	(%)	
Drip loss (%)	6.85[b]	7.37[b]	10.24[a]	34.04	0.01	8.07	8.16	7.60	36.27	0.31
*L**	59.76	59.91	59.46	4.59	0.75	60.33	59.65	59.16	4.66	0.25
a*	6.22	7.25	6.58	19.05	0.71	7.24	6.02	6.78	18.36	0.08
b*	14.97	15.62	14.93	7.01	0.74	15.28	15.14	15.10	6.97	0.29
Ether extract (%)	2.35	2.23	1.94	25.59	0.49	2.53[a]	2.01[ab]	1.97[b]	22.81	0.01

CV: Coefficient of variation.
G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds.
Means with different letters differ at P≤0.05 by Tukey`s test.

3.2.2. Gene expression of lipid metabolism-related genes in Longissimus muscle

There was a higher expression of the *FAS* gene of Piau crossbred compared to Duroc and Pietrain crossbred gilts (Figure 4). However, there was a higher expression of the *SCD* gene of Duroc and Pietrain crossbred compared to Piau crossbred gilts (Figure 4). Piau crossbred showed higher expression of *PRKAG1* compared to Pietrain crossbred (Figure 4). The nutritional plan did not affect the expression of any of the genes evaluated.

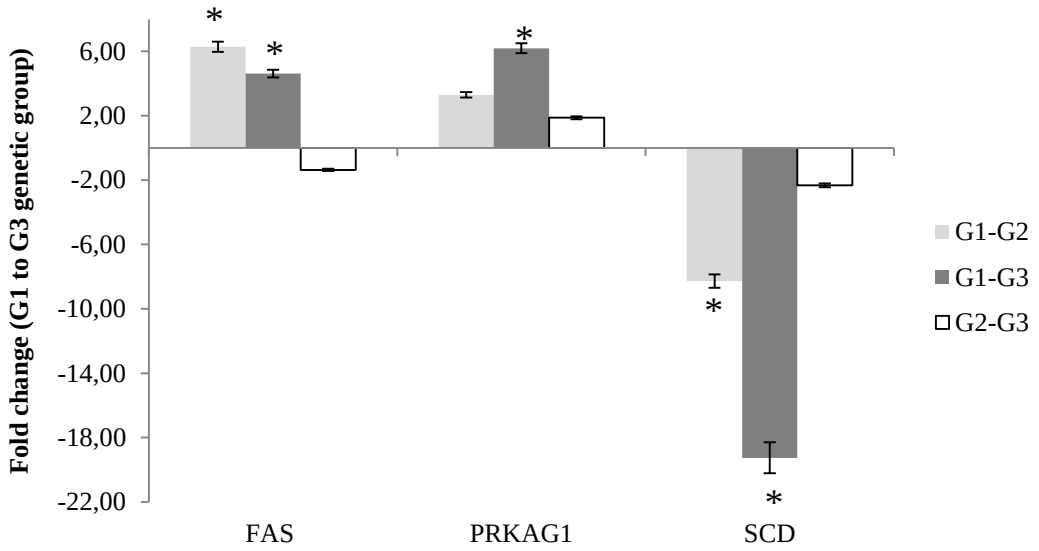

Figure 4. Fold change values for four genes that exhibited differential expression between G1, G2 and G3 (G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds) in gilts. Bars above the origin means higher expression at first level relatively to second. *$P < 0.05$.

3.2.3. Gene expression of muscle fiber type in Longissimus and Psoas muscle

There was a higher expression of the *MyHC IIa* gene in Longissimus muscle of Duroc and Pietrain crossbred compared to Piau crossbred gilts (Figure 5-A). The nutritional plan did affect the expression of the *MyHC IIb* and *IIa* genes in Longissimus muscle. A higher expression of the *MyHC IIb* gene was greater in gilts fed High nutritional plan compared to those fed Medium nutritional plan (Figure 5-B). A higher expression of the *MyHC IIa* gene was observed in gilts fed High nutritional plan compared to those fed Low and Medium nutritional plan (Figure 5-B).

