Research Article

Association of MYF5 gene allelic variants with production traits in pigs

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Abstract

We studied the phenotypic effects of polymorphisms at the MYF5 gene in a divergent F_2 swine population and found that one polymorphism was due to an insertion and another to a deletion. The genotypes of 359 F2 animals were obtained and the Normal/Normal (NN) and Normal/Insertion (NI) genotypes analyzed to determine associations with phenotypic data for performance, carcass and meat quality traits. Significant differences were observed ($p < 0.05$) between NN and NI animals for drip (NN = 3.14 ± 1.56; NI = 3.69 ± 2.78%), cooking (NN = 32.26 ± 2.41; NI = 33.21 ± 2.31%) and total loss (NN = 34.16 ± 2.63 and NI = 34.97 ± 2.08%). The Deletion marker was not statistically tested. The results indicate that the allelic variant Insertion is associated with a deleterious effect on meat quality traits and should be monitored in marker assisted selection programs.

Key words: myogenesis, DNA sequencing, pig production, PCR.

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Introduction

Meat is the result of the transformation of muscle cells into an edible product after an animal has been slaughtered. Muscle consists of a large number of fibers which themselves are made up of smaller fibrils, muscle mass being the sum of all the individual cellular processes and cellular growth occurring within the muscle. During the fetal period muscles grow due to cell proliferation (hyperplasia) but from birth until slaughter the growth of muscle cells no longer depends on the division of the cell nucleus but on an increase in the size and diameter of the cytoplasm (a hypertrophy). Cell proliferation may occur during hypertrophy but only in satellite cells.

Genes in the MyoD family (MYOD, MYF5, myogenin and MYF6) are related to mammalian muscle development, with the MYOD, MYF5 and myogenin genes being involved in prenatal hyperplastic processes and the MYF6 gene to postnatal muscle growth (te Pas *et al* ., 1999a). Myogenin is required for the myoblast differentiation established by the initial expression of the MYF5 or MYOD genes which are thus responsible for the determination and specialization of myoblasts, so myogenin could be considered to be a differentiation factor (Bergstrom and Tapscott 2001). Studies on the MyoD gene family (especially the MYOD and MYF5 genes) are still rare in pigs, although a study has been carried out on two commercial pig lines by te Pas *et al.* (1999a) who analyzed a *Hinf* I polymorphic site in the second intron of the MYF5 gene to determine the effects of genetic variation at this site on desirable traits but found no genotypically associated differences for any of the traits investigated (birth weight, weight at slaughter, growth rate, meat weight and subcutaneous fat thickness).

During the study reported in the present paper, we sequenced the MYF5 gene from both sexes of the parental generation of an F_2 crossed pig population. This parental population was constituted of Brazilian Piau boars and commercial white females. The F_2 offspring were genotyped to identify the allelic variants and the genotypes were evaluated statistically for associations with various performance, carcass and meat quality traits.

Material and Methods

The experiment was conducted on the Pig Breeding Farm of the Animal Science Department, Universidade Federal deViçosa (UFV), Viçosa, Minas Gerais, Brazil. Except for both parental boars (Brazilian Piau Breed), all other

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animals (18 parental sows, 11 Landrace x Large White and 7 Landrace x Large White x Pietrain, F_1 and F_2 animals) were maintained on the farm from birth until the time of slaughter. Animals were slaughtered by first applying an approved electric shock and then bled immediately using cardiac puncture. After cleaning and evisceration, the carcasses were divided into two halves, the left half being refrigerated at 4 °C for 24 h. Genetic analyses were performed at the Laboratory of Animal Biotechnology, Animal Science Department, UFV.

Blood was collected at the time of slaughter from each animal (F_0 parents and their F_1 and F_2 offspring), treated with proteinase K and the genomic DNA extracted from white blood cells using the phenol-chloroform technique. The DNA in each sample was quantified spectrophotometrically ($\lambda = 260$ and 280 nm) and 200 to 300 µL aliquots diluted in TE buffer (10 mmol/L Tris-HCl, pH 8.0, and 1 mmol/L EDTA, pH 8.0) to a concentration of 25 ng DNA/µL and kept at 4 °C. A DNA stock solution from each animal was also diluted in TE and stored at -20 °C.

