NICOLA VERGARA LOPES SERÃO

ASSOCIATION OF CANDIDATE GENE EXPRESSION WITH INTRAMUSCULAR FAT CONTENT IN THE PORCINE *Longissimus dorsi* MUSCLE

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de *Magister Scientiae.*

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Aprovada: 9 de julho de 2009.

Prof. Paulo Sávio Lopes
(Co-orientador)

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(Co-orientador)

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Profª. Simone Eliza Facioni Guimarães
(Orientadora)

"The further we go
And older we grow
The more we know
The less we show"

(Primary)

Robert Smith, Simon Gallup and Laurence Tolhurst (The Cure, 1981)

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BIOGRAFIA

Nicola Vergara Lopes Serão, filho de Teresa Cristina Vergara Lopes Serão e Leopoldo Serão Júnior, nasceu no Rio de Janeiro, Rio de Janeiro, no dia 22 de junho de 1982. É casado com Mariana Cruz Rossoni Serão e pai de Clara Rossoni Serão.

Em maio de 2002 ingressou no curso de Zootecnia da Universidade Federal de Viçosa, Viçosa, Minas Gerais, concluindo a graduação em agosto de 2007.

Em setembro do mesmo ano, iniciou o curso de Mestrado em Genética e Melhoramento na UFV, obtendo o título de *Magister Scientea* no dia 9 de julho de 2009.

SUMÁRIO

RESUMO

SERÃO, Nicola Vergara Lopes, M.Sc., Universidade Federal de Viçosa, julho de 2009. **Associação da expressão de genes candidatos com teor de gordura intramuscular no músculo *Longissimus dorsi* de suínos**. Orientadora: Simone Eliza Facioni Guimarães. Co-orientadores: José Braccini Neto e Paulo Sávio Lopes.

Este trabalho foi realizado com o objetivo de promover informação sobre o padrão de expressão de genes candidatos, por meio da reação em cadeia da polimerase em tempo real (*q*PCR), e correlacioná-los com o conteúdo de gordura intramuscular (GIM) em suínos de ambos os sexos e dos grupos genéticos Piau, Comercial e Cruzado, abatidos aos 30, 60, 90 e 120 kg. Amostras do músculo *longissimus dorsi* foram retiradas para medição dos níveis de GIM e extração de RNA. Os genes estudados foram ATN1, EEF1A2, FABP3, LDLR, MGP, OBSCN, PDHB, RYR1 e TRDN. Níveis superiores de GIM ($P<0.05$) foram observados para suínos da raça Piau e Cruzados aos 120 kg de peso ao abate, indicando que alelos provenientes de animais Piau têm papel importante na deposição de gordura. Independentemente do grupo genético, expressões dos genes ATN1, FABP3 e PDHB foram influenciadas ($P<0.05$) pelo peso ao abate. PDHB sofreu efeito do grupo genético ($P<0.05$), onde animais Piau e Cruzados mostraram maiores valores que Comerciais ($P<0.05$). No gene EEF1A2, o grupo genético foi significativo ($P<0.05$) e sua expressão foi maior em suínos Cruzados ($P<0.05$). Os Níveis de expressão dos genes LDLR, MGP, OBSCN, RYR1 e TRDN foram afetados pela interação de grupo genético e peso ao abate ($P<0.05$). Entre grupos genéticos, foi possível determinar equações de predição ($P<0.05$) dos níveis de expressão dos genes LDLR, OBSCN, RYR1 e TRDN para animais da raça Piau. Além disso, Piau foi o único grupo genético que mostrou um padrão de expressão para o gene LDLR. O Peso ao abate afetou a expressão do gene MGP ($P<0.05$) em suínos Comerciais e Cruzados. Também foi possível estabelecer um padrão de expressão para os genes OBSCN, RYR1 e TRDN em animais Cruzados. Além disso, esses três genes foram influenciados pelo grupo genético dentro de peso ao abate ($P<0.05$). Animais Comerciais apresentaram maiores valores de expressão de OBSCN ($P<0.05$) aos 30 e 90 kg de peso ao abate, mas os níveis de expressão em suínos Piau não foram diferente ($P>0.05$) daqueles aos 90 kg. Para o gene RYR1, foi observado maiores níveis de expressão ($P<0.05$) em suínos aos 30, 90 e 120 kg, mas não houve significância ($P>0.05$) entre as médias de expressão entre suínos Piau e Comercial aos 30 kg. Animais Piau

apresentarem maiores níveis de expressão de TRDN que suínos Comerciais ($P<0.05$) aos 60 kg de peso ao abate. Usando valores preditos, os níveis de expressão de todos os genes foram correlacionados com GIM, a partir de dados de todos os animais e apenas de suínos Piau. Usando todos os dados, os níveis de expressão dos genes FABP3, LDLR, TRDN, OBSCN, RYR1 e PDHB foram positivamente correlacionados ($P<0.05$) com GIM, enquanto que o gene EEF1A2 teve correlação negativa ($P<0.05$). Com os dados dos animais Piau, o gene MGP teve correlação negativa ($P<0.05$) com GIM e os genes LDLR, FABP3, TRDN, RYR1 e OBSCN positiva ($P<0.05$). Uma equação de predição de conteúdo de GIM, para todos os grupos genéticos, sexos e pesos ao abate, foi modelada usando valores ajustados de todos os genes. O coeficiente de determinação (R^2) associado à equação foi de 90,29%, onde os genes ATN1, EEF1A2, MGP e PDHB atuaram diminuindo os níveis de GIM e os genes LDLR, OBSCN e TRDN aumentando a deposição de gordura no músculo *longissimus dorsi*. O maior potencial para deposição de GIM observado em suínos Piau indica que esta raça deve carregar genes valiosos que determinam a deposição de lipídeos no músculo. Embora alguns genes foram correlacionados com GIM e incluídos na equação de predição, o gene LDLR mostrou ter um papel importante na determinação dos níveis de GIM, devido aos seus alto valores de correlação e coeficiente de regressão, e função biológica na captação de lipídeos para dentro da célula. Além disso, mais estudos são necessários para se atribuir a este gene como gene candidato para deposição de GIM no músculo *longissimus dorsi* em suínos.

ABSTRACT

SERÃO, Nicola Vergara Lopes, M.Sc., Universidade Federal de Viçosa, July, 2009. **Association of candidate gene expression with intramuscular fat content in the porcine *Longissimus dorsi* muscle**. Adviser: Simone Eliza Facioni Guimarães. Co-advisers: José Braccini Neto and Paulo Sávio Lopes.

This work was realized with the objective to provide information about the expression pattern of candidate genes, by real-time polymerase chain reaction (*q*PCR), and correlate it to intramuscular fat (IMF) content in pigs from both sexes, of Piau, Commercial and Crossbred genetic groups, slaughtered at 30, 60, 90 and 120Kg. *Longissimus dorsi* muscle samples were taken for IMF measurement and RNA extraction. The studied genes were ATN1, EEF1A2, FABP3, LDLR, MGP, OBSCN, PDHB, RYR1 and TRDN. Higher levels of IMF ($P<0.05$) were reported for Piau and Crossbred pigs slaughtered at 120Kg, indicating that alleles from Piau animals have important role in fat deposition. Irrespective of genetic group, expression of ATN1, FABP3 and PDHB genes were affected ($P<0.05$) by slaughter weight. PDHB was affected by genetic group ($P<0.05$) whereas Piau and Crossbred showed higher expression values than Commercial animals ($P<0.05$). In EEF1A2 gene, genetic group was significant ($P<0.05$) and its expression was higher in Crossbred pigs ($P<0.05$). Expression levels of LDLR, MGP, OBSCN, RYR1 and TRDN were affected by the interaction of genetic group and slaughter weight ($P<0.05$). Among genetic groups, it was possible to determine predicting equations ($P<0.05$) of expression levels of LDLR, OBSCN, RYR1 and TRDN genes for Piau animals. Moreover, Piau was the only genetic group that showed an expression pattern on LDLR gene. Slaughter weight affected MGP expression ($P<0.05$) in Commercial and Crossbred pigs. It was also possible to establish ($P<0.05$) a pattern of expression for genes OBSCN, RYR1 and TRDN in Crossbred animals. Moreover, these three genes were influenced by genetic group within slaughter weight ($P<0.05$). Commercial animals showed higher values of OBSCN expression ($P<0.05$) at 30 and 90Kg of slaughter weight, but expression levels in Piau pigs were not different ($P>0.05$) from them at 90Kg. For RYR1 gene, it was observed higher expression levels ($P<0.05$) in Piau pigs at 30, 90 e 120Kg, but no significance ($P>0.05$) was observed between expression means between Piau and Commercial pigs at 30Kg. Piau animals presented higher levels of TRDN expression than Commercial pigs ($P<0.05$) at 60Kg of slaughter weight. Using predicted values, levels of expression

from all genes were correlated to IMF content using data from all animals and only from Piau pigs. Using the whole data, expression levels of FABP3, LDLR, TRDN, OBSCN, RYR1 and PDHB genes were positively correlated ($P<0.05$) with IMF content while EEF1A2 showed negative correlation ($P<0.05$). In Piau animals, MGP was negatively correlated ($P<0.05$) with IMF and, LDLR, FABP3, TRDN, RYR1 and OBSCN genes positively ($P<0.05$). A prediction equation for IMF content, for all genetic groups, sexes and slaughter weights, was modeled using adjusted values of all genes. The coefficient of determination (R^2) associated to this equation was 90.29%, where ATN1, EEF1A2, MGP and PDHB genes act lowering IMF levels and LDLR, OBSCN and TRDN increasing fat deposition in *longissimus dorsi* muscle. The higher potential of IMF deposition observed in Piau pigs, indicates that this breed may carry valuable genes for determine lipid deposition in muscle. Although some genes were correlated and included in the prediction equation, LDLR gene showed to play an important role on the determination of IMF levels, due its high values of correlation and regression coefficient, and biological function of lipid recruitment into the cell. Thus, more studies are required to assign this gene as a candidate gene for intramuscular fat deposition in *longissimus dorsi* muscle in pigs.

1. Introduction

A lot of progress in livestock breeding has been made in the last few decades, but the process of achieving greater understanding of how to improve meat quality was slow before molecular technology markers became accessible with wide applications in breeding schemes (Gao et al., 2007). Meat quality is an economically important trait in farm animals. Quality requires analysis and classification of fat content, composition, tenderness, water-holding capacity, color, oxidative stability and uniformity. It is influenced by several factors, including breed, genotype, gender, feeding, fasting, pre-slaughter handling, stunning, slaughter weight, slaughter methods, chilling and storage conditions (Rosenvold & Andersen, 2003).

Pork is the most consumed meat worldwide, and its consumption is dependent on consumer satisfaction. Consequently, high pork quality stimulates consumption, which is beneficial to the meat industry. However, the term "meat quality" includes a variety of different aspects, and the most important of these are hygiene, toxicology, nutrition, technology (function) and sensory (eating) quality (Hullberg, 2004). For a successful pork industry, meat products must satisfy the consumer. The satisfaction of the consumers can be achieved at the point of purchase as well as at the point of consumption (Fortin et al., 2005). Once a product has been purchased, consumers respond to the smell, taste, and texture of the meat, which result in hedonic or value judgments, based upon past personal experience (Jeremiah, 1998). It is generally accepted that a higher content of intramuscular fat, or marbling, has a positive influence on the sensory experience associated with eating pork (Fortin et al., 2005).

Many pig breeds and lines have different contents of intramuscular fat. Hence, this genetic variability within and across breeds, is important for the improvement of pork quality. Moreover, breeds that have never undergone genetic progress may be able to act as suitable reservoirs of genes capable of promoting desirable changes in this trait. Thus, knowledge of mechanisms underlying intramuscular fat deposition requires information about DNA sequences, genetic and physiologic aspects that include sets of transcripts, proteins and phenotypic

data, using a broad variety of techniques such as sequencing, mutational, transgenic and expression analysis (Pomp et al., 2001).

The objective of this work is to provide information about the expression pattern of candidate genes, through real-time polymerase chain reactions, and to correlate it with intramuscular fat (IMF) content in pigs of different genetic groups at four slaughter weights.

2. Review

2.1. Intramuscular fat

Intramuscular fat (IMF) refers to the chemically extractable fat in a muscle sample, predominantly from adipocytes (extramyocellular lipids, EMyLs) and myocytes (intramyocellular lipids, IMyLs). Morphologically, IMF is the total lipid associated with all cells present in a meat sample, excluding subcutaneous fat and adipocytes from the intermuscular fat (Figure 2.1.1). IMyLs localize within muscle fiber cytoplasm and EMyLs localize in adipocytes interlaced between muscle fibers (Shi-Zheng and Su-Mei, 2008). These lipids are sources of energy for cell metabolism, and are mainly made up of phospholipids and triacylglycerols (TG). Increased TG content implies a higher IMF percentage and its concentration varies within muscles fibers (Essen-Gustavsson et al., 1994).

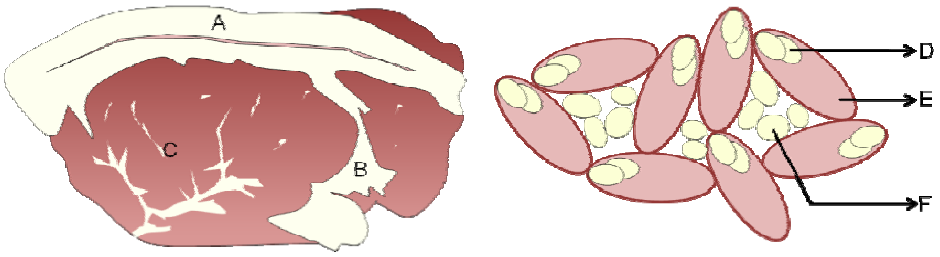

Figure 2.1.1 – Representation of subcutaneous fat (A), intermuscular fat (B) and intramuscular fat (C) in pork *longissimus dorsi* cut, and differences between intramyocellular (D) and extramyocellular lipids (F), within and between muscle fibers (E). Source: Adapted from Shi-Zheng and Su-Mei, 2008.

Muscle fiber classification is based on the ability to cope with energy consumption due to contractile activity with adequate ATP production and the parameters of the contractile response, which are determined by the protein composition of the myofibrils and sarcoplasmic reticulum. Muscle fibers of *longissimus dorsi* are classified as type IIB – fast, white and glycolytic – and present a lower intracellular concentration of TG than type I muscle fibers (Gerbens, 2004; Reggiani & Mascarello, 2004).

The fatty acids intake by intramuscular adipocytes and myocytes is facilitated by the enzyme lipoprotein lipase (LPL) located at the capillary endothelial cells which binds and hydrolyses lipoprotein TG. Inside the cell, fatty acids are bound by fatty acid-binding proteins (FABPs), and transported from the plasma membrane to the sites of fatty acid oxidation or fatty acid esterification into TGs or phospholipids (Gerbens, 2004). Hence, understanding the biological

mechanism that provides a higher deposition of TG in adipocytes and myocytes may be beneficial for improving pork quality.

There have been numerous studies comparing sensory characteristics of different pig breeds. Intramuscular fat content is an important factor that influences sensory quality (Klont et al., 1998). The amount of visible fat is the strongest visual stimulus for the purchase decision process (Fortin et al., 2005). It may be expected that the presence of more observed marbling in a pork chop would be a positive visual cue, resulting in the purchase of that product from the retail case. In contrast, it was observed that 80% of consumers who had the opportunity to select packages of pork chops from a retail case containing low (1.1% total fat), medium (2.3% fat), and high marbled pork loin chops (3.5% fat) have chosen medium or low marbled chops (Brewer et al., 2001; Berg, 2002). Although lipid content is not the single source of variation in determining the sensory quality of pork (Lonergan et al., 2007), its variation explains an important part of the genetic variation in eating quality of pork. An IMF content (Figure 2.1.2) below the recommended optimum range of 2.5–3% diminishes eating quality, whereas a higher IMF content will not only not improve this parameter; but it may have adverse effects on consumer acceptance due to increased visibility of fat in the meat (Gerbens, 2004). Hence, many of these aspects of consumers' acceptance, like color, appearance, juiciness, tenderness and flavor, should be taken into account to improve levels of IMF.

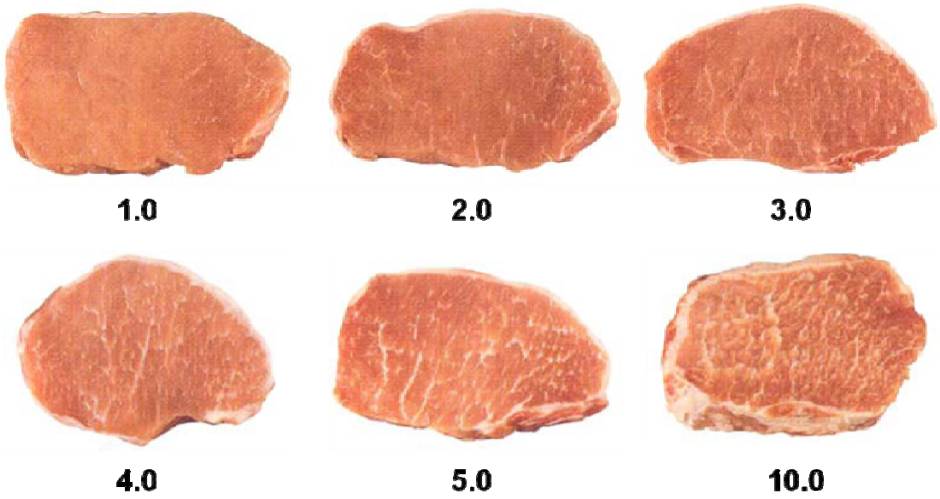

Figure 2.1.2 – Marbling pattern from the National Pork Producers Council (NPPC). Scores represent the percentage of intramuscular fat. Source: AMSA (2001).

Breeding programs have resulted in genetic changes in pigs. These genetic changes are generally desired, but some of the genetic alterations made in pursuit of breeding goals seem to have unintended negative side-effects. In order to reduce fatness in pigs, over the years many companies have begun selecting animals with lower backfat thickness (BF). From the year 1900 to 2000, BF reduced 62% (23.5 mm to 9.0 mm; Merks, 2000). Genetic correlation between BF and IMF ranges from 0.30 to 0.37 (de Koning et al., 1999; Pringle & Williams, 2000; Newcom et al., 2005), and since the 1960s, IMF content has been reduced from 2 – 4% to less than 1% (Wood, 2008). A genetic correlation between carcass and meat quality traits has also been reported (Table 2.1.1). These results indicate that genetic gain for IMF affects other meat quality traits as well, and it is feasible that in some situations these meat quality traits would be more economically attractive than production traits. Due to consumer demand, high heritability (h^2=0.5), and economic value, there will still be room for genetic improvement in terms of intramuscular fat over the next few years (Gerbens, 2004).

In order to detect the responsible genes underlying IMF content, many research groups have developed pig populations to identify quantitative trait loci (QTL) for marbling and backfat thickness (Table 2.1.2). A major objective of QTL analysis in livestock is to find genetic markers that can be implemented in breeding programs by marker-assisted selection (MAS; Kim et al., 2008). Moreover, the size of QTL regions is a major concern, since smaller QTL regions increase the genetic response from MAS (Spelman & Bovenhuis, 1998) and substantially reduce the number of positional candidate genes. In addition to the positional candidate genes underlying the QTL regions affecting IMF content (see section 2.4 in this review for some examples), genes may also be candidates based on existing knowledge of physiological and biochemical processes, through the candidate gene approach (Gerbens, 2004).

Genetic variation plays an important role in breeding systems. When there is no genetic variation across animals, genetic gains in that population are no longer possible. Breeds with non-recognized value may carry useful genes. In pig production, the Chinese breed Meishan, well-known due to its reproductive traits, may also be used for meat quality traits (Janss et al., 1997; Müller et al., 2000). Other breeds like Piau from Brazil, Creole from Haiti, Tongcheng from China, Turopolje from Croatia and pigs from the Basque region, have lower drip loss,

higher intramuscular fat content and rusticity (Benevenuto Jr., 2001; Goméz Fernández, 2003; Renaudeau et al., 2005; Fan et al., 2006; Harcet et al., 2006), all traits that may be interesting for genetic improvement in coming years. Thus, indigenous breeds are important resources for genetic variation.

Table 2.1.1 – Average genetic correlation of intramuscular fat (IMF) content with carcass and meat quality traits

	Carcass traits		Meat quality traits			
	Fatness	Leanness	Overall acceptability	Firmness	Color	Fiber type
IMF Content	0.3	-0.34	0.61	0.31	0.43	-0.05 – 0.06

Source: Shi-Zeng & Su-Mei, 2008.