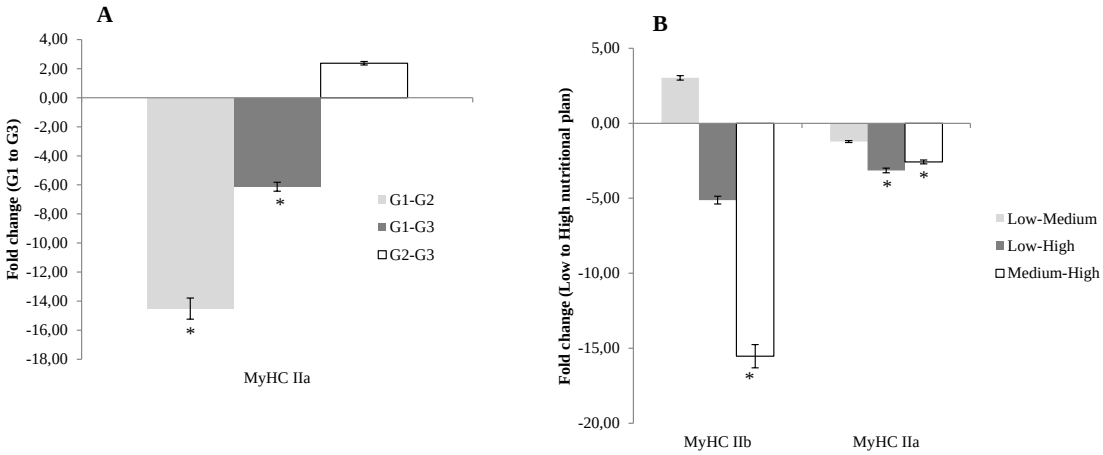

Figure 5. Fold change values for *MyHC IIa* gene that exhibited differential expression between G1, G2 and G3 (G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds) (A) or between nutritional plan Low, Medium and High (B) in Longissimus muscle (*MyHC IIa* and *IIb*). Bars above the origin means higher expression at first level relatively to second. **P* < 0.05.

There was a higher expression of the *MyHC IIx* gene in Psoas muscle of Duroc crossbred compared to Piau crossbred gilts (Figure 6) and higher expression of the *MyHC IIx* gene in Psoas muscle of Pietrain crossbred compared to Duroc crossbred gilts (Figure 6).

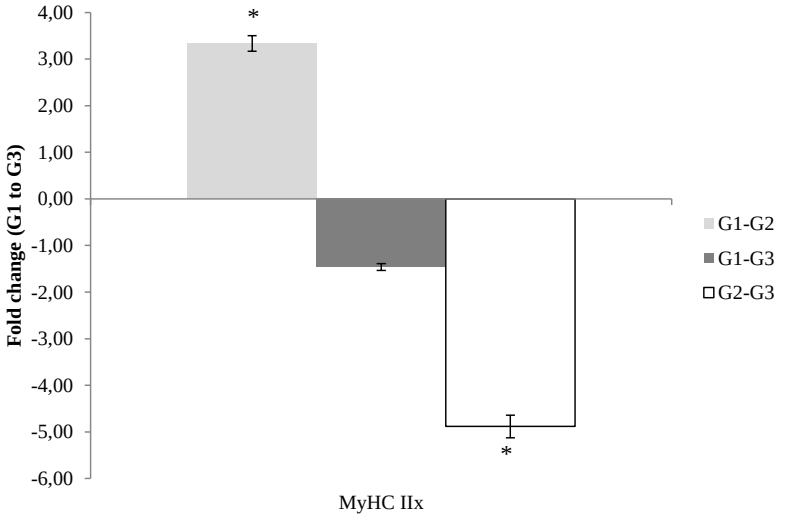

Figure 6. Fold change values for *MyHC IIx* gene that exhibited differential expression between G1, G2 and G3 (G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain

crossbreds) in Psoas muscle. Bars above the origin means higher expression at first level relatively to second. *$P < 0.05$.

4. Discussion

4.1. mRNA expression of MyHC isoforms in Longissimus muscle

Intensive selection for lean muscle growth in modern pigs induced a shift in muscle metabolism toward a more glycolytic and less oxidative fiber type (CHANG et al., 2003). Our result support this hypothesis as Duroc and Pietrain crossbred barrows possessed a more glycolytic and less oxidative metabolism, respectively, as well as a smaller capacity to use lipids as an energetic substrate. In the present study, results showed that Longissimus muscle of Duroc and Pietrain crossbred barrows exhibited increased expression of glycolytic fibers (*MyHC IIb*) compared with Piau. Moreover, a greater expression of *MyHC IIa* was observed in Duroc crossbred barrows compared to Piau.