The primers were designed using the WebPrimers program (http://alces.med.umn.edu/websub.html) and covered exon I (665 bp), exon II (75 bp) and exon III (791 bp) at the end of the gene. The MYF5 gene sequence (GenBank accession number Y17154) was used as the consensus sequence. Six primer pairs were constructed, the primers being selected according to Rychlik's (1993) criteria based on their position in the gene, annealing temperatures and variation in free energy (ΔG).

Each amplification reaction consisted of 1 U Taq DNA polymerase, 0.2 mmol/L dNTPs, 0.2 µmol/L of each forward and reverse primer, 20 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 2 to 4 mmol/L $MgCl_2$, and 25 ng genomic DNA in a total volume of 100 µL. The amplification program varied according to each primer pair and basically consisted of the following steps: denaturation at 94 °C/1 min, mean annealing temperature of 64 °C/1 min (according to the primer pair), and extension temperature of 72 °C/1 min, with 35 cycles of repetition. Successful amplification was determined on polyacrylamide gels stained with silver nitrate. The primers used in the amplification reactions and for sequencing are listed in Table 1.

To optimize the sequencing reactions, three pools (1, 2 and 3) of amplified DNA from parental sows were obtained for each fragment after amplification, sow DNA being used because it was expected that the MYF5 gene sequence would be conserved in these animals. Both parental boars were sequenced individually. Approximately 100 µL of amplified material from each pool and from both parental boars was purified on GFX^{TM} PCR DNA columns using the Gel Band Purification kit (Amersham Pharmacia Biotech). After purification, the DNA sample was quanti-

Table 1 - Primer sets used in the study, annealing position within the *MYF5* gene and expected fragment size.

Primer	Sequence	Position*	Size** (bp)
F1	AGAAGTCGGAGCTTCGGAGC	179-198	449
R1	CCCCGCTTACTTGCGCTGTTTA	606-627	
F2	AACGTTGGATTTCCTTCGTTCC	544-565	566
R2	CCACTCGTGGCTCAAACTCG	1090-1109	
F3	GGACGAGTTTGAGCCACGAGTGG	1087-1109	495
R3	GGTTFCTACACCTGCTTAAACG	1562-1581	
F4	CAGAGCACCTTTCGCCAAGA	2249-2268	159
R4	TCCTCTAAAGGCGTTCGTTCT	2387-2407	
F5	AGGGCAACGTAAGCAAGTCTACC	2729-2751	536
R5	CCCTTGGATGAATTGCCCCTATAAG	3240-3264	
F6	TTCTTTTCCTTTGTCTTGGCATTAA	3190-3214	479
R6	ACGTATCAATCACTGCCAATGCTA	3646-3669	

*The position of the primers was established based on GenBank sequence Y17154.
**Expected size of the fragments after amplification according to the same sequence.

fied in a Genequant II DNA/RNA spectrophotometer (Amersham Pharmacia Biotech) at $\lambda = 260$ and 280 nm using a wavelength of 320 nm for background compensation. The remaining purified DNA was stored at -20 °C until sequencing.

The DNA from the parental animals was sequenced by the dideoxynucleotide (ddNTPs) chain termination technique using the ABI PRISM BigDye Terminator Cycle Sequence Ready Reaction kit (Applied Biosystems). Briefly, in each reaction 20 ng of sample DNA was mixed with 2 µL of the sequencing solution provided with the Applied Biosystems Sequencing kit, 5.0 pmol of the same direct primer as was used in the amplification reaction and 2 µL of buffer (200 mM Tris-HCl, pH 9.0, 5 mM $MgCl_2$) in a total reaction volume of 10 µL. A similar reaction mix was prepared for the reverse primer. After the sequencing reaction, the pellet was resuspended in 15 µL hi-di formamide (Applied Biosystems), denatured at 95 °C for 5 min and kept on ice until application to an automatic ABI PRISM 310 sequencer. The generated sequences were edited and mounted in contigs using the SEQ MAN II program (DNASTAR, Inc.), and then aligned and compared to each other and to the Y17154 sequence using the DNASTAR program (DNASTAR Inc).