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Table 2.1.2 – Reported main QTL for intramuscular fat (IMF) content or its related traits, total muscle lipid percentage and marbling, and backfat thickness (BT) in various experimental crosses of pigs

Pig population	Chromosome(s) scanned	Quantitative trait loci		Reference
		IMF	BT	
B × LW	1 – 18 and X	1	Yes	Malek et al. (2001)
		2	No	
		8	No	
		10	No	
		13	Yes	
D × LW	1 – 18 and X	1		Sanchez et al. (2007)
		13	ND	
		15		
M × LW	1 – 18 and X	4p	No	Paszek et al. (2001)
		4q	Yes	
		6p	Yes	
		6q	Yes	
		12	No	
M × LW or L	1 – 18 and X	4	No	Rattink et al. (2000)
		6p	No	de Koning et al. (1999)
		6q	Yes	
		X	Yes	Harlizius et al. (2000)
D × N-L × LW	4, 6 and 7	6	No	Grindflek et al. (2001)
I × L	1 – 18	6	Yes	Óvilo et al. (2002a)
	X	X	ND	Perez-Enciso et al. (2002)
M × LW	1 – 18 and X	7	Yes	Bidanel et al. (1998)
M × D or H or L	13	13	Yes	Yu et al. (1999)

B, Berkshire; D, Duroc; H, Hampshire; I, Iberian; L, Landrace; LW, Large White; M, Meishan; N-L, Norwegian Landrace.

ND, not determined.

Source: Adapted from Gerbens, 2004.

2.2. Piau

Farm animal genetic resources are those species that are used, or may be used, for the production of food and agriculture, and the populations within each of them. “These populations within each species can be classified as wild and feral, landraces and primary populations, standardized breeds, selected lines, and any conserved genetic material” (FAO, 1999).

The word Piau has aboriginal origin, and means “spotted”. According to Castro et al. (2000) Piau (Figure 2.2.1) pigs have white or cream colored coat with black or red spots, smooth and uniformly distributed bristles, rectilinear or sub-

concave head profiles and Iberian ear types. This Brazilian naturalized pig breed originated from other breeds introduced by Portuguese settlers in the 16th century, and is also influenced by Dutch and African pig breeds (Vianna, 1985). These animals are considered to be fat-type, used to be reared in small farms, supplying farmers with meat and a large amount of fat.

Figure 2.2.1 – Piau boar from the Pig Breeding Farm (*Universidade Federal de Viçosa*, Viçosa, Minas Gerais, Brazil).

In 1956 an agreement was signed between the *Departamento Nacional de Produção Animal* (National Department of Livestock Production) and the *Departamento de Produção Animal da Secretaria de Agricultura do Estado de São Paulo* (Livestock Production Department of the Agriculture Secretariate of the State of São Paulo) proposing the development of genetic improvement projects for pig production. The Piau project was developed in Sertãozinho, in the State of São Paulo. However, during the 1960s, exotic breeds were imported from the USA and Europe. Introduction of breeds such as Duroc, Yorkshire, Hampshire, from the USA, and Landrace and Large White from Europe, were responsible for the replacement of naturalized animals (fat-type) and the beginning of the meat-type pig production era. After the 1970s, when national's nucleus herds were settled, there were modifications on the structure and organization of pig production, and large companies, like Agroceres-PIC and Seghars-Humus, started commercial activities in Brazil during the 1980s (Euclides Filho, 1999). This situation, as well as changes in consumer demand has placed naturalized pig breeds on their way to extinction (Lopes et al., 2002).

Piau was the first Brazilian breed to be registered at *Associação Brasileira de Criadores de Suínos* (Brazilian Association of Pig Breeders or ABCS) in 1986. Between 1989 and 1995, 1,250 Piau pigs were registered. Since then, there has been no information about the registration of new animals. Mariante et al. (2003a) reported that the conservation status of the Piau is threatened. The last information about the population of this breed was made by Mariante et al. (2003b). These authors reported that there were 119 animals (22 adult males, 50 adult females and 47 young) in the State of Distrito Federal. Sollero et al. (2008) observed through the use of DNA microsatellite analysis, a high rate of inbreeding not only with the Distrito Federal State animals, but also in the Piau herd from *Universidade Federal de Viçosa*.

Since 1998 the Pig Breeding Farm at *Universidade Federal de Viçosa* allocates Piau pigs for research purposes. Many trials have been carried out using this breed, such as QTL mapping using divergent crosses (Guimarães & Lopes, 2000; Pires et al., 2007, 2008a, 2008b; Paixão et al., 2008a, 2008b; Silva et al., 2008, 2009), polymorphism analysis of candidate genes (Soares et al., 2006; Figueiredo et al., 2008; Peixoto et al.; 2009; Faria et al., 2009), meat quality, carcass and performance traits trials (Band et al., 2005a, 2005b; Carmo et al., 2005; Peixoto et al., 2006; Faria et al., 2006), phylogeny and genetic diversity (Schierholt et al., 2008; Sollero et al., 2008; Souza et al., 2009) and expression patterns of candidate genes (Moraes, 2006). Although many Piau pigs have been used in these trials, the founder population consisted of only eight animals, which led to a high rate of inbreeding (Veroneze et al., 2007). Moreover, these animals may carry non-recognized valuable genes, and its conservation is an important strategy to maintain genetic diversity.

2.3. Real-time polymerase chain reaction

Function and structure of various cell types differ due to the transcribed mRNA and ultimately, the translated proteins. In other words, it is the cellular protein expression profile that differentiates them. Presently, the most practical way to study the specific genes expressed in a particular cell type is to analyze the mRNA content. Considering RNA liability, in a lab setting it is always converted into complementary DNA (cDNA) by a process known as *reverse transcription* (Zander et al., 2008). The methods used to quantify mRNA include techniques

based upon hybridization (e.g., Northern blotting, solution hybridization, and RNase protection assays) as well as amplification of individual RNA molecules by combining reverse transcription and the polymerase chain reaction (Schmittgen et al., 2000).

Real-time polymerase chain reaction (qPCR) is an *in vitro* technique used, simultaneously, to quantify and amplify DNA or cDNA molecules. qPCR is an efficient method for quantification of mRNA transcription levels due to its high sensitivity, reproducibility and large dynamic range; in addition, real-time PCR is fast, easy to use and provides simultaneous measurement of gene expression in many different samples for a limited number of genes (Nygard et al., 2007).

Generally, a PCR consists of four different kinetic stages. First, there is a lag phase where exponential amplification is already ongoing within the PCR tube, but no fluorescence signal above the background level is measurable. Secondly, in the logarithmic (*log*) phase an exponential growth in PCR production (ideally, there is a doubling of PCR product every cycle) is measurable as a fluorescent signal. Thirdly, in the retardation phase, there is an accumulation of PCR inhibiting factors and a loss of enzyme and substrates as the PCR decelerates the reaction. Fourthly, PCR reaches a steady state in the stationary phase, and no more amplicons are produced. For real-time PCR data analysis, the second phase – *log* phase, with its measurable exponential growth conditions – is crucial (Pannetier et al., 1993; Freeman et al., 1999; Wilhelm & Pingoud 2003; Schefe et al., 2006), and its kinetics can be observed in Figure 2.3.1 and described by the following equation:

$$R_n = R_0 \times (1 + E)^n$$

The amount of fluorescence signal after n and 0 cycles is R_n and R_0, expressed in arbitrary units. The PCR efficiency E ($0 \leq E \leq 1$) is a major issue in qPCR data analysis. In a perfect situation, it would always achieve perfect PCR amplification. A PCR with a perfect setup would theoretically double the gene-specific amplicons from cycle to cycle, which would be equivalent to $E=1=100\%$ (Schefe et al., 2006).

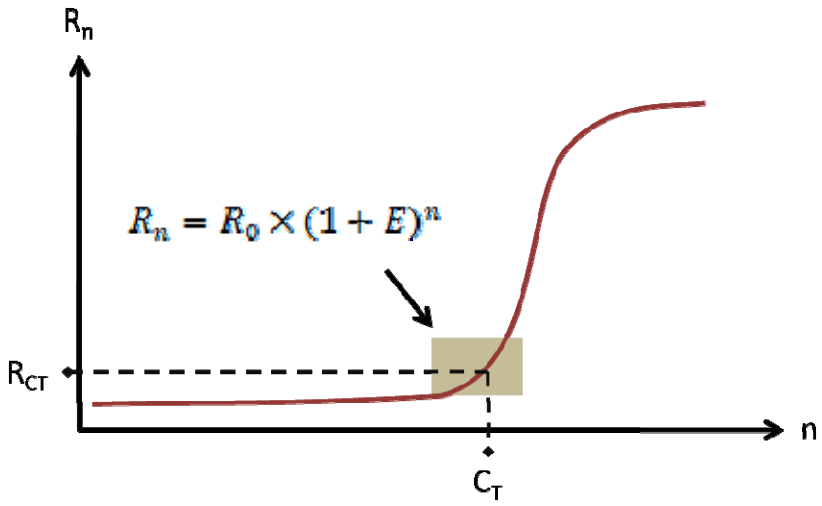

Figure 2.3.1 – qPCR amplification plot. The gray square indicates the exponential region, where the threshold must be set. C_T is the threshold cycle. (Adapted from Schefe et al., 2006)

Real-time reactions are carried out in a thermocycler that permits measurement of a fluorescent detector molecule, which decreases post-processing steps and minimizes experimental error. This is most commonly achieved through the use of fluorescence based technologies, including: probe sequences that fluoresce upon hydrolysis (TaqMan®) or hybridization (LightCycler); (*ii*) fluorescent hairpins; or (*iii*) intercalating dyes (SYBR® Green) (VanGuilder et al., 2008).

SYBR® Green detection methods use a dye (SYBR Green I) that fluoresces upon binding to double-stranded DNA during amplification. Therefore, fluorescence enhances as amplified products (amplicons) accumulate. This system has the advantages of being sensitive, simple and not requiring a specific probe, only specific primers that will determine the target of amplification. "Disadvantages include their indiscriminate binding to any double-stranded DNA", which can result in fluorescence readings in the "no template controls" due to dye molecules binding to primer dimmers (Bustin & Nolan 2004).

Quantification of mRNA transcription can be either relative or absolute. Relative quantification determines the changes in steady state transcription of a gene and is often adequate. Absolute quantification of transcription allows the precise determination of number of copies per cell, total RNA concentration, or unit mass of tissue. It requires the construction of an absolute standard curve for each individual amplicon to ensure accurate reverse transcription and PCR amplification profiles. In contrast, a relative standard consists of a sample, the

calibrator, which is used to create a dilution series with arbitrary units. During the qPCR assay, the target threshold cycle (C_T) is compared directly with the calibrator C_T and is recorded as containing either more or less mRNA (Bustin, 2000).

Relative quantification has the advantage of not requiring the construction of a standard curve. However, efficiencies of target and endogenous control amplifications must be approximately equal to accurate quantification. The efficiency is calculated using serial dilutions of cDNA. The results are represented in a graph ($Log_{10} \times C_T$ concentration) and the slope is used to determine the efficiency of reaction (Livak & Schimittigen, 2001), as the following equation (Pfaffl, 2001):

$$E = 10^{-\frac{1}{slope}} - 1$$

One of the critical steps in comparing transcription profiles is accurate normalization, adjusting for differences in amount and quality of starting material and differences in RNA preparation and cDNA synthesis, since the reference gene is exposed to the same preparation steps as the gene of interest. Recently a set of reference genes (ACTB – actin, beta; HPRT1 – hypoxanthine phosphoribosyltransferase 1; RPL4 – mitochondrial ribosomal protein L4; TBP – TATA box binding protein; TOP2B – topoisomerase (DNA) II beta 180kDa) have proven suitable for normalization of qPCR data in pigs (Erkens et al., 2006; Nygard et al., 2007).

2.4. Candidate genes for pork intramuscular fat

Various candidate genes in livestock have been successfully identified as having linkage associations (ESR – Estrogen receptor; A-FABP – Adipocyte fatty acid binding protein; H-FABP – Heart fatty acid binding protein; FUT1 – Fucosyltransferase 1; MC4R – Melanocortin-4 receptor; MYOG – Myogenin; PRLR – Prolactin receptor) or even causative relationships (GDF-8 – Myostatin; RYR1 – Ryanodine receptor 1; KIT – mast/stem cell growth factor receptor; MSHR – Melanocyte-stimulating hormone receptor) with many traits such as (re)production, disease resistance, meat quality and coat color. Seven candidate genes have been reported to have associations with IMF content: H-FABP, A-FABP,

ADRP (adipose differentiation-related protein), LEPR (leptin receptor), MC4R, SREBP1 (sterol regulatory element binding transcription factor 1), MYOD (myogenic differentiation), and there are possibly others (Rothschild, 1998; Shi-Zeng & Su-Mei, 2008).

The genes analyzed in this study were chosen from Sasaki et al. (2006), where MGP (matrix Gla protein), PDHB (pyruvate dehydrogenase (lipoamide) beta) and TRDN (Triadin) genes were related to changes in intramuscular fat deposition in cattle and from a Piau/Large White EST library developed at the *Universidade Federal de Viçosa* (Federal University of Viçosa), where ATN1 (atrophin 1), EEF1A2 (Eukaryotic translation elongation factor 1 alpha 2), FABP3 (Fatty acid binding protein 3), LDLR (low density lipoprotein receptor) and OBSCN (obscurin) genes were differently expressed (Peixoto, 2007). The RYR1 (ryanodine receptor type 1) gene was chosen due to its importance in terms of determining pork quality.

2.4.1. ATN1

In humans, unstable expansion of CAG repeat in atrophin 1 gene (ATN1) is responsible for Dentatorubral-pallidoluysian atrophy (DRPLA), a progressive neurodegenerative disorder (Li et al., 1993). ATN1 in knockout mice indicates that this gene is not essential during mouse development, because there are no differences between wild-type and knockout mice (Shen et al., 2007). Atrophin 1 proteins are designed to act as versatile transcriptional co-repressors (Zhang et al., 2002).

2.4.2. EEF1A2

Eukaryotic translation elongation factor 1 alpha 2 (EEF1A2) is one of two members of the EEF1A family of proteins (EEF1A1 and EEF1A2) that bind aminoacylated tRNA and facilitate their recruitment to the ribosome during translation elongation (Kulkarni et al., 2007). EEF1A proteins have other functions not related to translation such as inducing actin and tubulin cytoskeleton rearrangements (Jeganathan et al., 2008). These characteristics are consistent with the idea that the proteins regulate cytoskeletal organization (Anand et al., 2002).

It has been also reported that EEF1A2 interacts with phospholipase C beta 1 (PLC-β1), a key player in the regulation of nuclear inositol lipid signaling a wide range of cellular functions, such as proliferation and differentiation (Piazzi et al., 2008). Moreover, elongation factor EEF1A2 binds to phosphatidylinositol-4 kinase β, stimulating lipid kinase activity and phosphatidylinositol-4 phosphate generation, a minor phospholipid component of cell membranes (Jeganathan & Lee, 2007).

2.4.3. FABP3

Intracellular fatty acids in intramuscular adipocytes are bounded by fatty acid-binding proteins (FABPs), which are considered to be the most important carriers of intracellular fatty acids. FABPs facilitate the transport of fatty acids from plasma membranes to the sites of fatty acid oxidation or esterification into TG or phospholipids (Shi-Zeng & Su-Mei 2008). FABPs have been isolated from cytosols of vertebrates and invertebrates tissues as 13-15 kDa proteins. Nine FABP types have been named up to now after the first tissue from which they were isolated. They show a characteristic tissue and cellular distribution. They are postulated to be involved in fatty acid uptake and targeting, in modulation of fatty acid concentration and, in this way, in regulation of metabolism, signal transduction, gene transcription and detoxification (Veerkamp & Maatman, 1995; Glatz & van der Vusse 1996; Veerkamp et al., 2000).

Fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor) gene (FABP3) is located on SSC6 (Gerbens et al., 1997), within or close to a QTL affecting IMF content (de Koning et al., 1999; Grindflek et al., 2001; Óvilo et al., 2002b). It is predominantly expressed in heart and skeletal muscle, although its expression is verified in other tissues, such as renal cells, testis, mammary gland, adrenal gland and brain (Hertzel & Bernlohr, 2000).

Many authors have studied genetic variants of this gene and IMF content. Some reports indicate that FABP3 acts on pork fatness traits in diverse pig breeds (Gerbens et al., 1999, 2000, 2001; Grindflek et al., 2001; Óvilo et al., 2002b; Urban et al., 2002; Pang et al., 2006). On the other hand, no correlation was found between FABP3 alleles and IMF content in other trials (Nechtelberger et al., 2001; Urban et al., 2002). Recent investigations using the Brazilian naturalized breed Piau, indicates that polymorphism (Figueiredo et al., 2008) and mRNA expression

levels (Moraes, 2006) of FABP3 may not affect IMF content. Thus, current information does not provide satisfactory insights to use FABP3 as candidate gene.

2.4.4. LDLR

The low density lipoprotein receptor gene (LDLR) family code for a class of structurally closely related cell surface receptors that fulfill diverse biological functions in different organs, tissues, and cell types (Nykjaer & Willnow, 2002). The role that is most commonly associated with this evolutionarily ancient family is cholesterol homeostasis (maintenance of appropriate concentration of cholesterol). In humans, excess cholesterol in the blood is captured by low-density lipoprotein (LDL) and removed at the liver by the endocytosis of the LDL receptor (Li et al., 2001). In pigs, elevated blood plasma cholesterol (hypercholesterolemia) is associated with a mutation in the low density lipoprotein receptor gene located on chromosome 2 (Hasler-Rapacz et al., 1998).

2.4.5. MGP

Matrix Gla protein (MGP) gene is located on chromosome 5 of pigs (SSC5), and its mRNA was sequenced by Laize et al., (2005). Also called Matrix Gamma-Carboxyclutamic Acid, the MGP is a skeletal member of the family of extracellular mineral-binding Gla proteins (Luo et al., 1995), it is secreted by smooth vascular muscle cells and chondrocytes and accumulates in significant amounts in bone, cartilage, and dentin. MGP gene is related to tissue mineralization, and acts as a calcification inhibitor (Luo et al., 1997). Under normal circumstances, it seems that the accumulation of MGP in mineralized bone and calcified cartilage may be due its calcium-binding properties (Fraser & Price, 1988; Pinto et al., 2002).

In livestock research, some investigations of MGP expression patterns indicate that this gene may act on porcine mammary gland development (Su et al., 2005), cattle adipogenesis (Sasaki et al., 2006; Tan et al., 2006), reproduction (Ushizawa et al., 2007) and milk production (Ron et al., 2007).

2.4.6. OBSCN

Obscurin is a recently discovered giant protein (800–900kDa) of striated muscle, which received its name due to its difficult characterization (Young et al., 2001; Bowman et al., 2007). Obscurin, cytoskeletal calmodulin and titin-interacting

RhoGEF gene (OBSCN) is located on bovine chromosome 7 and chicken chromosome 2, but has not yet been mapped on porcine genome.

This protein is part of the family of giant sarcomeric signaling proteins, along with titin and nebulin, and appears to mediate interactions between the sarcoplasmic reticulum and myofibrils. Furthermore, it binds to the sarcoplasmic reticulum by interaction with small ankyrin-1 and to the contractile myofibril via titin and sarcomeric myosin (Price et al., 2006). Unlike titin, which is an integral component of sarcomeres, obscurin intimately surrounds myofibrils at the level of the Z-disk and M-band, where it is appropriately positioned to participate in their assembly and integration with elements of the sarcoplasmic reticulum (Kontrogianni-Konstantopoulos et al., 2003).

The precise functional role of obscurin in muscle tissue is still poorly characterized, and its importance for progression of myofibrillogenesis, stability of structural organization and integrity of myofibrils remains unclear (Borisov et al., 2006).

2.4.7. PDHB

The mammalian mitochondrial pyruvate dehydrogenase complex (PDC) catalyzes an oxidative decarboxylation of pyruvate (Koike et al., 1990), transforming pyruvate into acetyl-CoA, an important substrate in metabolism. This complex is composed of three enzymes: pyruvate dehydrogenase (PDH), dehydrolipoate transacetylase, and dihydrolipoate dehydrogenase. PDH is composed of two α and β units. The pyruvate dehydrogenase (lipoamide) beta gene (PDHB) is located at HSA 3p13-q23 (Olson et al., 1990), and is responsible for the two β–units (Ciszak et al., 2003).

Expression of PDHB is related to energy expenditure due to its role in metabolism, and lower levels of expression are observed in high-marbled steers (Sasaki et al., 2006).

2.4.8. RYR1

Ca^{2+} in striated muscle is a key player in the development of contractile force. In cardiac and skeletal muscle, depolarization of the sarcolemma and its infoldings, the T-tubules, trigger a massive release of Ca^{2+} from the sarcoplasmic reticulum, which then bathes the contractile filaments, binds to Ca^{2+}-binding

proteins, and finally induces contraction (Bers, 2001). The amount of Ca^{2+} released from the sarcoplasmic reticulum is determined by the efficiency of communication between voltage-dependent Ca^{2+} channels/dihydropyridine receptors and Ca^{2+} release channels/ryanodine receptors. Intrinsic alteration in these proteins, or in the environment that promotes their functional coupling, is known to cause contractile dysfunction and/or the pathological remodeling of cellular structures (Benkusky et al., 2004). In pigs, the ryanodine receptor type 1 (skeletal) gene (RYR1) is located at SSC 6q1.2 (Harbitz et al., 1990) and a single missense mutation (Fuji et al., 1991), where a cytosine is replaced by a thymine at nucleotide 1843 (C1843T) changing amino acid sequence from Arg^{615} to Cys^{615}, results in increased cytosolic Ca^{2+} levels in muscles, leading to an increased rate of muscle metabolism and lactic acid accumulation (Bowker at al. 2000).