We have also observed that Piau crossbred gilts exhibited increased expression of *MyHC IIx* compared with Duroc. These results suggested the higher expression of *MyHC IIa* was consistent with other local breeds such as Jinhua (GUO et al., 2011) and Meishan (LEFAUCHEUR et al., 2004) compared with commercial breeds. When protein deposition rate decreases, lipid deposition becomes the major component of weight gain and energy request to fat tissues increases (HOCQUETTE et al., 2000). Consequently, less energy substrate to contraction will be available to muscles fibers and energy metabolism of glycolytic fibers switch towards to oxidative energy metabolism, which is a more efficient pathway to produce energy (HOCQUETTE et al., 1998).

Collectively, different expression patterns of *MyHC* observed in the present study explains the differences observed for drip loss among genetic groups, therefore the frequency of skeletal muscle fibers can be predicted by their predominant *MyHC* isoforms (*I*, *IIa*, *IIx* and *IIb*) (KIM et al., 2013; LEE et al., 2012; RUUSUNEN; PUOLANNE, 2004). These *MyHC* isoforms have particular metabolic and contractile characteristics and are differently distributed among muscles, suggesting that *MyHC* distribution is related to their function (LEFAUCHEUR et al., 2002). Type I muscle fibers have greater oxidative capacity to support sustained contraction, whereas type *IIb* fibers are predominantly glycolytic and rapidly use glycogen for short bursts of

activity. The *IIa* and *IIx* fibers are intermediate to type *I* and *IIb* fibers (CHANG et al., 2003). Thus, the greater the frequency of type *IIb* fibers, greater is the chance to have a rapid decline in muscle pH during its conversion into meat, which decreases the water-holding capacity of meat and leads to a greater drip loss of pork. In the present study, lower drip loss observed in pork from Piau crossbred pigs may have occurred due to lower frequency of type *IIb* muscle fibers, in terms that mRNA expression of *MyHC IIb* was lower in Piau compared to other genetic groups.

With regard to nutritional plans effects, we have observed a higher mRNA expression of *MyHC IIb* in Longissimus muscle of crossbred pigs fed with Medium and High nutritional plan compared to crossbred pigs that received Low nutritional plan. Higher mRNA expression of *MyHC IIb* in Longissimus muscle caused by nutritional plan suggests that lysine stimulates the synthesis of body proteins at a transcriptional level (MORALES et al., 2015). Lysine is the most abundant amino acid in pig muscle proteins (BIKKER et al., 1994). The greater mRNA expression of *MyHC IIb* in Longissimus muscle observed in pigs fed a nutritional plan containing different lysine levels support the hypothesis that lysine may stimulate, either direct or indirectly, the transcription of *MyHC IIb* (MORALES et al., 2015).

Our result demonstrates that reduction of lysine promotes lower expression of *MyHC IIa* in Longissimus muscle of gilts. Oxidative fibers (*MyHC I*, *IIa* and *IIx*) are rich in mitochondria, which are related to relative capacity of muscle tissue to oxidize fatty acids (GUO et al., 2011). A previously study have reported that carnitine content in Longissimus muscle was lower in pigs fed with dietary of low lysine levels, promoting increase of IMF. Carnitine plays a pivotal role in β-oxidation of fatty acids by transporting fatty acyl-CoA across the inner mitochondrial membrane (KATSUMATA et al., 2012). Moreover, we may also speculate that a potential decrease of β-oxidation may be related to lower expression of *MyHC IIa* in Longissimus muscle of gilts and may have contributed to a higher intramuscular fat content in gilts fed with low lysine levels.

We also observed that *MyHC IIa* showed higher expression in barrows feeding lower lysine levels dietary. Our finding suggests that lower lysine dietary may affect the expression levels of *MyHC IIa* mRNA in skeletal muscle. Furthermore, lipids are also deposited in fibers with higher oxidative capacity (ESSEN-GUSTAVSSON et al., 1994). Expression of *PGC-1α* (PPARγ coactivator-1α) in muscle induces the conversion of type *II* fibers to type *I* (LIN et al., 2002). *PGC-1α* coactivates members

of type *II* class nuclear hormone receptors, including PPARγ, responsible for concerted activation of genes determining mitochondrial respiratory capacity (PUIGSERVER; SPIEGELMAN, 2003).