To produce the 359 pigs in the F_2 generation, 54 F_1 sows and 11 F_1 boars were randomly selected and intercrossed. Although the F_1 animals were not phenotypically evaluated, their MYF5 genotypes were determined in a 20 µL reaction volume, under similar PCR conditions as those used for the parental animals. For this experiment, phenotypic data from 359 F_2 animals resulting

from the initial crossing of commercial sows with native Brazilian boars were analyzed with the MYF5 gene fragments being amplified in a 20 μL reaction volume under similar conditions to those used for the parental generation. After amplification 5 μL of each amplification product was subjected to silver nitrate-stained polyacrylamide gel electrophoreses (AgPAGE).

Performance traits measured in the F$_2$ generation were: birth weight (BW); weight at 21 days (W$_{21}$), 77 days (W$_{77}$) and 105 (W$_{105}$); weight at slaughter (WS); average daily gain (ADG); feed intake (FI); feed-gain ratio (FG) from 77 to 105 days of age and age at slaughter (AS). Carcass traits measured in the F$_2$ generation: carcass length (MLC); backfat thickness at last 2nd-3rd thoracic vertebrae (SH); backfat thickness after last rib, at 6.5 cm from the midline (P2); loin depth (LD); loin eye area (LEA); total ham weight (THW); skinless and fatless ham weight (HW); total (bone in) loin weight (TLW); loin weight (LW) and carcass yield (CY). The 359 F$_2$ animals were slaughtered at approximately 65 kg of live weight. The meat quality traits determined in the *longissimus dorsi* muscle were: pH 45 min after slaughter (pH45), pH 24 hours after slaughter (pH24), intramuscular fat content (IMF), drip loss (DL), cooking loss (CL), total loss (TL), objective tenderness (OT), and color. Meat color was determined with the Hunter Lab system and included the spectrophotometric measurement of lightness (L), redness (A), yellowness (B), hue angle (h), and chroma (c).

Correlation between genotype and traits was performed using the PROC GLM module of the SAS program based on the model

$$y_{ijkl} = S_i + G_j + L_k + (C_{ijkl} - \overline{C})b + e_{ijkl}$$

where y_{ijkl} equals the observation, S_i the sex effect I ($_i$ being 1 for boars, 2 for sows), G_j the genotype effect ($_j$ being 1 for NN, 2 for NI), L_k the batch effect j ($_j$ being 1, 2, 3, 4, 5), $(C_{ijkl} - \overline{C})b$ the covariate and e_{ijkl} the random error. The covariates were litter size at birth weight, litter size at weaning for W$_{21}$, W$_{77}$, W$_{105}$ and WS; weight at 77 days for ADG, FG, FI, carcass weight for all carcass traits and AS for the meat quality traits. The difference between genotypes was tested by analysis of variance (F test) at the with the 5% significance level.

Results

Identification of mutations in the porcine MYF5 gene

We sequenced three exons of the MYF5 gene in the 18 commercial sows and 2 native Brazilian boars which made up the parental generation. Polymorphisms were only detected for fragments amplified using primer pair number 6 (F6 and R6). Even before sequencing, AgPAGE showed not only an amplified product differing in size from the expected fragment (479 bp) in one of the parental boars and in pools 1 and 3, but also heteroduplex formations. In view of the electrophoretic differences in pools 1 and 3, these pools were examined and the product of each female was individually sequenced. No variation in the electrophoretic or sequencing pattern were detected for pool 2.