RYR1 gene, also called Halothane gene (HAL), is responsible for malignant hyperthermia or porcine stress syndrome (PSS), a genetic disorder that results in a pale, soft and exudative meat (PSE meat). This disorder may be triggered by stressful conditions, such as transportation, mating, pre-slaughter handling, or halothane anesthesia in homozygous halothane positive (HAL^{nn}) pigs (Fisher et al., 2000). PSE meat is characterized by a pale, lean, soft texture and a low water-binding capacity (Bowker et al., 2000), and is a problematic issue for the pork industry. These adverse affects result in poor meat appearance (which is unacceptable for consumers), reduced production yield of cooked ham and increased seasoning loss of dry-cured hams (Davoli & Braglia, 2007). Although HAL^{nn} pigs result in low-quality meat products, they provide desirable carcass traits, with leaner, shorter and heavier carcasses than halothane negative pigs (Zhang et al., 1992). Worldwide, the frequency of this defective allele has decreased to nearly zero, though some lines maintain the gene, in order to capture the increased lean meat produced from heterozygous pigs (Davoli & Braglia, 2007).

Although many surveys have demonstrated the effect of Halothane genotypes on intramuscular fat content (Zhang et al., 1992; Hamilton et al., 2000; Brewer et al., 2002; Band et al., 2005), insights on how RYR1 gene expression patterns affect pork quality traits are scarce.

2.4.9. TRDN

In skeletal muscle, depolarization-initiated calcium release, and the subsequent muscle contraction, depends on the direct interaction between the slow voltage-gated L-type Ca^{2+} channel (dihydropyridine receptor, DHPR) and the sarcoplasmic reticulum Ca^{2+} release channel (RYR) in the triad junction. Release of Ca^{2+} from sarcoplasmic reticulum is also influenced by a key Ca^{2+} binding protein, calsequestrin (CSQ). However, an increasing number of other proteins associated with RYRs have been identified in the triadic region and have emerged as important regulators of the Ca^{2+} release mediated by these two channels (Shen et al., 2007; Goonasekera et al., 2007). Expressed both in heart and skeletal muscle, triadin (TRDN) is a major membrane protein that is specifically localized in the junction sarcoplasmic reticulum of skeletal muscle and is thought to play an important role in muscle excitation-contraction coupling (Guo & Campbell, 1995).

In mice, it has been reported that altered binding properties of RYR channels and a triadin isoform (Trisk 95) reduce sarcoplasmic reticulum calcium release, indicating that triadin is crucial for RYR function (Goonasekera et al., 2007). Moreover, decreased calcium release from the sarcoplasmic reticulum is associated with overexpression of TRDN, indicating that triadin negatively regulates RYR opening (Fodor et al., 2008). In contrast, triadin knock-out mice survived to adulthood, indicating that excitation-contraction coupling does not require this protein.

Although effects of this gene on pork quality are not well documented, low-marbled steers exhibit higher expression of TRDN than high-marbled steers (Sasaki et al., 2006).

3. Materials and Methods

3.1. Animals

Seventy-two pigs from the *Granja de Melhoramento de Suínos* (Pig Breeding Farm) of *Universidade Federal de Viçosa* (Federal University of Viçosa, Viçosa, Minas Gerais, Brazil) were used in this trial. These animals consisted of three genetic groups: the Brazilian naturalized breed Piau, the Commercial Line (Landrace x Large White x Pietrain; Com) and a Crossbred group (produced from Piau males and Commercial Line females; CrB). For each genetic group, 24 animals (boars and gilts) were slaughtered by electrical stunning at four slaughter weights: 30, 60, 90 and 120Kg. Therefore, for each combination of genetic group and slaughter weight, six animals (three males and three females) were harvested (Table 3.1.1). For Piau pigs at 30 and 120 kg of slaughter weight, four boars and two gilts were used. The pigs were fed with a recommended diet *ad libitum* twice daily with free access to water.

Table 3.1.1 – Number of replicates of each source of variation

Slaughter weight (Kg)	Piau		Crossbred (Crb)		Commercial (Com)		Total
	B	G	B	G	B	G	
30	4	2	3	3	3	3	18
60	3	3	3	3	3	3	18
90	3	3	3	3	3	3	18
120	4	2	3	3	3	3	18
Total	24		24		24		72

B, boars; G, gilts

3.2. Sampling

After slaughter, samples from muscle *longissimus dorsi* were collected at the P2 site (between the 12th and 13th rib) for intramuscular fat (IMF) measurement and RNA extraction. Samples used for IMF measurements were taken with approximately 20g, fat trimmed, identified, wrapped in aluminum foil, stored in plastic bags and kept frozen at -20^{o}C until analysis.

For RNA extraction samples, small pieces of muscle (0.5 x 0.2 cm) were immersed in new 15ml tubes containing RNAholder (BioAgency) to protect and conserve expression profiles of this tissue. In order to avoid RNA degradation, all

materials used were sprayed with RNase Exterminator (BioAgency). Afterwards, samples were kept at -20ºC until RNA extraction.

In order to correct the data, slaughter age (in days) from each animal was recorded to be tested as a covariate.

3.3. Intramuscular fat content measurement

Samples were thawed overnight 4ºC then processed using a tissue homogenizer (Homogeneizador de Tecidos Tipo Turrax CT-138, CIENTEC). Fat content was determined by the ether extract method (AOAC, 1990) and expressed as a percentage of IMF in fresh meat.

3.4. Total RNA extraction and first strand cDNA synthesis

Frozen samples were thawed at 4ºC one hour before total RNA extraction. Afterwards, samples were disrupted using TissueRuptor (QIAGEN) and total RNA was isolated from skeletal muscle tissue with RNeasy® Mini Kit (QIAGEN). As indicated for fibrous tissues, a Proteinase K (Fungal – Invitrogen) step was added in the protocol, to enhance cell lyses. On column DNase digestion was performed using RNase-Free DNase Set (QIAGEN). Total RNA concentration was estimated by NanoDrop ND-1000 (Thermo Fisher Scientific Inc.) spectrophotometer and quality analyzed by 1.2% agarose gel electrophoresis. RNA samples were kept at -70ºC until being used for reverse transcriptase reactions.

First-strand cDNA synthesis was performed using SuperScript™ III First-Strand kit (invitrogen™) and its concentration was estimated through spectrophotometry. Afterwards, cDNA samples were kept at -20ºC until real-time PCR reactions.

3.5. Primers design for Real-time PCR

Primers were designed using PrimerQuest software (www.idtdna.com/Scitools/Aplications/PrimerQuest) from IDT Scitools (Integrated DNA Technologies, Inc.). When mRNA sequences from Pigs (*Sus scrofa*) for the studied genes were unavailable, sequences from Humans (*Homo sapiens*) were used as substitutes. Ten primers were designed, nine for target genes and one as endogenous control (Beta-actin gene). From these target genes, four (Table 3.5.1) were chosen from Sasaki et al. (2006) and five (Table 3.5.2) from a

Piau/Large White Expressed Sequence Tags Library developed at *Universidade Federal de Viçosa* (Peixoto, 2007).

3.6. Real-time PCR reactions

All reactions were performed in an SDS ABI PRISM 7300 thermocycler (Applied Biosystems). The relative quantification method and the SYBR® Green detection system were used. Before expression profile analysis, each gene had its efficiency calculated (Livak & Schmittgen, 2001) using different serial concentrations of primers (100, 200 and 400 nM) and cDNA (1, 10, 20 and 40 ng). A cDNA pool of twelve animals, one from each treatment (genetic group x slaughter weight interaction), was used for the efficiency calculation.

Each reaction consisted of 12.5μL of Power SYBR® GREEN PCR Master Mix (Applied Biosystem), 0.5μL of each primer (forward and reverse), 5μL of cDNA and nuclease-free water to complete 25μL, and was performed following this program: 95°C for 3 min, 40 cycles of 95°C for 15 sec and 60°C for 1. After 40 cycles, all samples were submitted to a dissociation curve analysis for validation of non-specific products; for that, samples were heated 1°C per 30 sec, from 60°C until 94°C. Each reaction was performed in replicate. Data from relative quantification was transformed using the $2^{-\Delta Ct}$ method, according to Livak & Schmittgen (2001).

Table 3.5.1 – Primers designed from GenBank sequences, based on Sasaki et al (2006) findings for bovines

Gene name	Gene symbol	Primer sequence (5'-3')	Tm (°C)	NCBI access code	Species
Matrix Gla protein	MGP	F–TAAACCGGGAAGCTTGCGATGACT	63.6	AF525316	*Sus scrofa*
		R–GCGCTGCCGGAAATAACGATTGTA	64.5		
Pyruvate dehydrogenase (lipoamide) beta	PDHB	F–TCTGCTTGGAGAAGAAGTTGCCCA	62.9	NM_000925	*Homo sapiens*
		R–CAGCAATTCCAGCAAAGCCCATCT	64.1		
Ryanodine receptor 1 (skeletal)*	RYR1	F–GGCGATTGTTTCCGCAAGCAGTAT	60.0	NM_001001534	*Sus scrofa*
		R–TTTCTCCACTGCATGGTCACCTCT	59.9		
Triadin	TRDN	F–GTCAGCTGTGGCCGTTGTTATGTT	60.9	NM_006073	*Homo sapiens*
		R–AAAGAAGCCATAGACCCAGTCCGT	60.1		
Beta-actin†	ACTB	F–GAAGCATGTGGGCAACCAGCAATA	60.2	AY550071	*Sus scrofa*
		R–CCAATTTCACGGCCCAGAACGTAA	59.8		

†-Endogenous control; * RYR1 was not analyzed by Sasaki et al., (2006), but it was included in the present work due to its relevance in pork production.

Table 3.5.2 – Primers designed from a Piau/Large White Expressed Sequence Tags Library‡ developed at *Universidade Federal de Viçosa*

Gene name	Gene symbol	Primer sequence (5'-3')	Tm (°C)
Atrophin 1	ATN1	F–TAAACCGGGAAGCTTGCGATGACT	63.6
		R–GCGCTGCCGGAAATAACGATTGTA	64.5
Eukaryotic translation elongation factor 1 alpha 2	EEF1A2	F–TCTGCTTGGAGAAGAAGTTGCCCA	62.9
		R–CAGCAATTCCAGCAAAGCCCATCT	64.1
Fatty acid binding protein 3, muscle and heart	FABP3	F–GGCGATTGTTTCCGCAAGCAGTAT	60.0
		R–TTTCTCCACTGCATGGTCACCTCT	59.9
Low density lipoprotein receptor	LDLR	F–GTCAGCTGTGGCCGTTGTTATGTT	60.9
		R–AAAGAAGCCATAGACCCAGTCCGT	60.1
Obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	OBSCN	F–GAAGCATGTGGGCAACCAGCAATA	60.2
		R–CCAATTTCACGGCCCAGAACGTAA	59.8

‡ - Peixoto (2007)

3.7. Statistical analysis

All statistical procedures were performed using SAS 9.0 for Windows (Statistical Analysis System Institute, Inc., Cary, NC, USA).

3.7.1. Normality test

The UNIVARIATE procedure was used to verify normal distribution of IMF (expressed as percentage), slaughter age (A; expressed as days) and expression of each gene (expressed as $2^{-\Delta Ct}$). Although IMF and SA showed a normal distribution, none of the expression of studied genes showed a normal distribution. Therefore, the gene expression data was transformed using ln(x+1) as proposed by Vogel et al., (2004) and normal distribution was verified. The transformed gene expression for each gene is called EXP_{GENE} (expressed as arbitrary units; a.u.), where GENE is the gene symbol.

3.7.2. Analysis of covariance (ANCOVA)

In order to check the influence of the quantitative variable slaughter age (A) on IMF and transformed gene expressions (EXP_{GENE}), an analysis of covariance (ANCOVA) was performed. The ANCOVA is a method by which the influence of the covariates on the treatment means is reduced (Ott & Longnecker, 2001). Moreover, a covariate must be correlated to the dependent variable and cannot be influenced by independent variables. Therefore, the covariate slaughter age was tested within genetic group x slaughter weight interaction.

The ANCOVA was performed using the GLM procedure, according to the following statistical model:

$$Y_{ijkl} = \mu + G_i + W_j + S_k + (G*W)_{ij} + \left[\beta_{ij}\left(A_{ijl} - \bar{A}(\square) * (G*W)\right)_{ij}\right] + \varepsilon_{ijkl}$$

Where:

Y_{ijkl} is the observed trait; μ is the general constant; G_i is the ith level of genetic group, i=1, 2 or 3 (Piau, CrB or Com); W_j is the jth level of slaughter weight, j=1, 2, 3 or 4 (30, 60, 90 or 120Kg); S_k is the kth level of sex, k=1 or 2 (male or female); $(G*W)_{ij}$ is the interaction of the ith level of genetic group and the jth level of slaughter weight; β_{ij} is ith level of the genetic group and the jth level of slaughter

weight regression coefficient associated to the covariate A; A_{ijl} is the slaughter age of the lth observation at the ith level of genetic group and the jth level of slaughter weight, l=1 to 6; $\bar{A}$ is the average slaughter age; and ε_{ijkl} is the random error.

Using a significance level of 5%, the covariate slaughter age (A) had no effect on IMF and EXP_{GENE}. Therefore, slaughter age was removed from the model.

3.7.3. Analysis of variance (ANOVA) and multiple comparison test

Using a significance level of 5%, the ANOVA was performed using the GLM procedure, according to the following statistical model:

$$Y_{ijkl} = \mu + G_i + W_j + S_k + (G * W)_{ij} + \varepsilon_{ijkl}$$

Where:

Y_{ijkl} is the observed trait; μ is the general constant; G_i is the ith level of genetic group, i=1, 2 or 3 (Piau, CrB or Com); W_j is the jth level of slaughter weight, j=1, 2, 3 or 4 (30, 60, 90 or 120Kg); S_k is the kth level of sex, k=1 or 2 (male or female); $(G * W)_{ij}$ is the interaction of the ith level of genetic group and the jth level of slaughter weight; and ε_{ijkl} is the random error.

Genetic group and sex-adjusted means were obtained by LSMEANS statement and adjusted Tukey-Kramer multiple comparison tests were performed at a 5% level of significance. When the slaughter weight effect was significant, regression equations were adjusted.

3.7.4. Correlations

Using the option PREDICTED of the OUTPUT statement from GLM procedure, predicted values of IMF and EXP_{GENE} were obtained and the Pearson's product-moment correlation coefficient and the partial correlation between these two traits were calculated. These values were analyzed using two sets of data: the whole population and Piau pigs only. A 5% level of significance was used in these analyses.

3.7.5. Prediction equation for IMF

In order to adjust a multiple regression equation of intramuscular fat content with all genes, using predicted values, the STEPWISE method of the SELECTION option at the MODEL statement from the REG procedure was used.

In the stepwise method, variables are added one by one to the model, and the F statistic for a variable to be added must be significant. After a variable is added, the stepwise method looks at all the variables already included in the model and deletes any variable that does not produce a significant F statistic. This process ends when none of the variables outside the model have a significant F statistic and every variable in the model is significant. The default significance level of 15% was used (Copyright(c) 2009, SAS Institute Inc., Cary, NC, USA. All Rights Reserved. Reproduced with permission of SAS Institute Inc., Cary, NC).

4. Results and Discussion

The average OD_{260}/OD_{280} ratio from the total RNA samples was 2.08±0.011. Presence of 28S and 18S bands indicates good RNA quality (Figure 4.1).

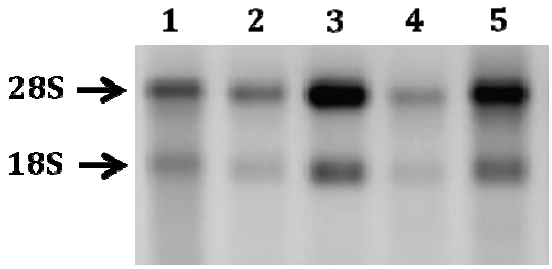

Figure 4.1 – Total RNA from five samples of *longissimus dorsi* muscle in a 1.2% agarose gel stained with ethidium bromide.

The amplified efficiency of target and endogenous genes were similar. Concentration of primers and cDNA are displayed in Table 4.1.

Table 4.1 – Concentration of primers, cDNA and reaction efficiency (E) for analyzed genes

Gene symbol	Primer (nM)	cDNA (ng/reaction)	E (%)
ATN1	400	200	95.19
EEF1A2	100	100	87.16
FABP3	400	200	82.53
LDLR	100	200	90.19
MGP	400	200	88.09
OBSCN	400	200	99.83
PDHB	400	200	92.28
RYR1	100	100	91.61
TRDN	400	200	98.76
ACTB†	400	200	90.18

†-Endogenous control

Dissociation curves were generated after amplification cycles and showed that only one fragment was amplified, lacking unspecified amplification and primer dimmers for all genes. Figure 4.2 illustrates dissociation curves from OBSCN gene.

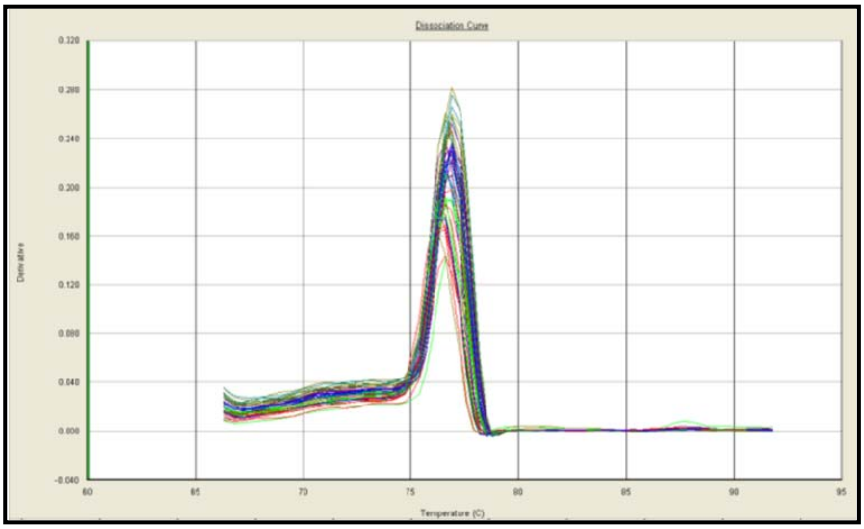

Figure 4.2 – OBSCN gene dissociation curve.

The coefficient of variation (CV) of Cycle Threshold (C_t) from replicates within each sample was low at less than 5%, indicating precision and reproducibility (data not shown). However, for some genes, higher CV values of Ct for different slaughter weights (W) within genetic groups (G) were observed, indicating individual variation between animals (Table 4.2).

Table 4.2 – Number of animals (*n*), Average C_t, C_t standard deviation (STD) and C_t coefficient of variation (CV) for each target gene in different slaughter weights (W) within genetic groups (G)

Gene symbol	G	W (Kg)	*n*	Average C_t	STD	CV (%)
ATN1	Piau	30	6	30.238	0.729	2.411
		60	6	32.142	1.701	5.292
		90	6	31.713	0.446	1.405
		120	5	33.179	1.138	3.429
	Com	30	6	33.100	1.690	5.105
		60	6	32.639	1.117	3.422
		90	6	32.114	0.831	2.587
		120	6	30.972	0.907	2.929
	CrB	30	6	31.237	0.477	1.527
		60	6	31.705	0.629	1.984
		90	6	31.497	0.466	1.480
		120	6	31.988	0.646	2.020
EEF1A2	Piau	30	6	21.648	1.874	8.657
		60	6	23.801	1.993	8.376
		90	6	23.068	1.174	5.087
		120	5	23.991	2.329	9.708

Cont.

Gene symbol	G	W (Kg)	*n*	Average C_t	STD	CV (%)
EEF1A2	Com	30	6	22.441	1.496	6.668
		60	6	22.720	0.991	4.361
		90	6	23.171	1.115	4.813
		120	6	22.922	0.644	2.810
	CrB	30	6	23.998	1.694	7.061
		60	6	24.027	1.369	5.696
		90	6	23.552	1.070	4.543
		120	6	24.638	1.504	6.102
FABP3	Piau	30	6	21.753	0.996	4.578
		60	6	23.171	0.841	3.628
		90	6	23.593	0.820	3.474
		120	5	22.879	1.706	7.459
	Com	30	6	23.978	1.027	4.282
		60	6	22.912	1.326	5.788
		90	6	24.364	1.273	5.225
		120	6	22.460	1.084	4.826
	CrB	30	6	22.536	1.272	5.647
		60	6	23.842	0.916	3.840
		90	6	23.650	0.808	3.418
		120	6	22.593	1.185	5.243
LDLR	Piau	30	6	30.892	1.013	3.278
		60	6	32.286	0.888	2.749
		90	6	32.503	0.739	2.273
		120	5	32.116	1.252	3.898
	Com	30	6	33.630	1.142	3.395
		60	6	32.158	2.147	6.675
		90	6	32.899	1.816	5.519
		120	6	32.007	0.991	3.097
	CrB	30	6	32.012	1.391	4.345
		60	6	32.939	0.696	2.114
		90	6	32.654	0.577	1.768
		120	6	32.244	1.000	3.103
MGP	Piau	30	6	22.070	1.616	7.324
		60	6	24.442	0.996	4.076
		90	6	23.911	0.763	3.190
		120	5	25.625	2.181	8.513
	Com	30	6	22.058	1.059	4.801
		60	6	22.148	1.835	8.286
		90	6	23.464	1.325	5.648

Cont.