4.2.Expression of lipid metabolism-related genes in Longissimus muscle

Intramuscular fat (IMF) measured by quantification of ether extract content on meat was greater with the decreasing of lysine in nutritional plans, which has been previously observed (BIDNER et al., 2004; KATSUMATA et al., 2005). Collectively, these results indicate that IMF may vary according to variation of digestible lysine on nutritional plan when dietary content of this amino acid is lower than required by the gilts.

Drip loss was smaller in Piau and Duroc compared to Pietrain crossbred gilts, in agreement to GJERLAUG-ENGER et al., (2010) that previously reported negative genetic correlation between IMF content and drip loss in meat. However, IMF did not differ between genotypes, although several authors have found greater IMF content for local breeds compared to commercial pigs (GUO et al., 2011; ZHAO et al., 2009).

We can assume that, compared with Piau crossbred, Duroc and Pietrain crossbred pigs showed satisfactory *de novo* lipogenesis capacity, although these pigs have higher lipid mobilization capacity in skeletal muscle (GUO et al., 2011; ZHAO et al., 2009). Therefore, in the present study, the reasons that may explain why genetic groups showed no differences in IMF according to relative mRNA expression levels of those key lipid metabolism pathways-related genes were assessed.

Based on intramuscular fat content results, we have evaluated mRNA expression of genes that encodes key enzymes related to lipid metabolism to understand the possible molecular factor underlying IMF deposition. Regarding lipogenesis, we have found differences in mRNA expression levels of *FAS*, *H-FABP*, *SCD* and *PRKAG1* between Piau, Duroc and Pietrain crossbred. *FAS* enzymes catalyzes all reaction steps in palmitate synthesis of acetyl-CoA and malonyl-CoA in the presence of NADPH (MUÑOZ et al., 2003; SUL; WANG, 1998), ie, *FAS* is key determinant of the maximal capacity of a tissue to synthesize fatty acids by *de novo* pathways (SMITH et al., 2003). Our present results showed that relative mRNA expression levels of *FAS* were greater in Piau crossbred pigs than in Duroc and Pietrain crossbred pigs.

We observed higher mRNA expression levels of *H-FABP* and *SCD* in Duroc and Pietrain crossbred compared with Piau crossbred. *H-FABP* is located on pig chromosome six and is expressed in various tissues, predominantly in cardiac and skeletal muscle cells (ZHAO et al., 2010). *H-FABP*, a protein of 15 kDa, is a major member of fatty acid binding protein (FABP) family, which plays a critical role in intracellular trafficking of long-chain fatty acids and metabolic homeostasis (CHMURZYŃSKA, 2006). This data suggested that Duroc and Pietrain crossbred pigs could have more fatty acid transport into intracellular trafficking compared to Piau crossbred pigs, facilitating the transport of fatty acids from membrane to sites of fatty acid oxidation.

Studies suggest that fatty acid composition in muscle tissue is different in fatty and lean pigs, in terms that local breeds showed a higher unsaturated fatty acid concentration compared with commercial pigs (SMITH et al., 1999; ZHAO et al., 2009). This difference may be due to *SCD* variance, in terms that *SCD* is a rate-limiting enzyme in biosynthesis of unsaturated fatty acid (DORAN et al., 2006). *SCD* catalyzes the conversion of saturated to unsaturated fatty acids of muscle lipids (RUDEL et al., 1995). However, the present study showed that *SCD* mRNA abundance in Duroc and Pietrain crossbred was higher compared with Piau crossbred pigs. Therefore, the results suggested that different expression of *SCD* between Duroc and Pietrain crossbred and Piau crossbred pigs may result in variation of muscle fatty acid composition.

PRKAG1 gene encodes a protein involved in fatty acid metabolism and is one of the three isoforms of AMPK γ subunit (BERNARD et al., 2007). At cellular level, AMPK is a regulatory mechanism that switches off ATP-consuming processes (lipogenesis, neoglucogenesis) and switches on catabolic processes that produce ATP (fatty acid oxidation, glycogenolysis, glycolysis) (HARDIE, 2011). Indeed, by inhibiting acetyl-CoA carboxylase (ACC) enzyme (HARDIE, 2003), AMPK activates fatty acid oxidation and reduces fatty acid synthesis (HARDIE, 2003). Our present results showed that the relative mRNA expression levels of *PRKAG1* were greater in Piau crossbred pigs than in Duroc and Pietrain crossbred pigs. This suggested that the fatty acid oxidation in muscles of Piau crossbred pigs was higher compared with Duroc and Pietrain crossbred pigs.