Multiple alignment of the parental sow and boar sequences with sequence Y17154 revealed two previously unreported variants, both demonstrable by AgPAGE and confirmable by fragment sequencing. The first variant was identified in a parental boar denominated 'boar C' and in sow number 211 and corresponded to a heterozygous TTT deletion (Figure 1) at positions 3420-3422 and characterized by the occurrence of heteroduplex bands. In addition to the 479 bp expected fragment, we also amplified a second 476 bp fragment which had not been previously identified and which we named the 'Deletion' (D) sequence before submitting it to the GenBank database where it was assigned the accession number AY312362.

The second variant was identified in two parental sows (numbers 151 and 154 from the Landrace x Large White x Pietrain genetic group), from which we obtained a larger amplified fragment. Sequencing and alignment demonstrated that both sows were homozygous for an ATG trinucleotide insertion at position 3326 and for an A nucleotide deletion at position 3357, which resulted in a previously unreported 481 bp amplified fragment (Figure 2) which we named the 'Insertion' (I) sequence before submitting it to the GenBank database where it was assigned the accession number AY285781.

We had established that the genotypes would be classified according to the allelic variants as: normal/normal (NN); normal/insertion (NI); normal/deletion (ND); insertion/deletion (ID); insertion/insertion (II); and deletion/deletion (DD).

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1) GenBank sequence      GAAATCAAGTTATAGGGAGTTTTAAATATATTTAACTTTTTTGCTTCTCTTTAATC
2) p69_exonIIIfrag6      GAAATCA*GTTATAGGGAGTTTTAAATATATTTAACTTTTTTGCTTCTCTTTAATC
3) p211_exonIIIfrag6     GAAATCA*GTTATAGGGAGTTTTAAATATATTTAACTTT---GCTTCTCTTTAATC
4) C_exonIIIfrag6        GAAATCA*GTTATAGGGAGTTTTAAATATATTTAACTTT---GCTTCTCTTTAATC
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Figure 1 - Partially sequenced region of the fragment amplified with the F6-R6 primer pair. The region comprises nucleotides 3382 to 3438 according to the Y17154 GenBank sequence. Line 1: GenBank sequence; line 2: sequence found in parental animals represented here by the sequence of female p69; lines 3 and 4: sequences obtained for female p211 and male C, respectively. Gray shadow indicates the region of deleted nucleotides. A deletion (*) is present in the three parental sequences compared to the consensus sequence (line 1). Since this supposed deletion was present in all animals sequenced, it was not considered for the analysis.

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1) GenBank sequence      ACCTCTG---TTTAGAGCTTTATTTTTTTCCCCTTTAAAAATATTAGAACAAATACTAGA
2) p147_exonIIIfrag6     ACCTCTG---TTTAGAGCTTTATTTTTTTCCCCTTTAAAAATATTAGAACAAATACTAGA
3) p151_exonIIIfrag6     ACCTCTGATGTTTAGAGCTTTATTTTTTTCCCCTTTAAAA-TATTAGAACAAATACTAGA
4) p154_exonIIIfrag6     ACCTCTGATGTTTAGAGCTTTATTTTTTTCCCCTTTAAAA-TATTASAACAAATACTAGA
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Figure 2 - Partially sequenced region of the fragment amplified with the F6-R6 primer pair. The region comprises nucleotides 3319 to 3378 according to the Y17154 GenBank sequence. Line 1: GenBank sequence; line 2: sequence found in parental animals represented here by the sequence of female p147; lines 3 and 4: sequences obtained for females p151 and p154, respectively. Inserted nucleotides are underlined and the region of deleted nucleotides is indicated by gray shadow.

Genotyping of the F_1 generation

The F2 generation was produced by randomly mating 11 F_1 boars (none of which had ND genotypes) with 54 F_1 sows (only 12 of which had ND genotypes). Only three F_1 sows were mated to NN boars and two F_1 sows were mated with NI boars to produce ND F_2 piglets, this small number being partly explainable by the fact that of the nearly 800 F_2 animals born, only 359 were genotyped and had their phenotypes evaluated during the study. The DD genotype did not occur in the F_2 generation because no ND boars were used in the F_1 matings. Also, no II genotype was found in the analyzed F_2 animals.