Gene symbol	G	W (Kg)	*n*	Average C_t	STD	CV (%)
MGP	Com	120	6	23.374	1.028	4.397
	CrB	30	6	23.184	1.748	7.538
		60	6	23.022	1.367	5.939
		90	6	23.535	1.476	6.272
		120	6	23.558	1.021	4.335
OBSCN	Piau	30	6	21.463	0.442	2.061
		60	6	21.891	0.769	3.513
		90	6	22.066	1.035	4.689
		120	5	22.184	0.735	3.314
	Com	30	6	22.975	0.755	3.285
		60	6	22.908	0.737	3.215
		90	6	22.916	0.462	2.017
		120	6	21.969	0.549	2.497
	CrB	30	6	22.808	0.959	4.205
		60	6	22.946	0.531	2.312
		90	6	23.118	0.606	2.620
		120	6	22.819	0.422	1.851
PDHB	Piau	30	6	32.158	1.276	3.968
		60	6	32.310	1.245	3.853
		90	6	32.023	0.688	2.150
		120	5	34.372	2.921	8.497
	Com	30	6	35.051	1.368	3.904
		60	6	33.549	1.245	3.710
		90	6	32.353	1.439	4.447
		120	6	31.843	0.660	2.074
	CrB	30	6	31.943	1.433	4.485
		60	6	32.388	1.294	3.995
		90	6	32.743	1.613	4.927
		120	6	33.124	0.483	1.458
RYR1	Piau	30	6	20.882	0.175	0.754
		60	6	20.715	0.893	4.309
		90	6	20.843	0.669	3.209
		120	5	20.918	0.920	4.389
	Com	30	6	21.214	0.430	2.026
		60	6	21.396	0.782	3.655
		90	6	21.639	0.696	3.217
		120	6	20.337	0.419	2.060
	CrB	30	6	22.509	0.564	2.505
		60	6	21.932	0.706	3.220

Cont.

Gene symbol	G	W (Kg)	*n*	Average C_t	STD	CV (%)
RYR1	CrB	90	6	21.963	0.668	3.043
		120	6	21.830	0.957	4.385
TRDN	Piau	30	6	19.706	1.116	5.663
		60	6	19.368	0.956	4.935
		90	6	19.313	1.065	5.513
		120	5	19.817	1.511	7.624
	Com	30	6	20.943	1.054	5.032
		60	6	20.679	1.425	6.893
		90	6	19.665	0.634	3.223
		120	6	19.190	0.269	1.400
	CrB	30	6	19.986	1.065	5.329
		60	6	20.049	1.243	6.198
		90	6	19.660	1.081	5.501
		120	6	20.111	0.826	4.108

An overall Analysis of Variance (ANOVA) table is illustrated below (Table 4.3). Genetic group x slaughter weight interaction (G*W) was significant (P<0.05) for six traits, genetic group (G) for two traits, slaughter weight (W) for three traits and sex (S) was only significant for intramuscular fat content (IMF).

Table 4.3 – Overall ANOVA for all studied traits, with genetic group x slaughter weight interaction (G*W), genetic group (G), slaughter weight (W) and sex (S) as sources of variation

Trait	Sources of variation			
	(G*W)	G	W	S
IMF	**	N/A	N/A	*
EXP_{ATN1}			*	
EXP_{EEF1A2}		**		
EXP_{FABP3}			**	
EXP_{LDLR}	*	N/A	N/A	
EXP_{MGP}	**	N/A	N/A	
EXP_{OBSCN}	**	N/A	N/A	
EXP_{PDHB}		**	**	
EXP_{RYR1}	**	N/A	N/A	
EXP_{TRDN}	*	N/A	N/A	

IMF, intramuscular fat content; EXP_{GENE}, expression of studied gene;

**, P<0.01; *, P<0.05; N/A, not applicable; Empty spaces, P>0.05.

4.1. IMF

Least square means (LSM) and standard error (SE) of IMF for each genetic group within a slaughter weight [G(W)] are provided in Table 4.1.1, and for Sex (S) in Table 4.1.2.

Table 4.1.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of intramuscular fat content (IMF), for each genetic group within slaughter weight (W)

Slaughter weight (Kg)	Genetic group	*n*	IMF (%) LSM	SE
30	Piau	6	0.96	0.17
	CrB	6	1.59	0.17
	Com	5	0.87	0.19
60	Piau	5	1.22	0.19
	CrB	5	1.70	0.19
	Com	6	1.45	0.17
90	Piau	5	1.48	0.19
	CrB	5	1.35	0.19
	Com	5	0.82	0.19
120	Piau	6	2.48[a]	0.17
	CrB	6	2.08[a]	0.17
	Com	4	1.00[b]	0.21

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

Within 30, 60 and 90Kg of slaughter weight, there was no statistical difference (P>0.05) across genetic groups. Within 120Kg, IMF content was 2.48 and 2.08% for Piau and Crossbred (CrB) animals, respectively, which differed statistically (P<0.05) from Commercial (Com) animals (1.00%). Like many indigenous breeds, Piau pigs have never undergone a genetic progression. Thus, it can be expected that there is a higher level of fatness in these animals than in commercial ones.

Intramuscular fat content in *longissimus dorsi* samples from Piau pigs are considered to be at a medium level (Brewer et al., 2001) and it may be correlated to other meat sensory properties. Shear force has a high and negative correlation (r=–0.86) with IMF content, improving juiciness and tenderness, due to the easiest muscle fiber separation (Lawrie, 1998; Jeleníková et al., 2008). Medium marbled pork also received high scores for juiciness compared to low marbled pork, but showed no differences in flavor and tenderness compared to low and high marbled

pork (Brewer et al., 2001). An increase in the fat content was correlated with a tendency towards a lower water-holding capacity, an undesirable characteristic for the pork industry. Improvement of palatability, juiciness and tenderness in pork loin was noted for IMF content above 3.0% (Daszkiewicz et al., 2005). Although a recent survey reported no correlation between these two pork characteristics (Rincker et al., 2007), it is usually accepted that IMF influences pork flavor and some studies reported that it is positively correlated to flavor. (Brewer et al., 2001; Channon et al., 2004; Fortin et al., 2005). However, a recent survey reported no correlation between these two characteristics of pork (Rincker et al., 2007). The amount of visible fat is one of the strongest visual cues for retail consumers considering purchasing pork (Levy & Hanna, 1994; Brewer et al., 2001). According to Brewer et al. (2001), medium marbled pork has an appearance that encourages purchase much more than high marbled pork.

Although a minimum desirable IMF level is constantly reported in several studies, it generally ranges from 2 to 3% of lipids (Rincker et al., 2008). Moreover, the higher IMF content presented in *longissimus dorsi* muscles from Piau pigs may be beneficial for pork quality than in leaner pigs.

In Table 4.1.2 the least square means of IMF for boars and gilts are reported. Boars had a higher IMF content than gilts (1.54 and 1.30%, respectively). This result is generally agreed upon by most authors (D'Souza & Mullan, 2002; Correa et al., 2006; Renaudeau & Mourot, 2007).

Table 4.1.2 – Least square means (LSM) and standard error (SE) of intramuscular fat content (IMF), expressed in a percentage, for each sex

Sex	*n*	IMF(%) LSM	SE
Boars	37	1.54[a]	0.0698
Gilts	27	1.30[b]	0.0825

Least square means without a common superscript letter are statistically different (P<0.05).

Considering IMF, slaughter weight within a genetic group [W(G)] was only significant (P<0.05) for Piau breed (Table 4.1.3). An illustration of IMF deposition according to slaughter weight for this breed is provided in Figure 4.1.1.

Table 4.1.3 – Significance levels (p-value) of slaughter weight within each genetic group for IMF

Source of Variation	Genetic group (p-value)		
	Piau	Com	CrB
Slaughter weight	<.0001	0.1073	0.1898

Linear regression equation was modeled when P<0.05.

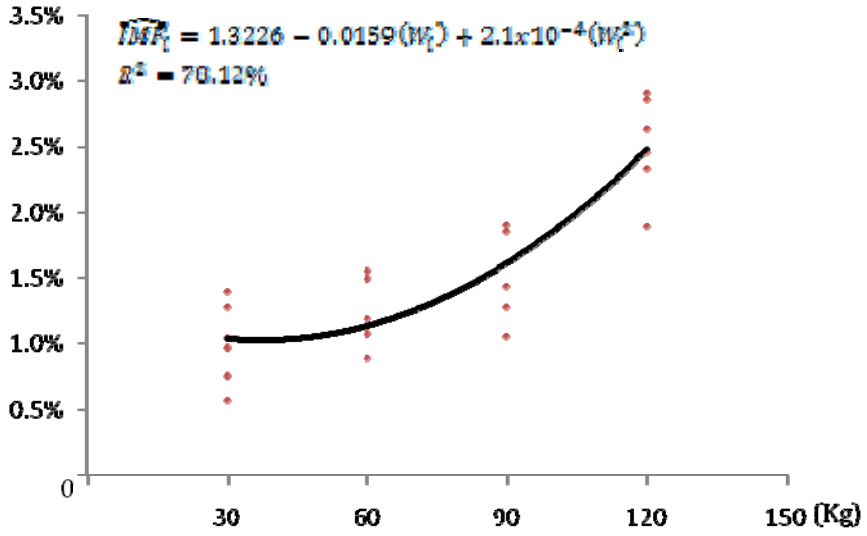

Figure 4.1.1 – Deposition pattern, prediction equation ($\widehat{IMF}_i$) and coefficient of determination (R^2) of intramuscular fat content in Piau pigs from 30 to 120Kg of slaughter weight.

To date, this is the only prediction equation that describes intramuscular fat deposition in Piau pigs. According to this plot, after reaching the lower value (1.02%) at 38Kg, IMF levels increase with slaughter weight, and show an interesting potential for IMF deposition in heavier pigs. It can also be observed that Piau pigs develop IMF depositions late. This might be clearer through a comparison of raw values in Table 4.1.1, where Piau pigs presented lower levels of IMF at 30 and 60Kg of slaughter weight and reached higher levels at 90 and 120Kg. Therefore, for the Piau breed, little increasing on weight provides good improvement of IMF content in heavier pigs.

Compared to other indigenous breeds, in a general way, the Brazilian naturalized breed Piau has lower levels of IMF than the Caribbean Creole (Renaudeau & Mourot, 2007), the Chinese Laiwu (5.91%; Lu et al., 2008) and Taihu (3.27%; Chu et al., 2003), the Iberian Lampinõ and Retinto (3.34 and 3.17%, respectively; Estévez et al., 2003) and the Polish Pulawska (3.56%; Grześkowiak et al., 2006), but has levels that are similar to those of the Chinese Tongcheng (2.66%; Lu et al., 2008), the Iberian Torbiscal (2.51%; Estévez et al., 2003) and the

French Gascon pig (2.60%; Sans et al., 2004). Many of these breeds are well-regarded for their superior pork quality in relation to lean pigs.

4.2. ATN1

Although there was no statistical effect of genetic group x slaughter weight interaction (P>0.05), the least square means of ANT1 gene expression (EXP_{ATN1}) are represented in Table 4.2.1. Within each slaughter weight, there was no statistical difference (P>0.05) between EXP_{ATN1} levels among genetic groups.

Table 4.2.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of normalized ATN1 gene expression (EXP_{ATN1}), in arbitrary units (a.u.), for each genetic group within slaughter weight (W)

Slaughter weight (Kg)	Genetic group	*n*	EXP_{ATN1}(a.u.) LSM	SE
30	Piau	6	0.80	0.20
	CrB	6	1.05	0.20
	Com	6	0.85	0.20
60	Piau	6	1.15	0.20
	CrB	6	1.38	0.20
	Com	6	1.04	0.20
90	Piau	6	1.23	0.20
	CrB	6	1.63	0.20
	Com	6	1.49	0.20
120	Piau	5	1.06	0.22
	CrB	6	1.15	0.20
	Com	6	1.22	0.20

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

Irrespective of genetic group, slaughter weight had a significant effect on EXP_{ATN1} (Figure 4.2.1).

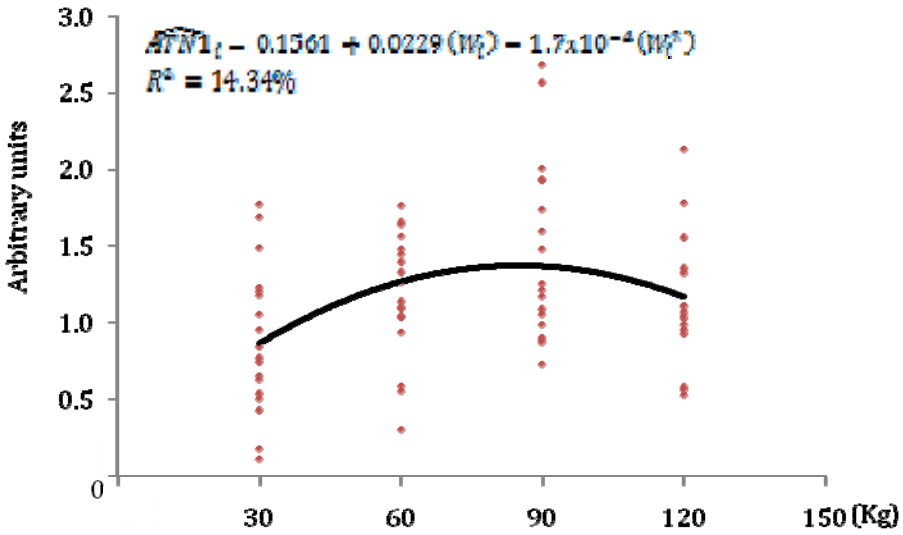

Figure 4.2.1 – Expression pattern, prediction equation ($\widehat{ATN1}_i$) and coefficient of determination (R^2) of EXP_{ATN1} in Piau, Commercial and Crossbred pigs from 30 to 120Kg of slaughter weight.

This gene showed higher expression in Duroc than Large White and Piau (36.5 and 13.2 times respectively) pigs who were slaughtered at 180 days (Peixoto, 2007), but in the present study there was no effect made by the category of genetic group (P>0.05).

The low value of a coefficient of determination (R^2) associated was with the prediction equation of EXP_{ATN1} and may be explained by a great variability of replicates (red dots in Figure 4.2.1) within slaughter weights that consist of all three genetic groups. Analyzing the expression pattern of an ATN1 gene, lower levels are observed on the ends of the curve, and the highest value is 1.20a.u. at 67Kg of slaughter weight.

Atrophin 1 proteins are proposed to act as versatile transcriptional corepressors (Zhang et al., 2002). In Table 4.1.1, higher IMF content was reported in pigs at 120Kg of slaughter weight, when expression levels of ATN1 were diminishing. Thus, ATN1 may act on biochemistry pathways related to fat deposition in early stages of development preventing adipose hypertrophy. As levels of IMF are increasing with slaughter weight, this gene might have minor role at early stages of development.

4.3. EEF1A2

Within each slaughter weight, there was no statistical difference (P>0.05) between EXP_{EEF1A2} levels among different genetic groups (Table 4.3.1).

Table 4.3.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of EEF1A2 gene expression (EXP_{EEF1A2}), in arbitrary units (a.u.), for each genetic group within slaughter weight (W)

Slaughter weight (Kg)	Genetic group	*n*	EXP_{EEF1A2}(a.u.) LSM	SE
30	Piau	6	7.093	0.431
	CrB	6	5.837	0.428
	Com	6	7.918	0.428
60	Piau	6	6.769	0.428
	CrB	6	6.298	0.428
	Com	6	7.293	0.428
90	Piau	6	7.468	0.428
	CrB	6	6..770	0.428
	Com	6	7.303	0.428
120	Piau	5	7.355	0.471
	CrB	6	5.942	0.428
	Com	6	6.645	0.428

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

There was genetic group effected (P<0.05) expression levels of the EEF1A2 gene (Table 4.3.2). Least square means in Commercial (7.290 a.u.) and Piau pigs (7.171 a.u.) were statistically equal, and higher (P<0.05) than in Crossbred (6.212 a.u.) pigs.

There was no significant effect (P>0.05) of slaughter weight, indicating that this gene is expressed at similar levels from 30 to 120Kg. Although EXP_{EEF1A2} between Piau and Commercial pigs was statistically equal (P>0.05), differences were expected because they are genetically divergent.

Table 4.3.2 – Number of animals (*n*), least square means (LSM) and standard error (SE) of EEF1A2 gene expression (EXP_{EEF1A2}), in arbitrary units (a.u.), for each genetic group

Genetic group	*n*	EXP_{EEF1A2}(a.u.) LSM	SE
Com	24	7.290^{a}	0.214
Piau	23	7.171^{a}	0.220
CrB	24	6.212^{b}	0.214

Least square means without a common superscript letter are statistically different (P<0.05).

The main role of eukaryotic translation elongation factor 1 alpha 2 (EEF1A2) is to bind aminoacylated tRNA and facilitate their recruitment to the

ribosome during translation elongation (Kulkarni et al., 2007). Moreover, it has been reported that EEF1A2 also acts on cell structure, regulating cytoskeletal organization (Anand et al., 2002) and stimulating phosphatidylinositol-4 phosphate generation, a minor phospholipid component of cell membranes (Jeganathan & Lee, 2007).

The ether extract represents all extractable material by ether from the sample. Besides the true fat (triglycerides), other material such as phospholipids, sterols, essential oils, fat-soluble pigments, etc., may be extracted (Triebold, 2007).

As phospholipids from cell membranes are also part of extracted fat, differences on IMF content may not be related to EEF1A2 expression, but to other factors such as cell hyperplasia and/or hypertrophy. If EXP_{EEF1A2} is the same for Piau and Commercial pigs, they must have similar performance of the EEF1A2 gene in cell membrane, but a different number or size of cells.

4.4. FABP3

Within each slaughter weight, there was no statistical difference (P>0.05) of EXP_{FABP3} levels among genetic groups (Table 4.4.1).

Table 4.4.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of FABP3 gene expression (EXP_{FABP3}), in arbitrary units (a.u.), for each genetic group within slaughter weight (W)

Slaughter weight (Kg)	Genetic group	*n*	EXP_{FABP3}(a.u.) LSM	SE
30	Piau	6	6.155	0.256
	CrB	6	6.399	0.255
	Com	6	6.320	0.255
60	Piau	6	6.762	0.255
	CrB	6	6.764	0.255
	Com	6	7.072	0.255
90	Piau	6	6.539	0.255
	CrB	6	7.138	0.255
	Com	6	6.736	0.255
120	Piau	5	7.499	0.279
	CrB	6	7.588	0.255
	Com	6	6.729	0.255

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

Slaughter weight had significant effect (P<0.05) on EXP_{FABP3} considering all three genetic groups. Figure 4.4.1 illustrates EXP_{FABP3}, in arbitrary units, according to slaughter weights.

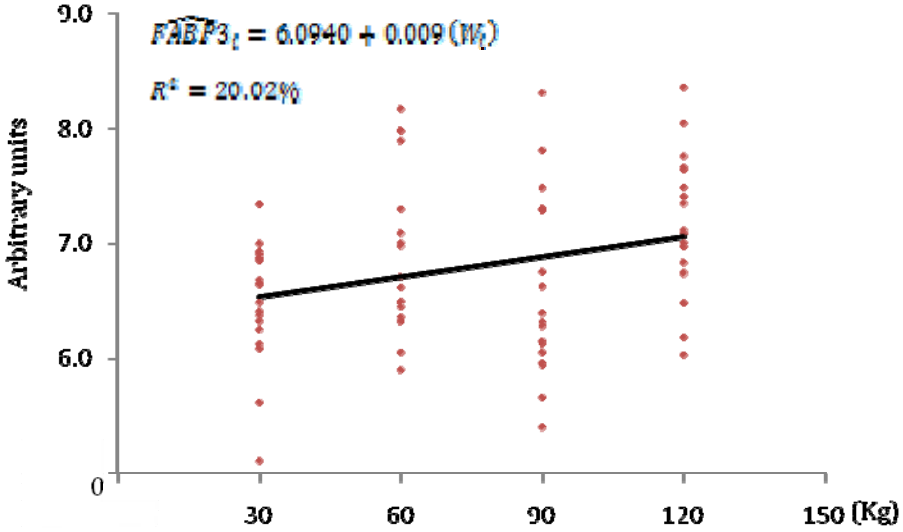

Figure 4.4.1 – Expression pattern, prediction equation ($\widehat{FABP3}_i$) and coefficient of determination (R^2) of EXP_{FABP3} in Piau, Commercial and Crossbred pigs from 30 to 120Kg of slaughter weight.