Our results showed that the relative mRNA expression levels of *FAS* and *PRKAG1* in Longissimus muscle were higher in Piau crossbred than in Duroc and

Pietrain crossbred pigs. However, relative mRNA expression levels of *H-FABP* and *SCD* were higher in Duroc and Pietrain crossbred than in Piau crossbred pigs. Probably, these differences may have been the cause of differences absence in IMF amount. These data indicated that IMF content between pig genotypes would partially occur by different lipid mobilization and fatty acid oxidation capacity.

We have found differences on relative mRNA expression levels of *PRKAG3* between Low, Medium and High nutritional plans in barrows. *PRKAG3* (AMPK γ3 subunit) gene is considered to be essential in energy homoeostasis due to its ability to 'sense' the levels of ATP, ADP and AMP (HARDIE, 2011). One of its target proteins is the glycogen synthase, a key regulatory enzyme in glycogen biosynthetic pathway, which is inhibited by phosphorylating activity of AMPK (CARLING; HARDIE, 1989). Due to its effects on glycogen synthase, AMPK exerts a strong influence on glycogen content in muscle tissue (GALVE et al., 2013). AMPK γ3 subunit has also been associated with enhanced oxidative metabolism, indicating higher relative area of IIA fibers (oxidative) and lower relative area of IIB fibers (glycolytic) (LEBRET et al., 1999). Oxidative fibers may be associated to increased IMF content (GUO et al., 2011). Furthermore, low dietary lysine level decreases the transcription of fibers IIB, but there is no available report showing the mechanism by which lysine causes this effect (MORALES et al., 2015) and increase IMF content (KATSUMATA et al., 2012; MADEIRA et al., 2013). In our study, it was observed a higher gene expression of *PRKAG3* in barrows feeding Low nutritional plan compared with barrows feeding Medium and High nutritional plan. Thus, the higher expression of *PRKAG3* gene in barrows feeding low lysine dietary may affect the increased IMF content probably due to higher increased of oxidative fibers. However, we did not observe differences in IMF amount between nutritional plans in barrows. These results suggest that gilts may have differences in lipid metabolism, such as enhancement of lipogenesis, reduction in β-oxidation of fatty acids, or both metabolic processes in Longissimus muscle. Higher IMF content in gilts fed with low lysine levels dietary confirms these differences in lipid metabolism.

5. Conclusions

The present study indicated that genotype has a strong influence on deposition of intramuscular fat content mainly by up-regulation of intramuscular lipogenic gene

expression. Differentially expression of *MyHC* isoforms in muscles may be the most important factors affecting intramuscular fat content and drip loss in pig genotypes. These results may provide valuable information for understanding the differences of meat quality in pig genotypes. Therefore, based on this information, it could be possible to manipulate meat quality forming process to produce high-quality pork.

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Supplementary Data

Supplementary Table 1. Fold change results for each contrast of genetic groups within each nutritional plan for *FAS* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	-2.3137	0.8374	4.9716	0.0128
	G1-G3	-2.5852	0.8374	6.0010	0.0064
	G2-G3	-0.2714	0.8374	1.2070	0.7495
	Low-Medium	0.2289	0.8374	-1.1719	0.7877
	Low-High	1.3212	0.8374	-2.4987	0.1320
	Medium-High	1.0922	0.8374	-2.1320	0.2085
Gilts					
	G1-G2	-2.6539	1.0333	6.2937	0.0193
	G1-G3	-2.2080	1.0333	4.6203	0.0466
	G2-G3	0.4459	1.0333	-1.3622	0.6712
	Low-Medium	0.4614	1.0333	-1.3769	0.6605
	Low-High	1.6210	1.0333	-3.0759	0.1341
	Medium-High	1.1596	1.0333	-2.2340	0.2765