Genotyping of the F_2 generation

The observed F_2 genotypes were NN = 264 (73.5%), NI = 86 (24%), ND = 5 (1.4%) and ID = 4 (1.1%), no type II or DD genotypes being detected in our sample. Since the NN and NI genotypes were the most frequent we excluded the other genotypes from the statistical analysis of the association with phenotypic traits.

The effect of the MYF5 variant on performance, carcass and meat quality traits

There were no statistically significant differences between the NN and NI genotypes on performance or carcass traits but there were statistically significant differences between the genotypes for the following meat quality traits: drip loss, 3.69% for NN compared with 3.14%, for NI (p = 0.028); cooking loss, 33.21% for NN compared with 32.26% for NI (p = 0.003); and total loss 34.97% for NN compared with 34.16% for NI (p = 0.024) (Table 2).

Discussion

Our study demonstrates that native Brazilian pigs have not yet been fully evaluated using genetic analysis. Little is known regarding the origin of Brazilian pig breeds but it has been speculated that during colonization of Brazil the Portuguese brought pigs which received genetic input from crossings with Dutch pigs and perhaps pigs originating from Africa during the slave trade (Vianna, 1985). Brazilian native breeds are used to provide not only meat but also fat to local communities, and are reared on small rural properties and adapt well to precarious management conditions, and due to their hardiness they require little nutritional and sanitary care (França, 1991). However, in the last decade the tendency has been towards other imported commercial pig breeds and relatively few native Brazilian pigs stocks remain because their primary phenotypic trait, fat production, is no longer of interest to the consumer and has become a limiting fact in the marketing of native breeds (Lopes *et al.*, 2002).

Native breeds possess a great but unexplored potential for the formation of reference populations and for the study of linkage maps and of the effects of quantitative trait loci and candidate genes. In addition to their extreme phenotype in terms of fat and meat deposition compared to that of commercial breeds, native breeds can be characterized by their extreme resistance to precarious management conditions and diseases (Guimarães and Lopes, 2001). Irgang (1986) stated that the preservation of the genetic potential of these breeds might be useful for eventual crossings aimed at increasing the hardiness of animals derived exclusively from imported breeds.

Pooling amplified DNA from parental sows has been reported to be an useful technique for sample sequencing (Rothschild and Soller 1999) and in our study pooling 6 animals per pool allowed us to optimize our experiments but did not impair the AgPAGE identification of polymorphisms even before samples sequencing. Despite our satisfactory results, it should be kept in mind that PCR is a competitive method, and fragments of various origins containing nucleotide variations at the primers annealing sites or even higher or lower degrees of purity differ in amplification efficiency. In these cases, important nucleotide variation might remain undetected.

Because F_1 animals were randomly selected it was not possible to have more F_2 animals carrying the I and, especially, D allelic variants, further work being needed to genotype another population to find these variants and then proceed with selective matings to produce a larger number of pigs with mutant MYF5 alleles.

The primers used in our study were designed based on the Y17154 GenBank sequence according to which exon 1 is located between nucleotides 836 and 1501, exon 2 between nucleotides 2308 and 2383, and exon 3 between nucleotides 2802 and 3593. This suggests that the nucleotide changes described by us are situated within exon 3. However, according to the sequence described by te Pas *et al.* (1999) exon 1 is located between nucleotides 1001 and 1501, exon 2 between nucleotides 2307 and 2387, and exon 3 between nucleotides 2801 and 2990 and, therefore, the

Table 2 - Performance, carcass and meat quality traits for F_2 generation normal/normal (NN) and normal/insertion (NI) genotypes in.