The low value of the coefficient of determination (R^2) associated with the prediction equation of EXP_{FABP3} may be explained by the great variability of replicates (red dots in Figure 4.4.1) within slaughter weights, which consist ofall three genetic groups. Analyzing the expression pattern of FABP3 gene, its levels are increased linearly within a slaughter weight, reaching the highest value at 120Kg of slaughter weight.

Heart fatty acid-binding protein (H-FABP) is a member of the FABPs family, and is a product of the fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor) gene (FABP3). H-FABP is responsible for long-chain fatty acid intake in cells and it is generally regarded as a candidate gene for IMF content (Chmurzyńska, 2006). Some reports indicate that variants of FABP3 act on IMF content in diverse pig breeds (Gerbens et al., 1999, 2000, 2001; Grindflek et al., 2001; Óvilo et al., 2002b; Urban et al., 2002; Pang et al., 2006). On the other hand, no significance was found between FABP3 alleles and IMF content in other trials (Nechtelberger et al., 2001; Urban et al., 2002).

Irrespective of slaughter weight, genetic group had no effect on EXP_{FABP3} (P>0.05). Although IMF content was influenced by genetic group within 120Kg of

slaughter weight (Table 4.1.1), FABP3 showed a slightly increasing patter of expression for all genetic groups. Thus, variations on IMF content may not suffer or suffer little effect due to an augmentation of FABP3 expression. Furthermore, investigation on FABP3 genotypes and mRNA levels influencing IMF content in Piau pigs demonstrates no association (Moraes, 2006; Figueiredo et al., 2008). Nevertheless, assignment of FABP3 as a candidate gene for IMF might be risky.

4.5. LDLR

Within each slaughter weight, there was no statistical difference (P>0.05) between LDLR gene expression (EXP_{LDLR}) levels among genetic groups (Table 4.5.1).

Table 4.5.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of LDLR gene expression (EXP_{LDLR}), in arbitrary units (a.u.), for each genetic group within slaughter weight (W).

Slaughter weight (Kg)	Genetic group	*n*	EXP_{LDLR}(a.u.) LSM	SE
30	Piau	6	0.818	0.209
	CrB	6	0.667	0.208
	Com	6	0.786	0.208
60	Piau	6	1.012	0.208
	CrB	6	1.210	0.208
	Com	6	1.653	0.208
90	Piau	6	1.402	0.208
	CrB	6	1.119	0.208
	Com	6	0.970	0.208
120	Piau	5	1.825	0.228
	CrB	6	1.273	0.208
	Com	6	0.881	0.208

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

Slaughter weight within a genetic group [W(G)] was only significant (P<0.05) for the Piau breed (Table 4.5.2). An illustration of LDLR expression according to slaughter weight in Piau pigs is provided in Figure 4.5.1.

Table 4.5.2 – Significance level (p-value) of slaughter weight in each genetic group for EXP_{LDLR}

Source of Variation	Genetic group (p-value)		
	Piau	Com	CrB
Slaughter weight	0.0141	0.0799	0.0860

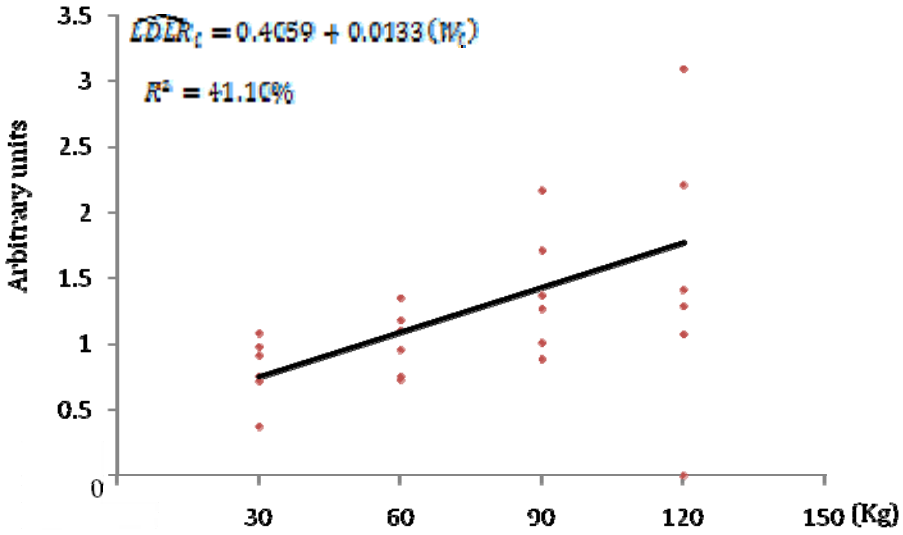

Figure 4.5.1 – Expression pattern, prediction equation ($\widehat{LDLR}_i$) and coefficient of determination (R^2) of EXP_{LDLR} in Piau pigs from 30 to 120Kg of slaughter weight.

Low-density lipoprotein (LDL) transports endogenous triacylglycerols and cholesterol from liver to other tissues. LDLs are edocytosed by LDL receptors that are located on the cell surface. Inside the cell, LDL particles are degraded in amino acids, cholesterol and fatty acids. These fatty acids are stored in a cell to be transterificated (Voet et al., 2000). The synthesis of the LDL receptor is controlled by the delivery of lipoprotein cholesterol; free cholesterol inside the cell suppresses LDL receptor synthesis in the endoplasmic reticulum (Horst, 1991). Thus, the LDLR gene is trigged by a lack of cholesterol in the cell.

The expression pattern of LDLR gene in Piau pigs (Figure 4.5.1) indicated that LDL receptors were synthesized as slaughter weight increased. Therefore, more LDLs were degraded and fatty acids were stored in *longissimus dorsi* cells. Hence, high levels of EXP_{LDLR} may indicate a higher deposition of intramyocellular than extramyocellular lipids.

4.6. MGP

Least square means of MGP gene expression (EXP_{MGP}) are represented in Table 4.6.1. Within each slaughter weight, there was no statistical difference ($P>0.05$) of EXP_{MGP} levels among genetic groups.

Table 4.6.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of normalized MGP gene expression (EXP_{MGP}), in arbitrary units (a.u.), for each genetic group within slaughter weight (W)

Slaughter weight (Kg)	Genetic group	*n*	EXP_{MGP}(a.u.) LSM	SE
30	Piau	6	6.601	0.284
	CrB	6	6.358	0.283
	Com	6	7.572	0.283
60	Piau	6	6.613	0.283
	CrB	6	6.604	0.283
	Com	6	6.511	0.283
90	Piau	6	6.283	0.283
	CrB	6	6.956	0.283
	Com	6	6.511	0.283
120	Piau	5	6.188	0.310
	CrB	6	7.294	0.283
	Com	6	6.151	0.283

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

Slaughter weight within genetic group [W(G)] had significant effect (P<0.05) in Commercial and Crossbred pigs (Table 4.6.2), while in Piau pigs there was no influence of slaughter weight on EXP_{MGP} (P>0.05). An illustration of MGP expression according to slaughter weight in Commercial and Crossbred pigs is provided in Figure 4.6.1.

Expression patterns of MGP gene were contrasting in both genetic groups. In Crossbred pigs, EXP_{MGP} increased with slaughter weight, while in Commercial pigs, levels of MGP decreased as slaughter weights increase (Figure 4.6.1).

Table 4.6.2 – Significance level (p-value) of slaughter weight in each genetic group for EXP_{MGP}

Source of Variation	Genetic group (p-value)		
	Piau	Com	CrB
Slaughter weight	0.7146	0.0071	0.0474

MGP gene is related to tissue mineralization, acting as a calcification inhibitor. Mice lacking the MGP gene went through arterial calcification and died of blood-vessel rupture (Luo et al., 1997). Moreover, a higher expression of MGP gene in high-marbled steers was observed probably due to the enhancement of intramuscular vascularization which provided energy for cells (Sasaki et al., 2006).

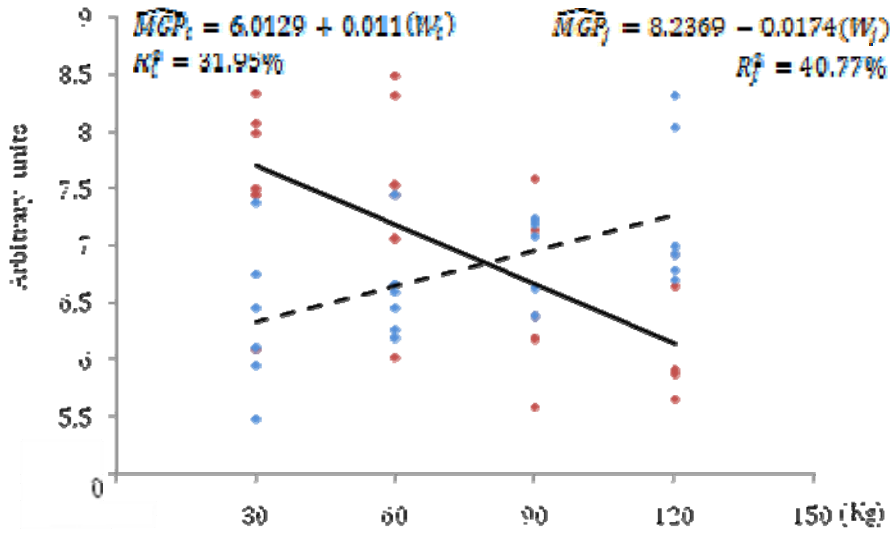

Figure 4.6.1 – Expression pattern, prediction equation and coefficient of determination of EXP_{MGP} in Crossbred (dashed line, $\widehat{MGP}_i$ and R_i^2, respectively) and Commercial (solid line, $\widehat{MGP}_j$ and R_j^2, respectively) pigs from 30 to 120Kg of slaughter weight. Blue and red dots represent, respectively, Crossbred and Commercial replicates within each slaughter weight.

Results of EXP_{MGP} and IMF content in Crossbred pigs at 120Kg are in concordance with Sasaki et al. (2006), where higher levels of MGP expression were reported in animals with more intramuscular fat content. Nevertheless, Piau pigs that showed the highest IMF content, did not exhibit variations of EXP_{MGP}, regardless of slaughter weight. Therefore, this gene might not influence IMF content in pigs as much as it has in cattle.

4.7. OBSCN

Least square means (LSM) and standard error (SE) of EXP_{OBSCN} for each genetic group within slaughter weight [G(W)] can be observed in Table 4.7.1.

Within 60 and 120Kg of slaughter weight, there was no statistical difference ($P>0.05$) across genetic groups for levels of OBSCN gene expression (Table 4.7.1). Within 30Kg, EXP_{OBSCN} was 7.392 a.u. in Commercial pigs, differing statistically ($P<0.05$) from Piau (6.077 a.u.) and Crossbred (5.693 a.u.) pigs. Piau and Commercial pigs had the higher EXP_{OBSCN} levels ($P<0.05$), with 8.194 and 7.189 a.u., respectively, at 90Kg of slaughter weight.

Table 4.7.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of normalized OBSCN gene expression (EXP_{OBSCN}), in arbitrary units (a.u.), for each genetic group within slaughter weight (W)

Slaughter weight (Kg)	Genetic group	*N*	EXP_{OBSCN}(a.u.) LSM	SE
30	Piau	6	6.077[b]	0.222
	CrB	6	5.693[b]	0.221
	Com	6	7.392[a]	0.221
60	Piau	6	7.490	0.221
	CrB	6	7.401	0.221
	Com	6	7.340	0.221
90	Piau	6	8.194[a]	0.221
	CrB	6	6.961[b]	0.221
	Com	6	7.189[ab]	0.221
120	Piau	5	7.826	0.242
	CrB	6	7.330	0.221
	Com	6	7.014	0.221

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

Obscurin protein is part of the family of giant sarcomeric signaling proteins, as titin and nebulin, and appears to mediate interactions between the sarcoplasmic reticulum and myofibrils (Price et al., 2006). These regulatory proteins may affect the amount of muscle contraction and sarcomere length, altering its proteolytic degradation susceptibility (Wick & Marriott, 1999). Therefore, these regulatory proteins might be important factors for meat tenderness, and their affects on IMF remain unclear.

Slaughter weight within a genetic group [W(G)] had significant effect (P<0.05) in Piau and Crossbred pigs (Table 4.7.2). Representation of OBSCN expression according to slaughter weight in Piau and Crossbred pigs is provided in Figure 4.7.1.

Table 4.7.2 – Significance level (p-value) of slaughter weight in each genetic group for EXP_{OBSCN}

Source of Variation	Genetic group (p-value)		
	Piau	Com	CrB
Slaughter weight	<.0001	0.6046	<.0001

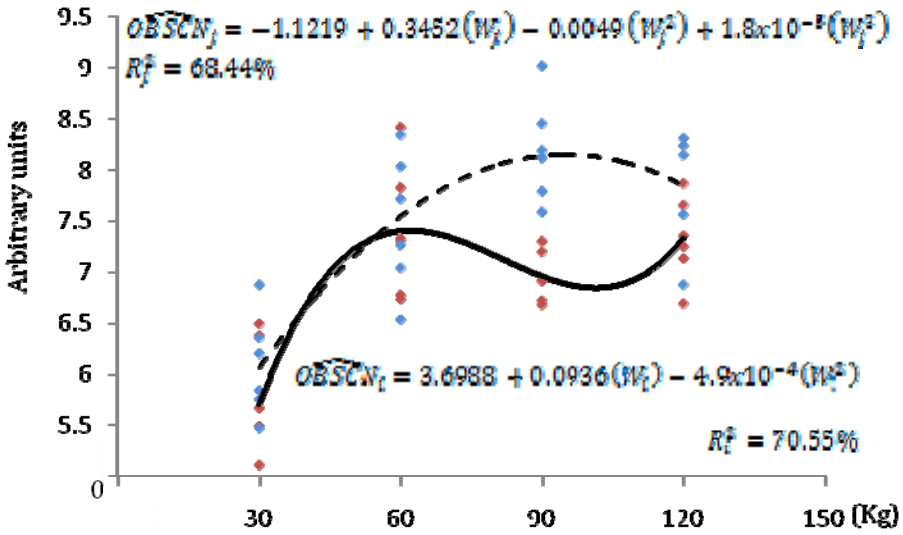

Figure 4.7.1 – Expression pattern, prediction equation and coefficient of determination of EXP_{OBSCN} in Piau (dashed line, $\widehat{OBSCN}_t$ and R_t^2, respectively) and Crossbred (solid line, $\widehat{OBSCN}_j$ and R_j^2, respectively) pigs from 30 to 120Kg of slaughter weight. Blue and red dots represent, respectively, Piau and Crossbred replicates within each slaughter weight.

Both genetic groups presented a moderate coefficient of determination (70.55% for Piau and 68.44% for Crossbred pigs) and different expression patterns. EXP_{OBSCN} in Piau pigs shows a slight decrease after a slaughter weight of 95Kg (8.16 a.u.).

Like other indigenous pigs, the Brazilian naturalized breed Piau may produce desirable pork. Thus, trials relating EXP_{OBSCN} to meat tenderness should provide insights into this gene's impact on pork quality.

4.8. PDHB

Within each slaughter weight, there was no statistical difference (P>0.05) in the EXP_{PDHB} levels within each genetic group (Table 4.8.1).

Table 4.8.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of normalized PDHB gene expression (EXP_{PDHB}), in arbitrary units (a.u.), for each genetic group within slaughter weight (W)

Slaughter weight (Kg)	Genetic group	*n*	EXP_{PDHB}(a.u.) LSM	SE
30	Piau	6	0.554	0.162
	CrB	6	0.523	0.161
	Com	6	0.203	0.161
60	Piau	6	1.556	0.161
	CrB	6	0.931	0.161
	Com	6	0.535	0.161
90	Piau	6	1.286	0.161
	CrB	6	1.199	0.161
	Com	6	1.020	0.161
120	Piau	5	1.090	0.176
	CrB	6	1.092	0.161
	Com	6	0.903	0.161

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

Genetic group affected (P<0.05) the expression levels of PDHB gene (Table 4.8.2). Least square means in Piau (1.122 a.u.) were statistically higher (P<0.05) than in Commercial (0.665 a.u.) pigs.

Table 4.8.2 – Number of animals (*n*), least square means (LSM) and standard error (SE) of normalized PDHB gene expression (EXP_{PDHB}), in arbitrary units (a.u.), for each genetic group

Genetic group	*n*	EXP_{PDHB}(a.u.) LSM	SE
Piau	23	1.122^{a}	0.082
CrB	24	0.937^{ab}	0.080
Com	24	0.665^{b}	0.080

Least square means without a common superscript letter are statistically different (P<0.05).

PDHB gene encodes the two β–units of pyruvate dehydrogenase, one of the three constituent enzymes of the mammalian mitochondrial pyruvate dehydrogenase complex. This complex transforms pyruvate into acetyl-CoA. This gene plays an important role im metabolism supplying acetyl-CoA to be used in the citric acid cycle (Koike et al., 1990; Ciszak et al., 2003).

Intramuscular fat is predominantly composed as extramyocellular and intramyocellular lipids. Piau pigs may not use all acetyl-CoA in metabolism and

store it as intramyocellular lipids, which may explain their higher level of IMF content.

Slaughter weight had significant affect (P<0.05) on EXP_{PDHB} in all three genetic groups. Figure 4.8.1 illustrates EXP_{PDHB}, in arbitrary units, according to slaughter weight.

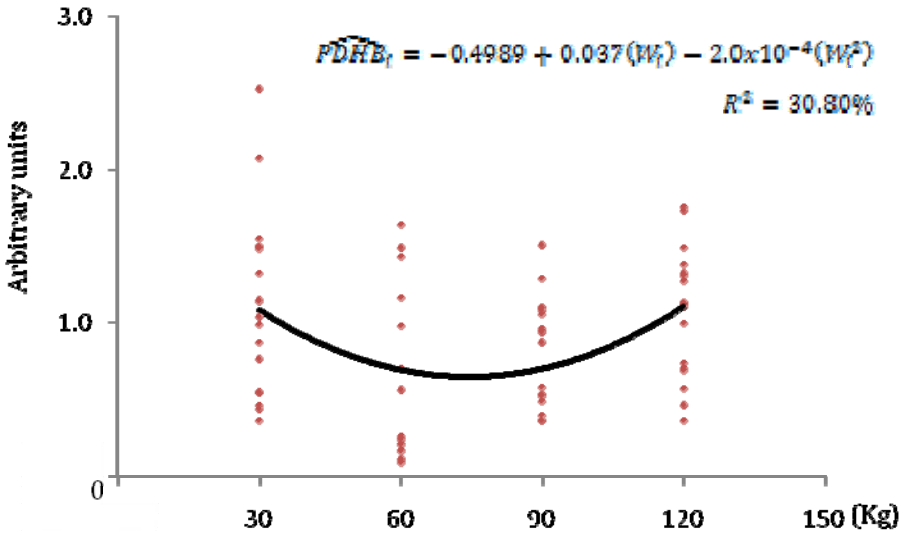

Figure 4.8.1 – Expression pattern, prediction equation ($\widehat{PDHB}_i$) and coefficient of determination (R^2) of EXP_{PDHB} in Piau, Crossbred and Commercial pigs from 30 to 120Kg of slaughter weight.

According to Figure 4.8.1, PDHB is expressed more at 30 and 120Kg of slaughter weight. Probably, the main source of energy during the earlier periods is intracellular lipids, and during the latter stages the residual acetyl-CoA is transformed triacylglycerol and stored in myocytes.

4.9. RYR1

Least square means (LSM) and standard error (SE) of EXP_{RYR1} for each genetic group within slaughter weight [G(W)] is provided in Table 4.9.1.

Genetic group had significant effect (P<0.05) on EXP_{RYR1} within 30, 90 and 120Kg of slaughter weight. Within 30Kg, there was no statistical difference (P>0.05) between the levels of RYR1 in Commercial (5.865 a.u.) and Piau (5.188 a.u.) pigs, but levels were statistically different (P<0.05) between those two breeds and Crossbred (3.940 a.u.) pigs. Piau pigs showed higher least square means of EXP_{RYR1} than Crossbreed and Commercial animals within 90 and 120Kg of slaughter weight (Table 4.9.1).

Table 4.9.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of normalized RYR1 gene expression (EXP_{RYR1}), in arbitrary units (a.u.), for each genetic group within slaughter weight (W)

Slaughter weight (Kg)	Genetic group	*n*	EXP_{RYR1}(a.u.) LSM	SE
30	Piau	6	5.188[a]	0.250
	CrB	6	3.940[b]	0.249
	Com	6	5.865[a]	0.249
60	Piau	6	6.474	0.249
	CrB	6	5.452	0.249
	Com	6	5.693	0.249
90	Piau	6	6.842[a]	0.249
	CrB	6	5.638[b]	0.249
	Com	6	5.638[b]	0.249
120	Piau	5	7.302[a]	0.272
	CrB	6	5.541[b]	0.249
	Com	6	5.613[b]	0.249

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

Ryanodine receptor 1, also known as halothane gene (RYR1) is one of the most important genes in the pork industry. Its effects on pork quality have been well documented (Davoli & Braglia, 2007). A single missense mutation (Fuji et al., 1991), where a cytosine is replaced by a thymine at nucleotide 1843 (C1843T) leads to increased cytosolic Ca^{2+} levels in muscle (Bowker at al. 2000), resulting in pale, soft and exudative meat (PSE meat).