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 2. Fold change results for each contrast of genetic groups within each nutritional plan for *HFABP* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	1.3922	0.6142	-2.6248	0.0360
	G1-G3	2.0798	0.6142	-4.2275	0.0033
	G2-G3	0.6876	0.6142	-1.6106	0.2776
	Low-Medium	0.1413	0.6142	-1.1029	0.8207
	Low-High	-0.2471	0.6142	1.1868	0.6922
	Medium-High	-0.3884	0.6142	1.3089	0.5351
Gilts					
	G1-G2	0.8379	1.1173	-1.7874	0.4630
	G1-G3	1.4792	1.1173	-2.7879	0.2021
	G2-G3	0.6413	1.1173	-1.5597	0.5731
	Low-Medium	-0.06433	1.1173	1.0456	0.9547
	Low-High	0.6446	1.1173	-1.5633	0.5711
	Medium-High	0.7089	1.1173	-1.6346	0.5337

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 3. Fold change results for each contrast of genetic groups within each nutritional plan for *SCD* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	3.4147	1.3628	-10.6642	0.0220
	G1-G3	2.2579	1.3628	-4.7829	0.1149
	G2-G3	-1.1568	1.3628	2.2296	0.4071
	Low-Medium	-1.4017	1.3628	2.6421	0.3173
	Low-High	-0.4388	1.3628	1.3555	0.7512
	Medium-High	0.9628	1.3628	-1.9491	0.4889
Gilts					
	G1-G2	3.0481	1.1622	-8.2712	0.0173
	G1-G3	4.2671	1.1622	-19.2542	0.0017
	G2-G3	1.2191	1.1622	-2.3280	0.3081
	Low-Medium	-1.2272	1.1622	2.3411	0.3050
	Low-High	-0.2078	1.1622	1.1549	0.8601
	Medium-High	1.0193	1.1622	-2.0269	0.3920

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 4. Fold change results for each contrast of genetic groups within each nutritional plan for *PRKAG1* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	-2.2307	1.0391	4.6936	0.0457
	G1-G3	-1.9804	1.0391	3.9460	0.0728
	G2-G3	0.2503	1.0391	-1.1895	0.8124
	Low-Medium	0.3330	1.0391	-1.2596	0.7523
	Low-High	0.7549	1.0391	-1.6875	0.4769
	Medium-High	0.4219	1.0391	-1.3397	0.6895
Gilts					
	G1-G2	-1.7223	0.9416	3.2996	0.0840
	G1-G3	-2.6318	0.9416	6.1980	0.0120
	G2-G3	-0.9095	0.9416	1.8784	0.3469
	Low-Medium	0.8457	0.9416	-1.7971	0.3809
	Low-High	1.1834	0.9416	-2.2711	0.2249
	Medium-High	0.3377	0.9416	-1.2637	0.7241

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 5. Fold change results for each contrast of genetic groups within each nutritional plan for *PRKAG3* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	0.8604	1.7572	-1.8155	0.6283
	G1-G3	1.6828	1.7572	-3.2105	0.3467
	G2-G3	0.8224	1.7572	-1.7683	0.6435
	Low-Medium	-3.9402	1.7572	15.3504	0.0333
	Low-High	-3.6869	1.7572	12.8786	0.0453
	Medium-High	0.2533	1.7572	-1.1919	0.8865
Gilts					
	G1-G2	-0.3877	1.8617	1.308306	0.8365
	G1-G3	1.1405	1.8617	-2.20457	0.5451
	G2-G3	1.5282	1.8617	-2.88426	0.4186
	Low-Medium	-1.1214	1.8617	2.17558	0.5518
	Low-High	0.7487	1.8617	-1.68028	0.6906
	Medium-High	1.8701	1.8617	-3.65558	0.3237

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 6. Fold change results for each contrast of genetic groups within each nutritional plan for *HSL* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	0.2184	1.6001	-1.1634	0.8923
	G1-G3	-2.0531	1.6001	4.1500	0.2091
	G2-G3	-2.2715	1.6001	4.8282	0.1658
	Low-Medium	-0.2255	1.6001	1.1692	0.8889
	Low-High	-0.5387	1.6001	1.4527	0.7387
	Medium-High	-0.3132	1.6001	1.2425	0.8461
Gilts					
	G1-G2	0.8804	1.7184	-1.8409	0.6147
	G1-G3	-0.7328	1.7184	1.6619	0.6748
	G2-G3	-1.6132	1.7184	3.0593	0.3603
	Low-Medium	0.6518	1.7184	-1.5711	0.7089
	Low-High	-1.3951	1.7184	2.6301	0.4275
	Medium-High	-2.0469	1.7184	4.1322	0.2491