Trait	Genotypes					
	Normal/normal			Normal/insertion		
	n	Mean	sd	n	Mean	sd
Performance traits*						
Birth weight (BW)	250	1.20	0.27	84	1.17	0.26
Weight at 21 days (W_{21})	186	4.93	1.05	72	4.92	0.99
Weight 77 days (W_{77})	197	21.43	3.99	79	20.30	4.20
Weight at 105 days (W_{105})	193	35.95	6.06	78	34.95	5.98
weight at slaughter (WS)	220	65.10	5.53	71	65.98	4.97
Feed intake (FI)	240	40.07	7.93	83	39.10	8.25
Average daily weight gain (ADG)	236	0.52	0.14	81	0.54	0.11
Feed-gain ratio from 77 to 105 (FG)	236	2.91	0.76	81	2.70	0.58
Age at slaughter (AS)	229	148.91	10.00	72	150.24	10.23
Carcass traits						
Mean carcass length (MLC, cm)	216	71.79	3.29	68	71.68	3.05
Backfat thickness at last 2nd-3rd thoracic vertebrae (SH, mm)	216	41.32	5.83	70	40.91	5.44
Backfat thickness after last rib at 6.5 cm from the midline (P2, mm)	217	16.98	3.64	70	17.70	3.95
Loin depth (LD, mm)	194	43.30	4.33	63	44.08	4.41
Loin eye area (LEA, cm^3)	201	26.52	3.70	66	26.00	3.56
Total ham weight (THW, kg)	230	7.29	0.86	73	7.31	0.83
Skinless and fatless ham weight (HW) (kg)	228	5.03	0.61	72	5.03	0.60
Total (bone in) loin weight (TLW, kg)	227	3.52	0.47	73	3.50	0.50
Loin weight (LW, kg)	228	1.05	0.18	72	1.05	0.18
Carcass yield (CY, %)	213	82.03	2.28	67	82.04	2.08
Meat quality traits						
pH of the meat 45 min after slaughter (pH_{45})	212	6.52	0.26	69	6.50	0.28
pH 24 hours after slaughter (pH_{24})	214	5.72	0.17	69	5.74	0.16
Lightness (L)	197	44.98	1.78	60	45.41	2.18
Redness (A)	195	0.69	0.57	59	0.71	0.67
Yellowness (B)	197	6.61	0.58	60	6.59	0.55
Intramuscular fat (IMF, %)	201	1.55	0.53	61	1.64	0.69
Drip loss (DL, %)	216	3.14[a]	1.56	69	3.69[b]	2.08
Cooking loss (CL, %)	217	32.26[a]	2.41	69	33.21[b]	2.31
Total loss (TL, %)	192	34.16[a]	2.63	56	34.97[b]	2.78
Objective tenderness (OT, g/1.2 cm)	187	5551.4	823.67	56	5759.1	916.65
Hue angle (h)	189	83.71	5.19	54	83.63	6.31
Chroma (c)	192	6.68	0.55	56	6.67	0.48

*All in kg, except for FG which is in kg/kg. n = number of observations; sd = standard deviation of the mean.
Means in the same row followed by different letters were significantly different by the F test at $p < 0.05$.

changes seen by us would be located in the untranslated 3' region of the gene. Since no protein sequence derived from the translation of the pig MYF5 gene has been reported in the literature so far, comparison of the gene sequence with the translated amino acid sequence is not possible and, therefore, the protein alterations generated by the allelic variants cannot be verified. If the sequence described by te Pas *et al.* (1999) is correct, the changes observed would be markers without any expected phenotypic effect and, due to their proximity to exonic regions, would be in linkage disequilibrium with the translated region of the gene.

However, the chromosome region 5q25, in which the MYF5 gene locus is situated contains another gene, insulin-like growth factor I (IGFI), which might exert a similar

function. In addition, myogenic factor 6 has recently been located on chromosome 5 of pigs (Vykoukalova *et al.*, 2003) and also in humans and mice (Patapoutian etal, 1993; Floss *et al.*, 1996) on the same chromosome as MYF5. It should also be remembered that our study used a F_2 pig population which may have had a high degree of linkage disequilibrium not only for the MYF6 or IGFI genes but also for as yet undiscovered genes.