Although the exact mechanism that halothane positive homozygote provides heavily-muscled lean pigs is not yet understood, there is a proposal for this phenomenon: RYR1 hypersensitivity could stimulate spontaneous muscle contractions leading to muscle hypertrophy and consequently improving energy utilization which would limit fat deposition (MacLennan & Phillips, 1992; Jurkat-Rott et al., 2000). Moreover, it has been reported that expression of RYR1, at any level, has less affect on concentrations of Ca^{2+} in skeletal muscle than its isoform RYR3 does (Perez et al., 2005). Thus, the regulation of Ca^{+2} releases is still unclear.

Nevertheless, in this study, Piau pigs had the highest levels of EXP_{RYR1} at 90 and 120Kg of slaughter weight (P<0.05) and its influence on high IMF contents (Table 4.1.1) becomes unclear. It was expected that higher levels of EXP_{RYR1} would be observed in low-marbled pigs. Thus, this gene might suffer post-transcriptional regulation by other genes.

Slaughter weight within genetic group [W(G)] had significant effect (P<0.05) in Piau and Crossbred pigs (Table 4.9.2). An illustration of RYR1 expression according to slaughter weight in Piau and Crossbred pigs is provided in Figure 4.9.1.

Table 4.9.2 – Significance level (p-value) of slaughter weight in each genetic group for EXP_{RYR1}

Source of Variation	Genetic group (p-value)		
	Piau	Com	CrB
Slaughter weight	0.0006	0.8956	<.0001

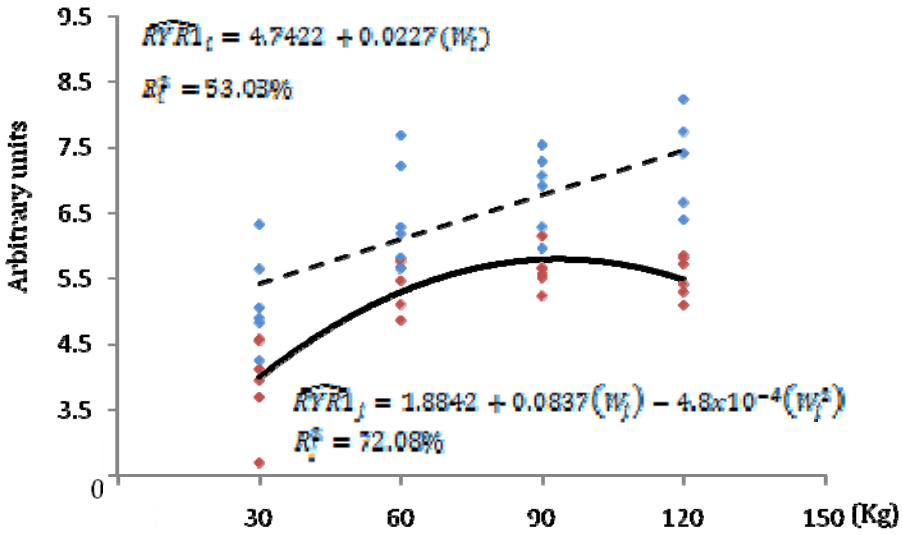

Figure 4.9.1 – Expression pattern, prediction equation and coefficient of determination of EXP_{RYR1} in Piau (dashed line, $\widehat{RYR1}_t$ and R_t^2, respectively) and Crossbred (solid line, $\widehat{RYR1}_j$ and R_j^2, respectively) pigs from 30 to 120Kg of slaughter weight. Blue and red dots represent, respectively, Piau and Crossbred pig replicate within each slaughter weight.

Both genetic groups exhibit different gene expression pattern. Crossbred pigs have low levels of EXP_{RYR1} at 30Kg of slaughter weight, reaching the higher value (5.53u.a.) at 87Kg of slaughter weight, and reducing afterwards. Piau pigs showed higher values of EXP_{RYR1} than Crossbred animals, and of expression levels increased linearly along with slaughter weight.

Measurements of resting Ca^{+2} content in cytosol of myocytes, may provide insights on how different concentrations of Ca^{+2} affect IMF content in Piau breed.

4.10. TRDN

Least square means (LSM) and standard error (SE) of EXP_{TRDN} for each genetic group within a slaughter weight [G(W)] is provided in Table 4.10.1.

Within 60Kg of slaughter weight, Piau and Commercial animals presented significant differences (P<0.05) between least square means of EXP_{TRDN} (10.116 and 8.430 a.u., respectively).

Table 4.10.1 – Number of animals (*n*), least square means (LSM) and standard error (SE) of normalized TRDN gene expression (EXP_{TRDN}), in arbitrary units (a.u.), for each genetic group within slaughter weight (W)

Slaughter weight (Kg)	Genetic group	*n*	EXP_{TRDN}(a.u.) LSM	SE
30	Piau	6	8.555	0.286
	CrB	6	8.066	0.285
	Com	6	8.405	0.285
60	Piau	6	10.116[a]	0.285
	CrB	6	8.938[ab]	0.285
	Com	6	8.430[b]	0.285
90	Piau	6	9.515	0.285
	CrB	6	9.900	0.285
	Com	6	9.144	0.285
120	Piau	5	10.499	0.313
	CrB	6	9.509	0.285
	Com	6	9.156	0.285

Within a slaughter weight, the least square means without a common superscript letter are statistically different (P<0.05).

Triadin is a major membrane protein that is specifically localized in the sarcoplasmic reticulum junction of skeletal muscle and is thought to play an important role in muscle excitation-contraction coupling (Guo & Campbell, 1995).

It is reported that decreased calcium release from the sarcoplasmic reticulum is associated with overexpression of TRDN, indicating that triadin negatively regulates RYR opening (Fodor et al., 2008). Thus, concentration of cytosolic Ca^{2+} in myocytes of Piau pigs must be lower than in Commercial animals, implying lower contraction activity and energy expenditure. Moreover, it is expected that within 60Kg of slaughter weight, Piau pigs present high levels of IMF. But in Table 4.1.1, it is shown that there was no significant difference across genetic groups within 60Kg of slaughter weight for IMF.

Slaughter weight within genetic group [W(G)] had significant effect (P<0.05) on Piau and Crossbred pigs (Table 4.10.2). An illustration of TRDN expression according to slaughter weight in Piau and Crossbred pigs is provided in Figure 4.10.1.

Table 4.10.2 – Significance level (p-value) of slaughter weight in each genetic group for EXP_{MGP}

Source of Variation	Genetic group (p-value)		
	Piau	Com	CrB
Slaughter weight	0.0022	0.1883	<.0001

Figure 4.10.1 indicates that this gene has different behavior in Piau and Crossbred pigs. The expression pattern on the TRDN gene in Piau pigs shows that after 60Kg of slaughter weight, levels of TRDN decrease until approximately 90Kg and then begins to increase again. This result at 90Kg of slaughter weight may not explain the biological pattern of the TRDN gene in Piau pigs as well as it explains the expression pattern in Crossbred animals.

Although the role of the TRDN gene in IMF deposition is unclear, knowledge of its behavior in many pig breeds may provide better understanding of Ca^{+2} release and muscle contraction.

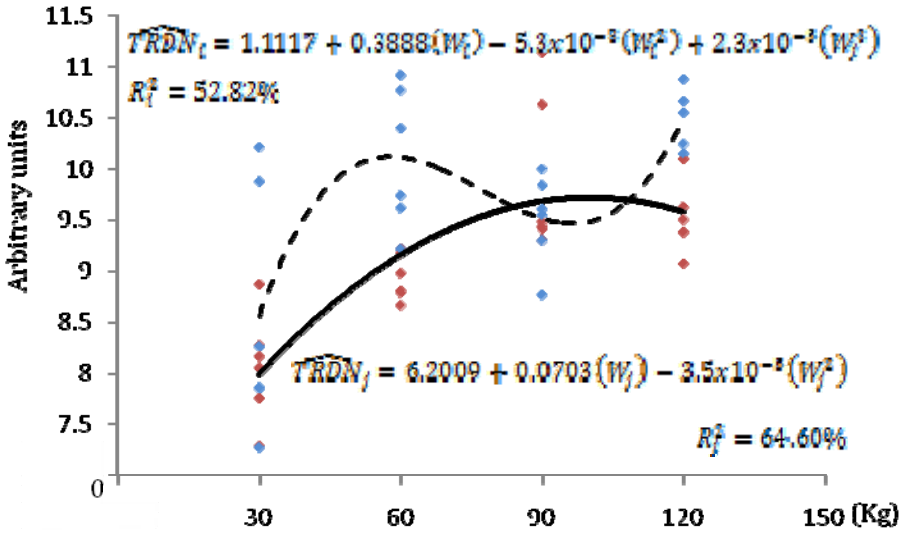

Figure 4.10.1 – Expression pattern, prediction equation and coefficient of determination of EXP_{TRDN} in Piau (dashed line, $\widehat{TRDN}_t$ and R_t^2, respectively) and Crossbred (solid line, $\widehat{TRDN}_j$ and R_j^2, respectively) pigs from 30 to 120Kg of slaughter weight. Blue and red dots represent, respectively, Piau and Crossbred pig replicate within each slaughter weight.

4.11. Correlation of IMF with expression of candidate genes

Using predicted values, levels of expression from all genes were correlated to IMF content in muscle *longissimus dorsi*, using all animals from the three different lines. Table 4.11.1 presents the correlation coefficient values between the levels of expression of each gene with the IMF content using the whole data. Using

the data from Piau animals only, these values are presented in Table 4.11.2. Correlation between all studied genes, using whole data, may be observed in Table 4.11.3 and for Piau pigs only at Table 4.11.4. Positive and negative partial correlation between the IMF content and the levels of candidate gene expression are presented in Table 4.11.5 and Table 4.11.6, respectively. Data from the whole population was used in this analysis.

Table 4.11.1 – Simple correlation values (%), and its probability (in parentheses), of candidate genes with IMF content*

	FABP3	LDLR	TRDN	EEF1A2	OBSCN	RYR1	PDHB	MGP	ATN1
IMF	0.6830 (<.0001)	0.6398 (<.0001)	0.4436 (<.0001)	-0.3964 (0.0006)	0.2724 (0.0206)	0.2648 (0.0246)	0.2334 (0.0485)	-0.1716 (0.1494)	-0.0962 (0.4213)

*, using adjusted values.

Table 4.11.2 – Simple correlation values (%), and its probability (in parentheses), of candidate genes with IMF content for Piau pigs only*

	LDLR	FABP3	MGP	RYR1	TRDN	OBSCN	EEF1A2	ATN1	PDHB
IMF	0.9094 (<.0001)	0.9012 (<.0001)	-0.8628 (<.0001)	0.7683 (<.0001)	0.7401 (<.0001)	0.5592 (0.0045)	0.2760 (0.1918)	0.1204 (0.5752)	0.1117 (0.6035)

*, using adjusted values.

Table 4.11.3. Correlations between expression pattern of all genes and its probability (in parentheses)*

	ATN1	EEF1A2	FABP3	LDLR	MGP	OBSCN	PDHB	RYR1	TRDN
ATN1	1								
EEF1A2	-0.0886 (0.4591)	1							
FABP3	0.3489 (0.0027)	-0.1966 (0.0979)	1						
LDLR	0.1353 (0.2572)	0.2539 (0.0314)	0.7170 (<.0001)	1					
MGP	-0.0965 (0.4200)	0.2284 (0.0537)	0.1553 (0.1929)	0.0625 (0.6022)	1				
OBSCN	0.2060 (0.0825)	0.4539 (<.0001)	0.4375 (0.0001)	0.6741 (<.0001)	0.0641 (0.5926)	1			
PDHB	0.5881 (<.0001)	-0.1371 (0.2509)	0.4563 (<.0001)	0.3389 (0.0036)	-0.3786 (0.0010)	0.4851 (<.0001)	1		
RYR1	0.0668 (0.5774)	0.5835 (<.0001)	0.3898 (0.0007)	0.6744 (<.0001)	-0.1179 (0.3240)	0.8770 (<.0001)	0.5327 (<.0001)	1	
TRDN	0.3588 (0.0020)	0.0522 (0.6632)	0.6284 (<.0001)	0.5071 (<.0001)	-0.3188 (0.0063)	0.5972 (<.0001)	0.8350 (<.0001)	0.7576 (<.0001)	1

*, using adjusted values.

Table 4.11.4. Correlations between expression pattern of all genes and its probability (in parentheses)* in Piau pigs only.

	ATN1	EEF1A2	FABP3	LDLR	MGP	OBSCN	PDHB	RYR1	TRDN
ATN1	1								
EEF1A2	0.4591 (0.0240)	1							
FABP3	0.3379 (0.1063)	0.1904 (0.3727)	1						
LDLR	0.4509 (0.0207)	0.5991 (0.0020)	0.8618 (<.0001)	1					
MGP	-0.0926 (0.6668)	-0.3679 (0.0770)	-0.5935 (0.0022)	-0.8088 (<.0001)	1				
OBSCN	0.8311 (<.0001)	0.4093 (0.0470)	0.6036 (0.0018)	0.7476 (<.0001)	-0.6025 (0.0018)	1			
PDHB	0.8663 (<.0001)	-0.0300 (0.8892)	0.3958 (<.0556)	0.2808 (0.1838)	-0.0007 (0.9975)	0.7621 (<.0001)	1		
RYR1	0.7233 (<.0001)	0.4209 (0.0406)	0.8464 (<.0001)	0.8996 (<.0001)	-0.6538 (0.0005)	0.9310 (<.0001)	0.6684 (0.0004)	1	
TRDN	0.5437 (0.0061)	0.0193 (0.9287)	0.9223 (<.0001)	0.7308 (<.0001)	-0.4604 (0.0236)	0.7407 (<.0001)	0.7004 (<.0001)	0.8878 (<.0001)	1

*, using adjusted values.

Table 4.11.5 – Positive partial correlation values (%), and its probability (in parentheses), of candidate genes with IMF content*

	LDLR	TRDN	OBSCN
IMF	0.7030 (<.0001)	0.4487 (0.0002)	0.2213 (0.0788)

*, using predicted values.

Table 4.11.6 – Negative partial correlation values (%), and its probability (in parentheses), of candidate genes with IMF content*

	PDHB	EEF1A2	FABP3	ATN1	RYR1	MGP
IMF	-0.5359 (<.0001)	-0.3228 (0.0093)	-0.2494 (0.0468)	-0.2245 (0.0745)	-0.1663 (0.1899)	-0.1430 (0.2596)

*, using predicted values.

The ATN1 and MGP genes did not show significant (P>0.05) simple or partial correlations (Table 4.11.1 and Table 4.11.6, respectively) between their expression and IMF content, when analyzed using all data. In Piau pigs, no significant correlation (P>0.05) between ATN1 and IMF was found (P>0.05), but MGP was –0.8628 correlated (P<0.05) with IMF in Piau pigs (Table 4.11.2). In this study, the pattern of expression of ATN1 indicates that the atrophin 1 proteins may act on biochemistry pathways related to fat deposition in early stages of development preventing adipose hypertrophy, due to its role as a transcriptional co-repressor (Zhang et al., 2002). Thus, its role in adipose deposition could not be assigned. Regarding MGP, its calcification inhibitor activity (Luo et al., 1997) does not seem to affect lipid deposition in the *longissimus dorsi* muscle, when analyzed using the data from all animals. Moreover, in Piau pigs, the high negative correlation between the expression of the MGP gene and IMF levels does not provide insights into how this gene may affect the lipid content in this muscle.

Using the whole data set, the only gene that showed negative and significant (P<0.05) correlation with the IMF content was EEF1A2 (–0.3964; Table 4.11.1). The similar value of the partial correlation coefficient (–0.3228; Table 4.11.6) and the fact that this gene is not highly correlated with other studied genes in this trial Table 4.11.3) may indicate that EEF1A2 does not undergo interference from other RNA transcripts. In Piau pigs, no significant correlation (P>0.05) between EEF1A2 and the IMF content was found. This gene is related to mRNA translation; its proteins bind aminoacylated tRNA and facilitates its recruitment by the ribosome

during the translation elongation (Kulkarni et al., 2007). Although elevated expressions of this gene result in more protein synthesis, it does not imply a higher muscle deposition. Therefore, this gene may translate proteins negatively related to IMF deposition in the whole population.

From analysis of the whole data set (Table 4.11.1), the relationship of all positively correlated genes with IMF content was statistically significant ($P<0.05$). Of these, three showed similar values: 0.2724 (OBSCN), 0.2648 (RYR1) and 0.2334 (PDHB). Using partial correlation analysis (Tables 4.11.5 and 4.11.6), these values were: –0.5359 for PDHB ($P<0.05$), 0.2231 for OBSCN ($P>0.05$) and 0.1663 for RYR1 ($P>0.05$). Moreover, in Piau pigs no correlation was observed between PDHB and IMF content ($P>0.05$; Table 4.11.2). Therefore, these results suggest that this gene has a confused behavior. Comparing the simple with the partial correlation coefficient, from the analysis of the whole data, the modification of signal and magnitude (from 0.2334 to –0.5359) indicates that higher levels of expression of PDHB would actually decrease the IMF content. Nevertheless, this gene may be undergoing genetic interactions that imply a positive simple correlation coefficient in the whole population and the lack of correlation in Piau pigs.

Like the PDHB gene, different correlation coefficients values between the IMF content and the expression levels of RYR1 are observed. Although there was significant ($P<0.05$) simple correlation between these two traits analyzing both the data sets, the partial correlation analysis indicates that this gene was not statistically correlated ($P<0.05$) with the deposition of IMF (Table 4.11.6). Moreover, the expression level of this gene is highly correlated with TRDN. The correlation coefficient ($P<0.05$) between RYR1 and TRDN was 0.7576 (Table 4.11.3) for the whole population and 0.8878 (Table 4.11.4) for Piau pigs only. The TRDN gene showed correlation of 0.4436 with IMF, using data from all animals, and of 0.7401 using Piau data only. It has been reported that triadin proteins interact with RYR1 (Zhang et al., 1997; Sasaki et al., 2006). Decreased calcium release from the sarcoplasmic reticulum is associated with an overexpression of TRDN, indicating that triadin negatively regulates RYR opening (Fodor et al., 2008). However, in the present study, these two genes were positively correlated ($P<0.05$). Calcium is a key player in muscle contraction (Bers, 2001), but the function of the RYR1 and TRDN genes in the release of calcium could not provide conclusive insights into lipid deposition.

The expression levels of the OBSCN gene were only significantly correlated ($P<0.05$) to the IMF content in the Piau population (Table 4.11.2). Obscurin is appropriately positioned to participate in the assembly and integration of the Z-disk and M-band with elements of the sarcoplasmic reticulum (Kontrogianni-Konstantopoulos et al., 2003), and may change the sarcomeric structure, somehow facilitating deposition of lipid.

The other two correlated genes have important roles in lipid transportation inside the cell (FABP3) and tissues (LDLR). Their simple correlation coefficient ($P<0.05$) with IMF was, respectively, 0.6830 and 0.6398 using the whole data set (Table 4.11.1), and 0.9012 and 0.9094 using data from Piau animals (Table 4.11.2). Moreover, they were 0.7170 correlated ($P<0.05$) to each other, using data from the whole population (Table 4.11.3), and 0.8618 correlated ($P<0.05$) analyzing only Piau animals (Table 4.11.4). In addition, their partial correlation coefficient ($P<0.05$) with IMF was 0.7030 for LDLR (Table 4.11.5) and –0.2494 for FABP3 (Table 4.11.6). LDL receptors bind to LDL particles in the bloodstream and initiate endocytosis in the cell, converting LDL into cholesterol, amino acids and fatty acids. These fatty acids are transported by fatty-acid binding proteins in the cell that are used in metabolism and lipid storage (Nelson & Cox, 2004). Thus, due to the high correlation values between the IMF content and the LDLR gene among all analysis, this gene may promote lipid deposition.

Significant correlation between expression of these genes and IMF content does not necessarily explain biological function. Some genes, such as TRDN and RYR1, were highly correlated with IMF content, but their biological roles could not be related to fat deposition. Other genes had different relationships with IMF content according to the data set used. Although in Piau pigs the EEF1A2 gene and IMF content are not correlated, using the whole data set, a negative correlation between these two traits is observed. Moreover, MGP was negatively correlated with IMF content in Piau pigs, but using data from all the animals no correlation was observed. Furthermore, the FABP3 and LDLR genes are known for their roles in the transportation of fatty acids and lipids, respectively, and they showed high positive values of correlation with IMF content, but FABP3 showed a different behavior using a partial correlation analysis.