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 7. Fold change results for each contrast of genetic groups within each nutritional plan for *MyHC I* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	-2.0621	2.0681	4.1759	0.3319
	G1-G3	-3.5446	2.0681	11.6689	0.1037
	G2-G3	-1.4825	2.0681	2.7943	0.4827
	Low-Medium	-0.2934	2.0681	1.2255	0.8888
	Low-High	1.6264	2.0681	-3.0874	0.4418
	Medium-High	1.9194	2.0681	-3.7837	0.3655
Gilts					
	G1-G2	-1.0312	1.9966	2.0437	0.6118
	G1-G3	-2.7992	1.9966	6.9605	0.1779
	G2-G3	-1.7680	1.9966	3.4058	0.3876
	Low-Medium	2.6518	1.9966	-6.2845	0.2007
	Low-High	2.8702	1.9966	-7.3117	0.1677
	Medium-High	0.2183	1.9966	-1.1634	0.9141

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 8. Fold change results for each contrast of genetic groups within each nutritional plan for *MyHC IIa* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	-0.4052	0.7650	1.3243	0.6028
	G1-G3	-0.1053	0.7650	1.0757	0.8920
	G2-G3	0.2998	0.7650	-1.2310	0.6997
	Low-Medium	0.0951	0.7650	-1.0682	0.9024
	Low-High	0.4883	0.7650	-1.4028	0.5313
	Medium-High	0.3932	0.7650	-1.3133	0.6135
Gilts					
	G1-G2	-0.4783	0.5510	1.3931	0.3968
	G1-G3	-0.2906	0.5510	1.2231	0.6043
	G2-G3	0.1877	0.5510	-1.1389	0.7373
	Low-Medium	0.2907	0.5510	-1.2232	0.6042
	Low-High	1.6552	0.5510	-3.1497	0.0076
	Medium-High	1.3645	0.5510	-2.5749	0.0234

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 9. Fold change results for each contrast of genetic groups within each nutritional plan for *MyHC IIx* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	-0.2181	0.5714	1.1632	0.7072
	G1-G3	-0.3492	0.5714	1.2739	0.5487
	G2-G3	-0.1312	0.5714	1.0952	0.8210
	Low-Medium	0.5368	0.5714	-1.4508	0.3600
	Low-High	0.1764	0.5714	-1.1301	0.7610
	Medium-High	-0.3603	0.5714	1.2837	0.5362
Gilts					
	G1-G2	3.8593	0.7387	-14.5133	0.0001
	G1-G3	2.6160	0.7387	-6.1305	0.0023
	G2-G3	-1.2433	0.7387	2.3674	0.1096
	Low-Medium	0.8496	0.7387	-1.8020	0.2652
	Low-High	0.7386	0.7387	-1.6686	0.3306
	Medium-High	-0.1109	0.7387	1.0799	0.8823

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 10. Fold change results for each contrast of genetic groups within each nutritional plan for *MyHC IIb* gene target in Longissimus muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	2.0259	0.7589	-4.0725	0.0156
	G1-G3	2.4834	0.7589	-5.5921	0.0042
	G2-G3	0.4575	0.7589	-1.3732	0.5541
	Low-Medium	2.0704	0.7589	-4.2000	0.0138
	Low-High	2.1260	0.7589	-4.3651	0.0118
	Medium-High	0.0556	0.7589	-1.0393	0.9424
Gilts					
	G1-G2	1.0928	1.7659	-2.1329	0.5438
	G1-G3	-0.6051	1.7659	1.5211	0.7358
	G2-G3	-1.6979	1.7659	3.2443	0.3490
	Low-Medium	-1.5984	1.7659	3.0281	0.3773
	Low-High	2.3583	1.7659	-5.1277	0.1984
	Medium-High	3.9567	1.7659	-15.5269	0.0379

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 11. Fold change results for each contrast of genetic groups within each nutritional plan for *MyHC I* gene target in Psoas muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	2.8384	1.5715	-7.1523	0.0876
	G1-G3	0.3168	1.5715	-1.2456	0.8425
	G2-G3	-2.5216	1.5715	5.7422	0.1260
	Low-Medium	-1.0235	1.5715	2.0328	0.5231
	Low-High	-0.9794	1.5715	1.9716	0.5409
	Medium-High	0.0440	1.5715	-1.0310	0.9779
Gilts					
	G1-G2	0.0642	0.6787	-1.0455	0.9256
	G1-G3	-0.1102	0.6787	1.0794	0.8728
	G2-G3	-0.1744	0.6787	1.1285	0.8001
	Low-Medium	0.6560	0.6787	-1.5757	0.3465
	Low-High	0.8116	0.6787	-1.7552	0.2472
	Medium-High	0.1556	0.6787	-1.1139	0.8213