Our study shows that in the F_2 population studied the Insertion (I) variation in the MYF5 gene is associated with effects on meat quality traits, with pigs carrying the MYF5 Insertion variant having meat with a lower water-holding capacity. The Insertion allele came not only from a native Piau boar but also from 2 parental sows that carried Pietrain breed genes so it needs to be verified whether or not this polymorphism is unique to the Piau breed. This could be achieved by analyzing commercial herds, especially those containing Pietrain pigs for the MYF5 Insertion variant and studying its association with meat quality traits. This analysis could provide information about a putative Quantitative Trait Loci (QTL) for meat quality traits in *Sus scrofa* Chromosome 5 (SSC5).

The difference in meat yield between carcasses is related to variation in the number of muscle fibers determined during the embryonic period (hyperplastic phase). As mentioned earlier, muscle fiber formation in mammals is an embryonic process mediated by the MyoD gene family. After slaughter, muscle becomes the major part of the meat produced and it may be assumed that the different genes that control the muscular hyperplastic process regulate variation in growth rate, carcass traits and meat quality of the animals. Consequently, if the number of muscle fibers is determined during embryonic life and regulated, in part, by the four genes of the MyoD family, polymorphisms in these genes can have an effect on traits related to pork production and quality.

Te Pas *et al.* (2000) found an increase in mRNA expression for myogenin, MYF5 and MYODI in the muscle tissue of pigs selected for rapid growth compared to those selected for lean growth. In another study, te Pas *et al.* (1999b), analyzed a polymorphism in the myogenin gene using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) using the *Msp I* enzyme and identified two alleles (A and B) and observed that the BB genotype was associated with increased birth weight, growth rate and lean meat content without affecting subcutaneous fat thickness. However, te Pas *et al.* (1999a) found no significant associations between the allelic forms of the MYF5 gene and meat production traits.

In our study, statistically significant differences ($p < 0.05$) were observed for drip loss, cooking loss and total loss between the NN and NI genotypes, with the NI genotype showing the highest values (*i.e.* undesirable traits). These traits might be related to the quantity or quality of muscle protein after slaughter. The Insertion variant was responsible for only a small proportion of the phenotypic variance in drip loss (2.2%), cooking loss (2.9%) and total loss (3.2%).

Since higher drip loss, cooking loss and total loss mean values were obtained for animals carrying the Insertion variant, this allele might be related to poor muscle protein quality or a reduction in the quantity of muscle protein in animals with this variant, this is supported by the fact that the normal allele was associated with a higher water-holding capacity. Meat with a lower pH shows, *a priori*, greater protein denaturation and since proteins are responsible for water retention in meat, more preserved proteins would reduce drip loss, cooking loss and total loss. In physical terms, a cut of meat with more preserved protein molecules would retain more water, while a smaller amount of intact protein leads to a lower water-holding capacity with greater losses during the handling processes (cutting, storage, cooking, etc.). Since no significant difference ($p = 0.42$ for pH_{45} and $p = 0.57$ for pH_{24}) in meat pH (Table 2) was observed between the genotypes, other factors such as the amount of muscle protein may have been responsible for the observed results.

Among the traits used to assess meat yield in pigs, the loin eye area (LEA) shows the best correlation with water-holding capacity. When analyzing the meat quality of the F_2 animals genotyped in our study, Benevenuto Júnior (2001) found a significant correlation ($p < 0.01$) between LEA and drip ($r = 0.22$) and cooking loss ($r = 0.15$). Since LEA is a predictor of meat yield, the lack of a significant difference ($p = 0.300$) for this trait between the NN and NI MYF5 genotypes investigated in our study suggests that the NN and NI genotypes do not influence protein deposition in muscle and, consequently, meat yield. We also found no statistical differences between the NN and NI genotypes (Table 2) in respect to carcass traits, so these genotypes may act through variation in the quality of muscle protein, which may show greater denaturation followed by a lower water-holding capacity even under similar pH conditions, as was the case of our study. The lower water-holding capacity of the NI genotype seems to be due to some kind of mechanism associated or linked with the allelic Insertion variant, although because we did not determine sarcomere length or other similar measurements more work is needed to confirm this hypothesis.