4.12. Prediction equation of IMF

Irrespective of significant correlation, a prediction equation of intramuscular fat deposition (IMF), for all genetic groups, sex and slaughter weight, was modeled using adjusted values of all genes:

$$\widehat{IMF} = 1.702 - 0.289(ATN1) - 0.57(EEF1A2) + 0.981(LDLR) - 0.114(MGP) + 0.098(OBSCN) - 0.794$$

$$R^2 = 90.29\%$$

From the nine studied genes, two of them (FABP3 and RYR1) were not significant (P>0.15) using the STEPWISE statement from REG procedure, but had significant (P<0.05) correlation with IMF content (Table 4.11.1). According to the prediction equation above, ATN1, EEF1A2, MGP and PDHB genes reduce IMF levels, while LDLR, OBSCN and TRDN increase it.

ATN1 and MGP genes were not significantly correlated (P>0.05) with IMF content (Table 4.11.2), but they were included in this equation (P<0.15). Although the expression of EEF1A2, in this equation, reduces IMF content, this gene was positively correlated (P<0.05) with IMF (Table 4.11.1).

Modification of signal and magnitude can be explained by the complexity of genetic interactions. Many of these genes may act on diverse metabolic pathways related directly or indirectly to fat deposition. Over-expression of ATN1, EEF1A2, PDHB and genes may reduce IMF content by repressing genes related to fat deposition and synthesizing more protein, resulting in higher energy expenditures and development of the vascular system. In contrast, LDLR, OBSCN and TRDN may allow increasing IMF levels by recruiting lipids from the blood stream into the cell and modifying the sarcomere structure, resulting in lower energy expenditure.

5. Implications

Piau pigs showed elevated IMF content in *longissimus dorsi* muscle, with 2.48% at 120Kg of slaughter weight. According to some authors, this value provides desirable characteristics in pork, like flavor, but sensory trials are required to ensure its quality.

Sex only had significant effect on IMF content. Expression levels of the studied genes demonstrated that this source of variation is not important in their control.

Some genes, like LDLR, MGP, OBSCN, RYR1 and TRDN, were affected by the interaction between genetic group and slaughter weight, indicating that these genes are triggered in different developmental stages and are influenced by the genetic background. For genes EEF1A2 and PDHB, the genetic diversity among groups had an effect on their expression levels, and these genes probably have different effects on IMF deposition due to correlation values. ATN1, FABP3 and PDHB may not be under the control of other genes due to their expression pattern not being affected by their genetic group.

Different correlation values for each gene from both data sets indicate that these genes have different behaviors, depending on the genetic background of genetic group. Moreover, the prediction equation for IMF content indicates that ATN1, EEF1A2, LDLR, MGP, OBSCN, PDHB and TRDN genes cooperate on the determination of IMF deposition.

The highly positive correlation values of the LDLR gene with IMF content, in both data sets, and its elevated coefficient of regression in the prediction equation of IMF, indicate that this gene is important for fat deposition in the porcine muscle *longissimus dorsi*, recruiting lipids from the blood stream into the cell. Moreover, it was possible to determine its expression pattern in Piau pigs, animals with higher IMF content, where increased levels of LDLR were accompanied with slaughter weight. Thus, more studies are required to assign this gene as a candidate gene for intramuscular fat deposition in the porcine muscle *longissimus dorsi*.

6. References

American Meat Science Association. (2001). *Meat Evaluation Handbook.* Savoy, IL: Author.

Anand, N., Murthy, S., Amann, G., Wernick, M., Porter, L. A., Cukier, I. H., et al. (2002). Gene encoding protein elongation factor EEF1A2 is a putative oncogene in ovarian cancer. *Nature Genetics 31*, 301 – 305.

AOAC. 1990. *Official Methods of Analysis* (15th Ed.). Association of Official Analytical Chemists. Arlington, VA.

Band, G. O., Guimarães, S. E. F., Lopes, P. S., Peixoto, J. O., Faria, D. A., Pires, A. V., et al. (2005). Relationship between the porcine stress syndrome gene and carcass and performance traits in F2 pigs resulting from divergent crosses. *Genetics and Molecular Biology*, *28*(1), 92-96.

Band, G. O., Guimarães, S. E. F., Lopes, P. S., Schierholt, A. S., Silva, K. M., Pires, A. V., et al. (2005). Relationship between the porcine stress syndrome gene and pork quality traits of F2 pigs resulting from divergent crosses. Genetics and Molecular Biology, *28*(1), 88-91.

Benkusky, N. A., Farrell, E. F., Valdivia, H. H. (2004). Ryanodine receptor channelopathies. *Biochemical and Biophysical Research Communications*, *322*, 1280–1285.

Berg, E. P. (2002). Fat = Flavor – Intramuscular Fat Baseline and Prediction. Third Annual Pork Quality Improvement Symposium. *Proceedings of the 3rd Annual Pork Quality Improvement Symposium*, Michigan State University, Michigan.

Bers, D. M. (2001). *Excitation–contraction coupling and cardiac contractile force* (2nd ed.), Dordrecht, Netherlands: Kluwer Academic.

Bidanel, J. P., Milan, D., Woloszyn, N., Bourgeois, F., Caritez, J. C., Gruand, J., et al. (1998). Mapping of QTLs in F2 crosses between Meishan and Large White pig breeds. *Proceedings of the XXVIth International Conference on Animal Genetics.* International Society for Animal Genetics, Auckland, pp. 105.

Borisov, A. B., Sutter, S. B., Kontrogianni-Konstantopoulos, A., Bloch, R.J., Westfall, M. V., Russell, M. W. (2006). Essential role of obscurin in cardiac myofibrillogenesis and hypertrophic response: evidence from small interfering RNA-mediated gene silencing. *Histochem Cell Biol*., 125:227–238.

Bowker, B. B., Grant, A. L., Forrest, J. C., Gerrard, D. E. (2000). Muscle metabolism and PSE pork. *Journal of Animal Science, 79*, 1-8.

Bowman, A. L., Kontrogianni-Konstantopoulos, A., Hirsch, H., et al. (2007). Different obscurin isoforms localize to distinct sites at sarcomeres. *FEBS Letters, 581*, 1549-1554.

Brewer, M. S., Jensen, J., Sosnicki, A. A., Fields, B., Wilson, E., McKeith, F. K. (2002). The effect of pig genetics on palatability, color and physical characteristics of fresh pork loin chops. *Meat Science, 61*, 249-256.

Brewer, M. S., Zhu, L. G., McKeith, F. K. (2001). Marbling effects on quality characteristics of pork loin chops: consumer purchase intent, visual and sensory characteristics. *Meat Science, 59*, 153–163.

Bustin, S. A. (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of Molecular Endocrinology, 25*, 169-193.

Bustin, S. A., & Nolan, T. (2004). Pitfalls of quantitative real-time reverse transcription polymerase chain reaction. *Journal of Biomolecular Techniques, 15*, 155-166.

Carmo, F. M. S., Guimarães, S. E. F., Lopes, P. S., Pires, A. V., Guimarães, M. F. M., Gomide, L. A. M. (2005). Association of Myf5 gene allelic variants with production traits in pigs. *Genetics and Molecular Biology, 28*(3), 363-369.

Castro, S. T. R., Albuquerque, M. S. M., Germano, J. L. Conservation of local pig breeds in Brasil. (2000). In: *Fifty Global Conference on Conservation of Domestic Animal Genetic Resources*. Fifty Global Conference on Conservation of Domestic Animal Genetic Resources. Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil.

Channon, H. A., Kerr, M. G., Walker, P. J. (2004). Effect of Duroc content, sex and ageing period on meat and eating quality attributes of pork loin. *Meat Science, 66*, 881–888.

Chmurzyńska, A. (2006). The multigene family of fatty acid-binding proteins (FABPs): Function, structure and polymorphism. *J Appl Genet, 47*, 39-48.

Chu, M., Wu, C., Zhang, J. (2003). The studies and application of high reproduction ability of Taihu pigs. *The Press of Chinese Agricultural Science and Technology*, 1-196.

Ciszak, E. M., Korotchkina, L. G., Dominiak, P. M., Sidhu, S., Patel, M. S. (2003). Structural basis for flip-flop action of thiamin pyrophosphate-dependent enzymes

revealed by human pyruvate dehydrogenase. *The Journal of Biological Chemistry, 278* (23), 21240–21246.

Correa, J. A., Faucitano, L., Laforest, J. P., Rivest, J., Marcoux, M., Gariépy, C. (2006). Effects of slaughter weight on carcass composition and meat quality in pigs of two different growth rates. *Meat Sci., 72*, 91–99.

Daszkiewicz, T., Bak, T., Denaburski, J. (2005). Quality of pork with a different intramuscular fat (imf) content. *Polish Journal of Food and Nutrition Sciences*, *14/55*(1), 31–36.

Davoli, R., & Braglia, S. (2007). Molecular approaches in pig breeding to improve meat quality. *Briefings in Functional Genomics and Proteomics 6*, 313-21.

de Koning, D. J., Janss, L. L. G., Rattink, A. P., van Oers, P. A. M., de Vries, B. J., Groenen, M. A. M. et al. (1999). Detection of Quantitative Trait Loci for Backfat Thickness and Intramuscular Fat Content in Pigs (*Sus scrofa*). *Genetics 152*, 1679–1690.

D'Souza, D. N., & Mullan, B. P. (2002). The effect of genotype, sex and management strategy on the eating quality of pork. *Meat Science, 60*, 95–101.

Erkens, T., Van Poucke, M., Vandesompele, J., Goossens, K., Van Zeveren, A. Peelman, L.J. (2006) Development of a new set of reference genes for normalization of real-time RT-PCR data of porcine backfat and *Longissimus dorsi* muscle, and evaluation with PPARGC1A. *BMC Biotechnology*, *6*.

Essen-Gustavsson, B., Karlsson, A., Lundstrom, K., Enfalt, A. C. (1994). Intramuscular fat and muscle fiber lipid contents in halothane gene free pigs fed high and low protein diets and its relation to meat quality. *Meat Science 38*, 269–277.

Estévez, M., Morcuende, D., López, R.C. (2003). Physico-chemical characteristics of M. Longissimus dorsi from three lines of free-range reared Iberian pigs slaughtered at 90 kg live-weight and commercial pigs: a comparative study. *Meat Science, 64*, 499–506.

Euclides Filho, K. (1999). *Melhoramento genético animal no Brasil. Fundamentos, história e importância* (1st ed.). Campo Grande: Embrapa, v. 500. 63 p.

Fan, B., Tang, Z. L., Xu, S. P., Liu, B., Peng, Z. Z., Li, K. (2006). Germplasm characteristics and conservation of Tongcheng pig: A case study for preservation and utilization of Chinese indigenous pig breeds. *AGRI, 39*, 51-63.

Food and Agriculture Organization. (1999). *Executive brief: The global strategy for the management of farm animal genetic resources*, p.49.

Faria D. A., Guimarães, S. E. F., Lopes, P. S., Pires, A. V., Paiva, S. R., Sollero, B. P. et al. (2006). Association between G316A growth hormone polymorphism and economic traits in pigs. *Genetics and Molecular Biology, 29*, 634-640.

Faria D. A., Peixoto, J. O., Lopes, P. S., Paiva, S. R., Silva, P. V., Guimarães, S. E. F. (2009). Association between insulin-like growth factor I microsatellite polymorphisms and important economic traits in pigs. *Revista Brasileira de Zootecnia / Brazilian Journal of Animal Science, 38*, 265-270.

Figueiredo, F. C., Lopes, P. S., Pinto, A.P.G., Faria, D. A., Mendonça, P. T., Guimarães, S. E. F. (2008). Association of the heart fatty acid-binding protein gene with quality of carcass and meat traits in pigs. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 60*, 408-413.

Fisher, P., Mellett, F. D., Hoffman, L. C. (2000). Halothane genotype and pork quality. 1. Carcass and meat quality characteristics of three halothane genotypes. *Meat Science, 54*, 97-105.

Fodor, J., Gönczi, M., Sztretye, M., Dienes, B., Oláh, T., Szabó, L., et al. (2008). Altered expression of triadin 95 causes parallel changes in localized Ca^{2+} release events and global Ca^{2+} signals in skeletal muscle cells in culture. *Journal of Physiology, 586*(23), 5803-5818.

Fortin, A., Robertson, W. M., Tong, A. K. W. (2005). The eating quality of Canadian pork and its relationship with intramuscular fat. *Meat Science 69*, 297–305.

Fraser, J. D., & Price, P. A. (1988). Lung, heart and kidney express high levels of mRNA for the vitamin K-dependent matrix Gla protein. *J Biol Chem, 263*, 11033–11036.

Freeman, W. M., Walker, S. J., Vrana, K. E. (1999). Quantitative RT-PCR: pitfalls and potential. *Biotechniques, 26*, 112-125.

Fujii, J., Otsu, K., Zorzato, F., de Leon, S., Khanna, V. K., Weiler, J. E., et al. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science, 253*, 448–451.

Gao, Y., Zhang, R., Hu, X., Li, N. (2007). Application of genomic technologies to the improvement of meat quality of farm animals. *Meat Science 77*, 36–45.

Gerbens, F., Rettenberger, G., Lenstra, J. A., Veerkamp, J. H., te Pas, M. F. (1997). Characterization, chromosomal localization, and genetic variation of the porcine heart fatty acid-binding protein gene. *Mammalian Genome, 8*(5), 328-32.

Gerbens, F., van Erp, A. J. M., Harders, F. L., Verburg, F. J., Meuwissen, T. H. E., Veerkamp, J. H., et al. (1999). Effect of genetic variants of the heart fatty acid-binding protein gene on intramuscular fat and performance traits in pigs. *Journal of Animal Science, 77*, 846–852.

Gerbens, F., de Koning, D.J., Harders, F.L., Meuwissen, T.H.E., Janss, L.L., Groenen, M.A., et al. (2000). The effect of adipocyte and heart fatty acid-binding protein genes on intramuscular fat and backfat content in Meishan crossbred pigs. *Journal of Animal Sciences, 78*, 552–559.

Gerbens, F., Verburg, F.J., Van Moerkerk, H.T.B., Engel, B., Buist, W., Veerkamp, J.H., et al. (2001). Associations of heart and adipocyte fatty acid-binding protein gene expression with intramuscular fat content in pigs. *Journal of Animal Science, 79*, 347–354.

Gerbens, F. (2004). Genetic control of intramuscular fat accretion. In: Muscle Development of Livestock Animals: Physiology, Genetics and Meat Quality. M. F. W. te Pas, M. E. Everts, and H. P. Haagsman, ed. CABI Publ., Cambridge, MA.

Glatz, J. F. C., & van der Vusse, G.J. (1996). Cellular fatty acid-binding proteins: their function and physiological significance. *Prog. Lipid Res. 35*, 43±282.

Gómez Fernández, M. (2003). The conservation programme for Basque pig breeds. *Arch. Zootec., 52*, 231-235.

Goonasekera, S. A., Beard, N. A., Groom, L., Kimura, T., Lyfenko, A. D., Rosenfeld, A., et al. (2007). Triadin Binding to the C-Terminal Luminal Loop of the Ryanodine Receptor is Important for Skeletal Muscle Excitation–Contraction Coupling. *Journal of General Physiology, 130*, 365-378.

Grindflek E., Szyda J., Liu Z., Lien S. (2001). Detection of quantitative trait loci for meat quality in a commercial slaughter pig cross. *Mammalian Genome, 12*(4), 299-304.

Grześkowiak, E., Borzuta, K., Strzelecki, J., Lisiak, D. (2006). Results of assessment of meat quality in fat-meat type pigs currently fattened on small farms. *Animal Science Papers and Reports, 24*(1), 113-118.

Guimarães, S. E. F., Lopes, P. S. (2000) Use of native genetic resources as a tool for the genomic mapping in swine. In: *Rare Breeds International Symposium*, Brasília, DF, Brazil.

Guo, W., & Campbell, K.P. (1995). Association of Triadin with the Ryanodine Receptor and Calsequestrin in the Lumen of Sarcoplasmic Reticulum. *Journal of Biological Chemistry, 270*(16), 9027-9030.

Hamilton, D. N., Ellis, M., Miller, K. D., McKeith, F. K., Parrett, D. F. (2000). The effect of the Halothane and Rendement Napole genes on carcass and meat quality characteristics of pigs. *Journal of Animal Science, 78*, 2862-2867.

Harbitz, I., Chowdhary, B., Thomsen, P. D., Davies, W., Kaufmann, U., Kran, S., et al. (1990). Assignment of the porcine calcium release channel gene, a candidate for the malignant hyperthermia locus, to the 6p11 → q21 segment of chromosome 6. *Genomics, 8*(2), 243-248.

Harcet, M., Đikić, M., Gamulin, V. (2006). Low genetic diversity of the Turopolje pig breed. *Food Technol. Biotechnol. 44*, 105-109.

Harlizius, B., Rattink, A. P., de Koning, D. J., Faivre, M., Joosten, R. G., van Arendonk, J. A. M., et al. (2000). The X chromosome harbors quantitative trait loci for backfat thickness and intramuscular fat content in pigs. *Mammalian Genome 11*, 800–802.

Hasler-Rapacz, J., Ellegren, H., Fridolfsson, A. K., Kirkpatrick, B., Kirk, S., Andersson, L., et al. (1998). Identification of a Mutation in the Low Density Lipoprotein Receptor Gene Associated With Recessive Familial Hypercholesterolemia in Swine. *American Journal of Medical Genetics 76*, 379-386.

Hertzel, A. V., & Bernlohr, D. A. (2000). The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. *Trends Endocrinol. Metab. 11*, 175–180.

Horst, A. (1991). *Molecular Pathology*. CRC Press.

Hullberg, A. (2004) *Quality of Processed Pork – Influence of RN genotype and processing conditions*. D.S. Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Janss, L. L. G., van Arendonk, J. A. M., Brascamp, E. W. (1997). Bayesian statistical analyses for presence of single major genes affecting meat quality traits in crossed pig population. *Genetics, 145*, 395–408.

Jeganathan, J., & Lee, J.M. (2007). Binding of elongation factor eEF1 A2 to phosphatidylinositol 4-kinase β stimulates lipid kinase activity and phosphatidylinositol 4-phosphate generation. *The Journal of biological chemistry, 282*(1), 372-380.

Jeganathan, S., Morrow, A., Amiri, A., Lee, J. M. (2008). Elongation factor eEF1A2 cooperates with phosphatidylinositol-4 kinaseIII β to stimulate filopodia production through increased PI(4,5)P_2 generation. *Molecular and Cell Biology,* doi:10.1128/MCB.00150-08.

Jeleníková, J., Pipek, P., Miyahara, M. (2008). The effects of breed, sex, intramuscular fat and ultimate pH on pork tenderness. *Eur Food Res Technol, 227*, 989–994.

Jeremiah, L. E. (1998). Marbling and pork tenderness. *Pork Fact Sheet*. Des Moines, IA: National Pork Producers Council and American Meat Science Association.

Jurkat-Rott, K., McCarthy, T., Lehmann-Horn, F. (2000). Genetics and pathogenesis of malignant hyperthermia. *Muscle Nerve 23*, 4–17.

Kim, J. H., Óvilo, C., Park, E. W., Fernndez, A. F., Lee, J. H., Jeon, J. T., et al. (2008). Minimizing a QTL region for intramuscular fat content by characterizing the porcine Phosphodiesterase 4B (*PDE4B*) gene. *BMB Reports, 41*(6), 466-71.

Klont, R. E., Brocks, L., Eikelenboom, G. (1998). Muscle Fibre Type and Meat Quality. *Meat Science, 49*(1), S219-S229.

Koike, K., Urata, Y., Koike, M. (1990). Molecular cloning and characterization of human pyruvate dehydrogenase β subunit gene. *Proceedings of the National Academy of Sciences of the United States of America, 87*(15), 5594-5597.

Kontrogianni-Konstantopoulos, A., Jones, E. M., Van Rossum, D. B., Bloch, R. J. (2003). Obscurin is a ligand for small ankyrin 1 in skeletal muscle. *Mol. Biol. Cell. 14*, 1138–1148.

Kulkarni, G., Turbin, D. A., Amiri, A., Jeganathan, S., Andrade-Navarro, M. A., Wu, T. D., et al. (2007). Expression of protein elongation factor eEF1A2 predicts favorable outcome in breast cancer. *Breast Cancer Res Treat 102*, 31–41.

Malek, M., Dekkers, J. C. M., Lee, H. K., Baas, T. J., Prusa, K., Huff-Lonergan, E., et al. (2001). A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. *Mammalian Genome, 12*, 673–645.

Laize, V., Martel, P., Viegas, C. S., Price, P. A., Cancela, M. L. (2005). Evolution of matrix and bone gamma-carboxyglutamic acid proteins in vertebrates. *J. Biol. Chem., 280*(29), 26659-26668.

Lawrie, R. A. (1998). *Lawrie's Meat Science*, 6th Ed., pp. 220–221, Woodhead Publishing Limited, Cambridge, U.K.

Levy, S., & Hanna, M. (1994). *Consumer quality audit summary.* Des Moines, IA: National Pork Producers Council.