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 12. Fold change results for each contrast of genetic groups within each nutritional plan for *MyHC IIa* gene target in Psoas muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	2.6384	0.7510	-6.2264	0.0025
	G1-G3	1.4506	0.7510	-2.7332	0.0693
	G2-G3	-1.1878	0.7510	2.2781	0.1311
	Low-Medium	-1.6249	0.7510	3.0842	0.0442
	Low-High	-1.6991	0.7510	3.2470	0.0363
	Medium-High	-0.0741	0.7510	1.0527	0.9225
Gilts					
	G1-G2	-2.2976	1.1593	4.9164	0.0630
	G1-G3	-0.4706	1.1593	1.3857	0.6896
	G2-G3	1.8271	1.1593	-3.5482	0.1324
	Low-Medium	-1.8832	1.1593	3.6889	0.1216
	Low-High	-0.0089	1.1593	1.0062	0.9939
	Medium-High	1.8743	1.1593	-3.6662	0.1233

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 13. Fold change results for each contrast of genetic groups within each nutritional plan for *MyHC IIx* gene target in Psoas muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	-0.6968	0.4884	1.6209	0.1707
	G1-G3	-0.2798	0.4884	1.2140	0.5738
	G2-G3	0.4171	0.4884	-1.3352	0.4044
	Low-Medium	-0.3655	0.4884	1.2883	0.4639
	Low-High	-0.0802	0.4884	1.0572	0.8713
	Medium-High	0.2852	0.4884	-1.2186	0.5665
Gilts					
	G1-G2	-1.7372	0.8199	3.3339	0.0482
	G1-G3	0.5507	0.8199	-1.4648	0.5103
	G2-G3	2.2879	0.8199	-4.8834	0.0121
	Low-Medium	-0.9644	0.8199	1.9513	0.2548
	Low-High	-0.4881	0.8199	1.4026	0.5590
	Medium-High	0.4764	0.8199	-1.3913	0.5684

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

Supplementary Table 14. Fold change results for each contrast of genetic groups within each nutritional plan for *MyHC IIb* gene target in Psoas muscle of pigs.

Sex	Comparison	Estimate	Std Err	Fold Change	p-value
Barrows	G1-G2	-1.1088	0.8529	2.1567	0.2100
	G1-G3	0.0057	0.8529	-1.0040	0.9947
	G2-G3	1.1146	0.8529	-2.1653	0.2078
	Low-Medium	0.2267	0.8529	-1.1702	0.7934
	Low-High	-0.7096	0.8529	1.6354	0.4164
	Medium-High	-0.9363	0.8529	1.9136	0.2868
Gilts					
	G1-G2	-1.8384	0.9692	3.5761	0.0740
	G1-G3	-1.2847	0.9692	2.4363	0.2015
	G2-G3	0.5537	0.9692	-1.4678	0.5748
	Low-Medium	-0.1203	0.9692	1.0870	0.9026
	Low-High	0.0822	0.9692	-1.0586	0.9333
	Medium-High	0.2025	0.9692	-1.1507	0.8368

G1 = Piau crossbreds; G2 = Duroc crossbreds; G3 = Pietrain crossbreds. Std Err: standard error. Positive values of fold change indicate higher expression of first level of contrast, and negative values indicate higher expression of second level of contrast.

3. GENERAL CONCLUSION

In the present study, greater performance and carcass yield were observed to Duroc and Pietrain crossbred pigs compared to Piau crossbred pigs. Genetic group affected most of traits and few traits were affected by nutritional plan, however the genetic background during the growing-finishing phase seems to be dependent in response to dietary lysine.

Genotype has a strong influence on intramuscular fat content increase mainly by up-regulation of intramuscular lipogenic gene expression, mainly *FAS* (Fatty acid synthase). Differentially expression of fiber types in skeletal muscle may be the most important factors affecting intramuscular fat content and drip loss in pig genotypes. These results may provide valuable information for understanding the differences of meat quality in pig genotypes. Therefore, based on this information, it could be possible to manipulate meat quality developing process to produce high-quality pork.