The literature shows that the MYF5 gene is overridden by other genes such as the MYOD I gene, which results in the reduction of deleterious effects due to mutations in any of these genes. Therefore, in order to assess the real dimension of the effects of MYF5 and MYOD I on muscle development, the other genes of the MyoD family responsible for hyperplastic muscle development are currently being studied. The identification of allelic variants in the genes of the MyoD family will help in the analysis of the interactions between different genes, especially those ones of this family, acting on muscle development.

Acknowledgements

References

Benevenuto Júnior AA (2001) Avaliação de rendimento de carcaça e de qualidade da carne de suínos comerciais, de raça nativa e cruzados. Dissertação de Mestrado, Universidade Federal de Viçosa, Viçosa, 93 pp.

Bergstrom DA and Tapscott SJ (2001) Molecular distinction between specification and differentiation in the myogenic basic helix-loop-helix transcription factor family. Molecular Cell Biology 21:2404-2412.

França LR (1991) Análise morfofuncional da espermatogênese de suínos adultos da Raça Piau. Dissertação de Mestrado, Universidade Federal de Minas Gerais, Belo Horizonte, 185 pp.

Floss T, Arnold HH and Braun T (1996) MYF5(m1)/Myf-6(m1) compound heterozygous mouse mutants down-regulate MYF5 expression and exert rib defects: Evidence for long-range cis effects on MYF5 transcription. Developmental Biology 25:140-147.

Guimarães SEF and Lopes PS (2000) Use of native genetic resources as a tool for the genomic mapping in swine In Proceedings Rare Breeds International Simposium (Digital publishing), 20-24 November 2000, Brasília, Brasil.

Irgang R (1996) Suínos de raças nativas: O interesse da pesquisa. Suinocultura Industrial 87:13-20.

Lopes PS, Guimarães SEF, Pires AV, Soares MAM, Carmo FMS, Martins MF, Benevenuto Júnior AA and Gomide LAM (2002) Results of performance, carcass yield and meat quality traits of f2 crosses between brazilian native and commercial pigs for QTL mapping. In: Proceedings Word Congress in Genetics and Animal Livestock Production, v. 32:37-40, 17-23 August 2002, Montpellier, France.

Patapoutian A, Miner JH, Lyons GE and Wold B (1993) Isolated sequences from the linked MYF5 and MRF4 genes drive distinct patterns of muscle-specific expression in transgenic mice. Development 118:61-69.

Rothschild MF and Soller M (1999) Candidate gene analysis to detect traits controlling traits economic importance in domestic livestock. In: Proceedings Simposio Internacional de Genética e Melhoramento Animal, pp 219-242, 21-24 September 1999, Animal Science Department, UFV, Viçosa, MG.

Rychlik W (1993) Selection of primers for polymerase chain reaction. In: White BA (ed) Methods in Molecular Biology. Human Press, Totowa, pp 31-40.

te Pas MF, Verburg FJ, Gerritsen CL and de Greef KH (2000) Messenger ribonucleic acid expression of the MyoD gene family in muscle tissue at slaughter in relation to selection for porcine growth rate. Journal of Animal Science 78:69-77.

te Pas MF, Somillion A, Hardes FL, Verburg FJ, van der Bosch TJ, Galesloot P and Meuwissen TH (1999a) Genetic variation at the porcine MYF5 gene locus. Lack of association with meat production traits. Mammalian Genome 10:123-127.

te Pas MF, Somillion A, Hardes FL, Verburg FJ, van der Bosch TJ, Galesloot P and Meuwissen TH (1999b) Influences of myogenin genotypes on birth weight, growth rate, carcass weight, backfat thickness, and lean weight of pigs. Journal of Animal Science 77:2352-2356.

Vianna AT (1985) Os Suínos. 14ª edição. Editora Nobel, São Paulo, 384 pp.

Vykoukalova Z, Knoll A, Dvorak J, Rohrer GA and Cepica S (2003) Linkage and radiation hybrid mapping of the porcine Myf 6 gene to chromosome 5. Animal Genetics 34:238.

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