Li, S.-H., McInnis, M. G., Margolis, R. L., Antonarakis, S. E., Ross, C. A. (1993). Novel triplet repeat containing genes in human brain: Cloning, expression, and length polymorphisms. *Genomics, 16*, 572–579.

Li, Y., Lu, W., Marzolo, M. P., Bu, G. (2001). Differential Functions of Members of the Low Density Lipoprotein Receptor Family Suggested by Their Distinct Endocytosis Rates. *The Journal of Biological Chemistry, 276*(21), 18000-18006.

Livak, K. J., & Schmittigen T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2DDCt method. *Methods*, *25*, 402-408.

Lonergan, S. M., Stalder, K. J., Huff-Lonergan, E., Knight, T. J., Goodwin, R. N., Prusa, K. J., et al. (2007). Influence of lipid content on pork sensory quality within pH classification. *Journal of Animal Science, 85*, 1074-1079.

Lopes, P. S., Guimarães, S. E. F., Pires, A. V., Soares, M. A. M., Carmo, F. M. S., Martins, M. F., et al. (2002). Results of performance, carcass yield and meat quality traits of F2 crosses between Brazilian native and commercial pigs for QTL mapping. In: *Proceedings of the World Congress in Genetics and Animal Livestock Production*. Montpellier, France, 30: 155.

Lu, P., Li, D., Yin, J., Zhang, L., Wang, Z. (2008). Flavour differences of cooked Longissimus muscle from Chinese indigenous pig breeds and hybrid pig breed (Duroc x Landrace x Large White). *Food Chemistry, 107*, 1529–1537.

Luo, G., D'Souza, R., Hoque, D., Karsenty, G. (1995). The matrix Gla protein gene is a marker of the chondrogenesis cell lineage during mouse development. *J. Bone and Mineral Research, 10*(2), 325-334.

Luo, G., Ducy, P., McKee, M. D., Pinero, G. J., Loyer, E., Behringer, R. R., et al. (1997). Spontaneous calcification of arteries and cartilage in mice lacking MGP. *Nature 386*, 78–81.

MacLennan, D. H., & Philipps, M. S. (1992). Malignant hyperthermia. *Science, 256*, 789–794.

Mariante, A. S., McManus, C., Mendonça, J. F. (2003a). Country report on the state of animal genetic resources. Brasília. Brasília: Embrapa/Genetic Resources and Biotechnology, *Documentos*, *99*, 121p.

Mariante, A. S., Castro, S. T. R., Albuquerque, M. S. M., Paiva, S. R., Germano, J. L. (2003b). Pig Biodiversity in Brazil. *Archivos de Zootecnia da Universidade de Córdoba, 52*, 245-248.

Merks, J. W. M. (2000). One century of genetic changes in pigs and the future needs. In: *The challenge of genetic change in animal production* (W.G. Hill, S.C., Bishop, B., McGuirk, J.C., McKay, G., Simm, G, Webb, A.J., Eds). Occasional publication n°. 27. British Society of Animal Science, Edinburgh, 8-19.

Moraes, D. S. L. (2006). *Níveis de expressão dos genes FABP3 e FABP4 e conteúdo de gordura intramuscular em suínos.* D.S. thesis, Universidade Federal de Viçosa, Minas Gerais, Brazil.

Müller, E., Moser, G., Bartenschlager, H., Geldermann, H. (2000). Trait values of growth, carcass and meat quality in Wild Boar, Meishan and Pietrain pigs as well as their crossbred generations. *Journ. Anim. Breed. Genet., 117*, 189-202.

Nechtelberger D., Pires V., Sölkner J., Stur I., Brem G., Mueller M., Mueller S. (2001) Intramuscular fat content and genetic variants at fatty acid-binding protein loci in Austrian pigs. *J. Anim. Sci., 79*, 2798–2804.

Newcom, D. W., Baas, T. J., Schwab, C. R., Stalder, K. J. (2005). Genetic and phenotypic relationships between individual subcutaneous backfat layers and percentage of Longissimus intramuscular fat in Duroc swine. *Journal of Animal Sciences, 83*, 316-323.

Nygard, A. B., Jorgensen, C. B., Cirera, S., Fredholm, M. (2007). Selection of reference genes for gene expression studies in pig tissues using SYBR green qPCR. *BMC Molecular Biology., 8*, 67.

Nykjaer, A., & Willnow, T. E. (2002). The low-density lipoprotein receptor gene family: a cellular Swiss army knife? *Trends in Cellular Biology, 12* (6), 273-280.

Olson, S., Song, B. J., Huh, T. L., Chy, Y. T., Veech, R. L., McBride, O. W. (1990). Three genes for enzymes of the pyruvate dehydrogenase complex map to human chromosomes 3, 7 and X. *The American Journal of Human Genetics, 46*, 340-349.

Óvilo, C., Pérez-Enciso, M., Barragán, C., Clop, A., Rodríguez, C., Oliver, M. A., et al. (2000). A QTL for intramuscular fat and backfat thickness is located on porcine Chromosome 6. *Mammalian Genome 11*(4), 344-346.

Óvilo, C., Clop, A., Noguera, J. L., Oliver, M. A., Barragan, C., Rodriguez, C., et al. (2002a). Quantitative trait locus mapping for meat quality traits in an Iberian × Landrace F2 pig population. *Journal of Animal Science 80*, 2801–2808.

Óvilo C., Oliver A., Noguera J.L., Clop A., Barragan C., Varona L., et al. (2002b). Test for positional candidate genes for body composition on pig chromosome 6. *Genet. Sel. Evol., 34*, 465–479.

Paixão, D. M., Guimarães, S. E. F., Silva Filho, M.I. , Lopes, P. S., Pereira, M. S., Sollero, B. P. (2008). Detecção de Locos de características quantitativas nos cromossomos 16, 17 e 18 para características de desempenho em suínos. *Revista Brasileira de Zootecnia / Brazilian Journal of Animal Science, 37*, 1781-1787.

Paixão, D. M., Silva Filho, M. I., Pereira, M. S., Lopes, M. S., Barbosa, L., Souza, K. R. S., et al. (2008). Detection of quantitative trait loci on chromosomes 16, 17 and 18 for carcass, internal organs and meat quality traits is pigs. *Genetics and Molecular Biology, 31*, 898-901.

Pang W. J., Bai L., Yang G. S. (2006). Relationship among H-FABP gene polymorphism, intramuscular fat content, and adipocyte lipid droplet content in main pig breeds with different genotypes in western China. *Acta Genetica Sinica, 33*(6): 515-524.

Pannetier C., Delassus S., Darche S., Saucier C., Kourilsky P. (1993). Quantitative titration of nucleic acids by enzymatic amplification reactions run to saturation. *Nucleic Acids Res., 21*, 577–583.

Paszek, A. A., Wilkie, P. J., Flickinger G. H., Miller L. M., Louis, C. F., Rohrer G. A., Alexander, L. J., et al. (2001). Interval Mapping of Carcass and Meat Quality Traits In a Divergent Swine Cross. *Animal Biotechnology*, *12*, 155-165.

Peixoto, J. O., Guimarães, S. E. F., Lopes, P. S., Soares, M. A. M., Pires, A. V., Silva, M. V., et al. (2006). Associations of leptin gene polymorphisms with production traits in pigs. *Journal of Animal Breeding and Genetics, 123*, 378-383.

Peixoto, J. O. (2007). *Identifição e validação de seqüências expressas em tecido muscular de suínos.* D.S. thesis, Universidade Federal de Viçosa, Minas Gerais, Brazil.

Peixoto, J. O., Faria, D. A., Silva, P. V., Fonseca, I., Lopes, P. S., Guimarães, S. E. F. (2009). Association between leptin gene single nucleotide polymorphisms and Carcass Traits in Pigs. *Revista Brasileira de Zootecnia / Brazilian Journal of Animal Science, 38*, 271-276.

Perez, C. F., López, J. R., Allen, P. D. (2005). Expression levels of RyR1 and RyR3 control resting free Ca2+ in skeletal muscle. *Am J Physiol Cell Physiol., 288*, 640-649.

Pérez-Enciso, M., Clop, A., Folch, J. M., Sánchez, A., Oliver, M. A., Óvilo, C., Barragán, C., Varona, L., Noguera, J. L. (2002). Exploring alternative models for sex-linked quantitative trait loci in outbred populations: application to an iberian x landrace pig intercross. *Genetics*, *16*(4): 1625–1632.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in realtime RT-PCR. *Nucleic Acids Research., 29*, 45.

Piazzi, M., Bavelloni, A., Cioffi, G., Faenza, I., D'Aguanno, S., Urbani, A., et al. (2008). Nuclear PLC Beta 1 signaling: EEF1A2 is a novel phosphosubstrate of PKC Beta 1 in myoblast. 3rd Annual National Conference of Italian Proteomic Association. *Proceedings of the 3rd Annual National Conference of Italian Proteomic Association*, Selva di Fasano, Brindisi, Italy.

Pinto, J. P., Conceição, N. Gavaia, P. J., Cancela, M. L. (2002). Matrix Gla protein gene expression and protein accumulation colocalize with cartilage distribution during development of the teleost fish Sparus aurata. *Bone, 32*, 201-210.

Pires, A. V., Lopes, P. S., Guimarães, C. T., Guimarães, S. E. F. (2007). Mapping quantitative trait loci for performance traits on pig chromosome 6 (SSC6). *Archivos Latinoamericanos de Producción Animal, 15*, 25-32.

Pires, A. V., Lopes, P. S., Guimarães, S. E. F., Guimarães, C. T., Peixoto, J. O. (2008a). Mapeamento de locos de características quantitativas associados à composição de carcaça, no cromossomo 6 de suínos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 60*, 725-732.

Pires, A. V., Lopes, P. S., Guimarães, S. E. F., Guimarães, C. T., Peixoto, J. O. (2008b). Mapeamento de locos de características quantitativas, no cromossomo seis suíno, associados às características de cortes de carcaça. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 60*, 725-732.

Price, N. D., Trent, J., El-Naggar, A. K., Cogdell, D., Taylor, E., Hunt, K. K., et al. (2006). Highly accurate two-gene classifier for differentiating gastrointestinal stromal tumors and leiomyosarcomas. *Proceedings of the National Academy of Sciences of the United States of America, 104*(9), 3414-3419.

Pringle, T. D., Williams, S.E. (2000). Fat thickness and loin eye area effects on pork carcass quality measures. *Journal of Muscle Foods, 11*(4), 307-318.

Rattink, A. P., de Koning, D. J., Faivre, M., Harlizius, B., van Arendonk, J. A. M., Groenen, M. A. M. (2000). *Mammalian Genome* 11, 656-661.

Renaudeau, D., Hilaire, M., Mourot, J. (2005). A comparison of carcass and meat quality characteristics of Creole and Large White pigs slaughtered at 150 days of age. *Anim. Res., 54*, 43-54.

Reggiani , C., Mascarello, F. (2004). Fibre Type Identification and Functional Characterization in Adult Livestock Animals. In: Muscle Development of Livestock Animals: Physiology, Genetics and Meat Quality. M. F. W. te Pas, M. E. Everts, and H. P. Haagsman, ed. CABI Publ., Cambridge, MA.

Renaudeau, D., & Mourot, J. (2007). A comparison of carcass and meat quality characteristics of Creole and Large White pigs slaughtered at 90kg BW. *Meat Science, 76*, 165–171.

Rincker, P. J., Killefer, J., Ellis, M., Brewer, M. S., McKeith, F. K. (2007). Intramuscular fat content has little influence on the eating quality of fresh pork loin chops. *Journal of Animal Science 86*, 730-737.

Ron, M., Israeli, G., Seroussi, E., Weller, J. I., Gregg, J. P., Shani, M., et al. (2007). Combining mouse mammary gland gene expression and comparative mapping for the identification of candidate genes for QTL of milk production traits in cattle. *BMC Genomics, 8*, 183.

Rosenvold, K., & Andersen, H. J. (2003). Factors of significance for pork quality – A review. *Meat Science, 64*(3), 219–237.

Rothschild M. F. (1998) Identification of quantitative trait loci and interesting candidate genes in the pig. Progress and prospects. *Proceedings 6th World Congress on Genetics Applied to Livestock Production*, 26, 403-410.

Sanchez, M. P., Iannuccelli, N., Basso, B., Bidanel, J. P., Billon, Y., Gandemer, G., et al. (2007). Identification of QTL with effects on intramuscular fat content and fatty acid composition in a Duroc × Large White cross. *BMG Genomics, 8*, 55.

Sans, P., Andrade, M.J., Ventanas, S., Ruiz, J. (2004). Quality Characteristics of Fresh Meat from Pigs of the Gascon Breed. *Food Science and Technology International, 10*, 29-34.

Sasaki, Y., Nagai, K., Nagata, Y., Doronbekov, K., Nishimura, S., Yoshioka, S., et al. (2005). Exploration of genes showing intramuscular fat deposition associated expression changes in musculus Longissimus muscle. *Animal Genetics, 37*, 40-46.

Schierholt, A. S., Fonseca, I., Silva, P. V., Paiva, S. R., Lopes, P. S., Faria, D. A., et al. (2008). Análise filogenética do gene da miogenina (Phylogenetic analysis of the Myogenin gene). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 60*, 156-162.

Schiller, C. M., Fowler, B. F., Woods, J. S. (1977). Effects of Arsenic on Pyruvate Dehydrogenase Activation. *Environmental Health Perspectives, 19*, 205-207.

Schmittgen, T. D., Zakrajsek, B. A., Mills, A. G., Gorn, V., Singer, M. J., Reed, M. W. (2000). Quantitative reverse transcription-polymerase chain reaction to study mrna decay: comparison of endpoint and real-time methods. *Analytical Biochemistry 285*, 194–204.

Schefe, J. H., Lehmann, K. E., Buschmann, I. R., Unger, T.Funke-Kaiser, H. (2006). Quantitative real-time RT-PCR data analysis: current concepts and the novel "gene expression's CT difference" formula. *Journal of Molecular Medicine, 84*(11), 901-910.

Serra, X., Gil, F., Pérez-Enciso, M., Oliver, M. A., Vázquez, J. M., Gisper, M., et al. (1998). A comparison of carcass, meat quality and histochemical characteristics of Iberian (Guadyerbas line) and Landrace pigs. *Livestock Production Science, 56*, 215–223.

Shen, X., Franzini-Armstrong, C., Lopez, J. R., Jones, L. R., Kobayashi, Y. M., Wang, Y., et al. (2007). Triadins Modulate Intracellular Ca2_ Homeostasis but Are Not Essential for Excitation-Contraction Coupling in Skeletal Muscle. *Journal of Biological Chemistry, 282*(52), 37864–37874.

Shi-Zheng, G., & Su-Mei, Z. (2009). Physiology, Affecting Factors and Strategies for Control of Pig Meat Intramuscular Fat. *Recent Patents on Food, Nutrition & Agriculture, 1*, 59-74.

Silva, K. M., Paixão, D. M., Silva, P. V., Sollero, B. P., Pereira, M. S., Lopes, P. S., et al. (2008). Mapping of quantitative trait loci and confirmation of the FAT1 region on chromosome 4 in an F2 population of pigs. *Genetics and Molecular Biology, 31*, 475-480.

Silva, K. M. , Guimarães, S. E. F. , Lopes, P. S. , Nascimento, C. S. , Lopes, M. S. , Weller, M. M. d C. A. (2009). Mapeamento de Locos de Características Quantitativas para Desempenho no Cromossomo 4 de suínos. *Revista Brasileira de Zootecnia / Brazilian Journal of Animal Science, 38*, 474-479.

Soares, M. A. M., Guimarães, S. E. F., Euclydes, R. F., Lopes, P. S., Peixoto, J. O., Guimarães, M. F. M., et al. (2006). Novos polimorfismos no gene da obesidade em raças divergentes de suínos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 58*, 401-407.

Sollero, B. P., Paiva, S., Faria, D., Guimarães, S. E. F., Castro, S., Egito, A., et al. (2008). Genetic diversity of Brazilian pig breeds evidenced by microsatellite markers. *Livestock Science,* p. x,.

Souza, C. A. , Paiva, S. R. , Pereira, R. W. , Guimarães, S. E. F. , Dutra Jr, W. M. , Murata, L. S. , et al. (2009). Iberian origin of Brazilian local pig breeds based on Cytochrome b (MT-CYB) sequence. *Animal Genetics, 40*(5), 759-762.

Spelman, R.J., & Bovenhuis, H. (1998). Genetic response from marker assisted selection in an outbred population for differing marker bracket sizes and with two identified quantitative trait loci. *Genetics 148*, 1389–1396.

Su, Z., Dong, X., Zhang, B., Zeng, Y., Fu, Y., Yu, J., et al. (2006). Gene expression profiling in porcine mammary gland during lactation and identification of breed- and developmental-stage-specific genes. *Science in China Series C: Life Sciences*, *49*(1), 26-36.

Tan, S. H., Reverter, A., Wang, Y. H., Byrne, K.A., McWilliam, S. M., Lehnert, S. A. (2006). Gene expression profiling of bovine in vitro adipogenesis using a cDNA microarray. *Funct. Integr. Genomics 6*, 235–249.

Triebold, H. O. (2007). *Quantitative analysis*. Thompson Press.

Urban T., Mikolasova R., Kuciel J., Ernst M., Ingr I. (2002). A study of association of the FABP3 genotypes with fat and meat production of pigs. *J. Appl. Genet., 43*, 505–509.

Ushizawa, K., Takahashi, T., Hosoe, M., Ishiwata, H., Kaneyama, K., Kizaki, K., Hashazume, K. (2007). Global gene expression analysis and regulation of the principal genes expressed in bovine placenta in relation to the transcription factor AP-2 family. *Reproductive Biology and Endocrinology 5*, 17.

VanGuilder, H. D., Vrana, K. E., Freeman, W. M. (2008). Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques, 44*, 619–626.

Veerkamp, J. H. & Maatman, R. G. H. J. (1995). Cytoplasmic fatty acid binding proteins: their structure and genes. *Prog. Lipid Res. 34*, 17-52.

Veerkamp, J. H., van Moerkerk, H. T. B., Zimmerman, A. W. (2000). Effect of fatty acid-binding proteins on intermembrane fatty acid transport. Studies on different types and mutant proteins. *Eur. J. Biochem., 267*, 5959-5966.

Veroneze, R., Reis Filho, J. C., Lopes, P. S., Guimarães, S. E. F., Torres, R. A. (2007). Estudo da endogamia em população de suínos da raça nativa brasileira Piau. *Anais do ZOOTEC 2007,* Londrina, Brazil.

Vianna, A. T. (1985). *Os suínos*. (14th ed.). São Paulo, Brasil: Nobel.

Voge, J. L, Santiago, C. A. T., Aad, P. Y., Goad, D. W., Malayer, J. R., Spicer, L. J. (2004). Quantification of insulin-like growth factor binding protein mRNA using real-time PCR in bovine granulosa and theca cells: effect of estradiol, insulin, and gonadotropins. *Domestic Animal Endocrinology, 26*, 241-258.

Wick, M., & Marriott, N. G. (1999). The relationship of Sarcomeric Architecture to Meat Tenderness. The Ohio State University. *Research and Reviews: Meat, Special Circular,* 172-199.

Wilhelm, J., & Pingoud, A. (2003). Real-time polymerase chain reaction. *Chembiochem, 4*, 1120–1128.

Wood, J. D., Nute, G. R., Richardson, R. I., Whittington, F. M., Southwood, O., Plastow, G. ,et al. (2004). Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science*, *67*, 651-667.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., et al. (2008). Fat deposition, fatty acid composition and meat quality: a review. *Meat Science, 78*, 343-358.

Young, P., Ehler, E., Gautel, M. (2001). Obscurin, a giant sarcomeric Rho guanine nucleotide exchange factor protein involved in sarcomere assembly. *Journal of Cell Biology, 154*, 123-136.

Yu, T. P., Wang, L., Tuggle, C. K., Rothschild, M. F. (1999) Mapping genes for fatness and growth on pig chromosome 13: A search in the region close to the pig PIT1 gene. J Anim Breed Genet 116:269-280.

Zander, D. S., Popper, H. H., Jagirdar, J., Haque, A. K., Cagle, P. T., Barrios, R. (Eds.). (2008). *Molecular Pathology of Lung Diseases. Series: Molecular Pathology Library, Vol. 1*. 682 p.

Zhang, W., Kuhlers, D. L., Rempel, W. E. (1992). Halothane gene and swine performance. *Journal of Animal Science, 70*, 1307-1313.

Zhang L., Kelley J., Schmeisser G., Kobayashi Y. M., Jones L. R. (1997). Complex formation between junctin, triadin, calsequestrin, and the ryanodine receptor. Proteins of the cardiac junctional sarcoplasmic reticulum membrane. *Journal of Biological Chemistry, 272*, 23389–23397.

Zhang, S., Xu, L., Lee, J., Xu, T. (2002). Drosophila Atrophin homolog functions as a transcriptional co-repressor in multiple developmental processes. *Cell, 108*, 45-56.